# Inoculation with *Azospirillum* combined with nitrogen fertilization in sorghum intercropped with *Urochloa* in off-season[1]

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## ABSTRACT

The objective of this work was to evaluate the management of nitrogen fertilization in grains of sorghum inoculated or not with *Azospirillum brasilense* in single crop or intercropped with *Urochloa brizantha* (cv. Paiaguás) in the off-season. The experimental design was a completely randomized block design with four replications, in a 2 x 2 x 3 factorial scheme, with sorghum in single-cropped system or intercropped with grass; sorghum seeds inoculated or not with *A. brasilense*; and N management (application of 100% of the dose at sowing or only in topdressing or split - 30% at sowing and 70% in topdressing) at a dose of 120 kg ha$^{-1}$ N. Morphological components and sorghum grain yield and productivity of dry matter of the aerial part of the grass and the sorghum were evaluated. The splitting of nitrogen fertilization did not interfere in the yield of sorghum grain straw intercropped with *U. brizantha*. The intercropping with *U. brizantha* did not reduce sorghum grain yield. In dry climate conditions in the off-season, inoculation of sorghum seeds cv. Ranchero with *A. brasilense* increases grain yield.

Keywords: dizatrophic bacteria; nitrogen; paiaguás grass; *Sorghum bicolor*.

## INTRODUCTION

The integrated crop-livestock systems (ICLS) are an alternative for sustainable intensification of land use (FAO, 2010) as they are based on diversified agricultural and livestock production in the same area: intercropped, sequential or rotated cultivation (Macedo, 2009; Crusciol *et al.*, 2020).

In the Cerrado biome (Brazil), the intercropping of tropical *Urochloa* (*Syn. Brachiaria*) forage species with grain crops has stood out in the ICLS, whose objective is to produce grains and forage/or straw for the summer crop in the no-tillage system (NTS) (Ceccon *et al.*, 2013; Maia *et al.*, 2014). Sorghum growing has been evaluated for the inclusion in these systems as it is an excellent alternative for grain and forage production in situations where water deficit and poor soil fertility offers risk for the other cultivation of grass crops, such as corn, for example (Magalhães *et al.*, 2014; Bogiani & Ferreira, 2017; Hadebe *et al.*, 2017), which makes it necessary to evaluate the performance of the culture intercropped with *Urochloa* in the lowland Cerrado in the off-season (autumn-winter), due to the lack of available information.

Sorghum has two periods of intense nutrient uptake, the first is during the vegetative phase, when the plant has 7 to 12 expanded leaves, and the second, during grain formation (Coelho *et al.*, 2002). In the first period, 20 to 30 days after the emergency, the plant begins a rapid growth, with an increase in the nutrient uptake rate from soil. Such behavior justifies this period as the ideal moment to perform topdressing nitrogen fertilization (Lourenção & Bagega, 2012).

The need and response potential of sorghum to nitrogen fertilization depends on the genotype and environmental factors, such as water availability and the content of organic matter in the soil (Mateus *et al.*, 2011). Soil texture is also an important factor to consider,

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mainly in split-nitrogen fertilization, because it is directly associated with the potential of N losses due to leaching in soils (Tully & Ryals, 2017). In clay soils, this potential for losses may not be significant (Hallaq, 2010), which would explain total fertilization at sowing or in topdressing, according to preference or conditions of the farmer.

Therefore, to increase the competitiveness of sorghum in the market, its inclusion in the ICLS, associated with adequate management of fertilization can be added to the use of plant-growth promoting bacteria, such as those of the *Azospirillum* genus, aiming to optimize the yield of grains and straw with low economic and environmental cost because, besides the possibility of supplying part of the N via atmospheric fixation, this genus stimulates the synthesis of phytohormones that act positively on the effects of stresses on the plant (Bashan & Bashan, 2010; Cassán *et al.*, 2014; Hungria *et al.*, 2015).

However, it is necessary to evaluate whether the associated use of these practices can promote synergism or antagonism over each other, such as the negative effect of nitrogen fertilization on the efficiency of *Azospirillum* in grasses (Hungria, 2011; Repke *et al.*, 2013), and the effect of competition among intercropped plant species (Mateus *et al.*, 2016).

Thus, the hypotheses of this study are: inoculation with *Azospirillum brasilense* in grain sorghum increases its grain yield; the splitting or not of nitrogen fertilization on sorghum under rainfed conditions does not interfere with grain yield in clayey soil; and the inter-row intercropping of grain sorghum with Paiaguás (*Urochloa brizantha*) does not interfere on sorghum grain yield. The objective of this study was to evaluate nitrogen fertilization management on the grass sorghum crop, inoculated or not with *A. brasilense* in the seeds, intercropped or not with *Urochloa* brizantha (cv. Paiaguás) in the NTS in the Cerrado in the off-season.

## MATERIAL AND METHODS

The experiments were conducted on the Teaching, Research and Extension Farm – Plant Production Sector at Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Engenharia de Ilha Solteira, Selvíria, State of Mato Grosso do Sul (20° 18' S and 51° 22' W, 370 m above sea level), in the dry farming area over 2015 and 2016.

The soil of the area was classified as clay-textured dystrophic Red Latosol (Oxisol) (580 g kg$^{-1}$ clay), according to Santos *et al.* (2018). Before installation of the experiments, soil fertility was analyzed in the 0.00-0.20 m layer, according to Raij *et al.* (2001), and the results were as follows: 17 mg dm$^{-3}$ P (resin); 22 g dm$^{-3}$ O.M.; 5.5 pH ($CaCl_2$); 1.4 mmol$_c$ dm$^{-3}$ K; 26.0 mmol$_c$ dm$^{-3}$ Ca; 18.0 mmol$_c$ dm$^{-3}$ Mg and 28.0 mmol$_c$ dm$^{-3}$ H+Al; 44.9 and 73.1 mmol$_c$ dm$^{-3}$ SB and CEC, respectively; 62% V and zero Aluminum. Fertilization was performed according to Cantarella *et al.* (1997) based on the chemical analyses and according to the need of the sorghum crop.

