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Associative diazotrophic bacteria inoculated in sugarcane cultivars: implications on morphophysiological attributes and plant nutrition

Dalliane Nogueira de Souza Lira[(1)], **Adriana Miranda de Santana Arauco**[(1)*], **Cácio Luiz Boechat**[(1)], **Mara Regina Moitinho**[(2)], **Julian Junio de Jesus Lacerda**[(1)] **and Elaine da Costa Martins**[(1)]

[(1)] Universidade Federal do Piauí, Departamento de Agronomia: Solos e Nutrição de Plantas, *Campus* Professora Cinobelina Elvas, Bom Jesus, Piauí, Brasil.

[(2)] Centro Nacional de Pesquisa em Energia e Materiais, Laboratório Nacional de Biorrenováveis, Campinas, São Paulo, Brasil.

*** Corresponding author:**
E-mail: adrianamsarauco@gmail.com

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ABSTRACT: Sugarcane is one of the first activities of economic importance in Brazil. The understanding of the nutritional dynamics at different phenological stages of the sugarcane crop with the use of nitrogen-fixing bacteria has been one of the alternatives to decrease fertilizer consumption and increasing plant production. This study aimed to assess the morphophysiological attributes in the initial growth and nutrition of two sugarcane varieties inoculated with strains of diazotrophic bacteria, individually and in an association, in a greenhouse. The experimental design was a randomized block design in a 2 × 8 factorial arrangement with two cultivars (Co 1341-76 and RB 867515) and eight treatments consisting of bacteria inoculated individually with BR 11140^T (*Azospirillum amazonense*), BR 11175^T (*Herbaspirillum seropedicae*), BR 11192^T (*Herbaspirillum rubrisubalbicans*), BR 11281^T (*Gluconacetobacter diazotrophicus*), and BR 11366^T (*Burkholderia tropica*), inoculation associated with the studied bacteria, N fertilization with 120 kg ha^{-1} of N, and absolute control without inoculation and nitrogen fertilization. Plants were conducted for 75 days after germination, and biometric variables and nutrient accumulation were measured. The sprouting rate index and root fresh matter were significant for inoculation ($p<0.01$). Inoculation with the strains BR 11192^T (*H. rubrisubalbicans*), BR 11281T (*G. diazotrophicus*), and RB 11366^T (*B. tropica*) showed the best results for root fresh matter regardless of the cultivar. The strains RB 11366^T (*B. tropica*), BR 11192^T (*H. rubrisubalbicans*), and BR 11175^T (*H. seropedicae*) stood out regarding the sprouting rate index. Inoculation showed a significant effect for stem diameter ($p<0.01$), especially the strains BR 11140^T (*A. amazonense*) and RB 11366^T (*B. tropica*). All inoculations were efficient for the biometric variable related to leaf length. Nitrogen, Fe, and Zn contents showed a significant effect of the interaction between cultivars and inoculation. The inoculation with the strain RB 11366^T (*B. tropica*), total N fertilization, followed by the other inoculations and the control stood out compared to the cultivar Co 1341-76 within the factor inoculation, indicating that the association of this strain with the cultivar Co 1341-76 is more efficient regarding the recommended commercial N dose for sugarcane. The cultivars Co 1341-76 and RB 867515 responded positively to the individual inoculation of strains of diazotrophic bacteria. The strains promoted improvements in some morphological and/or nutritional attributes of sugarcane plants. The strain *B. tropica* showed the best interaction with the tested cultivars for the biological N fixation, resulting in gains of biomass productivity and nutrient contents when not inoculated.

Keywords: biological nitrogen fixation, growth-promoting bacteria, *Saccharum* spp.

INTRODUCTION

In the agricultural context, Brazil stands out as the world's largest sugarcane (*Saccharum* spp.) producer, with a production of about 626 million tons and a harvested area of 8.6 million hectares, distributed in all producing states (Conab, 2018). Sugar and ethanol productions are expected to reach 35 million tons and 28 billion liters, respectively (Conab, 2018). In this context, the use of chemical fertilizers is essential for agricultural production. However, their costs and limited availability, as well as associated environmental problems, create an urgent need to find alternative strategies to reduce the environmental impact and production costs of chemical fertilizers (Majeed et al., 2015; Chaves et al., 2019).

Sugarcane extracts large amounts of nutrients from soils, especially potassium (K) and nitrogen (N). Brazilian sugarcane cultivars are estimated to extract approximately 260 kg ha^{-1} of total N at the plant-cane phase and 120 kg ha^{-1} at the ratoon phase (Vitti et al., 2011; Baptista et al., 2014). However, in general, these cultivars present a low response to mineral N fertilizers, with an average application of 60-70 kg ha^{-1} yr^{-1} of N in sugarcane plantations (Urquiaga et al., 2012; Oliver and Silva, 2018). This low response is related, among other factors, to associations that occur between sugarcane and N-fixing bacteria.

A large diversity of diazotrophic bacteria can establish an association with sugarcane, including strains of the genera *Azospirillum*, *Burkholderia*, *Herbaspirillum*, *Gluconacetobacter*, and *Stenotrophomonas* (Baldani and Baldani, 2005; Silva et al., 2016). *Gluconacetobacter diazotrophicus* is an endophytic diazotrophic bacterium isolated from sugarcane roots and stems (Cavalcante and Döbereiner, 1988). According to Radwan et al. (2004), strains of *Azospirillum amazonense* produce indole compounds such as auxins. The genus *Herbaspirillum* is considered obligatory and endophytic, besides having a low survival in the soil (Baldani et al., 2009). *Burkholderia tropica* colonizes the root surface and, subsequently, intercellular spaces, being mainly found in Brazilian sugarcane fields (Baldani et al., 1996).

Most of these associative diazotrophic bacteria are also referred to as plant growth-promoting rhizobacteria (PGPR). In addition to the biological N fixation (BNF), some of these bacteria present other benefits to plants, such as the production of plant growth regulator (auxins), solubilization of inorganic phosphates, siderophore production, and/or action as control agents (Carvalho et al., 2011; Santi et al., 2013; Cassán et al., 2014; Pii et al., 2015; Schlemper et al., 2018).

The contribution of BNF to the N nutrition of the crop is quite variable, reaching at least 40 kg ha^{-1} of N in field experiments (Urquiaga et al., 2012). This variation may be correlated with plant genotype, altitude, temperature, humidity, and especially soil fertility conditions (Oliveira et al., 2006; Schultz et al., 2017; Lopes et al., 2019). However, BNF assessments using the selection of efficient strains for N supply to various sugarcane genotypes and adapted to different edaphoclimatic conditions could further reduce the doses of commercial nitrogen fertilizers in the sugarcane field, making the crop more competitive in the market.

