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RESEARCH ARTICLE

Azospirillum brasilense and macronutrients in the initial establishment of brachiaria

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ABSTRACT

Pasture production needs sustainable technologies, associating correct fertilization techniques that allow its maintenance aiming at productivity for quality animal feed production. The objective was to evaluate the initial responses of the inoculation with *Azospirillum brasilense* associated to macronutrients in *Brachiaria brizantha* cv. 'Xaraés'. In pots filled with Oxisoil, with combinations of macronutrients as formulates (10-10-10; 20-10-10; 10-20-10; and 10-10-20) associated or not to inoculation with growth promoting bacteria *A. brasilense*, the experiment was conducted in a randomized blocks design, with four replicates. At 10, 20 and 30 days after emergence the plants were evaluated for number of leaves, length of the biggest leaf, aerial length, number of tillers and chlorophyll relative content. At 30 days after emergence, plants were evaluated for dry mass of roots and aerial part, root length, leaves content of nitrogen, phosphorus and potassium. The use of the formula 10-20-10, in the absence or presence of inoculation with *A. brasilense*, increased the leaves initial development of plants of *B. brizantha* cv. 'Xaraés'. The formula 10-10-10, in the absence and presence of bacterial inoculation, resulted in plants with lower foliar development up to 30 days after emergence.

Highlighted Conclusions

The nutritional availability influences in the initial development of seedlings of *B. brizantha* cv. 'Xaraés'.

INTRODUCTION

The cultivation of pastures in the Brazilian territory stands out due to the high demand from the cattle raising sector, especially in the Midwest (Dias et al. 2016). *Brachiaria* is a genus of Poaceae with herbaceous growth, native from Africa, introduced in Brazil as forages, but that can also be considered invasive plants in many ecosystems, especially in the Brazilian "cerrado" (Florindo et al. 2014). For the cattle raising sector, the brachiaria is a nutritive pasture, used in cattle feed, resulting in high quality animals, guaranteeing Brazil the position of the world's largest exporter of beef (Cezário et al. 2015, Peres et al. 2015, Dias et al. 2016).

Forages from the genus *Brachiaria* are used as a food source not only for cattle but also for buffaloes, sheep and goats due to its high forage production, fast and high regrowth capacity, and to its tolerance to adverse environmental conditions (cold, drought and fire), besides the persistence and late flowering (Florindo et al. 2014, Cezário et al. 2015). However, it has a high demand for fertile and drained soils, needing some regular fertilizations for the obtention of an average production of 21 tones of dry mass per hectare (Bonfim-Silva et al. 2014, Martins et al. 2014).

In the process of choice of a forage for the cattle raising farms, the speed of its development is essential. In rain or drought periods, plants from the genus *Brachiaria* have a high rates of growth and mean crude protein (Castro et al. 2013, Galzerano et al. 2013). Of average requirement, brachiarias are very responsive to doses of phosphorus, especially when managed with three cuts during its cycle, directly reflecting on the dry mass production, in especial under average conditions of rainfall and temperature (Castro et al. 2013, Oliveira et al. 2013).

In this sense, the use of organisms that optimize the use of both nutrients that are already on the soil as for the ones from inorganic or organic fertilization, stands out as a viable and sustainable alternative for these goals. Plant growth promoting bacteria are symbiont microorganisms capable of promoting growth through the nitrogen biologic

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fixation, solubilization of nutrients, production of phytohormones, among others (Guimarães et al. 2017). Among them, the bacteria *Azospirillum brasilense* stands out due to positive results when it was associated to grasses such as maize (Guimarães et al. 2014, Bulegon et al. 2017b), wheat (Fukami et al. 2016; Santos et al. 2017a), sugarcane (Santos et al. 2017b, Santos et al. 2019) and brachiaria (Bulegon et al. 2017a).

Using the inoculation with *A. brasilense* in *Urochloa ruziziensis* as an alternative to mitigate the damages caused by drought, Bulegon et al. (2017a) report that the inoculation influenced in the tolerance of this crop to the hydric stress, besides influencing in the increase of photosynthetic rates. Hungria et al. (2016) report that the use of *A. brasilense* together with the nitrogen fertilization of *Brachiaria* spp., in different regions of Brazil, resulted in inoculated plants with final nitrogen accumulation similar to the obtained in plants that received a second nitrogen fertilization (40 kg ha^{-1} of N) along its cycle.

