Revista Brasileira de Milho e Sorgo
Brazilian Journal of Maize and Sorghum

ISSN 1980 - 6477
Journal homepage: www.abms.org.br/site/paginas

# *Azospirillum brasilense* INNOCULATION COMBINED WITH DIFFERENT LEVELS OF NITROGEN FERTILIZATION AND ITS EFFECTS IN THE GRAIN SORGHUM SUBJECTED TO WATER RESTRICTION

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**How to cite**

PAIVA. A. P. L.; MAGALHÃES. P. C.; CARVALHO, L. P. JALES, H. F.; GOMES JÚNIOR, C. C.; LANA, U. G. P.; MARRIEL. I. E. *Azospirillum brasilense* innoculation combined with different levels of nitrogen fertilization and its effects in the grain sorghum subjected to water restriction. **Revista Brasileira de Milho e Sorgo**, v. 20, e1215, 2021.

**Abstract** – One of the major problems in the present scenario is the effect of climate changes and their consequences to the agriculture, mainly due to decreasing water availability. Water restriction is a limiting factor on plants growth, and it can lead to morphophysiological modifications as well as alterations in the plant's development. Sorghum is a grass widely utilized in agriculture due to its outstanding characteristic of drought resistance, which is higher than the other grasses, therefore being the fifth most sowed grain in the world. The inoculation of growth-promoting rhizobacteria in plants can promote growth and remodel its root system, decreasing the impacts of water restriction. In this sense, this study aims to evaluate the effects of *Azospirillum brasilense* inoculation in mitigating the water restriction effects in the grain sorghum *BRS 332* subjected to two different levels of nitrogen fertilization. Our experiment was carried out in a greenhouse under monitored conditions of temperature and moisture. Plants were treated with two distinct irrigation conditions, two *A. brasilense* inoculants, one treatment without inoculation, and two levels of nitrogen fertilization (high and low nitrogen fertilization). It was evaluated the differences in their ecophysiological and crop production characteristics. The results showed that plants subjected to drought and associated to rhizobacteria invested in shoot parts, which provided this hybrid higher efficiency between water absorption and loss, and consequently higher stomata efficiency during drought when compared to the control treatment. There was also an increment in the production of the grains, particularly flagrant under lower doses of nitrogen, minimizing the effects caused by drought and decreasing the need to utilize fertilizers.

**Keywords:** *Sorghum bicolor*; drought; nitrogen; rhizobacteria; grain yield.

# EFEITO DA INOCULAÇÃO DE *Azospirillum brasilense* COMBINADO COM DOSES DE NITROGÊNIO NO DESENVOLVIMENTO DE SORGO GRANÍFERO SUBMETIDO AO DEFICIT HÍDRICO

**Resumo -** Entre os maiores problemas no cenário atual destacam-se os efeitos climáticos e o consequente impacto na agricultura, sobretudo decorrentes da redução da disponibilidade hídrica. A restrição hídrica é um fator limitante no crescimento das plantas, responsável por causar modificações morfofisiológicas e alterar seu desenvolvimento. O sorgo cuja característica marcante consiste em maior tolerância à seca em relação a outras gramíneas, é o quinto cereal mais produzido no mundo. A inoculação de plantas por rizobactérias promotoras do crescimento pode remodelar seu sistema radicular, mitigando os efeitos do déficit hídrico. Com isso, o objetivo deste trabalho foi avaliar o efeito de inoculantes à base de *Azospirillum brasilense* na mitigação do déficit hídrico sob diferentes níveis de adubação nitrogenada no sorgo granífero *BRS 332*. O experimento foi conduzido em casa de vegetação sob condições controladas de temperatura e umidade. As plantas foram submetidas a duas condições hídricas; dois diferentes inoculantes à base de *A. brasilense*, além do tratamento sem inoculação e, dois níveis de adubação nitrogenada. Foram avaliadas diferentes características ecofisiológicas e de produção. Os resultados mostraram que as plantas submetidas à seca associadas as rizobactérias investiram em parte aérea, o que garantiu a esse híbrido maior eficiência entre a absorção e perda d'agua e, consequentemente, maior condutância estomática durante a seca, em relação aos tratamentos controle. Verificou-se também maior aumento na produção de grãos especialmente na condição de baixa dose de nitrogênio, minimizando os efeitos da seca e o uso em demasia de fertilizantes.

**Palavras-chave:** *Sorghum bicolor*; seca; nitrogênio; rizobactérias; grãos.


DOI: https://doi.org/10.18512/rbms2021v20e1215

Understanding plant physiological responses to drought is a key aspect for agriculture in the current scenarios, since one of the most urgent problems of the present time are the combined effects and impacts of climate changes in the different biomes (Akman; Zhang; Ejeta, 2020). The imbalance triggered by adverse meteorological conditions such as alterations in the temperature patterns, humidity and precipitation are capable of promoting significant alterations in the water availability of a given region, an element that is key to agriculture (Moncada; Petersen; Munkholm, 2021). Recent studies report that water restrictions can decrease plants growth season and development, leading to decreased patterns of plant productivity (Ashraf et al., 2021). In this scenario, studies aiming to increase food production under low water availability are essential.

Sorghum yields are capable of facing those climate adversities due to its high drought tolerance (Akman; Zhang; Ejeta, 2020). Sorghum (*Sorghum bicolor* L. Moench) is a grass native from Africa, and belongs to the *Andropogeneae* tribe. Among the plants utilized in the human diet, it is one of the most versatile and efficient regarding its tolerance to water restriction, high levels of photosynthetic activity and maturation speed. Those plants are capable to produce individuals with high levels of biomass, and it is a source of nourishment for more than 500 million people in 30 countries. Only rice, wheat, corn and potato surpass the sorghum in terms of the amount of food consumed (Silva *et al*., 2015).

Another current research aim is to decrease the utilization of fertilizers, once one of the major problems of using excessive amounts of fertilizers is the entrance of large quantities of nitrogen in the underground water, which leads to severe contamination of the water sources in certain area (Wang *et al*., 2021). One option to decrease the fertilizing dependency is to utilize growth promoting bacteria (GPB), which has great results in mitigating the adverse effects caused by water restrictions as well as increasing crop yields and plants growth (Reis, 2019).

*Azospirillum brasilense* is one of these bacteria whose role in drought tolerance has been documented in several studies. They have been described as enhancers of plants morphological characteristics, leading to individuals with increased number of root ramifications, increased root biomass, increased density of root hairs and resulting in an overall wider exploration of the soil profile when searching for water sources (Hungria, 2011).