Sugarcane inoculation with diazotrophic bacteria is an innovative technology, with which it is expected to increase crop productivity. Studies have shown that sugarcane can be benefited both from individual inoculation and from co-inoculation with a mixture of diazotrophic bacteria (Baldani and Baldani, 2005; Pereira et al., 2013; Gírio et al., 2015; Schultz et al., 2014, 2017). Schultz et al. (2014) studied the inoculation of a mixture of diazotrophic bacteria of the genera *Azospirillum*, *Burkholderia*, *Herbaspirillum*, and *Gluconacetobacter* in sugarcane and verified an increase in its productive potential, indicating a possible reduction of N fertilization, which would result in profit for the sugarcane industry and environment preservation because it is an alternative product with a low cost and environmental risk.

Studies that make feasible the use of inoculation in the sugarcane crop is timely and should be conducted under different environments and production systems to contribute to the construction of safe recommendations regarding this practice, being essential the diffusion of this low-cost biotechnology. We hypothesize that inoculation with diazotrophic bacteria improves growth, physiology, and nutrition of sugarcane cultivars under the growing conditions of the semiarid region of Piauí, which has a warm and humid climate. Therefore, this study aimed to assess the morphophysiological attributes in the initial growth and nutrition of two sugarcane cultivars inoculated with strains of diazotrophic bacteria, individually and in an association.

MATERIALS AND METHODS

Study area and experimental design

The plants were grown in a greenhouse from July to September 2016 in Bom Jesus, Piauí, Brazil. This municipality is part of the semiarid region of Piauí, with a warm and humid climate, classified by Köppen classification system as Awa, i.e., a rainy tropical climate with a dry season in the winter and temperature in the warmest month of 22 °C (Andrade Júnior et al., 2004). The area is located at the geographic coordinates 09° 04' 28" S and 44° 21' 31" W, with an average altitude of 277 m, average precipitation between 900 and 1200 mm yr^{-1}, and an average temperature of 26.2 °C (Inmet, 2016).

The experimental design was a randomized block design in a 2 × 8 factorial arrangement with two cultivars (Co 1341-76 and RB 867515) and eight treatments: individual inoculation of five bacterial strains provided by the Johanna Döbereiner Biological Resource Center (CRB-JD) - Embrapa Agrobiology Seropédica-RJ, Brazil (BR 11140 – *Azospirillum amazonense*, BR 11175 – *Herbaspirillum seropedicae*, BR 11192 – *Herbaspirillum rubrisubalbicans*, BR 11281 – *Gluconacetobacter diazotrophicus*, and BR 11366 – *Burkholderia tropica*), inoculation of a mixture of these five isolated strains, N fertilization at a dose of 120 kg ha^{-1}, and absolute control without inoculation and nitrogen fertilization.

The cultivars Co 1341-76 and RB 867515 were selected for their adaptability characteristics in the region, in addition to their high yield, richness, precocity, and excellent sprouting (Hoffmann et al., 2008).

The soil used in the experimental test was collected from a native area in the 0.00-0.20 m layer after removing the litter and surface organic layer. Subsequently, the soil sample was air-dried, sieved in a 2-mm mesh sieve, homogenized, and chemically and physically characterized. The soil was classified as *Latossolo Amarelo distrófico* (Santos et al., 2013), which corresponds to Oxisol (Ustox, Soil Survey Staff, 2014) and presented the following characteristics: pH(H_2O) = 5.3, pH(KCl) = 4.91, K = 88 mg kg^{-1}, P = 28.18 mg kg^{-1}, Ca^{2+} = 1.6 $cmol_c$ dm^{-3}, Mg^{2+} = 0.2 $cmol_c$ dm^{-3}, Al^{3+} = 0.1 $cmol_c$ kg^{-1}, potential acidity (H+Al) = 2.32 $cmol_c$ kg^{-1}, sum of bases (SB) = 2.03 $cmol_c$ kg^{-1}, CEC effective (t) = 2.13 $cmol_c$ kg^{-1}, CEC potential (T) = 4.35 $cmol_c$ kg^{-1}, base saturation (V%) = 46 %, m = 4 %, OM = 20 g kg^{-1}, Cu = 0.28 mg kg^{-1}, Mn = 3.04 mg kg^{-1}, Fe = 61.21 mg kg^{-1}, Zn = 2.29 mg kg^{-1}, clay = 200 g kg^{-1}, silt = 20 g kg^{-1}, and sand = 780 g kg^{-1}.

Soil fertility correction was performed based on the recommendations for the use of correctives and fertilizers (van Raij et al., 1996). Liming was performed using 712 kg ha^{-1} of dolomitic limestone (85 % of total neutralizing power) at 90 days before planting to raise base saturation to 60 %. All the experimental units received phosphate and potassium fertilization at a dose of 177.5 kg ha^{-1} of potassium chloride (62 % K_2O) and 526 kg ha^{-1} of single superphosphate (18 % Ca, 11 % S, and 19 % of P_2O_5) at planting, split at equal doses (one at planting and the other 20 days after emergence) and incorporated into 6 cm of depth. The source used in the nitrogen fertilization was urea (45 % N), which was only carried out in the treatment with nitrogen fertilization, with an application of 120 kg ha^{-1} of N split into two doses of 60 kg (one at planting and the other 20 days after germination).

The 64 experimental units consisted of plastic containers with a capacity of 20 kg of soil. These containers were drilled to facilitate the drainage of excess water and keep a 70 % soil water retention capacity. Five 1-bud setts (mini-cuttings) were planted at each container, and thinning was performed at 15 days after planting, leaving the two more vigorous plants.

Preparing and inoculation

The inoculum was prepared from colonies isolated from each strain, which were individually cultured in test tubes with specific liquid culture media. The strain BR 11140 was cultured in LGI medium, BR 11175 and BR 11192 in 3x NFB medium, BR 11281 in LGI-P medium, and RB 11366 in JMV medium, under stirring at 175 rpm and 30 °C for 24 h (Döbereiner et al., 1999). The tubes were incubated at 30 °C for seven days, being considered positive for counting those with typical aerobic films near the culture medium surface. The counting of the population of diazotrophic bacteria was performed by the most probable number (MPN) technique using the McCrady table for the triplicates, by dilution.

Bacterium population in the inoculants for the application was estimated at approximately 10^8–10^9 cells mL^{-1}. Subsequently, 75 mL of the inoculum of bacterial suspension was added to 175 g of peat (sterilized and neutralized) and packed in 0.05 mm polyethylene plastic bags. The bags with peat were maintained at 30 °C for seven days. The suspension for inoculation was prepared according to Oliveira et al. (2006), with a dose of 1,250 g of inoculant at a volume of 100 L of distilled water.

Inoculation was carried out at planting time using the previously selected five 1-cm bud setts (mini-cuttings). The mini-cuttings were immersed into the inoculant for 30 min and then transferred to plastic containers filled with 18 kg of non-autoclaved soil.

Morphophysiological attributes

Sprouting was assessed daily and considered when the bud protruded the substrate surface. The germination rate index of Maguire (1962), here called as sprouting rate index (SRI), was obtained from daily sprouting count, using the following equation: SRI = (B1/N1 + B2/N2 + B3/N3 + Bn/Nn), where Bn is the number of buds computed in the n counts (Gírio et al., 2015).