The climatic type of the region is Aw, according to the classification of Köppen. Some climatic information collected over the conduction of the experiments is shown in Figure 1.

The experiments were set in the off-season of each year (2015 and 2016), in an area with a record of five years under no-tillage system, where cotton was cropped until mid-2013, and remained fallow until the end of the 2014. Spontaneous vegetation were desiccated before the setting of the experiments using the herbicide Glyphosate (1.44 kg ha$^{-1}$ a.i.); after, the plant residues were ground using a horizontal shredder (Triton). After the first experiment, the area was cultivated with soybean in the summer in 2015 and the second experiment was set in succession in 2016.

The experimental design was a randomized complete block design in a 2 x 2 x 3 factorial scheme, with four replications, consisting of sorghum single cropped or intercropped with *Urochloa brizantha*, sorghum inoculated or not with *Azospirillum brasilense* and application of nitrogen only at sowing or only in topdressing or split – 30% at sowing and 70% in topdressing at the beginning of the panicle initiation stage – at 120 kg ha$^{-1}$ N, using urea as source applied between the rows of sorghum.

Sorghum was mechanically sown (March 17, 2015 and April 6, 2016) using sowing-fertilizer equipment with a rod-type furrow opener (hoe) mechanism for NTS, at approximately 0.03-m depth and with a density of 10 m$^{-1}$ seeds, rows spaced at 0.45 m, 6 m long. The sowing fertilization consisted of 90 kg $P_2O_5$ and 30 kg $K_2O$ kg ha$^{-1}$, using simple superphosphate (18% $P_2O_5$) and potassium chloride (60% $K_2O$) as sources, respectively.

The experiments were composed of 48 plots with seven rows of sorghum. The hybrid Ranchero, with an aptitude for grain production was used. In the intercropping treatments, *U. brizantha* cv. BRS Paiaguás was used in both years. The diazotrophic bacteria were supplied by the AZO Total inoculant, developed for corn and wheat crops (registration number in MAPA: PR-93923-10074-1), physical nature: liquid, density: 1.0 g mL$^{-1}$; use dosage: 100 mL$^{-1}$ 20 kg seeds (guarantee of 2 x $10^8$ colony forming units mL$^{-1}$ of *A. brasilense*, AbV5 and AbV6 strains). The inoculation of sorghum seeds was carried out about 30 minutes before sowing in the shade.

Grass was sown simultaneously with sorghum using another seed-fertilizer between the rows of sorghum, at the same spacing of 0.45 m, using approximately 10 kg ha-

[1] of viable pure seeds (CV = 60%) of the *U. brizantha*. Grass seeds were sown at a 0.06-m depth, according to Kluthcouski *et al.* (2000), with the objective of delaying the emergence of grass in relation to sorghum.

Nitrogen topdressing fertilization was hand-performed approximately 10 cm from the sorghum plants, according to the treatments, approximately 30 days after emergence (DAE) (Cantarella *et al.*, 1997), when the plants were about 0.30 m high (04/24/2015 and (05/13/2016), at the panicle initiation stage (growth stage 2 - GS2) (Magalhães *et al.*, 2014).

The following were determined at harvest (06/18/2015 and 07/26/2016, approximately 90 and 110 DAE, respectively) in the sorghum crop: final stand of the plants, where the plants of the three central rows of the plot were counted, discarding 1.5 m from each end; the basal diameter of the stem; plant height and panicle length; the number of grains per panicle and the harvest index (ratio between dry mass of the grains and the dry mass of the entire plant). For these determinations, 10 plants were randomly collected in the useful area of the experimental plot. The mass of one thousand grains was determined by weighing four samples per plot and corrected for 13% moisture.

The material was collected for determination of grain yield, dry matter of sorghum and aerial part of the grass, carried out on the same day when sorghum was harvested, starting by the collection of the plants at the three central rows, discarding 1.5 m at each end, extrapolating it to one hectare. In the sorghum crop, the stem and leaf fractions were separated with pruning shears. Subsequently, this material was weighed and dried in an oven (65 ºC) to determine the dry matter. At sorghum harvesting, samples were taken to determine the dry matter yield of the aerial part of the grass in 1 $m^2$ (1.0 x 1.0-m metal square), in two samples per plot, adopting as cutting height close to the ground.

Data were submitted to the Shapiro-Wilk test to test normality and, due to climatic adversities and peculiar soil conditions, analysis of the data was carried out separately for each year, using the F test ($p < 0.05$) and the means compared by Tukey's test ($p < 0.05$) using SISVAR 5.3 computer software (Ferreira, 2008).

## RESULTS AND DISCUSSION

In the two years of evaluation, the final plant stand of sorghum (FPS) was not influenced by the interactions betwen factors or by the isolated effects of any of the treatments (Tables 1). It should be emphasized the absence of interference from the *U. brizantha* on the plant stand ($p > 0.05$). The sowing of *U. brizantha* between the rows and in greater depth in relation to the sorghum seeds are crop strategies that minimize their competition with sorghum plants in the establishment phase (Kluthcouski *et al.*, 2000; Silva *et al.*, 2014), keeping the final stand approximate to that obtained in monoculture. Additionally, early cycle cultivars as Ranchero may decrease competition between species (Pariz *et al.*, 2009; Crusciol *et al.*, 2013). These results corroborate those obtained by Crusciol *et al.* (2011) and Mateus *et al.* (2011).