In this sense, this study aims to evaluate the responses of sorghum (cultivar BRS 332) inoculated with bacteria from the genus *Azospirillum brasilense* subjected to different levels of water restriction and different doses of nitrogen fertilization. Additionally, we also aimed to observe if the different treatments would mitigate the adverse effects caused by drought in grain production.


DOI: https://doi.org/10.18512/rbms2021v20e1215

## MATERIAL AND METHODS

### I. Growth conditions and vegetal material

The experiment was carried out in a greenhouse at Embrapa Maize and Sorghum Research Center (Embrapa Milho e Sorgo - Brazilian Agency for Agricultural Research), at Sete Lagoas municipality, Minas Gerais State, (19º28' S, 44º15'08'' W, altitude of 732m), Brazil. The climate in the area is typical from the tropics (warm climate and rainy summers), with mean annual precipitation of 1335 mm and mean annual temperature of 21.6ºC.

Dystrophic red latosol was the soil used in the experiment, and it was followed the recommendations of soil correction and fertilization according to the soil chemical analysis, as described in table 1. Soil analysis was performed in the Soils Fertilization Laboratory, at Embrapa Maize and Sorghum. To fertilize the crop it was applied 300 kg $ha^{-1}$ of NPK fertilizer, with the proportion of 8-28-16 of each element.

### II. Description of the treatments and experimental design

The treatments were mainly constituted by:

- Three kinds of inoculation: two based on *Azospirillum brasilense* (strains CMS11 + CMS1626, and one commercial inoculant), and a control treatment without inoculation. The treatments were identified as A1 (CMS11 + CMS1626), A2 (commercial inoculant) and A0 (control without inoculation);
- Two kinds of irrigation: the first one was completely irrigated to field capacity (FC) and the other with water restriction (WR) at 50% field capacity from the moment that the plants were pre-flowering;
- Two levels of nitrogen fertilization: high nitrogen (HN) (180 kg of N $ha^{-1}$) applied at top dressing and low nitrogen (LN) (24 kg de N $ha^{-1}$) applied for soil correction. Urea was utilized as a source of nitrogen fertilization (45% of N in its composition).

A completely randomized design (CRD) with factorial design of 3x2x2, totalizing 12 treatments and 5 repetitions was utilized in this experiment, leading to 60 experimental units.

**Table 1** – Soil chemical analysis.

| $pH_{H20}$ | $P_{Mehlich-1}$ | K | Ca | Mg | Al | H+Al | SB* | V** | OM |
|---|---|---|---|---|---|---|---|---|---|
| - | mg $dm^{-3}$ | | $cmol_c\ dm^{-3}\ kg^{-1}$ | | | | | % | dag |
| 5.24 | 2.17 | 30.33 | 2.47 | 0.2 | 0.37 | 9.07 | 2.74 | 23.23 | 5.97 |
| *SB: sum-of-bases; **V: base saturation; OM: organic matter (Embrapa, 1999). | | | | | | | | | |


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**III. Preparation of the inoculant, nitrogen fertilization and imposition of water stress**

A mixture of two strains of *Azospirillum brasilense* inoculants (CMS11 and CMS1626) was utilized following a 1:1 proportion. The inoculants were part of the diazotroph bacteria collection from the Microbiology and Biochemistry Laboratory from Embrapa Maize and Sorghum. The selected strains were cultivated in a TSB (Tryptic Soy Broth) nutritive solution for 72h at 28ºC temperature, under constant agitation of 120 rpm. After the inoculation cycle, the strains were centrifuged for 10 minutes, at 6000 rpm rotation and resuspended in saline solution [0.85% (m/v) NaCl], and then the optical density was adjusted to 1.0 in 500 nm absorbance, which is equivalent to 108 viable cells per mL (REIS, 2015). The commercial *Azospirillum brasilense* inoculant was also utilized, which was prepared according to the manufacturer recommendations.

The sowing process was performed in 60 pots with the capacity of 20kg, filled with dystrophic red latosol. It was sowed nine seeds per pot disposed in three pits, and then applied 0.004 mL of inoculants by using a precision pipette, totalizing 0.012 mL per pot. It was used a proportion of 200 mL inoculant to 140,000 sorghum seeds, following the recommendations from the Microbiology and Biochemistry Laboratory from Embrapa Maize and Sorghum.

After the germination, the first thinning was performed, leaving three plants per pot and 45 days later the second thinning was performed, leaving only two plants per pot. As soon as the plants reached the stage V4 (four fully expanded leaves), the treatments HN (nitrogen) were fertilized as follow:

- At V4 stage, it was applied 36 Kg $ha^{-1}$of N (0.8g of urea per pot dissolved in 200 mL of water).
- At stages V8 (eight fully expanded leaves) and V12 (twelve fully expanded leaves) it was applied 60 kg of N $ha^{-1}$ (1.34g of urea dissolved in 200 mL of water).

The soil water content was monitored daily during the mornings and afternoons (9am and 3pm) utilizing a moisture sensor watermark (tensiometer model 200SS – 5'', IRROMETER, California – USA), installed in the center of the pots for each repetition at 0.2m depth. Those sensors detect the soil water tension based on the electric resistance by using digital gauges (Watermark meter) from the same company. The values ranged from 0 kPa (completely moist) to -200 kPa (completely dry).

The water reposition was performed according to the sensor scanning values, and the water was replaced to field capacity (FC – the soil water tension was approximately -18 kPa). This process happened before the imposition of the WR (water restriction) treatments. All calculus was performed utilizing an electronic spreadsheet, done as a function of the soil water retention curve. As plants reached the pre-flowering stage, the WR treatments were imposed. It was kept watering the irrigated treatment daily, and this irrigation was performed until the soil reached FC moisture. The non-irrigated treatments received 50% of the total water available, which means that water tension in the soil reached -138 kPa, a value that corresponds to the soils specified. The WR imposition was kept until the grains were harvested.

## IV. Morphophysiological analysis and crop production components

Three morphophysiological evaluations were performed during the imposition of the water restriction, aiming to obtain data from the treatments fully irrigated (day 0), partially stressed (day 7 after the imposition of the water deficit) and at the highest stress (14 days after the imposition of the water deficit).