Plant height (PH) (cm) was measured with a graduated ruler from the plant base to the insertion of the leaf +1. Leaf length (LL) (cm) was measured with a graduated ruler on the leaf +3. Stem diameter (SD) (mm) was measured with a caliper at the plant base. Stem length (SL) (cm) was measured with a graduated ruler. These variables were measured at 75 days after germination.

In the destructive quantitative analyses, plants were removed from plastic containers, and the shoot was separated from roots with a cut close to the soil surface. The shoot was washed in running water to remove dust particles and the roots to remove soil excess, being then weighed, to obtain the stem fresh matter (StFM) and root fresh matter (RFM). These plant materials were dried in an oven at 65 °C until constant weight to obtain the shoot dry matter (SDM).

Plant nutrition

Samples from the shoot (leaves and stem) were processed in a Willy mill. Nitrogen content was determined by sulfur digestion, and the other nutrients were extracted by nitro-perchloric digestion. The contents of copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn) were determined by atomic absorption spectrophotometry (Silva, 2009).

Data processing and analysis

The data were initially submitted to the multivariate exploratory analyses of hierarchical clustering and principal components, as the use of multivariate analysis allows selecting only the most expressive variables (attributes), that is, variables with the highest percentage of correlation and behavior characterization of the treatments under study (cultivars and inoculated bacterial strains).

In addition to the criterion of non-collinearity, only attributes with factor loads higher than 0.60 in absolute value were selected. For the clustering analysis, the similarity matrix was constructed with the Euclidean distance, and groups were linked using Ward's method (Hair et al., 2005). Hotelling's T^2 test was performed to test whether there was a significant difference between groups observed in the dendrogram.

The principal component analysis considered principal components whose eigenvalues were higher than the unity, as the criterion established by Kaiser (1958). The coefficients of linear functions that define the principal components were used in the interpretation of their meaning, using the signal and relative size of coefficients as an indication of the weight to be assigned for each variable. After the selection and standardization of variables (null mean and unit variance), the analysis was processed in the software Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA).

The data were also submitted to analysis of variance after the variables were selected, and multivariate exploratory analysis was performed. Also, the variables were submitted to the Scott-Knott test as a function of the significance level ($p \leq 0.05$) found in the F-test. These analyses were conducted using the software R (R Development Core Team, 2016).

RESULTS

Relationship of the interdependence between treatments and characteristics of the plant inoculated

Thirteen attributes selected by the multivariate analyses were analyzed in this study: shoot dry matter (SDM), fresh stem matter (StFM), root fresh matter (RFM), sprouting rate index (SRI), stem diameter (SD), length of the leaf 3+ (LL), stem length (SL), plant height (PH), and contents of N, Cu, Mn, Fe, and Zn.

This result indicates that these 13 variables were sufficient to identify the natural correlations and multiple influences of treatments (cultivars and associative bacteria) on the morphophysiological behavior and sugarcane nutrition.

When the multivariate clustering analysis was carried out by the hierarchical method, the dendrogram constructed from samples of biometric variables and nutrient concentration in the plant as a function of treatments showed the existence of possible patterns of groupings, in which there is evidence of separation into two groups according to the cultivar under study (Figure 1). Hotelling's T^2 test was performed to verify whether the groups composed of the cultivars are similar or dissimilar, in which a significant effect ($F = 107.23$; $p = 0.001$) was observed, confirming that the cultivars Co 1341-76 and BR 867515 have distinct characteristics.

A complementary analysis of principal components was performed aiming a better understanding of the clustering analysis. The same groups can be observed in the biplot graph (Figure 2). In addition, the variables responsible for characterizing the distinctions between groups were added to the graph, and there is a more apparent separation in which the effect of inoculations and N source can also be verified on the cultivars.

Figure 2 shows the biplot graph with the first two principal components (PC1 and PC2), and the variance assigned to them is shown in table 2. The PC1 represents 55.4 % of the total variance of the original data, and the PC2 represents 24.2 % of this variance (Figure 2). All the morphological attributes and manganese (Mn) were retained in PC1, and all other nutrients of the plant shoot and the sprouting rate index (SRI) were retained in PC2, according to the cut-off criterion (higher than 0.60 in value absolute) considered for interpreting each principal component (Table 1).

All variables retained in PC1 and PC2 were directly associated with each other according to their respective signals at each component (Table 2). Considering that PC1 and PC2 are independent considering their orthogonality, we can conclude that the morphophysiological variables are directly associated with the Mn content found in the plant shoot and that these morphophysiological characteristics are not related to the other micronutrient contents (Fe, Cu, and Zn) and N. Furthermore, SRI is directly associated with micronutrients and N concentration of the plant (Table 1 and Figure 2).

The direction of the arrows with the variables as a function of the direction of the dispersion of treatments shows that the cultivar BR 867515 presented the best response relative to the morphophysiological attributes compared to the cultivar Co 1341-76, regardless of the inoculations with bacteria or N source (Figure 2).

Among the treatments applied in the cultivar Co 1341-76, the inoculation with the strain RB 11366^{T} (*B. tropica*) stood out because it presented the highest contents

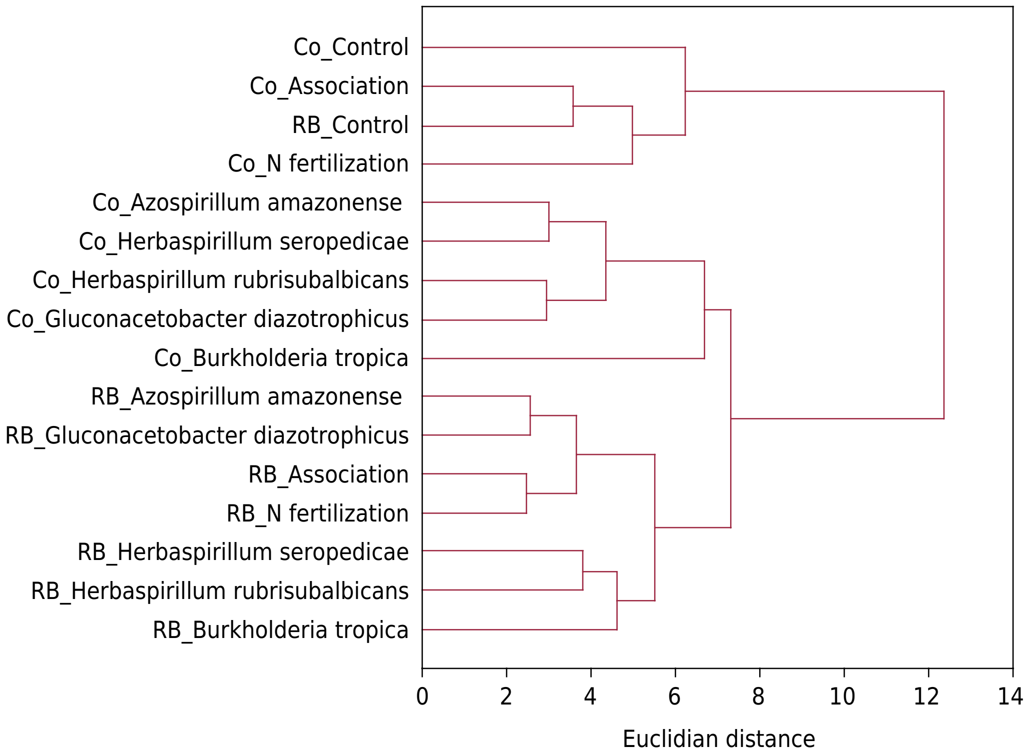

Figure 1. Dendrogram showing the hierarchy of groups (cultivars and associative bacteria) resulting from the clustering analysis by the hierarchical method.