In view of the foregoing, the hypothesis of this research is that the use of different doses of different formulas, with higher or lower availability of nitrogen, phosphorus and potassium, will reflect in the responses of the use of inoculation with plant growth promoting bacteria, responding in morphometric parameters along the plant development. Thus, the objective of this study was to evaluate the initial response of *Brachiaria brizantha* cv. 'Xaraés' to inoculation with *Azospirillum brasilense* associated to macronutrients.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse, under the geographical coordinates 54° 22' W, 24° 46' S, and altitude of 420 m. The region climate is classified as Cfa mesothermic humid (Alvares et al. 2013).

Seeds of brachiaria from the specie *B. brizantha* cv. 'Xaraés', obtained from the local commerce, were inoculated with the commercial inoculant 'Nitro1000 gramineas' containing the plant growth promoting bacteria (PGPB) *A. brasilense*, strains Abv5 and Abv6, in the concentration of 2,0 x 10^8 CFU mL^{-1}.

For the formulated production, it was used urea (CH_4N_2O), super phosphate simple (P_2O_5) and potassium chloride (KCl) as source of nitrogen (N), phosphorus (P) and potassium (K), respectively.

The experimental design used was of randomized blocks with a factorial scheme 2x4, with four replicates. The first factor constituted in the presence and absence of inoculation with the plant growth promoting bacteria *Azospirillum brasilense*. The second factor constituted in different formulates of fertilizers with nitrogen, phosphorus and potassium (10-10-10, 20-10-10, 10-20-10 and 10-10-20).

Seeds were inoculated in a laminar flux chamber, 30 minutes prior sowing, in the dose of 100 mL to each 25 kg of seeds. The experimental units constituted of 15 liters polyethylene pots, filled with 14 dm^3 of substrate. Sieved soil, from the experimental area, was used as substrate, being it characterized as an Oxisoil with clayey texture (Santos et al. 2013). It was chemically analyzed, presenting the following characteristics: pH ($CaCl_2$): 6.29; organic matter: 19.14 g dm^{-3}; P available ($Mehlich^{-1}$): 32.55 mg dm^{-3}; K ($Mehlich^{-1}$): 0.26 $cmol_c$ dm^{-3}; Ca^{2+} (KCl 1 mol L^{-1}): 5.71 $cmol_c$ dm^{-3}; Mg^{2+} (KCl 1 mol L^{-1}): 3.29 $cmol_c$ dm^{-3}; Al^{3+} (KCl 1 mol L^{-1}): 0.00 $cmol_c$ dm^{-3}; H+Al (pH SMP 7.5): 3.36 $cmol_c$ dm^{-3}; bases sum (BS): 9.22 $cmol_c$ dm^{-3}; cation exchange capacity (CEC): 12.63 $cmol_c$ dm^{-3}; bases saturation (V): 73.39%.

In the sequence, six seeds of *B. brizantha* cv 'Xaraés' were sowed per pot, according to the pre-determined treatment, being they thinned at six days after emergence, leaving five plants per pot. Parallel to the implementation, the application of different fertilizers with nitrogen, phosphorus and potassium was made. The cultural managements were made according to the necessity.

The experiment was conducted for 40 days. From the tenth day, every 10 days the following non-destructive evaluations were made: number of leaves, length of the longest leave (cm), number of tillers and SPAD index (Konica Minolta Plus 520).

At the end of the experiment were made the destructive analysis of root and aerial dry mass (g), root length (cm), foliar content of nitrogen, phosphorus and potassium, according to the methodology of Lana et al. (2016).

Data were tabulated and submitted to variance analysis. When significant compared by the Tukey test at 5% probability of error, for the macronutrient's comparison, and regression for the evolution along the periods. All analyses were made using the statistic software SISVAR (Ferreira 2014).

RESULTS AND DISCUSSION

Figure 1 presents the number of leaves of *Brachiaria brizantha* cv. 'Xaraés' in function of development periods, cultivated under different availabilities of macronutrients in the absence and presence of bacterial inoculation with *Azospirillum brasilense*. Plants fertilized with the formula 10-10-20, in the absence or presence of bacterial inoculation, presented regression curves in the polynomial model of the second order, with trend of development superior to the other treatments (Figure 1).