Plant height was measured by using a measuring tape and the stalks diameter by using a digital pachymeter. All physiological analysis was performed by using the last fully expanded leaf during the mornings, between 8am and 10am. The stomata conductance was measured using a porometer (Leaf Porometer - Decagon Devices), the relative amount of chlorophyll was measured by using a SPAD (Minolta SPAD 502 Osaka, Japan) and the efficiency of the photosystem II ($Fv/Fm$) by using a FluorPen FP 100 device. The leaf water potential ($\Psi_w$) was evaluated by using the Shollander pressure bomb - (1000 Pressure Chamber) in the 7$^{th}$ day of stress, performed at 12pm, and this water potential was kept until the 14$^{th}$ day of stress.

The plants were harvested and divided in three parts: shoot parts (SP – leaves and stalks), panicles and roots. It was determined the total leaf area for each treatment by using a digital leaf area scan (LI-3100C, Licor, Nebraska, USA). After scanning, the SP, plants were placed in paper bags and dried in a dry oven at 65ºC for 72h to obtain their dry mass. The production components determined were: panicle mass, length and diameter; grain total mass and 100 grains mass.

The root system in each pot was carefully cleaned (with minimum loss of the plant material) using running water and stored in plastic pots containers with 70% (v/v) ethanol to preserve the samples until they were analyzed. The morphometric analysis were performed using the WinRhizo system (WinRhizo Pro, Regent Inc. Instr., Canada) connected to a scanner (Epson, Expression 10000 XL, Epson America, Inc., USA) to measure: root length (cm), surface area ($cm^2$), mean diameter (Ø in mm) and root volume ($cm^3$). After finishing those measurements, the roots were placed in plastic bags and transferred to a dry oven; where they were dried at 65ºC for 72h to obtain the root dry mass.

## V. Data analysis

All data was submitted to variance analysis and then to Scoot Knott test to compare their mean values, using 5% of probability ($p \leq 0.05$). The statistical analyses were carried using SISVAR software (System of Analysis of Variance – Federal University of Lavras, Universidade Federal de Lavras, Lavras, Brazil).

## RESULTS

### I. Morphophysiological analysis

The sorghum plants (BRS 332 hybrid) inoculated with the strains of *Azospirillum brasilense* (A1 and A2) had an increment in their height when compared to the control treatment (A0) after 7 and 15 days of water restriction (Figure 1). Additionally, when analyzing the stalk diameter during the 15 days of drought, it was possible to notice that the treatments without the minimum dose of nitrogen (LN), when compared to the treatments subjected to high nitrogen fertilization (HN) without inoculation, presented a significant increment in the stalk diameter (Figure 2).

It was possible to notice in the ecophysiological analyses that the water restriction decreased the amount of chlorophyll in all the inoculation treatments irrespective of the nitrogen fertilization doses, when compared to the treatments that received persistent irrigation (Figure 3 A). It was also possible to verify that the treatments at FC that received HN presented a higher amount of chlorophyll than those under LN (Figure 3 B), highlighting a higher probability of chlorophyll degradation when subjected to water restriction and low doses of nitrogen fertilization.

On the 7th day of water restriction, the water deficit decreased the quantum efficiency of photosystem II (Fv/Fm) when compared to the different kinds of nitrogen fertilization (Figure 4

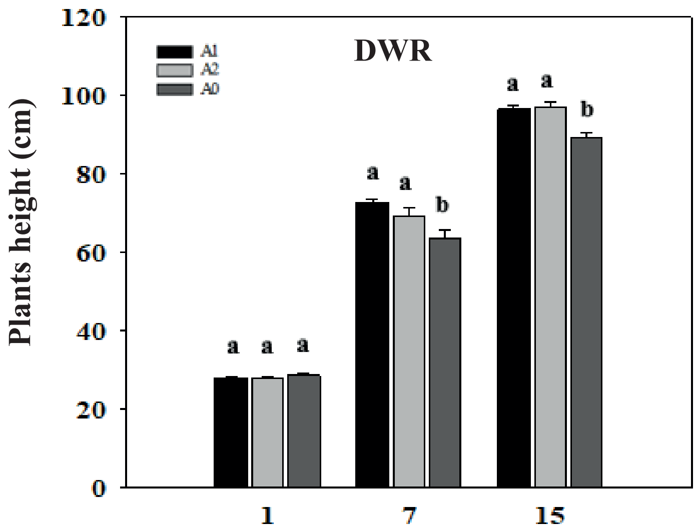


**Figure 1 –** SSorghum plants height inoculated with A1 and A2 (*Azospirillum brasilense*) and control (A0) during the 1st, 7th and 15th day of water restriction (DWR). Averages with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.

**The letters indicate a comparison between the inoculation conditions in a given day.*


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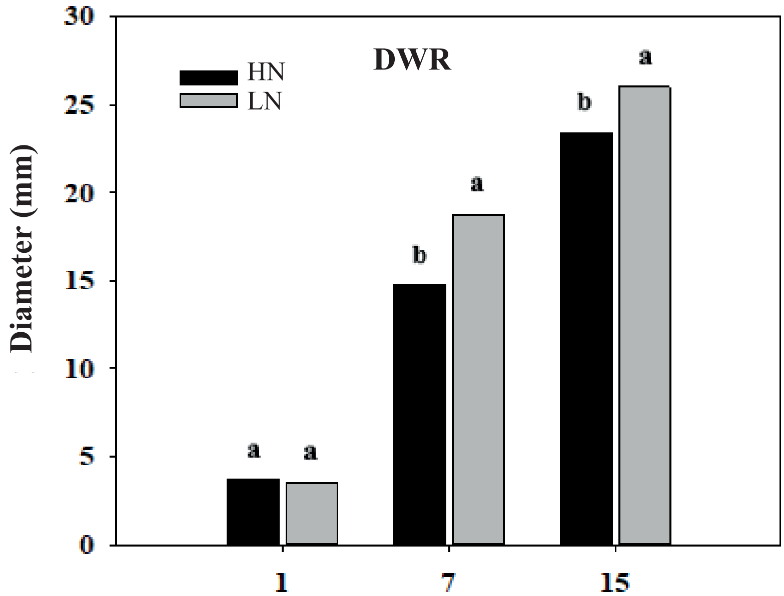


**Figure 2 –** Diameter of the sorghum stalks (mm) submitted to two different doses of nitrogen fertilization (High Nitrogen – HN: 180 kg of N $ha^{-1}$, represented by the black bars; and Low Nitrogen – LN: 24 kg of N $ha^{-1}$, represented by the grey bars) in the 1st, 7th and 15th day of water restriction (DWR). Averages with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.