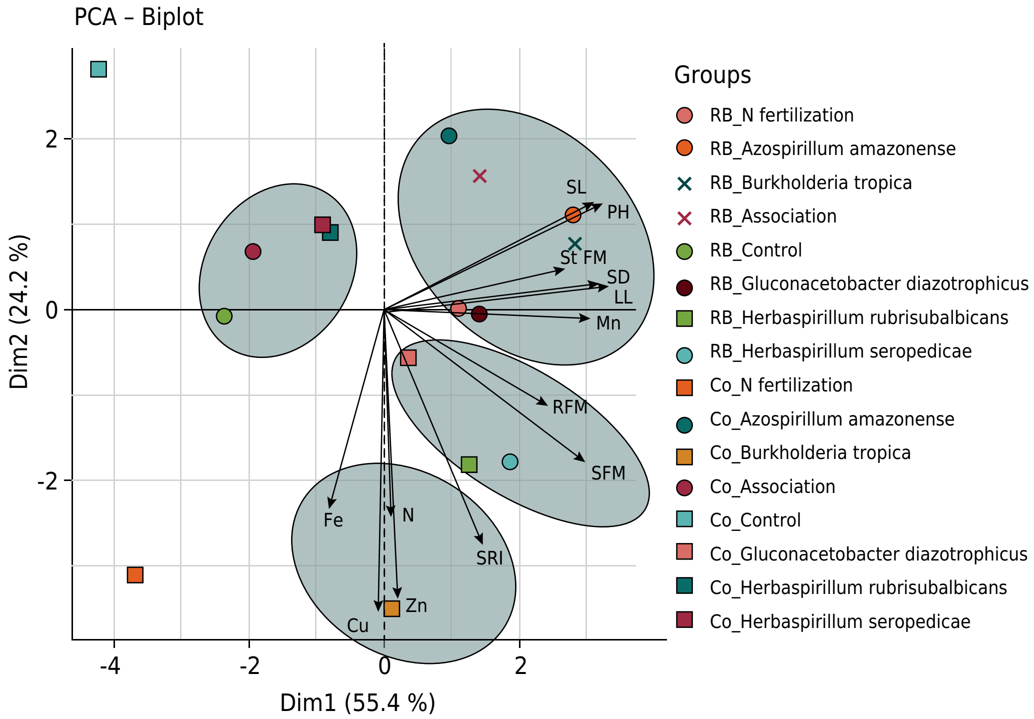

Figure 2. Biplot graph with the morphophysiological attributes, plant nutrients, and assessed treatments. SFM: shoot fresh matter; StFM: stem fresh matter; RFM: root fresh matter; SRI: sprouting rate index; PH: plant height; SD: stem diameter; LL: length of the leaf +3; SL: stem length; N: nitrogen; Fe: iron; Cu: copper; Zn: zinc; and Mn: manganese.

Table 1. Correlation between morphophysiological parameters and nutrients of sugarcane plants and the first two principal components (PC1 and PC2)

Principal component	PC1	PC2
Explained variance (%)	**55.4***	**24.2***
Correlation		
SDM	**−0.74**	0.44
StFM	**−0.66**	−0.12
RFM	**−0.61**	0.28
SRI	−0.36	**0.68**
SD	**−0.79**	−0.08
LL	**−0.82**	−0.07
SL	**−0.77**	−0.31
PH	**−0.80**	−0.31
N	−0.02	**0.60**
Cu	0.02	**0.88**
Mn	**−0.76**	0.03
Fe	0.20	**0.61**
Zn	−0.05	**0.85**

* Value referring to the percentage of variation of the original set of data retained by the respective principal components. Correlations in bold (higher than 0.60 in absolute value) were considered in the interpretation of the principal component. SDM: shoot dry matter; StFM: stem fresh matter; RFM: root fresh matter; SRI: sprouting rate index; SD: stem diameter; LL: length of the leaf 3+; SL: stem length; PH: plant height; N: nitrogen; Cu: copper; Mn: manganese; Fe: iron; and Zn: zinc.

of the micronutrients Fe, Cu, and Zn, in addition to N, also presenting the highest SRI. Treatments with the control of the cultivar BR 867515 and with the cultivar Co 1341-76 in association and inoculated with the strains BR 11175^T (*H. seropedicae*) and BR 11192ᵀ (*H. rubrisubalbicans*) had the lowest values of fresh stem matter, stem diameter, stem length, leaf length, manganese concentration, and hence a lower plant height among the other treatments under study (Figure 2).

All the assessed micronutrients were selected by the multivariate analysis, but only N was selected among the macronutrients. It indicates that the inclusion of these elements is important in studies aiming at understanding the behavior of sugarcane cultivars as a function of the inoculation with diazotrophic bacteria because they are more relevant in the analysis, which is verified by their load values in their respective principal components (Table 1).

Effect of varieties and strains of diazotrophic bacteria on morphophysiological parameters and plant nutrition

The analysis of variance (ANOVA) for attributes selected by the multivariate analysis showed that shoot dry matter (F = 22.18; p = 0.01) and plant height (F = 4.35; p = 0.03) were significant only in relation to cultivars (Figures 3a and 3b). Thus, regardless of the inoculant or N fertilization, the cultivar RB 867515 presented a higher height, consequently reflecting in a higher shoot dry matter compared to the cultivar Co 1341-76, corroborating the results presented in the biplot graph (Figure 2).