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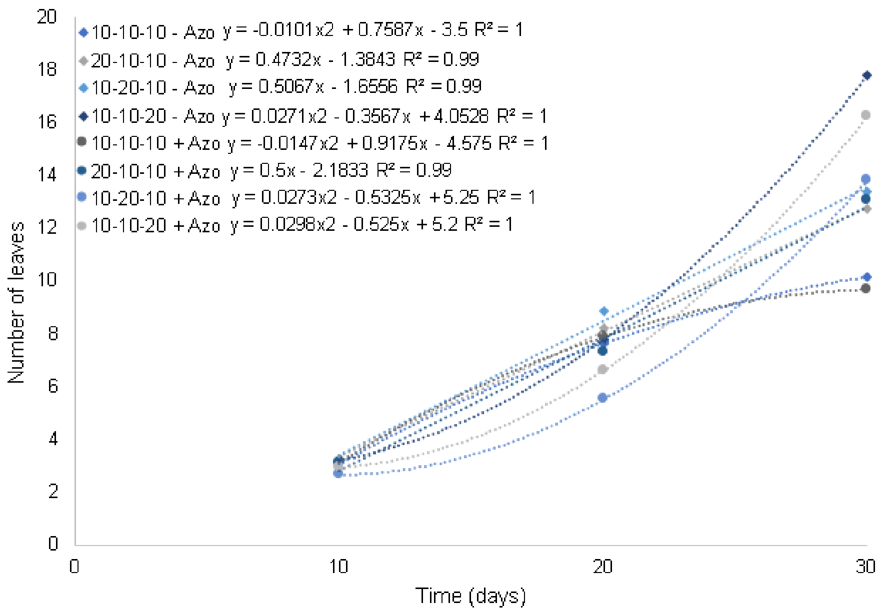

Figure 1. Number of leaves in plants of *Brachiaria brizantha* cv. 'Xaraés', at 10, 20 and 30 days after emergence, in function of fertilization with formulates in different proportions of macronutrients (10-10-10; 20-10-10; 10-20-10; and 10-10-20) in the presence (+ Azo) and absence (- Azo) of seed inoculation with *Azospirillum brasilense.*

At 30 days after emergence (DAE), non-inoculated plants and fertilized with the formula 10-10-20 presented average of 18 leaves per plant, while plants fertilized with the 10-10-10, in the presence and absence of inoculation with *A. brasilense*, presented average of 9 leaves per plant at 30 DAE. Plants fertilized with the formula 20-10-10 and 10-20-10 (Figure 1), in the presence and absence of inoculation, presented medium development in relation to the other regimen previously mentioned.

When choosing a pasture, the speed of leaf development is a decisive factor in the choice. Among many functions of the leaves in the plants is the realization of photosynthetic processes, which produce water, carbonic gas and sugars, using luminous energy collected by the photosynthetic pigments, being thus determinant the capacity of the luminous capture of these leaves in the photosynthesis (Taiz et al. 2014). In this sense, the number of healthy and photosynthetically active leaves is directly related to the photosynthetic potential of these pasture crops, guaranteeing the energy production for the recovery of organs removed due to pasture.

Comparing the development of *B. brizantha* cv. 'BRS' in full sun and shade with inoculation with *Burkholderia pyrrocinia* and *Pseudomonas fluorescens*, Lopes et al. (2018) mention that the use of bacterial coinoculation reflected on increase of the foliar area and in the SPAD index, independent of luminous availability. The use of coinoculation, in plants cultivated under plenty of sunlight, resulted in a better biomass production and in better photosynthetic performance than the non-inoculated, besides being an alternative as biofertilizers in forestry pasture due to the availability of light for growth to be limited (Lopes et al. 2018).

Figure 2 presents the length of leaves of *B. brizantha* cv. 'Xaraés' cultivated under different availabilities of macronutrients, in the absence and presence of bacterial inoculation with *A. brasilense,* in function of time. At 20 DAE, plants inoculated and fertilized with the formula 20-10-10 presented the highest averages, but they did not stand out at 30 DAE. Plants inoculated and fertilized with the formula 10-20-10 presented the smallest averages for the length of the biggest leaf at 20 days and stood out with higher averages at 30 days.