In 2015, the cropping modalities significantly influenced the basal stem diameter (BSD) and the

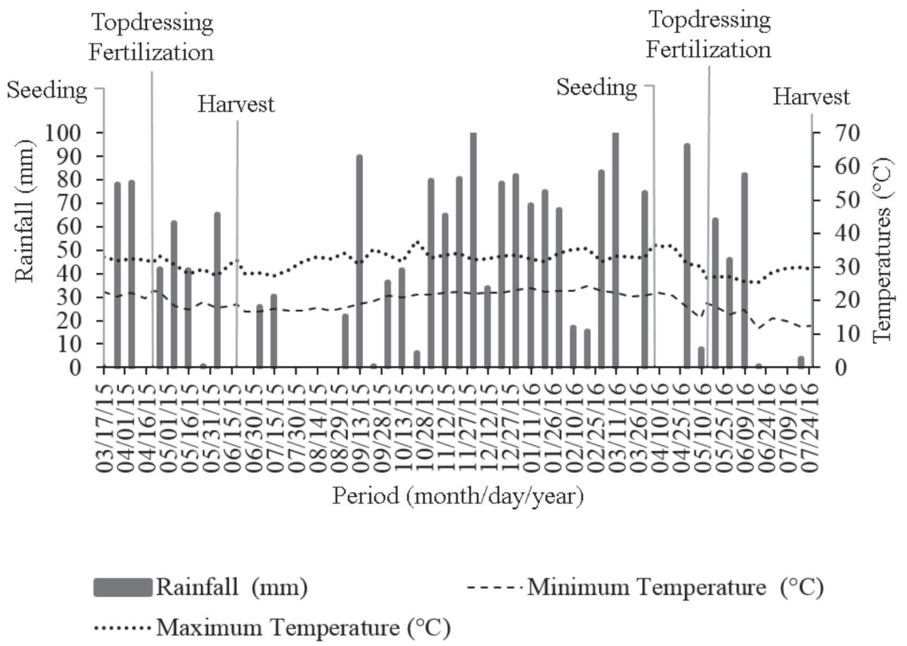


**Figure 1:** Decendial meteorological data of rainfall (mm), maximum and minimum temperatures (°C) over the experimental period. Selviria – MS, 2015 and 2016

management of nitrogen fertilization significantly influenced plant height (PH) (Table 1). Treatments with N application in topdressing provided higher plants, which was caused by the supply of N at the panicle initiation stage, when the plant begins a period of intense development (Lourenção & Bagega, 2012; Cavalcante *et al.*, 2018).

However, it is emphasized that the split fertilization 30% - 70% highlighted with higher average PH and SBD, which may be attributed to the top dressing (70% N) as to 30% at sowing, possibly benefited the crop in the first year due to the adequate water regime after sowing (Figure 1).

The lack of response in the second year, for the largest of the morphological characteristics of the plants (Table 1), suggests a residual effect of soybean cropping at the site over summer (2015/2016). Thus, the NTS record of the area is extremely important for the response of the crop to fertilization (Mateus *et al.*, 2011; Borghi *et al.* 2014, Fontes *et al.*, 2017).

As for the higher SBD in sorghum intercropped in 2015 (Table 1), it was probably a response to competition with grass, in which sorghum accumulates a greater amount of photoassimilates in the stem to excel in competition (Fernandes *et al.*, 2014). Similar results were obtained by Crusciol *et al.* (2011) and Mateus *et al.* (2011), who observed a basal stem diameter of the sorghum intercropped with *U. brizantha* (cv. Marandu) and *Panicum maximum* (cv. mombasa) equal to or greater than that of the plants in single cropping.

A significant interaction ($p < 0.05$) was found in 2015 between inoculation and cropping modalities, for PH and

**Table 1:** Averages final plant stand (FPS), plant height (PH), panicle length (PL), stem basal diameter (SBD) of sorghum under nitrogen fertilization management, with and without inoculation in the seeds with A. brasilense and single cropped or intercropped with U. brizantha. Means and their respective standard error, 2015 and 2016(1)

| Treatments | 2015 | | | |
|---|---|---|---|---|
| | **FPS (plants $ha^{-1}$)** | **PH(2) (m)** | **PL(2) (cm)** | **SBD (mm)** |
| Inoculation | ns | ** | ** | ns |
| With | 164.197±4.855 | 1.36±0.01 | 24.3±0.2 | 16±0.4 |
| Without | 177.002±4.444 | 1.35±0.02 | 24.3±0.2 | 16±0.3 |
| Cropping Modalities | ns | | | ** |
| Intercropped | 166.794±4.893 | 1.35±0.02 | 24.4±0.2 | 17±0.3a |
| Single | 174.405±4.656 | 1.36±0.01 | 24.2±0.2 | 16±0.3b |
| L.S.D. | 13.573 | 0.028 | 0.56 | 0.77 |
| Fertilization Management(3) | ns | ** | ns | ** |
| 0 % - 100 % | 173.181±5.758 | 1.39±0.01a | 24.1±0.3 | 15±0.3b |
| 30 % - 70 % | 174.883±6.308 | 1.38±0.02a | 24.5±0.3 | 16±0.3a |
| 100 % - 0 % | 163.734±5.483 | 1.29±0.01b | 24.3±0.3 | 17±0.3a |
| L.S.D | 20.057 | 0.042 | 0.83 | 1.13 |
| C.V. (%) | 14 | 4 | 4 | 8 |
| **Treatments** | **2016** | | | |
| | **FPS (plants $ha^{-1}$)** | **PH (m)** | **PL (cm)** | **SBD(2) (mm)** |
| Inoculation | ns | ns | ns | * |
| With | 174.328±5.141 | 1.27±0.01 | 26.2±0.2 | 17±0.2 |
| Without | 176.104±5.059 | 1.28±0.01 | 26.0±0.2 | 15±0.3 |
| Cropping Modalities | ns | ns | ns | |
| Intercropped | 173.489±5.255 | 1.28±0.01 | 26.0±0.2 | 16±0.3 |
| Single | 176.943±4.921 | 1.27±0.01 | 26.1±0.2 | 16±0.2 |
| L.S.D. | 15.914 | 0.020 | 0.61 | 0.73 |
| Fertilization Management(3) | ns | ns | ns | ns |
| 0 % - 100 % | 175.834±6.125 | 1.29±0.01 | 25.9±0.3 | 16±0.3 |
| 30 % - 70 % | 176.280±6.710 | 1.27±0.01 | 26.5±0.3 | 17±0.3 |
| 100 % - 0 % | 173.534±6.081 | 1.26±0.01 | 25.8±0.2 | 16±0.4 |
| L.S.D. | 23.517 | 0.031 | 0.91 | 1.08 |
| C.V (%) | 15 | 3 | 4 | 8 |