The sprouting rate index (F = 2.50; p = 0.03) and root fresh matter (F = 4.86; p<0.01) were significant for inoculation. The inoculation with the strains BR 11192ᵀ (*H. rubrisubalbicans*),

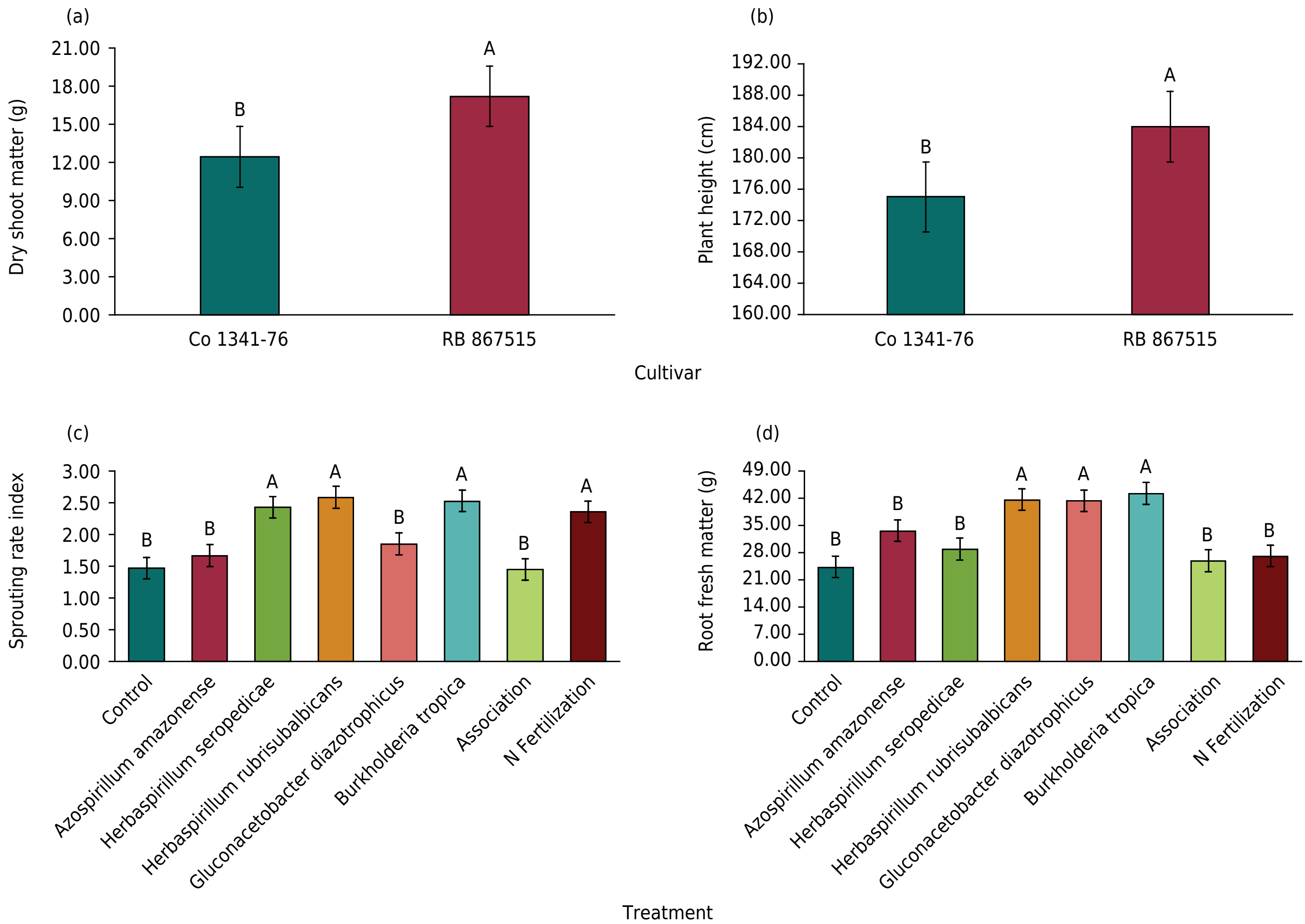

Figure 3. Means and standard error of dry shoot matter (a), plant height (b), sprouting rate index (c), and root fresh matter (d) of the cultivars BR 867515 and Co 1341-76 as a function of treatments. Means followed by the same letter do not differ from each other by the Scott-Knott test at a 5 % probability level.

BR 11281^T (*G. diazotrophicus*), and RB 11366^T (*B. tropica*) showed the best results for root fresh matter regardless of the cultivar (Figure 3d). The strains RB 11366^T (*B. tropica*), BR 11192^T (*H. rubrisubalbicans*), and BR 11175^T (*H. seropedicae*) (Figure 3c) stood out for the sprouting rate index (Figure 2).

Biometric variables related to stem measures (fresh matter, length, and diameter) stood out compared to the others (Figures 3 and 4), indicating that they should be considered in studies of this nature, with more responsive characteristics. The fresh matter (F = 12.63; p = 0.04) and stem length (F = 4.35; p = 0.03) were higher when the cultivar RB 867515 was assessed (Figures 4a and 4b), regardless of the inoculations.

However, the inoculation showed a significant effect for stem diameter (F = 4.21; p<0.01), especially the strains BR 11140 (*A. amazonense*) and RB 11366^T (Figure 4c). Moreover, all inoculations were efficient for the biometric variable related to leaf length (F=7.60; p<0.01) (Figure 4d).

Nitrogen (F = 141.97; p<0.01), iron (F = 15.71; p<0.01), and zinc contents (F = 5.13; p<0.01) showed a significant effect of the interaction between cultivars and inoculation (Figures 5a, 5b, and 5c, respectively). Copper (F = 7.88; p = 0.02) showed an effect only for inoculation, while manganese showed an effect for cultivar (Figures 6a and 6b, respectively). The cultivar RB 867515 was more efficient regarding the nitrogen assimilated by the plant shoot than the cultivar Co 1341-76 in all assessed inoculations, as well as in the total N fertilization and control.