Plants fertilized with the formula 10-10-10, in the presence of *A. brasilense*, showed the smallest length of the biggest leaf at 30 days (Figure 2). Fertilization with the formula 10-10-20, in the absence and presence of *A. brasilense*, resulted in highest length of the biggest leaf. The cultivation of *U. brizantha* associated to *A. brasilense* under regime of four different doses of nitrogen, in two years of study, resulted in a 20% reduction in the need for nitrogen fertilization, increase in the forage production and mitigation of effects from hydric stress (Leite et al. 2019).

Studying three forages (*Axonopus purpusii*, *Hymenachne amplexicaulis* and *Mesosetum chaseae*) inoculated with *Azospirillum,* Souza et al. (2017) report that the inoculation of *A. purpusii*, *H. amplexicaulis* resulted in higher root volume and higher aerial dry mass, and that the inoculation of *M. chaseae* reflected in higher plant height, dry mass and root volume.

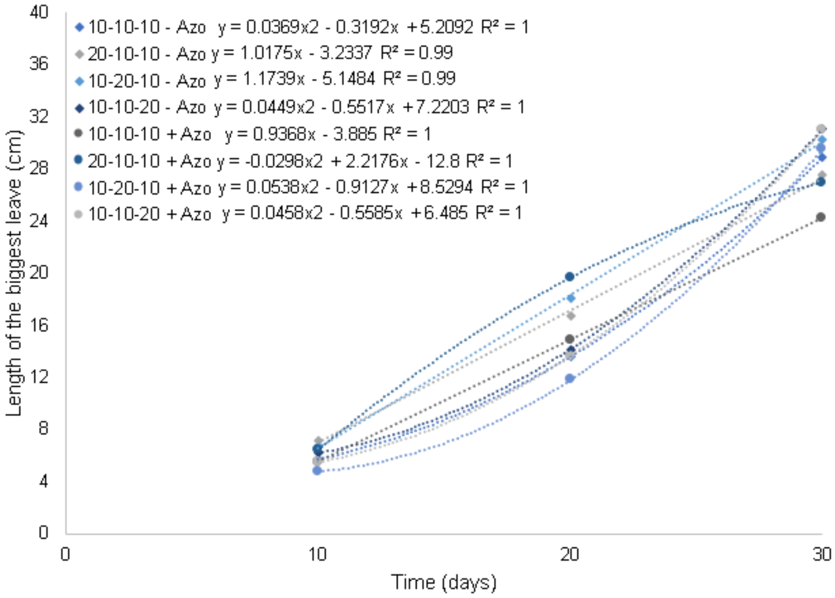

Figure 2. Length of the biggest leave in plants of *Brachiaria brizantha* cv. 'Xaraés', at 10, 20 and 30 days after emergence, in function of fertilization with formulates in different proportions of macronutrients (10-10-10; 20-10-10; 10-20-10; and 10-10-20) in the presence (+ Azo) and absence (- Azo) of seed inoculation with *Azospirillum brasilense.*

Figure 3 presents the plant height of *B. brizantha* cv. 'Xaraés' cultivated under different availability of macronutrients, in the absence and presence of inoculation with *A. brasilense,* in function of time. At 20 and 30 days, plants fertilized with the formula 10-20-10 presented greater averages for the parameter plant height. At 20 days, plants fertilized with the formula 10-20-10, independent of inoculation, presented the smallest averages (Figure 3).