(1)Different letters in the column differ from each other by the Tukey's test ($p < 0.05$); (2)Interaction between inoculation and cropping modalities factors; (3)Sowing and in topdressing, respectively. * and ** significant at 5 and 1% probability, respectively; nsNon-significant; L.S.D. = Least Significant Difference; C.V. = Coefficient of Variation.

panicle length (PL) (Table 2). The intercropped inoculated sorghum presented higher PH and PL than the single-cropped inoculated. The opposite occurred in sorghum without inoculation, where these variables were higher in sorghum in single crop. Evenly taller plants are likely to have higher panicle heights, which favors mechanical harvesting and results in a reduced percentage of panicles that are not harvested with the harvester-platform. Furthermore, a higher panicle height could be beneficial, as the sorghum yield is not reduced, the grass is not frequently mowed, and less time is required to close off the area to animals at the first grazing (Crusciol *et al.*, 2012; Borghi *et al.*, 2013).

This result evidences a synergism between *A. brasilense* and sorghum intercropped with *U. brizantha*. For inoculation within the cropping modalities, inoculated-intercropped sorghum presented higher PH and PL in comparison to the non-inoculated, whereas in single-cropped sorghum, these two variables were higher in the absence of inoculation (Table 2).

In 2016, single-cropped not-inoculated sorghum showed higher SBD when compared to intercropped sorghum without inoculation, while intercropped and inoculated sorghum showed the highest SBD in relation to the not-inoculated sorghum (Table 2). These results show a beneficial effect of inoculation on sorghum (Hungria, 2011; Nakao *et al.*, 2018), because thicker stems allows greater water and nutrient translocation capacity (Mateus *et al.*, 2011).

In the first year, the mass of one thousand grains (MTG) was lower in inoculated sorghum. On the other hand, the number of grains per panicle (NGP) was higher, demonstrating a relationship between the latter variable and grain yield, which has been reported in the literature (Mateus *et al.*, 2011; Magalhães *et al.*, 2014) (Table 3).

Nevertheless, in the first year, the aerial part dry matter yield (APDM) (stem + leaves) was significantly higher in inoculated sorghum (10%). Under the same conditions of this study, Nakao *et al.* (2018) also obtained an increment in straw (25% of the stem + leaves) in Ranchero sorghum inoculated with *A. brasilense*.

Although APDM was positive in the first year, studies carried out in the Cerrado with other grass species inoculated by *Azospirillum* reported the achievement of increments higher than those observed in the present study, and indicated that the potential response of the inoculated species depends on the hybrid used (Quadros *et al.*, 2014; Marini *et al.*, 2015; Pereira *et al.*, 2015). Quadros *et al.* (2014) evaluated three corn hybrids inoculated by Azospirillum, and observed a response to APDM in only one of the inoculated hybrids, with increase of 4.8 t $ha^{-1}$ (43%) of total dry matter of the aerial part (stem + leaves).

The inoculation incremented NGP and grain yield (GY) (Tables 3), presenting satisfactorys yield values for the condition of the study in both years (Freitas *et al.*, 2014). The increase in the number of grains as a result of the inoculation with *A. brasilense* has also been reported in studies with other grass species, such as Chaves *et al.* (2016), which verified an increase by 35% in the number of spikelets per rice panicle inoculated with *A. brasilense* (AbV5 and AbV6 strains).

Inoculation incremented grain yield (GY) by 15.5 and 12.5%, in both years, respectively, in comparison to the treatment without inoculation. These increases are higher than those obtained by corn inoculated with *A. brasilense*, reported by Puente *et al.* (2009), of 11%, within the range reported by Lana *et al.* (2012), from 7 to 15%, and below the range reported by Hungria *et al.* (2010), from 24 to 30%, in comparison to the not inoculated.

**Table 2:** Unfolding of the significant interactions between inoculation and cropping modalities for plant height (PH), panicle length (PL) (2015) and stem basal diameter (SBD) (2016) of sorghum under nitrogen fertilization management, with and without inoculation in the seed with A. brasilense and single cropped or intercropped with U. brizantha [(1)]. Means and their respective standard error

| Inoculation | Cropping Modalities | | |
|---|---|---|---|
| | **Intercropped** | **Single** | **L.S.D.** |
| | | PH (m) | |
| With | 1.38±0.02Aa | 1.33±0.01Bb | 0.04 |
| Without | 1.31±0.02Bb | 1.38±0.02Aa | |
| | | PL (cm) | |
| With | 24.9±0.3Aa | 23.7±0.2Bb | 0.80 |
| Without | 23.8±0.3Bb | 24.7±0.4Aa | |
| | | SBD (mm) | |
| With | 17±0.4Aa | 16±0.3Aa | 1.03 |
| Without | 15±0.4Bb | 16±0.4Aa | |

[(1)]Different upper-case letters in the column and different lowercase letters in the row differ from each other by the Tukey's test ($p < 0.05$). L.S.D. = Least Significant Difference.

