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

ISSN 1980 - 6477

Journal homepage: www.abms.org.br/site/paginas

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How to cite

MARQUES, D. M.; MAGALHÃES P. C.; MARRIEL, I. E.; GOMES JUNIOR, C. C.; SILVA, A. B.; MELO, I. G.; SOUZA, T. C. *Azospirillum brasilense* favors morphophysiological characteristics and nutrient accumulation in maize cultivated under two water regimes. **Revista Brasileira de Milho e Sorgo**, v. 19, e1152, 2020

Azospirillum brasilense FAVORS MORPHOPHYSIOLOGICAL CHARACTERISTICS AND NUTRIENT ACCUMULATION IN MAIZE CULTIVATED UNDER TWO WATER REGIMES

Abstract : The use of plant growth-promoting rhizobacteria (PGPR) is an important and promising tool for sustainable agriculture. The objective of this study was to evaluate the morphophysiological responses and nutrient uptake of maize plants inoculated with *A. brasilense* under two water conditions. The experiment was carried out in a greenhouse with ten treatments: five *A. brasilense* inoculants (Control, Az1, Az2, Az3 and Az4) inoculated in the seed and two water conditions - irrigated and water deficit. Treatments with water deficit were imposed at the V6 stage for a period of 15 days. The morphophysiological characteristics, gas exchange, root morphology, shoot, root and total dry matter, as well as nutrient analysis, were evaluated after water deficit. *Azospirillum brasilense* (Az1, Az2, Az3 and Az4) increased growth (height 10.5%, total dry weight 20%), gas exchange (Ci= 6%) and nutrient uptake (N= 19%, P= 20%, K= 24%) regarding control under irrigation conditions. Inoculation by Az1 and Az3 benefited the root architecture of maize plants, with a greater exploitation of the soil profile by these roots. Water deficit caused a reduction in the development of maize plants. Inoculation by Az1, Az2 and Az3 can improve plant growth, nutrient uptake and mitigate the effects of water deficit in the development of maize plants.

Keywords: *Zea mays* L.; Water deficit; WinRhizo; Leaf area; Rhizobacteria.

Azospirillum brasilense FAVORECE CARACTERÍSTICAS MORFOFISIOLÓGICAS E ACÚMULO DE NUTRIENTES EM MILHO CULTIVADO SOB DOIS REGIMES HÍDRICOS

Resumo : O emprego de rizobactérias promotoras do crescimento de plantas (PGPR) é uma ferramenta importante e promissora para a agricultura sustentável. O objetivo deste trabalho foi avaliar as respostas morfofisiológicas e a absorção de nutrientes das plantas de milho inoculadas com *A. brasilense* em duas condições hídricas. O experimento foi realizado em casa de vegetação, com dez tratamentos: cinco inoculantes de *A. brasilense* (Controle, Az1, Az2, Az3 e Az4) aplicados na semente e duas condições hídricas - irrigado e em déficit hídrico. Os tratamentos com déficit hídrico foram impostos no estádio V6 por um período de 15 dias. Foram avaliadas, após a imposição do déficit hídrico, as características fitotécnicas, trocas gasosas, morfologia radicular, massa seca da parte aérea, raiz e total e análise de nutrientes. *Azospirillum brasilense* (Az1, Az2, Az3 e Az4) proporcionou maior crescimento (altura 10,5%, massa seca total 20%), aumento nas trocas gasosas (Ci= 6%) e absorção de nutrientes (N= 19%, P= 20%, K= 24%) em relação ao controle na condição de irrigação. A inoculação por Az1 e Az3 beneficiou a arquitetura radicular das plantas de milho, com maior exploração do perfil do solo por estas raízes. O déficit hídrico causou redução no desenvolvimento das plantas de milho. A inoculação por Az1, Az2 e Az3 pode melhorar o crescimento vegetal, absorção de nutrientes e mitigar os efeitos do déficit hídrico no desenvolvimento das plantas de milho.

Palavras-chave: *Zea mays* L.; Déficit hídrico; WinRhizo; Área foliar; Rizobactéria.

DOI:https://doi.org/10.18512/1980-6477/rbms 2020.v19.e1152

Throughout their development, plants can be exposed to various environmental stresses. Water deficit is the abiotic stress that most reduces the yield of cultivated areas in the world, negatively impacting agricultural sustainability (Kerry et al., 2018). The plant response to water stress is a reduction in gas exchange, a decrease in leaf area and growth rate (Mutava et al., 2015; Dar et al., 2018). Under conditions of low water availability, plants absorb less nutrients due to decrease in mass flow transport (Vurukonda et al., 2016).

Maize is one of the crops most sensitive to water status (Djemel et al., 2019), and is frequently grown worldwide under water deficit conditions. Under cultivation conditions with a reduction in approximately 40% water availability (drought), maize reduces its yield by, on average, 39% (Daryanto et al., 2016). Therefore, the tolerance of maize plants to water deficit from drought needs to be improved in order to meet the food demand in areas with limited availability of water resources. In this context, the search for technological research that aligns water availability with soil management is gaining importance in the current global scenario.