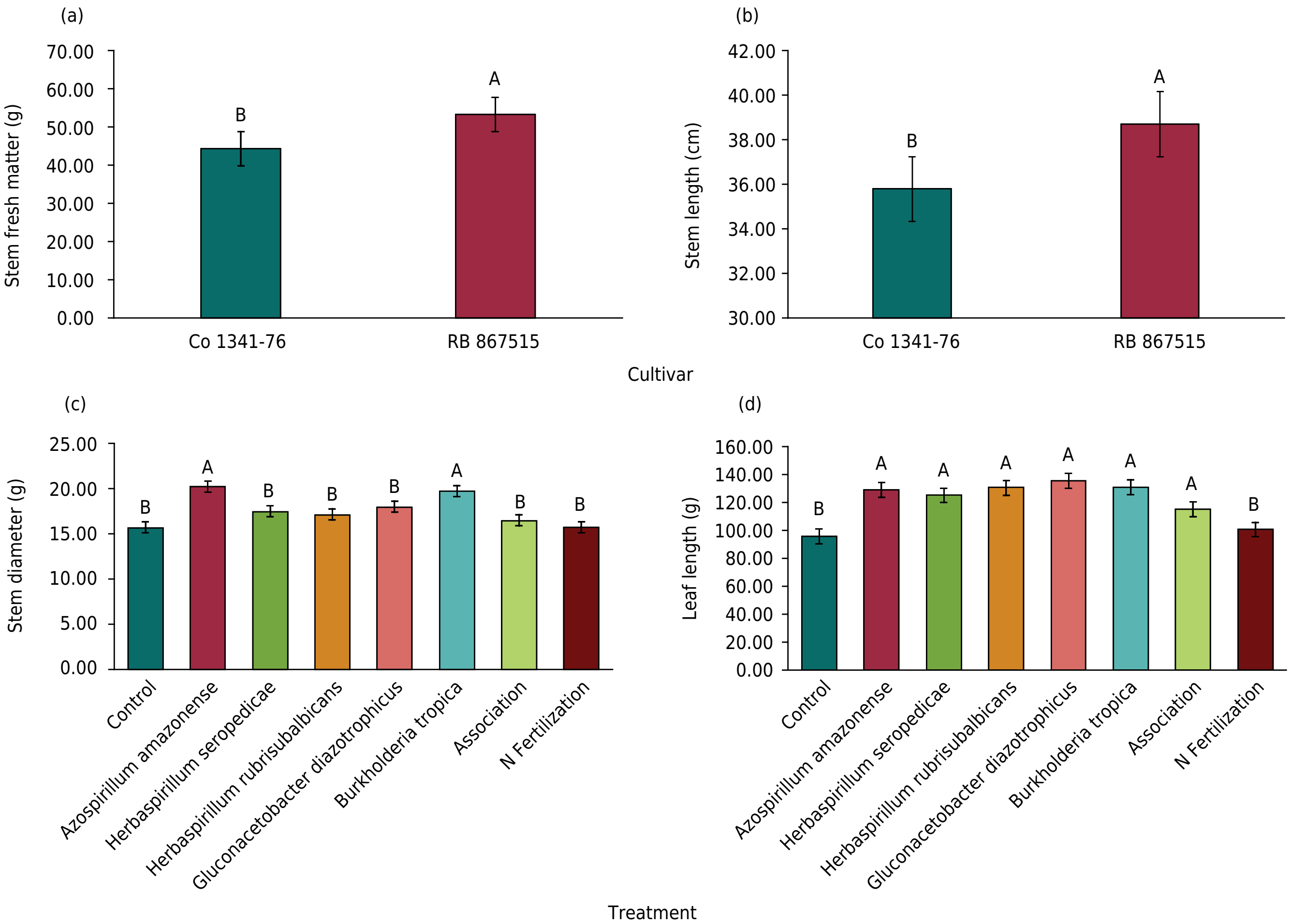

Figure 4. Means and standard error of stem fresh matter (a), stem length (b), stem diameter (c), and leaf length (d) of the cultivars BR 867515 and Co 1341-76 as a function of treatments. Means followed by the same letter do not differ from each other by the Scott-Knott test at a 5 % probability level.

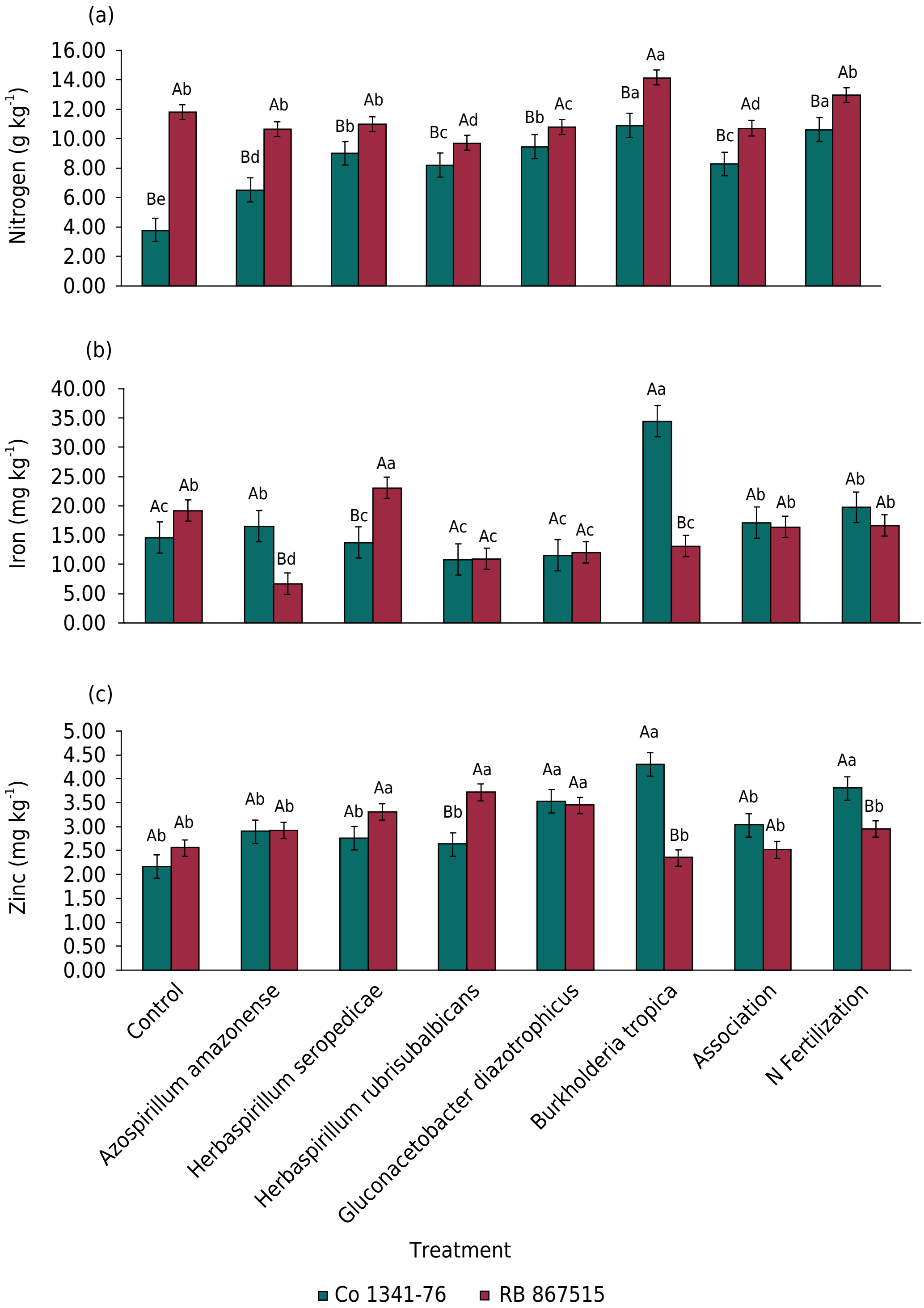

Figure 5. Means and standard error of nitrogen (a), iron (b), and zinc contents (c) in the plant shoot as a function of treatments. Means followed by the same letter do not differ from each other by the Scott-Knott test at a 5 % probability level. Means of the interactions followed by the same uppercase (cultivar) and lowercase letters (inoculations) do not differ from each other by the Scott-Knott test at a 5 % probability level.