Diazotrophic bacteria associated with grasses provides better soil exploitation and greater water and nutrient uptake by these species (Schultz *et al.*, 2012). Thus, the higher grain yield of sorghum inoculated with *A. brasilense* allows a better ability of the plant in exploiting the soil by it is roots because its inoculation via seed results in an increase in the dry mass of the root system of plants (Andrade *et al.*, 2019).

In addition, Assefa *et al.* (2010) report that the amount of water required during the sorghum cycle ranges from 450 to 650 mm, depending on the prevailing climatic conditions. Moreover, this study was conducted in dry farming conditions in the off-season, when there is a reduction in rainfall and the occurrence of high temperatures is common in the Cerrado. The rainfall accumulated over the crop cycles were 296 mm in 2015 and 364 mm in 2016 (Figures 1), poorly distributed over time and below the lower limit reported by the authors as mentioned above. However, despite these climatic inconveniences during the conduction of the experiments, crop grain yields were adequate for the season, according to Freitas *et al.* (2014).

Among the variables related to the yields of sorghum straw and grain, only APDM was significantly influenced by the cropping systems in 2015. In this year, the intercropped sorghum incremented APDM by approximately 10%, in comparison to the single crop (Table 3). This result is related to the higher SBD of intercropped sorghum (Table 1) because plants with thicker stems have a higher capacity for water translocation and accumulation of nutrients (Mateus *et al.*, 2011).

**Table 3:** Average number of grains per panicle (NGP), mass of one thousand grains (MTG), aerial part dry matter yield (APDM), grain yield (GY), harvest index (HI) of sorghum under nitrogen fertilization management, with and without inoculation in the seeds with A. brasilense single cropped or intercropped with U. brizantha. Means and their respective standard error, 2015 and 2016[(1)]

| **Treatments** | **2015** | | | | |
|---|---|---|---|---|---|
| | **NGP** | **MTG (g)** | **APDM (kg ha$^{-1}$)** | **GY (kg ha$^{-1}$)** | **HI** |
| Inoculation | * | ** | * | ** | ns |
| With | 1,397±42a | 18.7±0.4b | 6,888±220a | 6,138±169a | 0.47±0.01 |
| Without | 1,249±44b | 20.2±0.3a | 6,276±149b | 5,313±132b | 0.46±0.01 |
| Cropping modalities | ns | ns | * | ns | ns |
| Intercropped | 1,363±47 | 19.7±0.5 | 6,883±154a | 5,928±192 | 0.46±0.01 |
| Single | 1,283±43 | 19.2±0.2 | 6,282±214b | 5,523±143 | 0.47±0.01 |
| L.S.D. | 130 | 0.96 | 566 | 426 | 0,02 |
| Fertilization Management[(3)] | ns | ns | ns | ns | ns |
| 0 % - 100 % | 1,290±67 | 19.4±0.3 | 6,680±266 | 5,455±247 | 0.45±0.00 |
| 30 % - 70 % | 1,298±57 | 19.8±0.6 | 6,625±229 | 5,987±191 | 0.47±0.01 |
| 100 % - 0 % | 1,382±41 | 19.1±0.5 | 6,441±229 | 5,733±181 | 0.47±0.01 |
| L.S.D. | 192 | 1.41 | 837 | 630 | 0.03 |
| C.V. (%) | 17 | 8 | 15 | 13 | 7 |
| **Treatments** | **2016** | | | | |
| | **NGP** | **MTG[(2)] (g)** | **APDM (kg ha$^{-1}$)** | **GY (kg ha$^{-1}$)** | **HI[(2)]** |
| Inoculation | * | * | ns | * | * |
| With | 1,394±43a | 18.9±0.3 | 6,314±278 | 5,868±193a | 0.48±0.01 |
| Without | 1,265±40b | 20.0±0.3 | 6,575±306 | 5,215±215b | 0.45±0.01 |
| Cropping modalities | ns | | ns | ns | |
| Intercropped | 1,310±43 | 19.9±0.4 | 6,324±312 | 5,602±213 | 0.47±0.01 |
| Single | 1,349±45 | 19.0±0.3 | 6,566±272 | 5,481±218 | 0.46±0.01 |
| L.S.D. | 119.5 | 0.83 | 883 | 648 | 0.02 |
| Fertilization Management[(3)] | ns | ns | ns | ns | ns |
| 0 % - 100 % | 1,243±46 | 19.7±0.3 | 6,249±374 | 5,384±253 | 0.46±0.02 |
| 30 % - 70 % | 1,352±52 | 19.7±0.5 | 6,481±379 | 5,704±201 | 0.47±0.01 |
| 100 % - 0 % | 1,393±57 | 19.0±0.4 | 6,604±330 | 5,537±325 | 0.45±0.01 |
| L.S.D. | 176.6 | 1.23 | 1,305 | 958 | 0.04 |
| C.V. (%) | 15 | 7 | 23 | 20 | 9 |

[(1)]Different letters in the columns are different from each other by Tukey's test ($p < 0.05$); [(2)]Interaction between inoculation and cropping modalities factors; [(3)]Sowing and in topdressing, respectively; * and ** significant at 5 and 1% probability, respectively; [ns]Non-significant; L.S.D. = Least Significant Difference; C.V. = Coefficient of Variation.

In 2016, the interaction between inoculation and cropping modalities influenced the mass of one thousand grains (MTG) (Table 3). Intercropped-sorghum without inoculation presented the highest MTG, in relation to the single cropped, while intercropped-sorghum without inoculation presented higher MTG than the inoculated (Table 4).