The use of plant growth-promoting rhizobacteria (PGPR) is an important and promising tool for sustainable agriculture. PGPR offers an economically attractive and ecologically correct alternative to increase water and nutrient availability (Prasad et al., 2019). Due to their huge genetic pool, these microorganisms are the source of biochemical reactions that recycle nutrients for plant growth (Tkacz & Poole, 2015). *Azospirillum* is one of the best studied genera of bacteria of PGPR. These bacteria are able to colonize several species of monocotyledonous plants, including important crops such as wheat, maize and rice (Yadav & Sarkar, 2019).

The benefits arising from the association of this bacteria with plants are the synthesis of various growth regulating substances, such as phytohormones (Barnawal et al., 2019); biological fixation of nitrogen (Revolti et al., 2018); phosphate and iron solubilization (Galindo et al., 2016; Revolti et al., 2018); biocontrol of phytopathogens (Cassán & Diaz-Zorita, 2016) and plant protection against abiotic stress (Dar et al., 2018; Kaushal, 2019). Due to the positive effects on plant growth and nutrition, the use of *Azospirillum* can be considered a plant biostimulant (Van Oosten et al., 2017).

Inoculation by *Azospirillum brasilense* leads to plant growth due to the combination of several physiological, biochemical and morphological mechanisms (Bashan & Bashan, 2010). In the roots, *Azospirillum* causes modifications in the root architecture, which are related to the increase in the number of radicels and root length, leading to greater soil exploration (Vacheron et al., 2013) and nutrient absorption (Dar et al., 2018). Inoculation may also induce hormone signaling from root to shoot, regulating leaf growth and other plant physiological processes (Barnawal et al., 2019).

It is important to consider that the interaction of *A. brasilense* strains with plants is quite dynamic, considering the genetics of the bacteria itself, plant stage, type of soil, besides the environmental condition under study (Coelho et al., 2017). However, it is noteworthy to mention that, Galindo et al. (2018) found a 5 to 6% increase in corn yield when plants were inoculated with *A. brasilense*. In addition, Hungary et al. (2010) and Okon & Labandera-Gonzalez (1994) reported grain yield gains up to 30% in inoculated plants compared to those without inoculation. Thus, broadening these studies with new inoculants in drought tolerant (DKB 390) and tropical maize genotypes, as well as characterizing morphology concomitantly with physiology, may have great benefits for world agriculture. In addition, PGPR studies under abiotic stress such as water deficit are still poorly explored in maize. Coupled with these factors, the selection of strains tolerant and effective to water deficit is important and makes farming practices more sustainable.

In this context, the objective of this study was to evaluate the morphophysiological and nutrient uptake responses of maize plants inoculated with *A. brasilense* under two water conditions.

Material and Methods

Plant and microbiological material, growth conditions and experimental design

The experiment was carried out in a greenhouse at Embrapa Milho e Sorgo, in Sete Lagoas - MG, Brazil, located at the geographical coordinates: 19º28’ S, 44º15’08’’ W, and average altitude of 732 m. The temperature averages recorded during the evaluation period were maximum of 31.2ºC and minimum of 12.9ºC. Relative air humidity ranged from 30% to 72%. The maize hybrid used was DKB 390, which is tolerant to low water availability and adapted to different types of soil and managements. According to Souza et al. (2013) and Ávila et al. (2017), this hybrid has been classified as tolerant to water deficit due to its morphophysiological and biochemical adaptations. For the formulation of each inoculant, the combination of two homologous *Azospirillum brasilense* strains, belonging to the collection of the Laboratory of Microbiology and Soil Biochemistry of Embrapa Milho e Sorgo, was used at a 1:1 (v/v) ratio.

The experimental design was completely randomized (CRD), in a 5 x 2 factorial scheme, consisting of five *A. brasilense* inoculants: control (without inoculation), Az1 (CMS 7+26), Az2 (CMS 11+26), Az3 (CMS 26+42) and Az4 (CMS 7+11) and two contrasting water conditions (irrigated and water deficit), with four replications.

The selected strains were grown in trypcasein soy broth for 72 hours at 29°C under constant stirring. After this period, cultures of each strain were centrifuged, resuspended in saline solution (0.85% NaCl) and adjusted to an optical density of approximately 10^8 viable cells per mL, according to preliminary tests. Seed inoculation was carried out using ground charcoal and cassava starch paste as a carrier.

Sowing was carried out in 20 dcm^{-3} plastic pots, containing Oxisol. Five seeds were planted per pot and, after germination, thinning was done, leaving two plants per pot. Fertilization was carried out according to the recommendation of the soil chemical analysis, applying the formulation 08-28-16 (10 g) and FTE BR12 (2.5 g) to 20 dcm^{-3} of soil at planting. Nitrogen top dressing was performed applying 4 g of urea per pot 30 days after planting.

The water content in the soil was monitored daily between 9 a.m. and 3 p.m. with the aid of GB Reader N1535 (Measurement Engineering, Australia) moisture sensors installed in the center of each pot at a depth of 20 cm. All treatments were maintained at field capacity (FC) (water tension in the soil of –18 kPa) during the period prior to stress imposition. At the V6 growth stage (six fully expanded leaves), water deficit was imposed for a period of 15 days in the treatment undergoing water restriction. In this treatment, the water tension in the soil was reduced to -138 KPa, which corresponds to the application of 50% of the water available in the soil. The irrigated treatment did not undergo alterations under the water condition, maintaining field capacity. Water replenishment calculations were performed with the aid of a spreadsheet, made according to the water retention curve of the soil.