The inoculation with the strain RB 11366T (*B. tropica*), total N fertilization, followed by the other inoculations and the control stood out compared to the cultivar Co 1341-76 within the factor inoculation, indicating that the association of this strain with the cultivar Co 1341-76 is more efficient regarding the recommended commercial N dose for sugarcane. In addition, the strain RB 11366^T (*B. tropica*) stood out for the cultivar RB 867515 within the factor inoculation (Figure 5a). Similar behavior was observed for iron (Figure 5b) and zinc (Figure 5c) contents but considering the cultivar Co 1341-76.

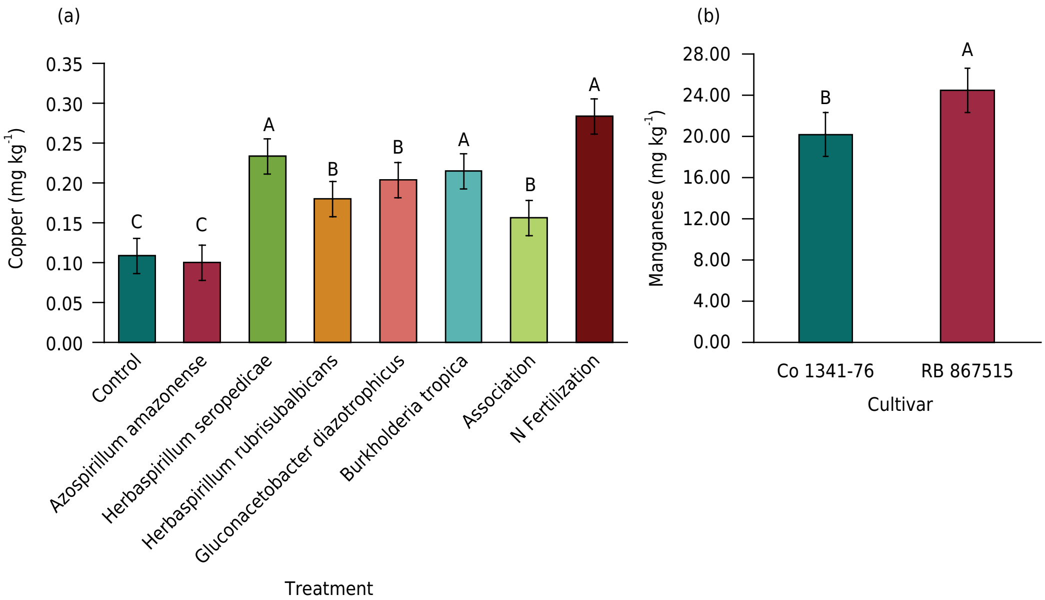

Figure 6. Means and standard error of copper (a) and manganese contents (b) in the plant shoot as a function of treatments. Means followed by the same letter do not differ from each other by the Scott-Knott test at a 5 % probability level.

A significant effect (F = 7.91; p<0.01) was observed for the micronutrient copper regarding the factor inoculation, with the highest values found in the inoculation with BR 11175^T (*H. seropedicae*) and RB 11366^T (*B. tropica*), not differing from the treatment composed of the total N fertilizer (Figure 6a). Moreover, a significant difference was only observed for the cultivar regarding the manganese content (F = 13.87; p<0.01), with the highest average values observed in plants of the cultivar RB 867515, as also verified in the multivariate analysis of principal components (Figure 2).

DISCUSSION

The characterization of a pattern or behavior depends on different interactions between the assessed factors. In this context, data multivariate analyses can be very efficient, allowing visualizing the natural correlations and multiple influences on behavior, especially when using multivariate techniques of interdependence, i.e., when no variable or group is treated as dependent or independent (Hair et al., 2005). Results obtained with multivariate exploratory analyses of clustering and principal components were corroborated, in general, by the analysis of univariate variance (ANOVA).

The results showed that an expressive group of morphophysiological variables was affected both by cultivars and by inoculations applied to them. In this context, the micronutrients deserve special attention since those assessed in this study were selected by the multivariate analysis and presented a strong relevance (Table 1), as well as the biometric variables related to plant stem measures (stem fresh matter, stem length, and stem diameter).

According to Cassán et al. (2014), changes in plant growth and transformation can be provided by the hormonal contribution of bacteria associated with it, mainly in the auxin, cytokinin, gibberellin, and ethylene production, which lead to plant growth. In addition, inoculation promotes biomass gains, with a different contribution between cultivars and inoculated strains, suggesting an interaction between these factors (Pereira et al., 2013).

In this study, the variable root fresh matter was selected by the multivariate analysis (Figure 2), indicating its expressiveness in the characterization of the behavior of cultivars as a function of inoculations (Figure 3d). According to Gosal et al. (2012), plants inoculated with diazotrophic bacteria present modifications in the root system architecture, which facilitates nutrient absorption since this characteristic is related to the synthesis of hormones that alter root morphology, favoring the increase of lateral roots and root hairs (Santi et al., 2013).

Other authors have already observed significant gains of biomass in sugarcane with the individual inoculation of diazotrophic strains. Marques Júnior et al. (2008) inoculated *H. seropedicae* in mini-cuttings of the cultivar RB72454 and observed a significant increase of root and shoot dry matter at 45 days after cultivation under greenhouse conditions. In our study, shoot dry matter was not influenced by inoculation, but only by the cultivar (Figure 3a). In a similar study, Schultz et al. (2014) used the joint inoculation of five strains of diazotrophic bacteria and different N doses in two commercial cultivars of sugarcane under field conditions and observed no significant differences for shoot dry matter in plant cane and ratoon.

The sprouting rate index (SRI) was positively influenced by individual inoculation with the strains RB 11366^T (*B. tropica*), BR 11192^T (*H. rubrisubalbicans*), and BR 11175^T (*H. seropedicae*) (Figure 3c). Landell et al. (2012) also observed an increase in the sprouting rate of buds provided by the inoculation of diazotrophic bacteria, favoring the emission of roots in sugarcane stems with the pre-sprout planting system.

The influence of individual inoculation with diazotrophic species on the sprouting of sugarcane seedlings was also assessed by Chaves et al. (2015), who used the cultivars RB867515 and IACSP95-5000. The authors found the highest sprouting rates indices for inoculation with the strains BR11366^T (*B. tropica*) and BR11504 (*H. rubrisubalbicans*), corroborating the results obtained in our study (Figure 3c). Suman et al. (2005) observed similar results with the inoculation of the diazotrophic species *G. diazotrophicus* in the cultivar CoSe92423 and obtained significant increases in the percentage of germination.

In our study, stem diameter showed the highest means regardless of the assessed cultivar in the inoculation treatments with the strains BR 11140 (*A. amazonense*) and RB 11366^T (*B. tropica*) (Figure 4c). Stem length and plant height, on the other hand, were influenced by plant genotype, standing out the cultivar RB 867515 for both variables (Figures 4b and 3b, respectively). These results demonstrate the difficulty of the phenotypic selection of responsive cultivars to the inoculation with diazotrophic bacteria, and that the promoted responses are variable for strains and cultivar (Pereira et al., 2013).