Stress condition during growth stages 1 (from sowing to panicle initiation) and 2 (from panicle initiation to flowering) impaired the differentiation of panicle resulting in a reduction in the number of grains, which was compensated by the increase of its mass during growth stage 3 (from flowering to physiological maturation) (Magalhães *et al.*, 2014). This behavior explains the higher MTG of inoculated sorghum as the lowest NGP was obtained in this treatment, which may have been caused by the stress of the plants under low water-availability conditions at a period of high demand by the crop (Sarig *et al.*, 1988) due to the poorly distribution over the cycle (Figure 1).

In contrast, the lower MTG obtained in inoculated sorghum was caused by the higher NGP in this treatment, which may have promoted competition between these drains for photoassimilates at the filling stage. Therefore, based on what has been reported, the higher NGP in the inoculated plants is attributed to the greater ability of these plants to resist environmental stresses, as observed in other studies with inoculation with *A. brasilense* for it is hormonal effect on root growth (Schultz *et al.*, 2012; Andrade *et al.*, 2019).

In 2016, the harvest index (HI) was significantly influenced by the interaction between inoculation and cropping modalities. The intercropped-inoculated sorghum presented higher HI than the single-cropped. In the analysis of inoculation within cropping modalities, the intercropped-inoculated sorghum presented higher HI than the sorghum without inoculation (Table 4). Although plants with a higher MTG have higher HI (Menezes *et al.*, 2015), the increase of the latter atribute in the inoculated sorghum resulted in compensation for the largest NGP.

No significant interactions were found between inoculation and nitrogen fertilization management for the dry matter yield of the aerial part of *U. brizantha* (ADMP). However, in 2015, ADMP was positively influenced by the intercropping with inoculated sorghum (Table 5).

This result can be attributed to the fact that in the first year, the experimental area was not used and in the second year, the experiment was set in succession to the soybean crop, that is, in a more adequate condition due to the residual effect of the legume cultivation. Thus, it is possible that less favorable soil conditions and better rainfall distribution in the first year (Figure 1) provided more favorable conditions for the response of the grass intercropped with inoculated sorghum. In this case, it is possible that the bacterium had migrated to the grass because, according to Hungria, (2011), *Urochloa* plants are hosts of this bacterium.

**Table 5:** Average aerial part dry matter yield (APDM) of U. brizantha grown intercropped with sorghum in nitrogen fertilization, inoculated or not in the seeds with A. brasilense. Means and their respective standard error, 2015 and 2016[(1)]

| Treatments | APDM (kg ha$^{-1}$) | |
|---|---|---|
| | **2015** | **2016** |
| Inoculation | * | ns |
| With | 1,922±130a | 1,440±81 |
| Without | 1,376±166b | 1,306±80 |
| L.S.D | 399 | 241 |
| Fertilization management [(2)] | ns | ns |
| 0 % - 100 % | 1,809±119 | 1,240±75 |
| 30 % - 70 % | 1,696±272 | 1,435±104 |
| 100 % - 0 % | 1,442±197 | 1,444±111 |
| L.S.D. | 596 | 361 |
| Coefficient of Variation (%) | 28 | 20 |

[(1)]Different letters are not different from each other by the Tukey's test ($p < 0.05$); [(2)]Sowing and in topdressing, respectively. L.S.D. = Least Significant Difference.

**Table 4:** Unfolding of the significant interactions between inoculation and cropping modalities, mass of one thousand grains (MTG) and harvest index (HI) of sorghum under nitrogen fertilization management with and without inoculation in the seeds with A. brasilense and single cropped or intercropped with U. brizantha. Means and their respective standard error, 2016[(1)]

| Inoculation | Cropping modalities | | |
|---|---|---|---|
| | **Intercropped** | **Single** | **L.S.D.** |
| | | MTG (g) | |
| With | 18.87±0.4Ba | 18.98±0.4Aa | 1.17 |
| Without | 20.98±0.4Aa | 19.07±0.5Ab | |
| | | HI | |
| With | 0.50±0.01Aa | 0.47±0.01Ab | 0.03 |
| Without | 0.44±0.02Ba | 0.45±0.01Aa | |

[(1)]Same uppercase letters in the same column and same lowercase letters in the row are not different from each other by the Tukey's test ($p < 0.05$). L.S.D. = Least Significant Difference.

## CONCLUSIONS

The total nitrogen fertilizer recommended and applied only at sowing or in topdressing or split (30% at sowing and 70% in topdressing) does not interfere in the yield of grain and straw of sorghum intercropped with *U. brizantha* (cv. Paiaguás).

In severe dry conditions in the off-season, the inoculation of sorghum seeds cv. Ranchero with *Azospirillum brasilense* increase grain yield by 14%.

The intercropping of grain sorghum with *U. brizantha* does not interfere in the sorghum grain yield.

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## REFERENCES

Andrade AF, Zoz T, Zoz A, Oliveira CE S & Witt TW (2019) *Azospirillum brasilense* inoculation methods in corn and sorghum. Pesquisa Agropecuária Tropical, 49:e53027.

Assefa Y, Staggenborg SA & Prasad VPV (2010) Grain Sorghum Water Requirement and Responses to Drought Stress: A Review. Crop Management, 9:1-11.

Bashan Y & De-Bashan LE (2010) How the plant growth-promoting bacterium *Azospirillum*/ promotes plant growth - a critical assessment. Advances in Agronomy, 108:77-136.

Bogiani JC & Ferreira QCB (2017) Plantas de cobertura no sistema soja-milho-algodão no Cerrado. Piracicaba, International Plant Nutrition Institute. 15p. (Technical Bulletin, 160).