Biometric variables and dry matter

After fifteen days of water deficit, the following biometric variables were evaluated: plant height, using a graduated ruler; stem diameter, using a digital caliper, measured at the soil level; total plant leaf area (LA), measured by a leaf area reader (LI-3100C, Licor, Nebraska, USA). Plant shoot was conditioned in paper bags and subjected to forced air drying at 65ºC for 72 hours, after which the dry matter was evaluated.

Echophysiological characteristics

Gas exchange was evaluated in the last fully expanded leaf, in the morning, between 8 a.m. and 10 a.m., on the first and last day (15 days) of water deficit imposition. The net photosynthetic rate (A), stomatal conductance (gs) and internal carbon (Ci) were evaluated. From the values of A and Ci, carboxylation efficiency (A/Ci) was obtained. An LI 6400 infrared gas analyzer (IRGA - LI-COR, Lincoln, NE, USA) was used, equipped with a chamber (LI-6400-40, LI-COR Inc.). Measurements were performed on a 1 cm^2 leaf area and the airflow in the chamber was at a CO_2 concentration of 380 mmol mol^{-1}. A photon flux density (PPFD) of 1500 µmol m^{-2} s^{-1} was used with a red-blue LED light source and the chamber temperature was 28°C.

The leaf water potential (midday, Ψ_{md}) was determined at 12 o'clock through a Scholander pressure chamber (3005 Soil Moisture Equipment Corp., Santa Barbara CA, USA) on a fully expanded leaf per replicate.

Root morphology and macronutrients in tissues

For the analysis of the morphology of the root system, the image analysis system WinRhizo Pro 2007a (Regent Instruments, Sainte-Foy, QC, Canada) was used, coupled to a professional scanner (Epson, Expression 10000 XL, Epson America, Inc., USA), equipped with an additional light unit (TPU). The procedures for obtaining the images were made according to Souza et al. (2012). The following characteristics were determined: root length (cm), root surface area (cm^2), mean root diameter (mm) and root volume (cm^3). The roots were then stored in paper bags and transported to a forced circulation oven at 65°C until a constant mass was obtained.

After shoot and root dry matter were evaluated, the samples were ground in a Willy mill. The ground material was used to determine the accumulation of nutrients N, P, K, Ca, Mg, S, Cu, Mn and Zn in the dry matter, according to the methodology proposed by Silva (2009).

Data analysis

For all analyzed variables, the means and the ± standard error (SE) were calculated. For the statistical analysis of the results, the analysis of variance (ANOVA) and the Scott Knott test at 0.05% significance ($p \leq 0.05$) were performed, using the Sisvar software, version 4.3 (Universidade Federal de Lavras, Lavras, Brazil).

Results

Under irrigation, the presence of all inoculants (Az1, Az2, Az3 and Az4) yielded an increase in height of 9 to 12% and LA of 15 to 35%, in comparison to the control (Fig. 1ad). The same pattern was observed for shoot dry matter, with inoculants increasing in Az1 (22%), Az2 (16%), Az3 (29%) and Az4 (15%) and higher total dry matter(Az1-21%, Az2-16%, Az3-29% and Az4-15%), when compared to uninoculated control plants (Fig. 1ef). Root dry matter did not differ among treatments (data not shown). There was no difference in mean stem diameter and Ψmd among treatments for the same condition ($p \leq 0.05$) (Fig. 1bc).

The water deficit condition reduced all the physiological characteristics (Fig.1abcdef). In relation to the inoculation by *A. brasilense*, the inoculants Az1 and Az2 increased the height of maize plants by 18% and 21.5%, respectively, in relation to the plants without inoculation (Fig. 1a). Stem diameter was higher with the presence of all inoculants, when compared to the control (Fig. 1b). LA was higher with inoculants Az1 (28%), Az2 (37%) and Az3 (23%) (Fig.1d). Ψmd, shoot dry matter and total dry matter did not differ statistically among treatments under the same condition ($p \leq 0.05$) (Fig. 1 cef).