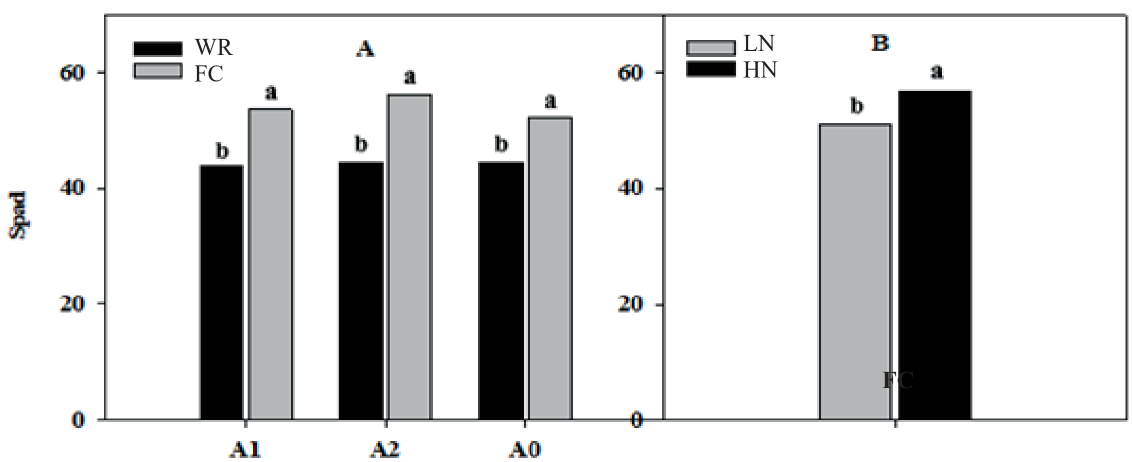


**Figure 3 – A)** Relative chlorophyll content in sorghum leaves inoculated with A1 and A2 (*Azospirillum brasiliense*) and control (A0) subjected to two water conditions: water restriction (WR, represented by the black bars) and field capacity (FC, represented by the grey bars). **B)** Mean values of relative chlorophyll content in plants under different regimens of fertilization (High Nitrogen – HN: 180 kg of N $ha^{-1}$, represented by the black bars; and Low Nitrogen – LN: 24 kg of N $ha^{-1}$, represented by the grey bars) under persistent irrigation. Averages with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.

A) as well as when compared to the treatments with inoculants, where it was not possible to find significant differences between the inoculants (Figure 4 B).

All sorghum plants subjected to water restriction had a decrement in their leaf water potential (Ψmd - MPa) when compared to the plants that were irrigated to FC, irrespective of the interaction (inoculation vs. nitrogen fertilization vs. irrigation kind – Table 2). Plants cultivated under FC presented higher rates of stomata conductance when compared to those under WR (irrespective of the interaction inoculation vs. nitrogen fertilization vs. irrigation kind – Figure 5 A). It is important to highlight that the sorghum plants treated with low nitrogen (LN) associated to *Azospirillum* (A1 and A2) presented a higher stomata conductance when compared to the plants with no inoculant (A0) and irrespective of the kind of irrigation (Figure 5 B).

On the 15th day of water restriction the efficiency of the photosystem II in the sorghum plants was similar to those found on the 7th day of water restriction (Table 3). There were also higher rates of stomata conductance in plants irrigated to FC irrespectively of the inoculant and nitrogen fertilization dose (Table 4). Treatments with low nitrogen fertilization (LN) associated

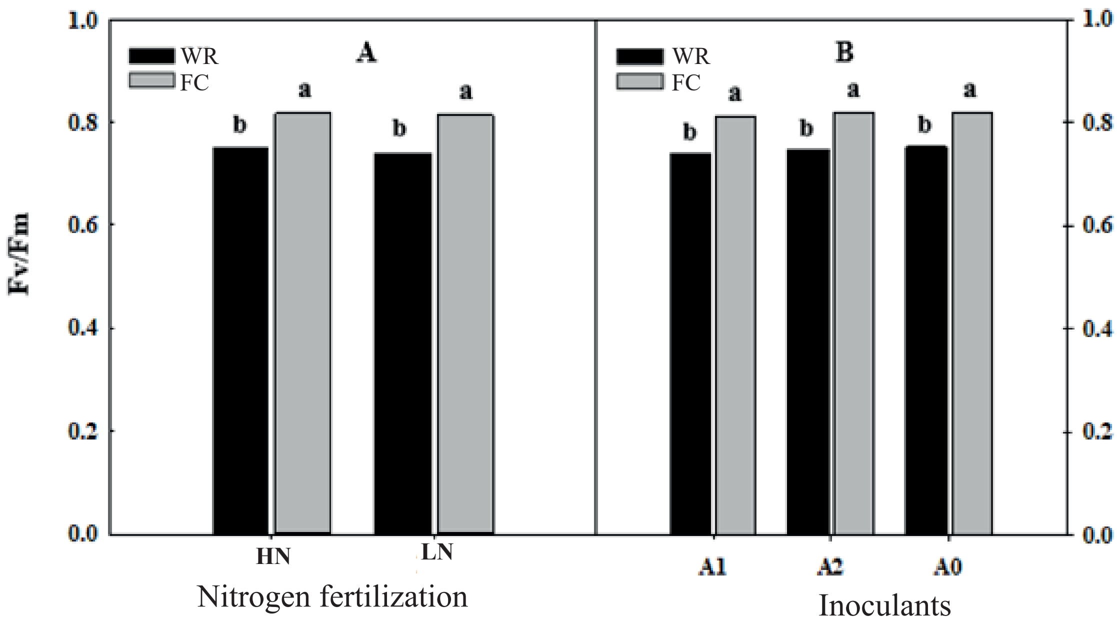


**Figure 4 – A)** Photosystem II efficiency (Fv/Fm) in sorghum plants on the 7th day of water restriction (WR) under different doses of nitrogen fertilization (High Nitrogen – HN: 180 kg of N $ha^{-1}$ and Low Nitrogen – LN: 24 kg of N $ha^{-1}$) subjected to two kinds of irrigation: WR (water restriction) and FC (field capacity). **B)** Photosystem II efficiency in plants inoculated with A1, A2 and with no inoculant (A0) in the interaction of irrigated and stressed water conditions. Averages with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.