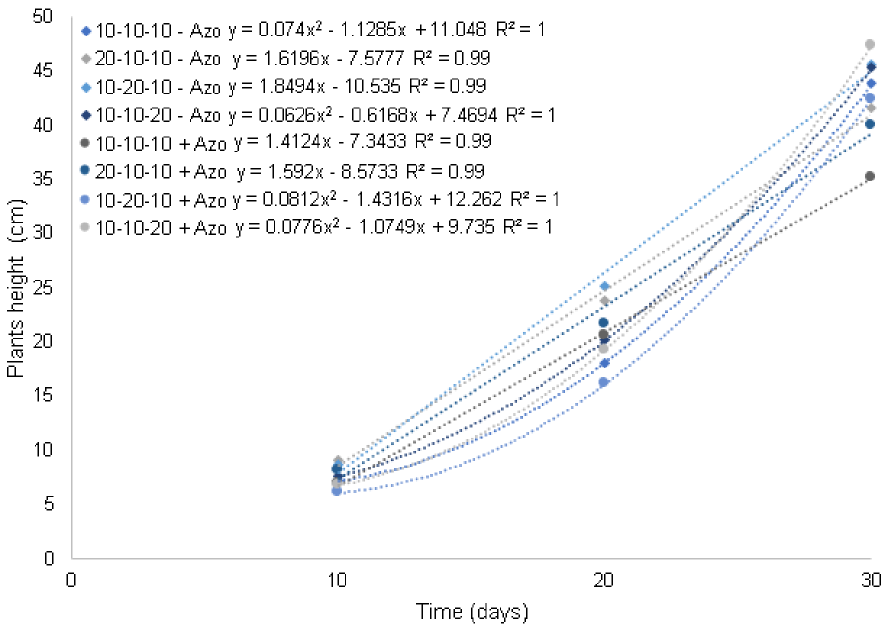

Figure 3. Plants height of plants of *Brachiaria brizantha* cv. 'Xaraés', at 10, 20 and 30 days after emergence, in function of fertilization with formulates in different proportions of macronutrients (10-10-10; 20-10-10; 10-20-10; and 10-10-20) in the presence (+ Azo) and absence (- Azo) of seed inoculation with *Azospirillum brasilense.*

Potassium solubilizing bacteria used together with stone powder in *U. brizantha* cv. 'Marandu' did not result in meaningful differences in the morphological parameters, such as width and length of leaves, density of tillers and dry mass. However, the stone powder promoted improvements in the bromatological properties, such as acid detergent fiber, hemicelluloses, dry mass digestibility, total digestible nutrients, digestible and metabolizable energy, contributing then to a nutritional enhancement, indicating the potential of this silicate rock as an alternative source to potassium chloride (KCl) (Miranda et al. 2018).

Figure 4 presents the SPAD index of plants of *B. brizantha* cv. 'Xaraés' cultivated under different availability of macronutrients, in the absence and presence of inoculation with *A. brasilense,* in function of time. The SPAD index was superior in plants inoculated and fertilized with the formula 20-10-10, being this fact predictable due to the higher availability of nitrogen of easy absorption in the soil, associated to the biological nitrogen fixation caused by the *A. brasilense.* But at 30 days, non-inoculated plants fertilized with the formula 20-10-10 presented the smallest averages, this fact may have occurred due to the high loss of nitrogen by volatilization, causing less significant effects in plants that had the nitrogen fixation bacteria as an alternative source of supply of this nutrient.

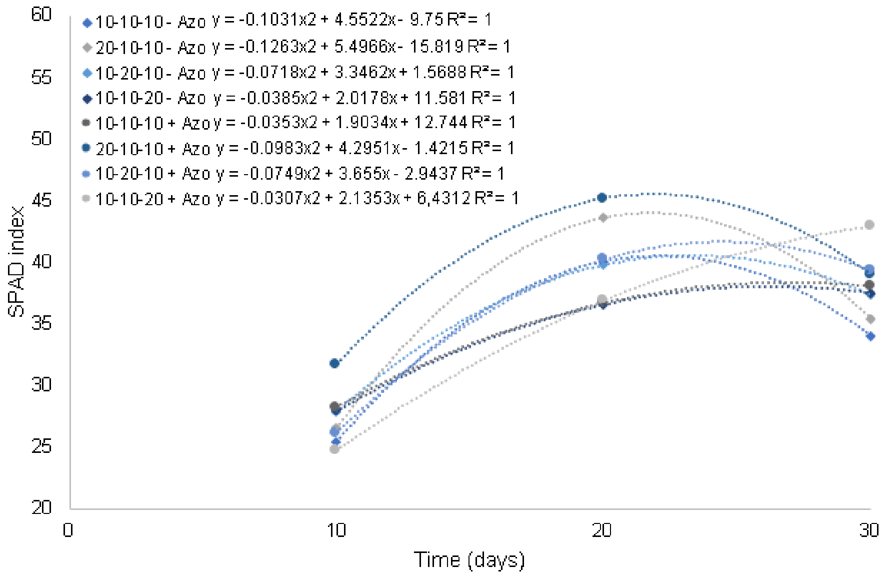

Figure 4. SPAD index of plants of *Brachiaria brizantha* cv. 'Xaraés', at 10, 20 and 30 days after emergence, in function of fertilization with formulates in different proportions of macronutrients (10-10-10; 20-10-10; 10-20-10; and 10-10-20) in the presence (+ Azo) and absence (- Azo) of seed inoculation with *Azospirillum brasilense.*

As mentioned in the parameter number of leaves, Lopes et al. (2018) comparing the development of *B. brizantha* cv. 'BRS' in different light availabilities, with inoculation with *B. pyrrocinia* and *P. fluorescens,* obtained that the bacterial coinoculation increase the SPAD index, independent of light availability. Testing the inoculation with strains from diazotrophic organisms and commercial inoculant based on Abv5 and Abv6 of *A. brasilense* in *B. brizantha* cv. 'Marandu' the authors report increase in the nitrogen content in the roots and in the aerial part, besides crude protein content in plants inoculated with diazotrophic strains (Guimarães et al. 2016).