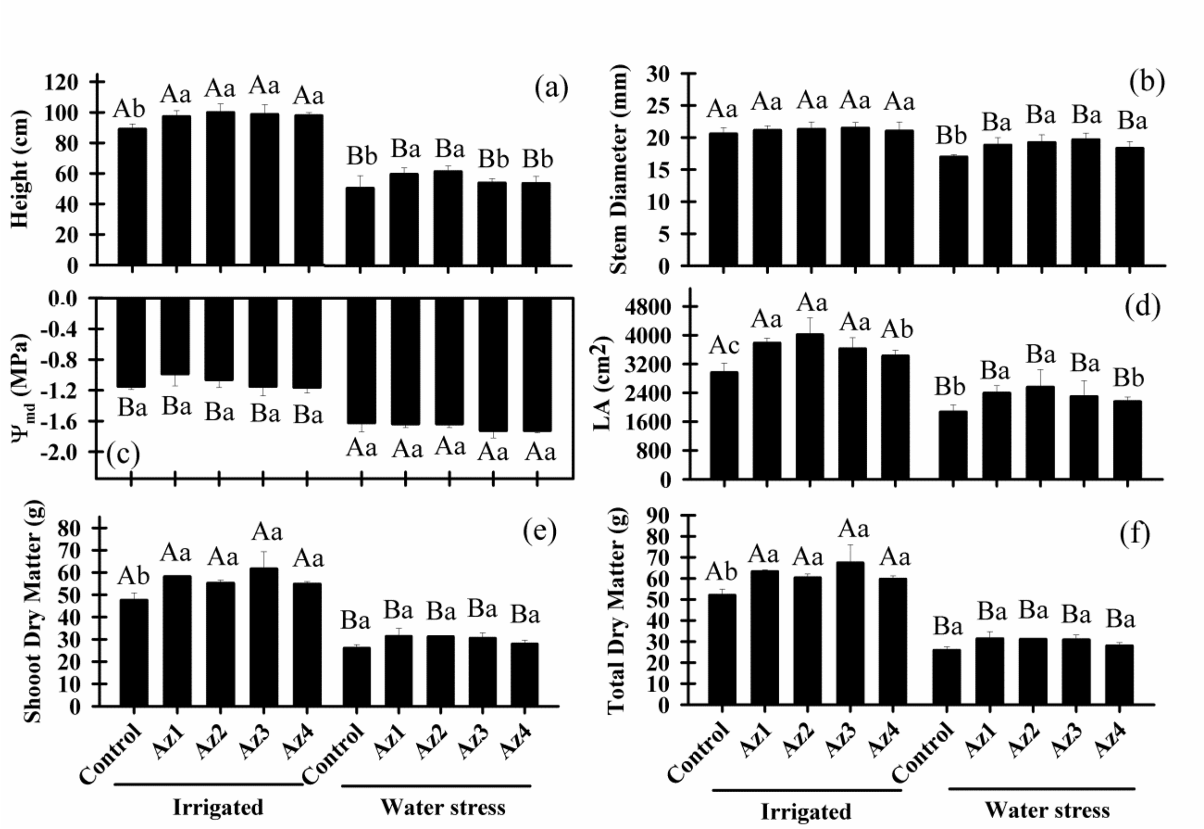

Figure 1. Maize plants submitted to irrigation and water deficit inoculated with *A. brasilense* (Az1, Az2, Az3 and Az4). Plant height (a); stem diameter (b); water potential (Ψmd) (c); leaf area (LA) (d); shoot dry matter (e) and total dry matter (f). Lowercase letters compare the treatments under the same condition (irrigated/water deficit). Uppercase letters compare the treatments within irrigation and water deficit. Means followed by the same letter do not differ by the Scott-Knott test at 5% probability ($p \leq 0.05$). Each value indicates the mean ± SE.

Leaf gas exchange (A, gs, Ci and E) was higher under irrigation, when compared to those under stress ($p \leq 0.05$) (Fig. 2abcd). For plants under irrigation, A, gs and E did not differ statistically with the imposition of treatments ($p \leq 0.05$) (Fig. 3abd). The presence of inoculant Az1 yielded an increase in Ci in maize leaf mesophyll (Fig. 3c) and a reduction in carboxylation efficiency (A/Ci) for the same condition, when compared to the other treatments.

In relation to the water deficit condition, A was higher with inoculants Az2 and Az4 (Fig. 3a). There was no statistical difference for the variables gs, Ci and E under the same condition ($p \leq 0.05$) (Fig. 2bcd). Carboxylation efficiency was higher with inoculant Az4 (Fig. 2e).

In general, inoculation by *A. brasilense* under irrigation yielded a better development of root architecture in maize in relation to water deficit (Fig. 3abcd). The inoculants Az1 and Az3