**Table 2 -** Leaf water potential (Ψmd - MPa) in sorghum plants subjected to water restriction (WR), persistent irrigation and nitrogen fertilization, inoculated with *Azospirillum brasilense* (A1 and A2) and with no inoculant (control treatment A0). Averages values with the same letters mean that there was no difference when using the Scott-Knott test at 5% probability.

| Treatments | High Nitrogen Doses (180 kg of N $ha^{-1}$) | | Low Nitrogen Doses (24 kg of N $ha^{-1}$) | |
|---|---|---|---|---|
| | Irrigated | Water restriction | Irrigated | Water restriction |
| ***A1*** | -1.00 A | -2.14 B | -0.98 A | -2.14 B |
| ***A2*** | -1.12 A | -1.90 B | -1.08 A | -2.16 B |
| ***A0*** | -1.04 A | -1.96 B | -1.13 A | -2.28 B |

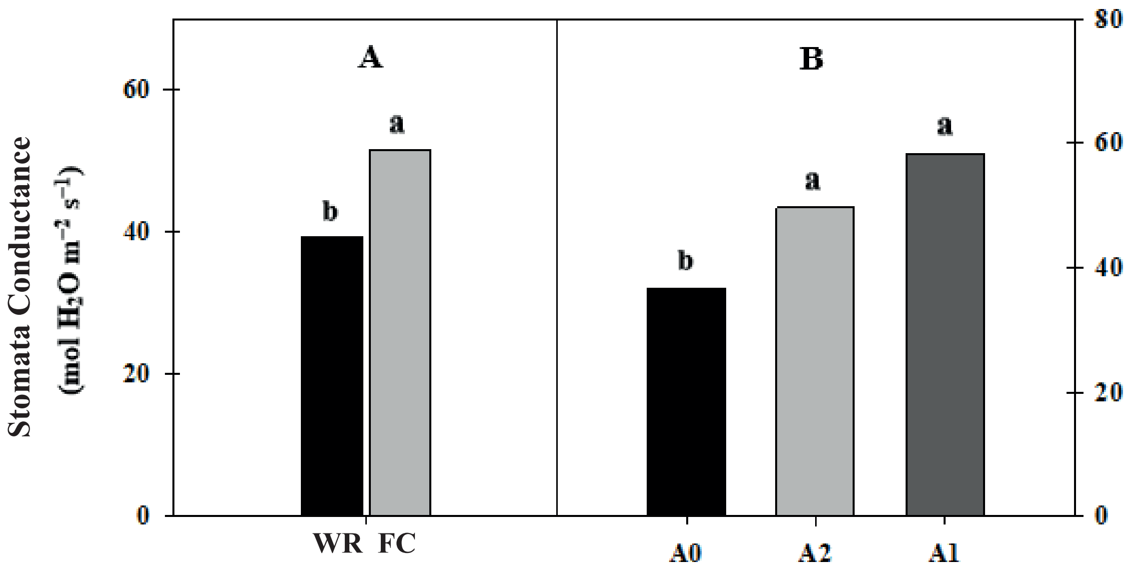


**Figure 5 – A)** Mean values for stomata conductance rates (mol H2O $m^{-2}$ $s^{-1}$) in sorghum plants under two kinds of water irrigation regimens: WR (water restriction, black bars) and FC (field capacity, grey bars). **B)** Mean values for stomata conductance rates (mol H2O $m^{-2}$ $s^{-1}$) in the treatments inoculated with *Azospirillum brasilense* (A1 and A2) and with no inoculant (A0 – black bars) under low nitrogen fertilization. Bars with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.


DOI: https://doi.org/10.18512/rbms2021v20e1215

**Table 3 –** Chlorophyll fluorescence in sorghum plants inoculated with rhizobacteria associated to nitrogen fertilization doses vs. irrigation levels and control. Averages values with the same letters mean that there was no difference when using the Scott-Knott test at 5% probability.

| Treatments on the15th day of WR | High Nitrogen Doses (180 kg of N $ha^{-1}$) | | Low Nitrogen Doses (24 kg of N $ha^{-1}$) | |
|---|---|---|---|---|
| | Irrigated | Water restriction | Irrigated | Water restriction |
| ***A1*** | 0.8120 B | 0.7220 A | 0.8160 B | 0.7260 A |
| ***A2*** | 0.8240 B | 0.7400 A | 0.8240 B | 0.7480 A |
| ***A0*** | 0.8180 B | 0.7590 A | 0.8160 B | 0.7360 A |

**Table 4 –** Mean values of stomata conductance rates (mol H2O $m^{-2}$ $s^{-1}$) in sorghum plants on the 15th day inoculated with *Azospirillum brasilense* and irrigation to field capacity (FC) and water restriction (WR) subjected to two nitrogen fertilization doses. Averages values with the same letters mean that there was no difference when using the Scott-Knott test at 5% probability.

| Treatments | High Nitrogen Doses (180 kg of N $ha^{-1}$) | | Low Nitrogen Doses (24 kg of N $ha^{-1}$) | |
|---|---|---|---|---|
| | Irrigated | Water restriction | Irrigated | Water restriction |
| ***A1*** | 160.16250 B | 57.112500 A | 121.55000 B | 64.787500 A |
| ***A2*** | 149.28750 B | 77.112500 A | 175.03750 B | 58.637500 A |
| ***A0*** | 136.83333 B | 46.225000 A | 142.86250 B | 72.033333 A |

to inoculant A2 presented high rates of stomata conductance (Figure 6).

**II. Sorghum yield data and root morphology**

All the irrigated treatments had higher values of leaf area, shoot parts fresh mass, and shoots parts dry mass, than the treatments subjected to water restriction, regardless of the inoculation type (Figure 7). On the other hand, the inoculation with *Azospirillum brasilense* seemed to decrease the adverse effects caused by the water restriction in the production of sorghum grains. The inoculation with *Azospirillum brasilense* had positive effects in the sorghum plants under water restriction, with increased panicle mass when compared to the control treatment (Figure 8). There was also a positive relation between water restriction (WR) vs. *Azospirillum brasilense* vs. LN related to the panicle fresh mass (Figure 9 A) and panicle diameter (Figure 9 B).