In relation to the destructive analysis performed at the end of the experiment, no statistical difference was observed ($p<0.05$) for the parameters root length ad foliar content of phosphorus and potassium. Figure 5 presents the number of tillers in plants of *B. brizantha* cv. 'Xaraés' cultivated under different availability of macronutrients, in the absence and presence of inoculation with *A. brasilense,* at 20 and 30 DAE. At 20 days, there was no statistical difference for the number of tillers. At 30 days, plants fertilized with the formula 10-10-20, in absence and presence of inoculation with *A. brasilense*, obtained higher averages on the number of tillers. Plants fertilized with the formula 10-10-10, independent of inoculation, presented the smallest averages (Figure 5).

Aiming to evaluate the forage production of *Cynodon dactylon* grasses, inoculated with *A. brasilense*, with nitrogen doses and different cutting regimes, Aguirre et al. (2018) report increases in the forage production when it was inoculated in the pasture implementation, even without nitrogen application, being unnecessary the re-inoculation.

Figures 6 and 7 present the dry mass of roots and aerial part of plants of *B. brizantha* cv. 'Xaraés' cultivated under different availability of macronutrients, in the absence and presence of inoculation with *A. brasilense,* at 30 days after emergence. The aerial dry mass and root dry mass differed statistically only for the presence and absence of inoculation in the treatment 10-20-10, where the absence of inoculation resulted in higher averages (Figures 6 and 7).

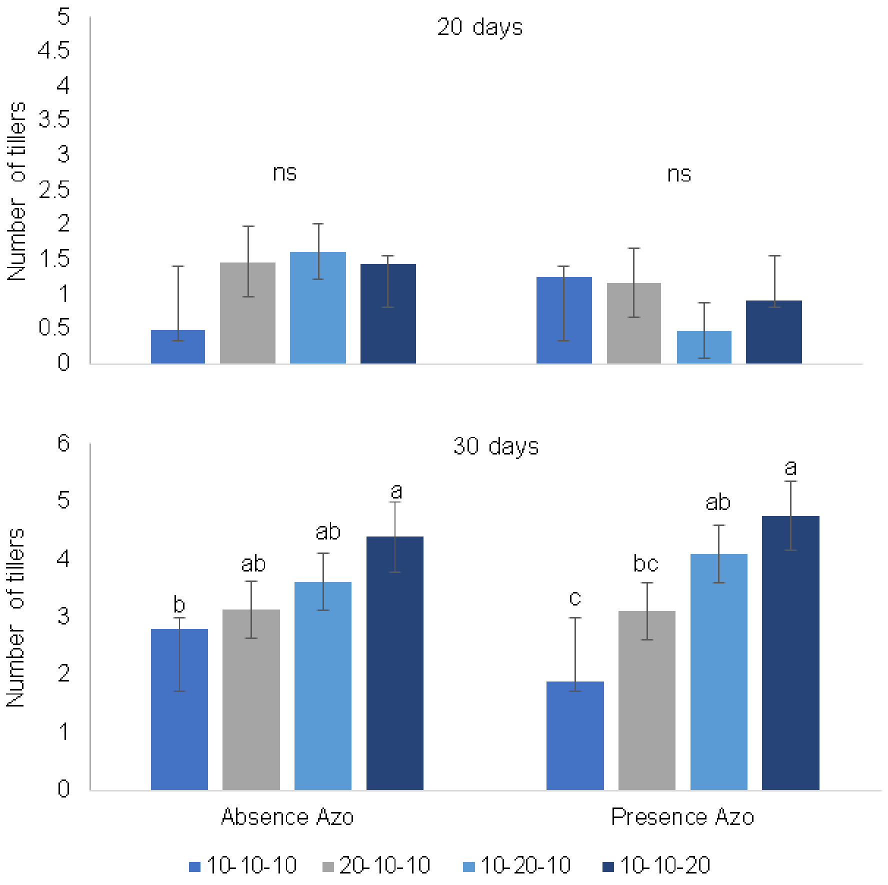

Figure 5. Number of tillers of plants of *Brachiaria brizantha* cv. 'Xaraés', at 20 and 30 days after emergence, in function of fertilization with formulates in different proportions of macronutrients (10-10-10; 20-10-10; 10-20-10; and 10-10-20) in the presence (+ Azo) and absence (- Azo) of seed inoculation with *Azospirillum brasilense*.