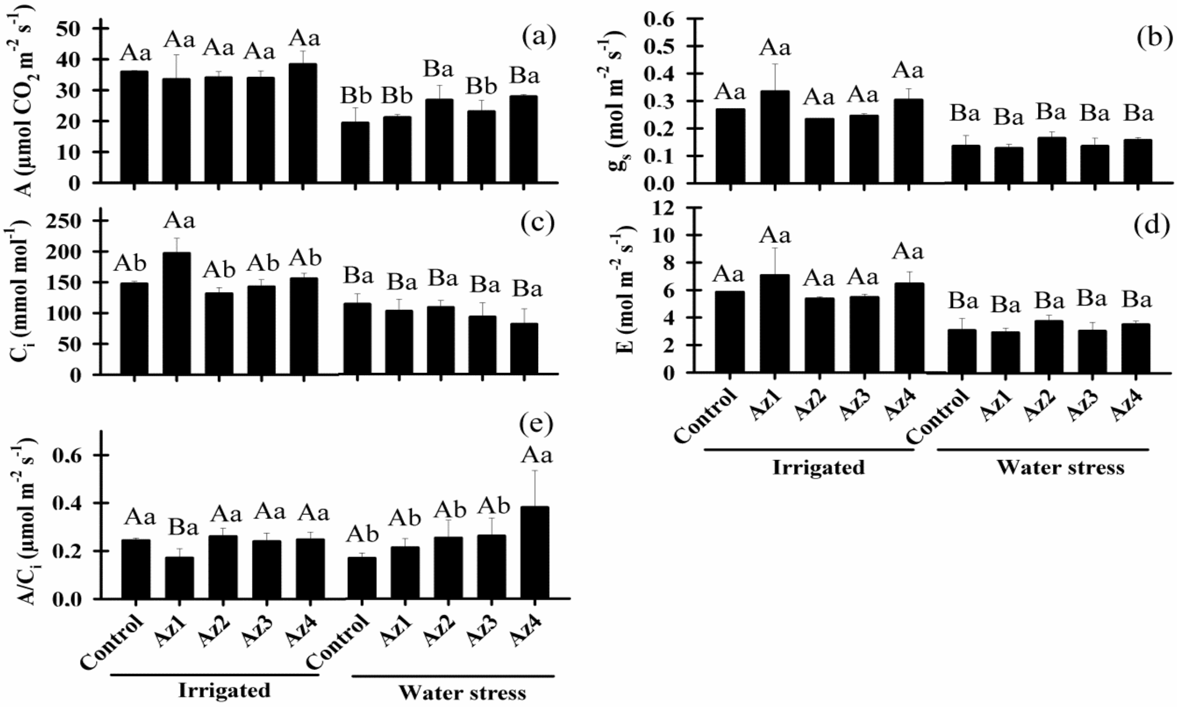

Figure 2. Leaf gas exchange of maize plants under irrigation and water deficit with *A. brasilens*e (Az1, Az2, Az3 and Az4). (A) photosynthesis (a); (gs) stomatal conductance (b); (Ci) internal carbon (c); (E) transpiration (d); (A/Ci) carboxylation efficiency (e). Lowercase letters compare the treatments under the same condition (irrigated/water deficit). Uppercase letters compare the treatments within irrigation and water deficit. Means followed by the same letter do not differ by the Scott-Knott test at 5% probability ($p \leq 0.05$). Each value indicates the mean ± SE.

showed longer length (Az1-33%, Az3-45%) root surface area (Az1-26%, Az3-37%), higher for the maize plants under irrigation, when compared to the other treatments ($p \leq 0.05$) (Fig. 3ab). The mean diameter and root volume were higher in 45% and 52%, respectively, with inoculant Az3, when compared to the other treatments (Fig. 3cd).

In relation to water deficit, there was no significant difference among treatments with the inoculants. In general, water deficit limited root development (Fig. 3abcd).

Under irrigation, all inoculants increased the accumulation of nutrients N, P, K, Ca, S, Mn and Zn in the dry matter of maize plants, with emphasis on inoculants Az1 and Az3 (Fig 4abcdfhi). Az3 yielded higher concentration of Mg in maize, when compared to the other treatments (Fig. 4e). The accumulation of Cu was higher in plants with inoculants Az1 and Az3 (Fig. 4g).

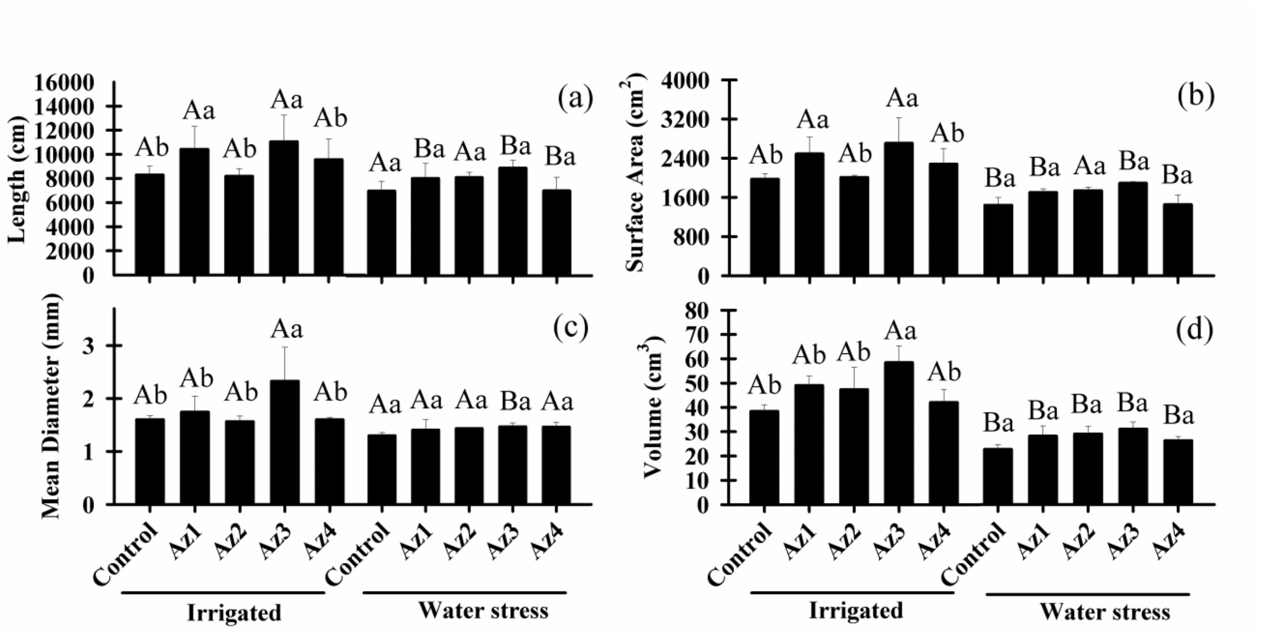

Figure 3. Root morphology of maize plants inoculated by *A. brasilense* (Az1, Az2, Az3 and Az4). Length (a); surface area (b); mean diameter (c); root volume (d). Lowercase letters compare the treatments under the same condition (irrigated/water deficit). Uppercase letters compare the treatments within irrigation and water deficit. Means followed by the same letter do not differ by the Scott-Knott test at 5% probability (p≤0.05). Each value indicates the mean ± SE.