The treatments subjected to field capacity


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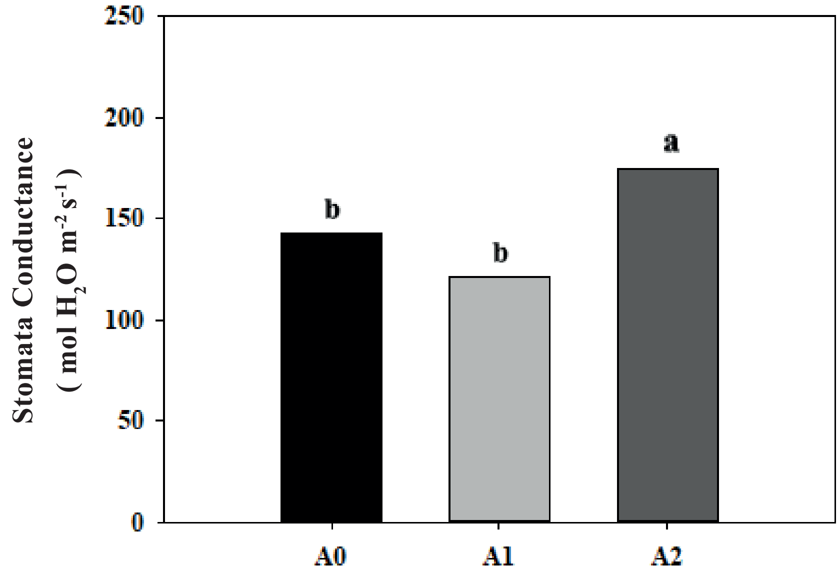


**Figure 6 –** Stomata conductance (mol H2O $m^{-2}$ $s^{-1}$) in sorghum plants inoculated with rhizobacteria subjected to low levels of nitrogen fertilization (24 kg of N $ha^{-1}$); A0 is the control treatment without inoculation (black bars), and treatments A1 (light grey bars) and A2 (dark grey bars) were inoculated with *Azospirillum brasilense*. Averages values with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.

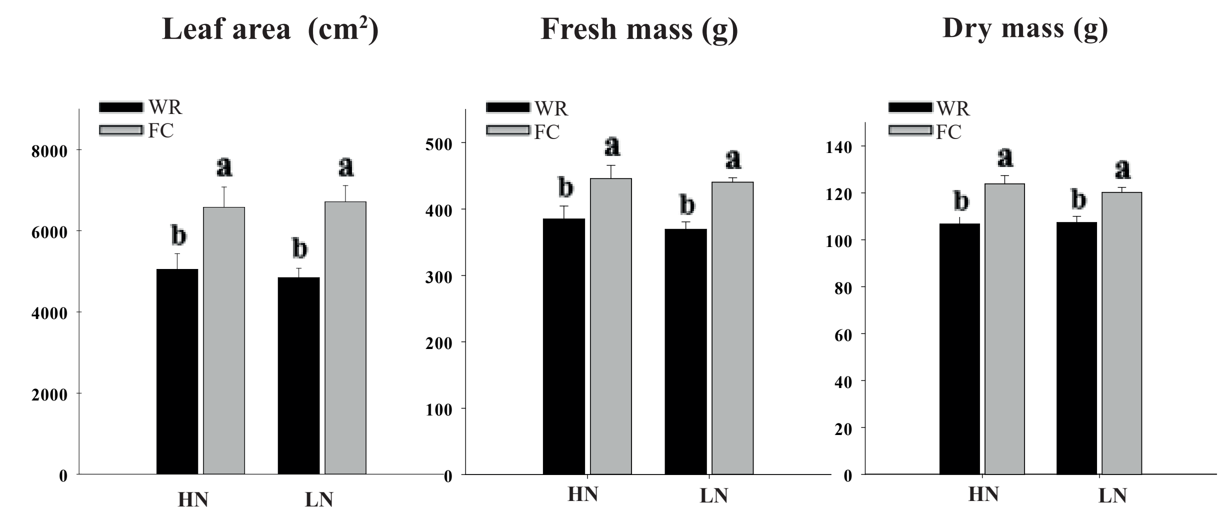


**Figure 7 –** Evaluation of the shoot parts of sorghum subjected to water restriction (WR, black bars) and field capacity (FC, grey bars) in two distinct nitrogen fertilization schemes (high nitrogen - HN:180 kg of N $ha^{-1}$ and low nitrogen - LN: 24 kg of N $ha^{-1}$). Averages values with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.


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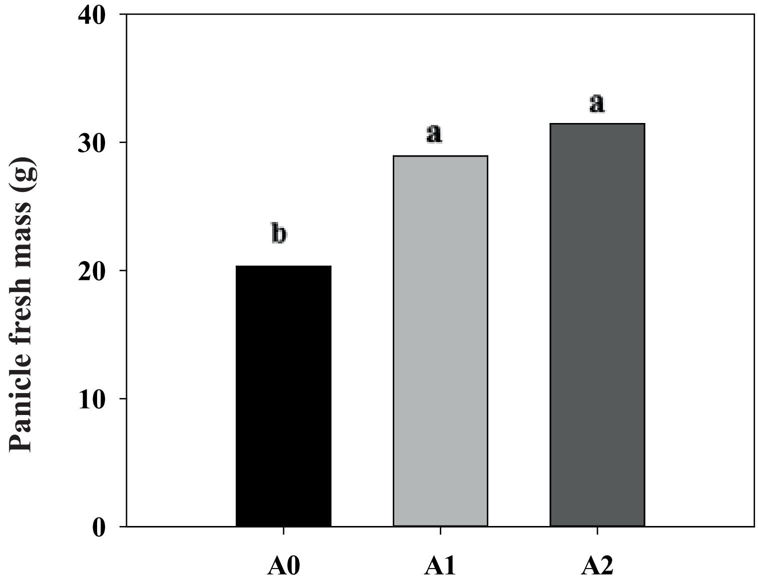


**Figure 8 –** Panicle fresh mass (g) from the control treatment (black bars, A0) and inoculated treatments (rhizobacteria *Azospirillum brasilense* – light grey A1, and dark grey A2) subjected to water restriction. Averages values with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.