Borghi E, Crusciol CAC, Nascente AS, Souza VV, Martins PO, Mateus GP & Costa C (2013) Sorghum grain yield, forage biomass production and revenue as affected by intercropping time. European Journal of Agronomy, 51:130-139.

Borghi E, Crusciol CAC, Trivelin PCO, Nascente AS, Costa Ciniro & Mateus GP (2014) Nitrogen fertilization ($^{15}NH_4NO_3$) of palisadegrass and residual effect on subsequent no-tillage corn. Revista Brasileira de Ciência do Solo, 38:1457-1468.

Cantarella H, Raij BV & Sawazaki E (1997) Sorgo-granífero, forrageiro e vassou. In: Raij BV, Cantarella H, Quaggio JÁ & Furlani AMC (Eds.) Boletim Técnico 100: Recomendação de Adubação e Calagem para o Estado de São Paulo. Campinas, Instituto Agronômico. p.66-67.

Cassán FD, Vanderleyden J & Spaepen S (2014) Physiological and agronomical aspects of phytohormone production by model plant-growth-promoting rhizobacteria (PGPR) belonging to the genus *Azospirillum*. Journal of Plant Growth Regulation, 33:440–459.

Cavalcante TJ, Castoldi G, Rodrigues CR, Nogueira MM & Albert AM (2018) Macro and micronutrients uptake in biomass sorghum. Pesquisa Agropecuário Tropical, 48:364-373.

Ceccon G, Borghi E & Crusciol CAC (2013) Modalidades e métodos de implantação do consórcio milho-braquiária. In: Ceccon G (Ed.) Consórcio Milho Braquiária. Brasília, Embrapa. p.27-46.

Chaves JS, Miranda AFM, Santana AS, Rodríguez CA & Silva ES (2016) Eficiência da inoculação na cultura do arroz (*Oryza sativa* L.) no sul do estado de Roraima. Revista Ambiente: Gestão e Desenvolvimento, 9:75-84.

Coelho AM, Waquil JM, Karam D, Casela CR & Ribas PM (2002) Seja doutor de seu sorgo. Sete Lagoas, Potafos. 24p. (Technical Bulletin, 14).

Crusciol CAC, Mateus GP, Momesso L, Pariz Cm, Castilhos AM, Calonego JC, Borghi E, Costa C, Franzluebbers AJ & Cantarella H (2020) Nitrogen-fertilized systems of maize intercropped with tropical grasses for enhanced yields and estimated land use and meat production. Frontiers in Sustainable Food Systems, 4:1-13.

Crusciol CAC, Mateus GP, Nascente AS, Martins PO, Borghi E & Pariz CM (2012) An innovative crop-forage intercrop system: early cycle soybean cultivars and palisade grass. Agronomy Journal, 104:1085–1095.

Crusciol CAC, Mateus GP, Pariz CM, Borghi E, Costa C & Silveira JPF (2011) Nutrição e produtividade de híbridos de sorgo granífero de ciclos contrastantes consorciados com capim Marandu. Pesquisa Agropecuária Brasileira, 46:1234-1240.

Crusciol CAC, Nascente AS, Mateus GP, Borghi E, Leles EP & Santos NCB (2013) Effect of intercropping on yields of corn with different relative maturities and palisadegrass. Agronomy Journal, 105:599–606.

FAO – Food and Agriculture Organization (2010) An international consultation on integrated croplivestock systems for development: The way forward for sustainable production intensification. Integrated Crop Management. Rome, Food agriculture organization of the United Nations. Available at: < http://www.fao.org/fileadmin/templates/agphome/images/iclsd/documents/crop_livestock_proceedings.pdf.> Accessed on: March 18th, 2016.

Fernandes PG, May A, Coelho FC, Abreu MC & Bertolino KM (2014) Influência do espaçamento e da população de plantas de sorgo sacarino em diferentes épocas semeadura. Ciência Rural, 44:975-981.

Ferreira DF (2008) SISVAR: um programa para análises e ensino de estatística. Revista Científica Symposium, 6:36-41.

Fontes GP, Tomlinson PJ, Roozeboom KL & Diaz DAR (2017) Grain sorghum response to nitrogen fertilizer following cover crops. Agronomy Journal, 109:2723–2737.

Freitas RS, Borges WLB & Ticelli M (2014) Sorgo Granífero – Desempenho Agronômico de Cultivares. Pesquisa & Tecnologia, 11:1-6.

Hadebe ST, Modi AT & Mabhaudh T (2017) Drought tolerance and water use of cereal crops: a focus on sorghum as a food security crop in Sub-Saharan Africa. Journal of Agronomy and Crop Science, 203:177–191.

Hallaq AHA (2010) The impact of soil texture on nitrates leaching into groundwater in the north governorate, Gaza strip. Journal of the Social Sciences, 38:1–37.

Hungria M (2011) Inoculação com *Azospirillum brasilense*: inovação em rendimento a baixo custo. Londrina, Embrapa Soja. 20p.

Hungria M, Campo RJ, Souza SEM & Pedrosa FO (2010) Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant and Soil, 331:413-425.

Hungria M, Nogueira MA & Araujo RS (2015) Soybean seed co-inoculation with *Bradyrhizobium* spp. and *Azospirillum brasilense*: A new biotechnological tool to improve yield and sustainability. American Journal of Plant Sciences, 6:811-817.

Kluthcouski J, Cobucci T, Aidar H, Yokoyama LP, Oliveira IP, Costa JLS, Silva JG, Vilela L, Bacellos AO & Magnabosco CU (2000) Sistema Santa Fé: Tecnologia Embrapa: integração lavoura pecuaria pelo consórcio de culturas anuais com forrageiras, em áreas de lavoura, nos sistemas plantio direto e convencional. Santo Antonio de Goiás, Embrapa/Arroz e Feijão. 28p. (Circular, 38).