In general, water deficit limited nutrient uptake by maize plants (Fig. 4). The accumulation of nutrients K, Ca and Mn was higher with the presence of inoculants (Fig. 4cdh). The concentration of N increased with Az1, Az2 and Az3 (Fig. 4a). The inoculants Az1 and Az2 yielded an increase in S concentration in maize plants (Fig. 4f). There was no statistical difference in the treatments with *A. brasilense* for the elements P, Mg, Cu and Zn under the same condition (Fig. 4begi).

Discussion

Azospirillum brasilense in maize under irrigation

In general, under irrigation, the presence of *Azospirillum brasilense* (Az1, Az2, Az3 and Az4) yielded higher growth, LA and increased nutrient uptake. There was also alteration in the root architecture of maize plants with greater soil exploration by these roots. The benefits provided by the inoculation with *Azospirillum* are related to the improvement in the acquisition

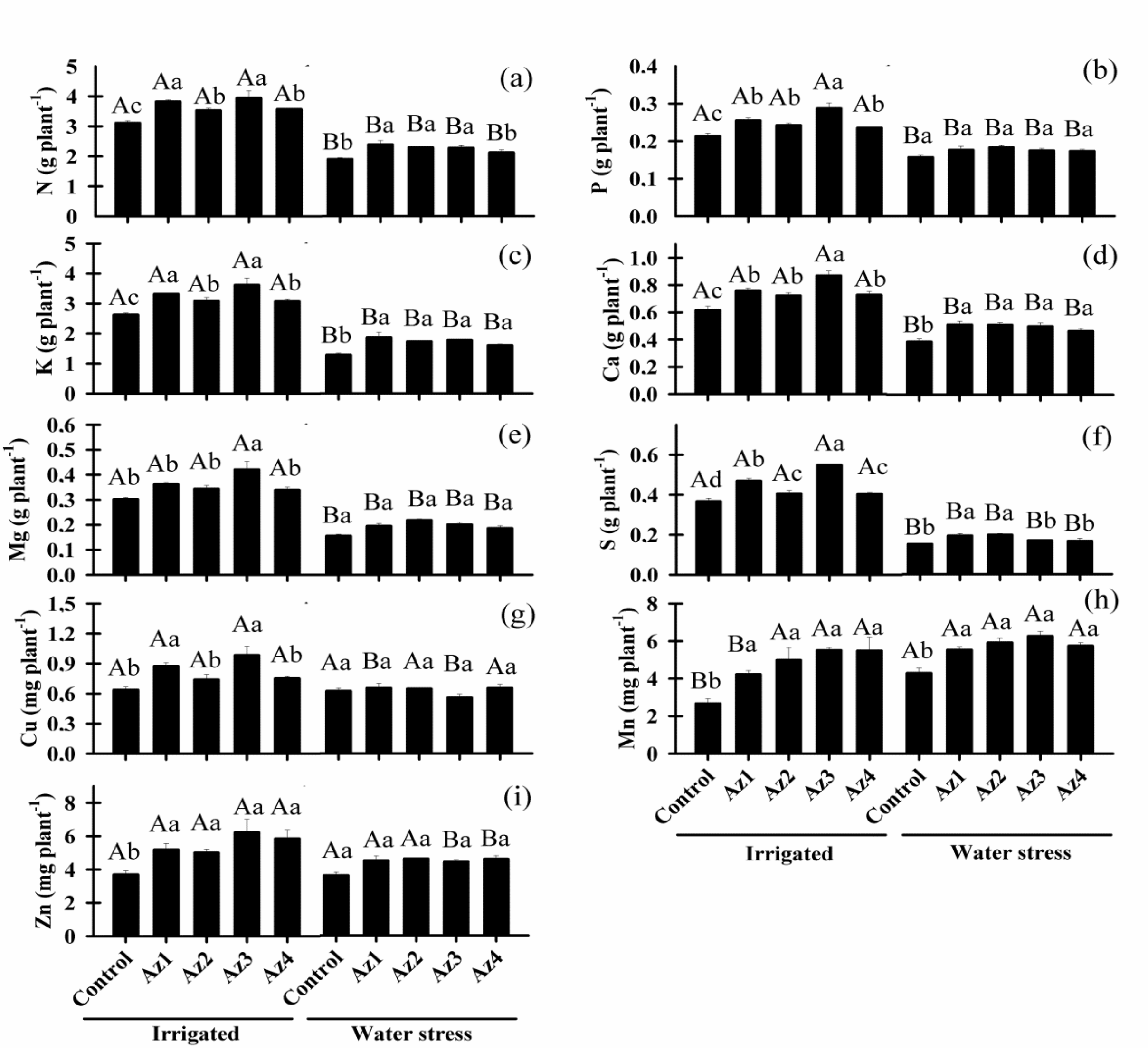

Figure 4. Concentration of nutrients in the tissues of maize plants inoculated by *A. brasilense*(Az1, Az2, Az3 and Az4). N (a); P (b); K (c); Ca(d); Mg (e); S (f); Cu (g); Mn (h) and Zn (i). Lowercase letters compare the treatments under the same condition (irrigated/water deficit). Uppercase letters compare the treatments within irrigation and water deficit. Means followed by the same letter do not differ by the Scott-Knott test at 5% probability (p≤0.05). Each value indicates the mean ± SE.

of important plant resources, such as increased uptake of water, nitrogen, phosphorus and other minerals (Fukami et al., 2017). In addition, these bacteria can modulate hormonal levels in plants, in addition to performing the biological fixation of N_2 (Dar et al., 2018).

Inoculation by *A. brasilense* did not statistically alter photosynthesis, gs and E of plants under irrigation, although more research examining a time profile is necessary to take more consistent conclusion. The nutritional status of plants, allied to good water status, (Ψmd) may confer the maximum photosynthetic activity. In addition, the similarity in the photosynthetic rate in all treatments with the control plants is probably related to the saturation of the rubisco activity (Kluge et al., 2015). Besides, Inagaki et al. (2014) reported that the assimilation rate of leaf CO_2 was not affected by the inoculation of maize seeds with *A. brasilense* and *H. seropedicae*.

The greater soil exploration by roots with *A. brasilense* led to an increase in the uptake of nutrients N, P, K, Ca, S, Mn and Zn in maize tissues in the absence of water deficit. This fact possibly occurred as an indirect consequence of the phytohormones produced by these bacteria (Galindo et al., 2016; Daret al., 2018). The production of indole-3-acetic acid (IAA) by *A. brasilense* has been considered as the most important characteristic in the promotion of plant growth through the changes caused as a consequence of the increase in root length and volume (Cassán & Diaz-Zorita, 2016).

The highest length, surface area, mean diameter and root volume in maize with *A. brasilense* found in this study may also be related to *Azospirillum* stimulating the formation of trichomes and root growth, increasing the volume of soil explored by the root system and the absorption of water and nutrients (Bulegon et al., 2017).