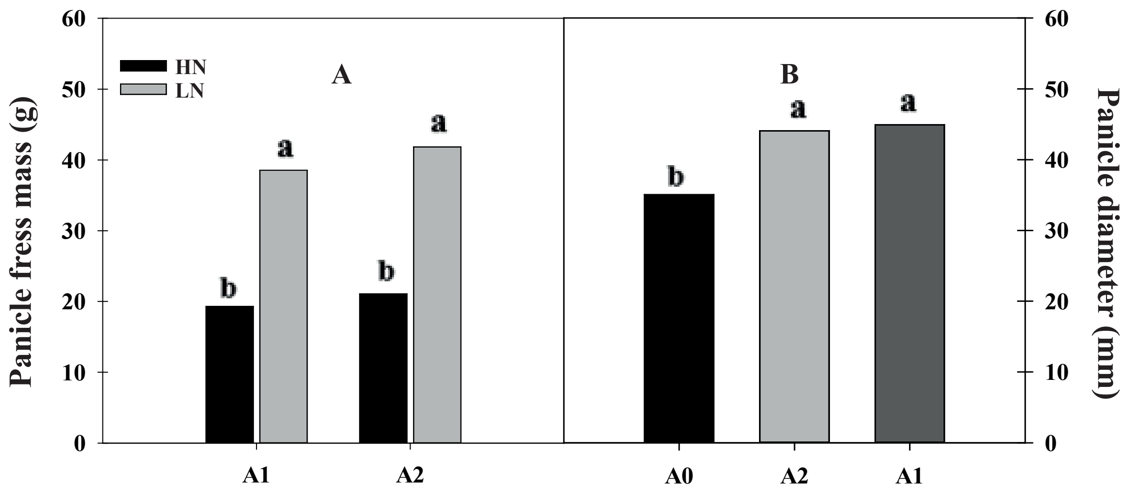


**Figure 9 –** Effect of the inoculation with *Azospirillum brasilense* rhizobacteria in sorghum plants. A) Panicle fresh mass of the plants cultivated using low (black bars with inoculant A1, and 24 kg of N ha$^{-1}$) and high doses of nitrogen fertilization (light grey bars with inoculant A2, 180 kg of N ha$^{-1}$) subjected to water restriction. B) Panicle diameter of the plants inoculated with *Azospirillum brasilense* rhizobacteria (A1dark grey bars and A2 light grey) and with no inoculant (A0 black bars) subjected to low doses of nitrogen fertilization. Averages values with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.

(FC) had a higher productivity of grains when compared to the treatments subjected to water restriction (WR). The total amount of fresh mass in the grains subjected to water stress was superior in the treatments that received the inoculant *Azospirillum brasilense* (Figure 10). Grains treated with low nitrogen (LN) fertilization had higher volume of grains than grains treated with high nitrogen (HN) fertilization when comparing the mass of 100 grains weight and total weight of the grains (Figure 11).

When analyzing the morphometry of the root system, the root length, mean root diameter and root dry mass values were higher when associated to high levels of nitrogen (HN) fertilization and were independent of the presence of inoculants (Table 5). Regarding the root dry mass, the treatments inoculated with *Azospirillum brasilense* had lower mass than the control treatment. There were also no differences in the root morphology in the irrigated treatments and non-irrigated treatments, which is interesting because it is possible to assume that the rhizobacteria increased the root growth in plants subjected to water restriction.

## DISCUSSION

Drought is a limiting event in plants life, and it can lead to morphophysiological and developmental modifications (Marques, 2019). In this study, it was demonstrated that the gas exchange rates, the shoot parts (i.e. leaf area, leaf fresh mass and leaf dry mass) and the productivity indexes were affected by water restriction. It was also demonstrated that the inoculation with the *Azospirillum brasilense* rhizobacteria increased the sorghum plants (BRS 332 hybrid) root system, plant's height and enhanced its leaf water potential. *Azospirillum brasilense* is a rhizobacteria capable of fixing atmospheric $N_2$ by using a complex of nitrogenase enzymes, working under low oxygen concentrations, which confers the plants a higher ability to thrive abiotic stress.

Sorghum plants inoculated with the strains of *Azospirillum brasilense* (A1 and A2) were higher than the control plants (A0). Nevertheless, there was no statistical difference

**Table 5 –** Sorghum plants root system morphometry subjected to two nitrogen fertilization doses. Averages values with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.

| | Root length (cm) | Root diameter (mm) | Root dry mass (g) |
|---|---|---|---|
| **High nitrogen doses (180 kg of N ha$^{-1}$)** | 1918.3029 a | 23218.003 a | 18.6180 a |
| **Low nitrogen doses (24 kg of N ha$^{-1}$)** | 1726.0492 b | 19519.148 b | 15.2470 b |

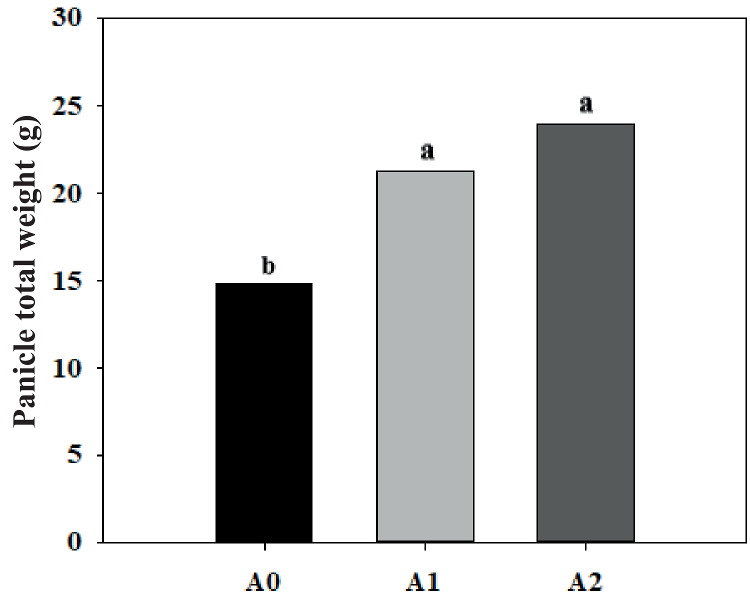


**Figure 10 –** Effect of the inoculation with *Azospirillum brasilense* rhizobacteria (A1 light grey bar and A2 dark grey bar) and without inoculation (A0 black bar) in the panicle total weight of sorghum grains subjected to water restriction. Averages values with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.