Lana MC, Dartora J, Marini D & Hann JEH (2012) Inoculation with Azospirillum, associated with nitrogen fertilization in maize. Revista Ceres, 59:399-405.

Lourenção ALF & Bagega D (2012) Tecnologias para a cultura do sorgo (*Sorghum bicolor* L. Moench). In: Roscoe R, Lourenção ALF, Grigolli JFJ, Melotto AM, Pitol C & Miranda RAS (Eds.) Tecnologia e produção: milho safrinha e culturas de inverno 2012. Campo Grande, Fundação MS. p.138-144.

Macedo MCM (2009) Integração lavoura e pecuária: o estado da arte e inovações tecnológicas. Revista Brasileira de Zootecnia, 38:133-146.

Magalhães PC, Souza TC, May A, Lima Filho OF, Santos FC, Moreira JAA, Leite CEP, Albuquerque CJB & Freitas RS (2014) Exigências edafoclimáticas e fisiologia da produção. In: Borém A, Pimentel LD & Parrella RAC (Eds) Sorgo: do plantio à colheita. Viçosa, Universidade Federal de Viçosa. p.58-88.

Maia GA, Costa KAP, Severiano EC, Epifanio PS, Neto JF, Ribeiro MG, Fernandes PB, Silva JFG & Gonçalves WG (2014) Yield and chemical composition of Brachiaria forage grasses in the offseason after corn harvest. American Journal of Plant Sciences. 5:33-941.

Marini D, Guimarães VF, Dartora J, Lana MC & Pinto Júnior AS (2015) Crescimento e produtividade de híbridos de milho em resposta à associação com *Azospirillum brasilense* e fertilização com nitrogênio. Revista Ceres, 62:117-123.

Mateus GP, Crusciol CAC, Borghi E, Pariz CM, Costa C & Silveira JPF (2011) Adubação nitrogenada de sorgo granífero consorciado com capim em sistema de plantio direto. Pesquisa Agropecuária Brasileira, 46:1161-1169.

Mateus GP, Crusciol CAC, Pariz CM, Borghi E, Costa C, Martello JM, Franzluebbers AJ & Castilhos AM (2016) Sidedress nitrogen application rates to sorghum intercropped with tropical perennial grasses. Agronomy Journal. 108:433–447.

Menezes CB, Saldanha DC , CV Santos, Andrade LC, Mingote Júlio MP, Portugal AF & Tardin FD (2015) Evaluation of grain yield in sorghum hybrids under water stress. Genetics and Molecular Research, 14:12675 12683.

Nakao AH, Andreotti M, Soares DA, Modesto VC & Dickmann L (2018) Intercropping *Urochloa brizantha* and sorghum inoculated with *Azospirillum brasilense* for silage. Revista Ciência Agronômica, 49:501-511.

Pariz CM, Andreotti M, Tarsitano MAA, Bergamaschine AF, Buzetti S & Chioderolli CA (2009) Technical and economical performance of corn intercropped with Panicum and Brachiaria forage in crop-livestock integration system. Pesquisa Agropecuária Tropical, 39:360–370.

Pereira LM, Pereira EM, Revolti LTM, Zingaretti SM & Môro GV (2015) Seed quality, chlorophyll content index and leaf nitrogen levels in maize inoculated with *Azospirillum brasilense*. Revista Ciência Agronômica, 46:630-637.

Puente ML, Garcia JE & Alejandro P (2009) Effect of the bacterial concentration of *Azospirillum brasiliense* in the inoculum and its plant growth regulator compounds on crop yieldof corn (*Zea mays* L.) in field. World Journal of Agriculture Sciences, 5:604-608.

Quadros PD, Roesch LFW, Silva PRF, Vieira VM, Roehrs DD & Camargo FAO (2014) Desempenho agronômico a campo de híbridos de milho inoculados com *Azospirillum*. Revista Ceres, 61:209-218.

Raij BV, Andrade JC, Cantarella H & Quaggio JA (2001) Análise química para avaliação da fertilidade de solos tropicais. Campinas, Instituto Agronômico. 284p.

Repke RA, Cruz SJS, Silva CJ, Figueiredo PG & Bicudo SJ (2013) Eficiência da *Azospirillum brasilense* combinada com doses de nitrogênio no desenvolvimento de plantas de milho. Revista Brasileira de Milho e Sorgo, 12:214-226.

Santos HG, Jacomine PKT, Anjos LHC, Oliveira VA, Lumbreras JF, Coelho MR, Almeida JÁ, Cunha TJF & Oliveira JB (2018) Sistema Brasileiro de Classificação de Solos. Brasília, Embrapa. 353p.

Sarig S, Blum A & Okon Y (1988) Improvement of the water status and yield of field-grown grain sorghum (*Sorghum bicolor*) by inoculation with *Azospirillum brasilense*. Journal of Agricultural Science, 110:271-277.

Schultz N, Morais RF, Silva JÁ, Baptista RB, Oliveira RP, Leite JM, Pereira W, Carneiro Júnior JB, Alves BJR, Baldani JI, Boddey RM, Urquiaga S & Reis VM (2012) Avaliação agronômica de variedades de cana de acucar inoculadas com bactérias diazotróficas e adubadas com nitrogênio. Pesquisa Agropecuária Brasileira, 47:261-268.

Silva AG, Moraes LE, Horvathy Neto A, Teixeira IR & Simon GA (2014) Consórcio sorgo e braquiária na entrelinha para produção de grãos, forragem e palhada na entressafra. Revista Ceres, 61:697-705.

Tully K & Ryals R (2017) Nutrient cycling in agroecosystems: Balancing food and environmental objectives. Agroecology and Sustainable Food Systems, 41:761–798..