Zemrany et al. (2007) discussed that the species *Azospirillum lipoferum* resulted in the accumulation of root biomass, which would be related to the greater number of meristematic tips, meaning more ramifications and alterations in root architecture. As a consequence, the inoculated roots had a higher root surface area, interacting with the soil particles, water and microorganisms. In addition, the same authors emphasized that the inoculated plants with greater root surface and number of meristematic tips tend to exude more organic acids in the rhizosphere, favoring the colonization of PGPR.

Not only can the presence of *Azospirillum* improve the absorption of various macro and micronutrients, but also increase the efficient use of nutrients by plants, thus favoring plant development (Galindo et al., 2016). Another explanation for this fact was the greater root surface area with inoculation by *A. brasilense*. The increase in root surface area may be directly related to the increase in nutrients in the tissues (Souza et al., 2016). Consequently, the inoculated DKB 390 plants showed higher height and LA, reflecting in a higher biomass accumulation (shoot and total dry matter).

The higher shoot growth of maize with *A. brasilense* corroborates the results found by Calzavara et al. (2018), who reported that the inoculation of the Ab-V5 strain (*A. brasilense*) increased maize shoot length, differing from the control plants, when cultivated with a total N supply. In addition, not only rhizobacteria such as *A. brasilense* have effect on root lengthening and architecture, but also induces hormonal signaling from root to shoot, regulating leaf growth and other physiological plant processes (Barnawal et al., 2019).

The increase in N concentration in plant tissues with the inoculation under irrigation is in agreement with the results found by Portugal et al. (2016), who reported positive influence of *A. brasilense*, resulting in higher leaf N in maize plants. In addition, Martins et al. (2018) found that inoculation by *A. brasilense* improved the efficiency of N uptake in maize and increased grain yield, when compared to uninoculated control plants.

Thus, in maize production with high technology and irrigation, approaches with the use of inoculants to improve yield is an effective technology due to the beneficial effects in promoting plant development. Therefore, the use of *A. brasilense* can be indicated for intensifying plant growth and nutrition.

Azospirillum brasilense **in maize under water deficit**

Drought restricts plant growth and development, reduces diffusion and mass flow of nutrients, besides reducing crop yield. As a consequence of water restriction, the maize plants undergoing water deficit decreased the leaf water potential (Ψmd), showing no difference among the treatments with *A. brasilense*. A lower Ψmd reflected in stomatal closure, decreasing gs and E. This limitation in stomatal opening under water deficit reduces CO_2 input and, consequently, a reduction in A is observed, independent of treatments (Mutava et al., 2015).