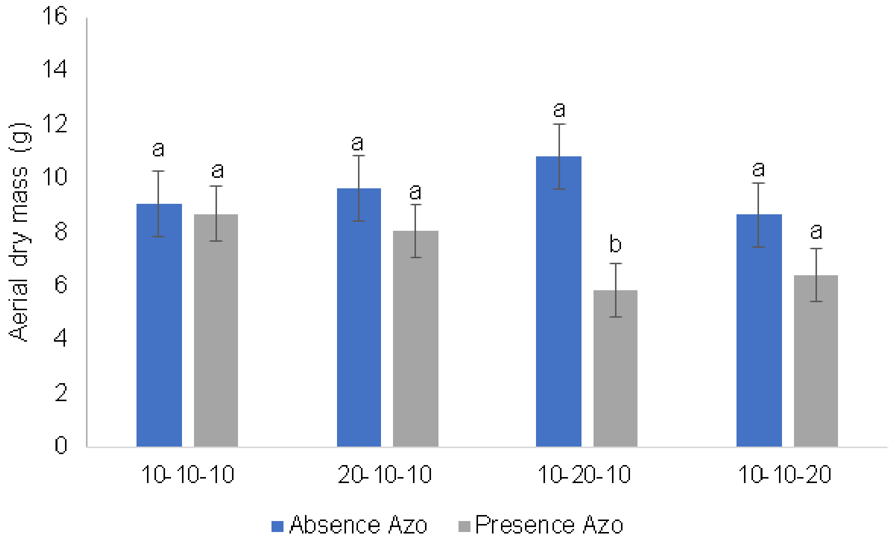

Figure 6. Aerial dry mass of plants of *Brachiaria brizantha* cv. 'Xaraés', at 30 days after emergence, in function of fertilization with formulates in different proportions of macronutrients (10-10-10; 20-10-10; 10-20-10; and 10-10-20) in the presence (+ Azo) and absence (- Azo) of seed inoculation with *Azospirillum brasilense*.

Evaluating the diversity in vitro of bacteria associated to *B. decumbens* and *B. humidicola,* Oliveira et al. (2018) tested 20 diazotriphic isolates from the rizosphere, identified as *Bacillus* sp., *Burkholderia* sp., *Enterobacter* sp., *Klebsiella* sp., *Microbacterium* sp., *Pantoea* sp., *Ralstonia* sp., *Rhizobium* sp., *Sinomonas* sp., and *Sphingomonas* sp., and except the genus *Rhizobium* sp., all organisms present capacity to produce indoleacetic acid in the presence of tryptophan, and 60% produced indoleacetic acid independent of tryptophan. From all the microorganisms evaluated, 70% solubilize phosphate, 30% produced cellulase, 15% produced polygalacturonates and 30% produced amylase. The authors concluded that the characteristics obtained indicated that the association of pastures with bacteria with different growth promoting mechanisms may reflect in different responses to the plant development.