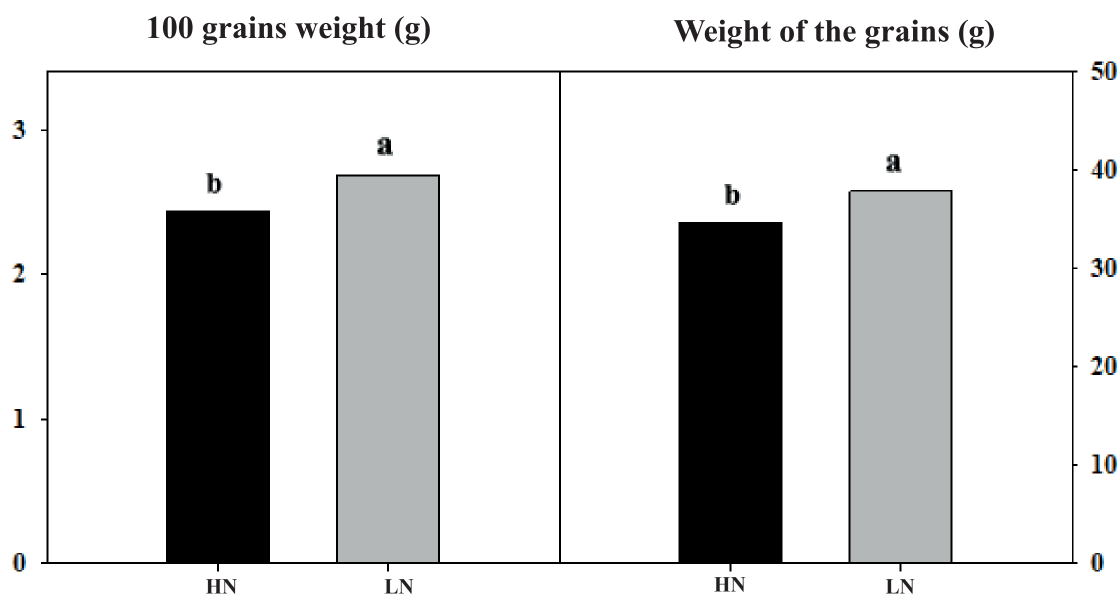


**Figure 11 –** Production of sorghum grains subjected to different doses of nitrogen fertilization (black bars, high nitrogen fertilization HN: 180 kg of N $ha^{-1}$ and light grey bars, low nitrogen fertilization LN: 24 kg of N $ha^{-1}$). Averages values with the same lower case letters mean that there was no difference when using the Scott-Knott test at 5% probability.

when comparing the interactions with the nitrogen fertilization doses. Our results diverge from the literature, where it is possible to find many studies that report the beneficial effects of using the association of diazotroph bacteria and nitrogen fertilization. Working with *Urochloa brizantha* cv Paiaguás Rocha and Costa (2018) found a 8% taller plants when comparing inoculated fertilized treatments to controls without those treatments. In a study using maize plants, Kappes *et al*. (2011) found similar results when analyzing the influences of *A. brasilense* inoculation, where the plants inoculated with the bacteria were taller than the control treatment.

Nitrogen is one of the most important macronutrients for plants. According to Vanderlip (1999), sorghum plants need approximately 70% of nitrogen until half of its growing cycle (sixty days after flower emergence). Nitrogen is crucial for the formation of proteins, amino acids and DNA (Goulart, 2016), and when absent can limit plant growth (Carvalho, 2005), once this nutrient acts directly in the process of cell expansion and division (Martins, 2013). Sorghum plants present high levels of nitrogen in its stalks and leaves, and it is later translocated to the grains (Albuquerque et al., 2013). It is possible to assume that the bacteria increased plant survival, particularly increasing the exploitation of the soil through the roots, as *Azospirillum brasilense.* It enhances the acquisition of essential resources such as water, nitrogen, phosphorus and other minerals (Fukami *et al.,* 2017), in addition to altering the morphology of the roots by producing growth regulatory substances such as phytohormones (Kaushal, 2019). Our results corroborate those found by Marques (2019).

It was possible to notice that the panicle production components of sorghum BRS 332 had a high output when fertilized with low nitrogen doses. Our results are similar to those of Mortate *et al*. (2020) when studying sorghum plants inoculated with *Azospirillum brasilense* subjected to different amounts of nitrogen fertilization at Ituitaba municipality, Minas Gerais State. Mortate *et al*. (2020) also found that sorghum productivity increased when the seeds were inoculated and there was a 25% reduction in the use of nitrogen fertilizers. A possible hypothesis is that there is a restrain of bacterial growth caused by presence of the nutrient. Roesch and colleagues (2006) observed that maize plants subjected to high amounts of nitrogen fertilization decreased the diazotroph bacteria colonization. According to Fonseca (2014), nitrogen is capable of altering plant's physiological conditions, and in this sense, it is possible that the interaction with bacteria could be affected. When studying plants in a greenhouse, Mortate *et al* (2020) found that treatments which received 200 kg of urea $ha^{-1}$ had an inhibitory effect in the proliferation of diazotroph bacteria. They also found that the population of diazotroph bacteria in the rhizosphere of sorghum plants originating from seeds inoculated with *A. brasilense* decreased when the amount of urea utilized in the fertilization increased.

When comparing the genotype of

sorghum utilized in our study, it was possible to find that imposing a water restriction regimen decreased the leaf water potential, but there was no difference between the treatments inoculated with the rhizobacteria. Marques (2019) found similar results when studying corn plants. Arzanesh *et al.* (2011), on the other hand, found different results for wheat plants under the same drought conditions.

The plants main response to a decreasing water potential is to close the stomata, a mechanism utilized to reduce the water loss to the atmosphere. Once the absorption of $CO_2$ and the photosynthetic rates are reduced, the overall plant development is affected (Marques, 2019). This pattern can be emphasized in the present study, since plants at FC presented higher stomata conductance values. It is also important to highlight that plants subjected to low nitrogen fertilization and associated to *Azospirillum brasilense* surpluses those that were not inoculated. These results suggest that the bacteria decreased the limitation of the non-stomatic mechanism that the water restriction reflects in plants of this hybrid.

Chapman and Barreto (1997) explains that the chlorophyll content is associated to the fact that more than 50% of the total amount of nitrogen in the leaves interact with the chloroplasts and chlorophyll components. In this sense, all plants subjected to water restriction (WR) also presented decreased levels of chlorophyll. Our results corroborate those from Ávila (2018) and Kalaji *et al.* (2016), as the fluorescence of the chlorophyll *a* is a great indicative of the integrity of photosystem II. The results obtained using flourpen were under 0.75, and this result indicates that the quinone (primary acceptor of electrons in the photosystem II – PSII) is completely oxidized. Then, the center of reaction in FSII is open, which means that it is possible that the PSII reaction center was damaged or decreased its capacity to transfer energy for the other components (Konrad, 2005). Thus, a lower fluorescence indicates a lower level of stress and a higher photochemical efficiency.

## CONCLUSIONS

Inoculating sorghum with *Azospirillum brasilense* strains increased the shoot parts growth as well as the production of sorghum grains (BRS 332 hybrid), particularly under low nitrogen doses. The inoculation also increased the root system growth, minimizing the adverse effects caused by the water restrictions.

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