Inoculation of *A. brasilense* by Az2 and Az4 yielded higher A. Plants with the inoculant Az4 also showed higher carboxylation efficiency (A/Ci), which shows an influence of this inoculant on the carboxylation activity of rubisco, which maintains the photosynthetic activity, even under water deficit conditions. Maize plants under water deficit show damage to photosystems (Reis et al., 2018), which is responsible for collecting radiant energy and converting it to chemical energy (ATP). Thus, plants under stress end up channeling the radiant energy for the production of Reactive Oxygen Species (ROS), leading to this damage.

The higher *A* in plants under water deficit and inoculated with *A. brasilense* could be the result of a better condition or stress relief caused by these rhizobacteria, such as an increase in defense mechanisms against ROS (Kaushal & Wani, 2016; Ngumbi & Kloepper, 2016). What could have resulted in the end of water deficit imposition were better growing conditions such as higher height, LA and plant diameter with *A. brasilense*. These results are in agreement with those found by Bulegon et al. (2016), who emphasized that *Urochloaruziziensis* plants under drought conditions, inoculated via leaf and seeds by *A. brasilense* increased the activity of SOD and catalase in the stress treatment, making the plants more efficient in the removal of ROS and tolerant to water deficit.

However, the use of inoculants did not result in greater dry matter accumulation (shoot and total). Although it did not increase leaf biomass, LA increased, possibly under the induction of hormonal signaling by this root-to-shoot rhizobacteria (Barnawal et al., 2019), providing greater leaf elongation in maize. As a consequence, with the same biomass, the plants could present higher LA (i.e., photosynthetic area). This may evidence reduced metabolic cost, since water deficit results in lack of energy and reduced growth. In addition, inoculation can bring benefits in increasing plant growth, as well as inducing plants to overcome environmental stresses (Chibebaet al., 2015).

The interaction of *A. brasilense* with plants can be quite dynamic, presenting variations in the mechanisms used by this rhizobacteria. Under water deficit conditions, the genotype DKB 390 presents physiological mechanisms mainly at the shoot level to minimize water loss and maintain the productivity (Lavinsky et al., 2015). As a result, it reduces the dependence of metabolic adjustments on the root system to increase water uptake (Lavinsky et al., 2015). It is possible to note that the influence of *A. brasilense* under drought was mainly on the shoot (height, stem diameter, LA, A), with lower effects on root characteristics. It can be inferred that the interaction of *A. brasilense* with this genotype (DKB 390) induced more effects on the energy producing organs (photosynthesis - leaves).

The presence of *A. brasilense* did not alter root morphology under water deficit. This fact can be justified by the characteristic of the (tolerant) genotype itself under water deficit conditions (Lavinsky et al., 2015; Souza et al., 2016). In addition, it is possible that the greater production of photoassimilates by plants can be translocated to the roots, and thus yield greater root exudation, that is, an energy source for the metabolism of this rhizobacteria.

Due to drought, water stress reduced the availability and transport of nutrients from the soil to the plants, since nutrient transportation to the roots is limited by water (Vurukonda et al., 2016). However, in spite of the non-alteration in the root morphology and dry matter of maize plants, the presence of *A. brasilense* increased the accumulation of nutrients (N, K, Ca, S and Mn). Moreover, it can be suggested that, with the same investment in roots, the presence of this rhizobacteria yielded greater efficiency in the absorption of these nutrients. Teixeira Filho et al. (2017) reported that inoculation with *A. brasilense* in wheat resulted in increases in N and S concentrations in straw (physiological maturity), when compared to the treatment without inoculation.

Interestingly, the inoculants (Az1, Az2, Az3 and Az4) increased nutrient uptake in maize plants under stress, but no influence on gs and E was observed. It is important to note that transpiratory flow is very important for nutrients to enter plants. However, with water deficit, there may be a reduction in this transpiratory flow. Thus, the presence of *A. brasilense* (PGPR) could benefit maize plants by mineralizing soil organic matter by releasing hydrolytic enzymes and thus increasing nutrient availability (Olliver et al., 2011; Pii et al., 2015), even under water deficit conditions.

Another probable explanation is that the activity of H$^+$-ATPase is important in the movement of solute in the cells and in the process of nutrient acquisition by plants (Pii et al. 2015). The PGPR, such as *A. brasilense*, can stimulate H$^+$-ATPase in the plasma membranes of the root cells, and this action can be attributed to phytohormones production (IAA) (Pii et al., 2015).

The interaction of bacteria with plants under different environmental conditions can result in divergent responses in plant development. However, from the results found in this work it is possible to indicate the use of inoculants with *A. brasilense* to provide more sustainable agricultural practices and increase the nutrition of maize plants.

Conclusions

The morphophysiological characteristics, growth and nutrient incorporation were reduced in maize plants grown under water deficit.

The use of *A. brasilense* by inoculants Az1, Az2, Az3 and Az4 in maize cultivation with irrigation can be indicated for intensifying plant growth.

Azospirillum brasilense leads to root growth and nutrient uptake in maize plants, with emphasis on the Az3 inoculant under the tested water conditions.

The inoculants Az1, Az2 and Az3 can attenuate the effects of water deficit, mainly by improving shoot growth and nutrient uptake. The use of these inoculants with *A. brasilense* can be a strategy to mitigate the effects of water deficit in the maize crop.

Acknowledgements

This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

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