In addition to biological nitrogen fixation, many associative diazotrophic bacteria can act in the synthesis of plant growth, regulating substances, and other processes (Santi et al., 2013; Chaves et al., 2015; Schlemper et al., 2018). The production of indole acetic acid (IAA) by the strains used in our study (*G. diazotrophicus*, *H. seropedicae*, *H. rubrisubalbicans*, *B. tropica*, and *A. amazonense*) was recently reported by Chaves et al. (2015).

Possibly, the strains used in this study have a different BNF capacity and IAA production, in addition to probably acting on other biological processes with different intensities, which may justify, at least in part, their differentiated behavior on some assessed attributes in sugarcane. These processes were not analyzed in this study, not allowing determining the effects that resulted in the improvement of the individual inoculation.

Even though variations are observed between responses and interactions between bacteria and sugarcane genotypes, several groups of bacteria have shown a significant contribution when inoculated due to BNF capacity or benefits such as growth promotion. However, endophytic bacteria may have an advantage over the bacteria that inhabit the rhizosphere since they live inside the plant tissue, which represents an opportunity to be in constant contact with plant cells and hence to exercise more easily a direct beneficial effect (Santoyo et al., 2016).

The significant interaction between cultivars and inoculations tested in our study for nitrogen content and the micronutrients iron and zinc of the plant shoot indicates that their efficiency of assimilation by plants depends on a combined effect between these two factors. Nitrogen is probably the key factor for the association of diazotrophic bacteria with sugarcane, and plants show pronouncedly thin, commonly woody stems when nitrogen deficiency develops slowly. This woody characteristic provides an excess of carbohydrate accumulation, which cannot be used in the synthesis of amino acids or other nitrogen compounds (Hawkesford et al., 2012).

The best N utilization was observed in both cultivars when inoculated with the strain RB 11366^T (*B. tropica*), indicating that this strain has better interaction with the tested cultivars for BNF although it has not influenced important characteristics such as shoot dry matter, plant height, stem fresh matter, and stem length. The experimental period was possibly not sufficient for a better response of these parameters to inoculation.

The strain RB 11366^T (*B. tropica*) provided a higher N content when compared to that obtained with the treatment of mineral N in the cultivar BR 867515, but equal to the cultivar Co 1341-76, suggesting the possibility of the total replacement of the mineral nitrogen fertilization by the inoculation in fields of these sugarcane cultivars in the assessed soil. In general, an average of 60-70 kg ha^{-1} yr^{-1} of nitrogen is applied in the Brazilian sugarcane fields as a commercial nitrogen fertilizer source, increasing production costs (Urquiaga et al., 2012). The substitution of these fertilizers by inoculation will allow a great reduction in the production costs of the sugar-energy sector, besides preserving the environment.

Among the assessed micronutrients, only Mn was not influenced by treatments of inoculation with diazotrophic bacteria. A high variation was observed in relation to Fe and Zn contents, with alternations in lower and higher values among cultivars depending on the strain used for inoculation (Figure 5b). The strain RB 11366^T (*B. tropica*) stood out for both nutrients in the cultivar Co 1341-76, providing high and equal Fe and Zn contents, respectively, compared to the treatment fertilized with N. This strain, together with the strain BR 11175^T (*H. seropedicae*), provided Cu contents similar to the treatment fertilized with N and higher to the other treatments, regardless of the cultivar.

The increased micronutrient uptake by crops inoculated with diazotrophic bacteria may be related to several factors. These bacteria can promote the root system growth or lead to changes in its architecture depending on the phytohormone production, increasing the contact surface of the plant with soil nutrients and/or acting in the release of compounds that reflect in the increased availability of nutrients in the rhizosphere (Pii et al., 2015; Dinesh et al., 2018; Schlemper et al., 2018).

Some diazotrophic rhizospheric bacteria, for example, can synthesize siderophores, which assist plants to uptake iron ions in a sufficient quantity to supply the necessary demand for the development of both bacteria and host (Sheng et al., 2008; Santi et al., 2013). Some bacteria are also able to release compounds that solubilize zinc oxide, which increases its availability to plants (Saravanan et al., 2007; Dinesh et al., 2018).

CONCLUSIONS

The cultivars Co 1341-76 and RB 867515 responded positively to the individual inoculation of strains of diazotrophic bacteria at the initial vegetative growth of sugarcane, but the cultivar RB 867515 showed the best performance under local edaphoclimatic conditions.

All individually tested strains (BR 11366^T - *B. tropica*, BR 11192^T - *H. rubrisubalbicans*, BR 11140 - *A. amazonense*, and BR 11175^T - *H. seropedicae*) promoted improvements in some morphological and/or nutritional attributes of sugarcane plants. However, the strain *B. tropica* presented the best interaction with the tested cultivars for biological nitrogen fixation.

Inoculation with the studied strains provided a higher nutrient content, especially for nitrogen, zinc, and iron in the tested sugarcane cultivars.

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AUTHOR CONTRIBUTIONS

Conceptualization: Dalliane Nogueira de Souza Lira (equal), Adriana Miranda de Santana Arauco (equal, lead), and Cácio Luiz Boechat (equal).

Methodology: Dalliane Nogueira de Souza Lira (equal), Adriana Miranda de Santana Arauco (lead), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (supporting), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

Software: Dalliane Nogueira de Souza Lira (equal), Adriana Miranda de Santana Arauco (equal), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (supporting), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

Validation: Dalliane Nogueira de Souza Lira (supporting), Adriana Miranda de Santana Arauco (lead), Cácio Luiz Boechat (equal), Mara Regina Moitinho (supporting), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

Formal analysis: Dalliane Nogueira de Souza Lira (equal), Adriana Miranda de Santana Arauco (supporting), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (supporting), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

Investigation: Dalliane Nogueira de Souza Lira (supporting), Adriana Miranda de Santana Arauco (lead), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (supporting), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

Resources: Dalliane Nogueira de Souza Lira (equal), Adriana Miranda de Santana Arauco (lead), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (supporting), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

Data curation: Dalliane Nogueira de Souza Lira (supporting), Adriana Miranda de Santana Arauco (lead), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (supporting), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

Writing - original draft: Dalliane Nogueira de Souza Lira (supporting), Adriana Miranda de Santana Arauco (lead), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (supporting), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

Writing – review and editing: Dalliane Nogueira de Souza Lira (supporting), Adriana Miranda de Santana Arauco (lead), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (supporting), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

Visualization: Dalliane Nogueira de Souza Lira (supporting), Adriana Miranda de Santana Arauco (lead), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (equal), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

Supervision: Adriana Miranda de Santana Arauco (lead), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (equal), and Julian Junio de Jesus Lacerda (supporting).

Project administration: Adriana Miranda de Santana Arauco (lead).

Funding acquisition: Dalliane Nogueira de Souza Lira (supporting), Adriana Miranda de Santana Arauco (lead), Cácio Luiz Boechat (supporting), Mara Regina Moitinho (supporting), Julian Junio de Jesus Lacerda (supporting), and Elaine da Costa Martins (supporting).

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