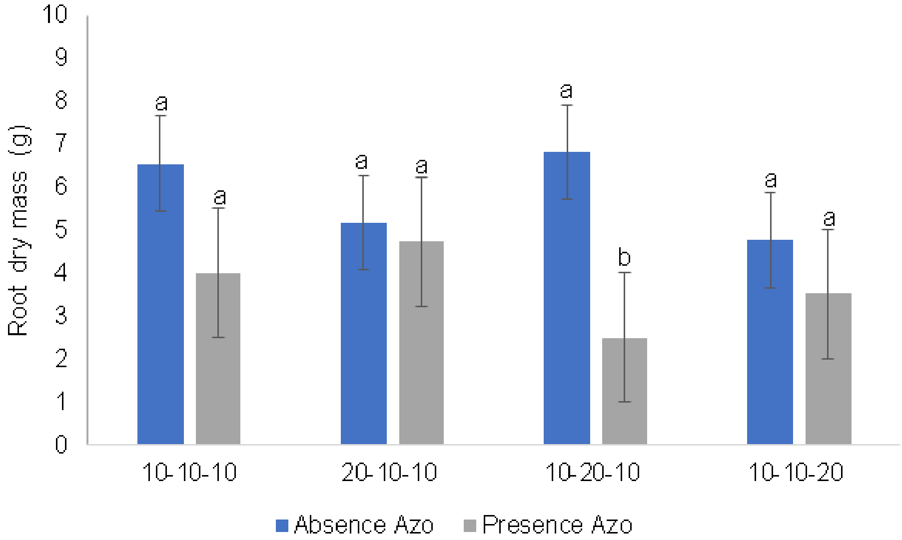

Figure 7. Root dry mass of plants of *Brachiaria brizantha* cv. 'Xaraés', at 30 days after emergence, in function of fertilization with formulates in different proportions of macronutrients (10-10-10; 20-10-10; 10-20-10; and 10-10-20) in the presence (+ Azo) and absence (- Azo) of seed inoculation with *Azospirillum brasilense.*

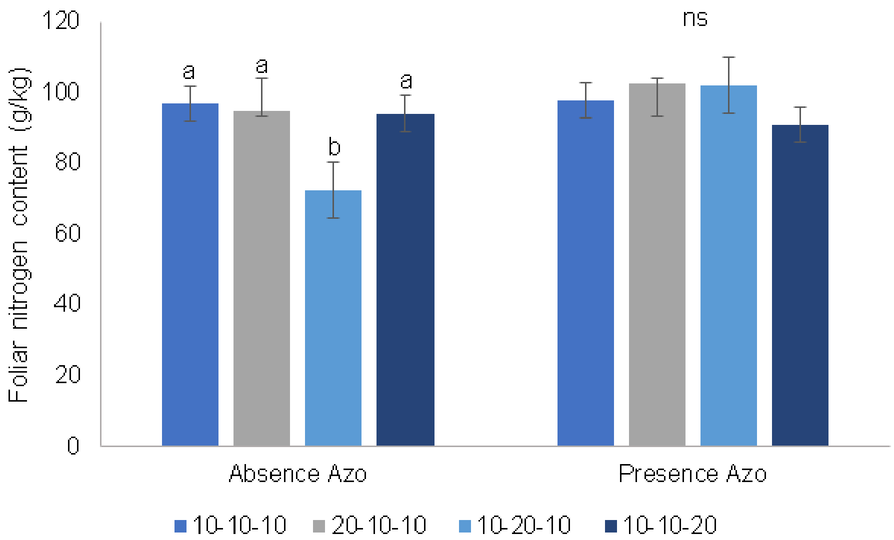

Figure 8. Foliar nitrogen content of plants of *Brachiaria brizantha* cv. 'Xaraés', at 30 days after emergence, in function of fertilization with formulates in different proportions of macronutrients (10-10-10; 20-10-10; 10-20-10; and 10-10-20) in the presence (+ Azo) and absence (- Azo) of seed inoculation with *Azospirillum brasilense*.

These metabolites reported by Oliveira et al. (2018) are directly related to the characteristics of growth of plants. The indoleacetic acid is essential for plant growth, being one of the auxin sources that plants use in the root development and aerial development, reflecting in biomass production (Glick 2012). The solubilization of mineral phosphates is a very important characteristic in relation to the high demand of pastures for phosphorus (Oliveira et al. 2013).

Figure 8 presents the foliar nitrogen content in plants of *B. brizantha* cv. 'Xaraés' cultivated under different availability of macronutrients, in the absence and presence of inoculation with *A. brasilense.* At 30 DAE there was difference only for non-inoculated plants, which fertilization 10-20-10 resulted in smaller averages of foliar nitrogen content.

The use of the formula 10-20-10, in the absence or presence of inoculation with *A. brasilense*, increased the leaves initial development of plants of *B. brizantha* cv. 'Xaraés'.

The formula 10-10-10, in the absence and presence of bacterial inoculation, resulted in plants with lower foliar development up to 30 days after emergence.

The nutritional availability influences in the initial development of seedlings of *B. brizantha* cv. 'Xaraés'.

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