Article - Agriculture, Agribusiness and Biotechnology

# Gas Exchange, Root Morphology and Nutrients in Maize Plants Inoculated with *Azospirillum brasilense* Cultivated Under Two Water Conditions

Daniele Maria Marques<sup>1,3</sup> https://orcid.org/0000-0003-1263-8290

Paulo César Magalhães<sup>2</sup> https://orcid.org/0000-0001-7842-4506

https://orcid.org/0000-0002-1670-0285

Ivanildo Evódio Marriel<sup>2</sup>

Carlos César Gomes Júnior<sup>3</sup> https://orcid.org/0000-0001-9379-5778

Adriano Bortolotti da Silva<sup>4</sup> https://orcid.org/0000-0003-1316-8243

Thiago Corrêa de Souza<sup>3\*</sup> https://orcid.org/0000-0002-4991-7704

<sup>1</sup>Federal University of Lavras-UFLA, Department of Biology, Laboratory of Plant Anatomy, Lavras, Minas Gerais, Brazil; <sup>2</sup>Maize and Sorghum National Research Center, Sete Lagoas, Minas Gerais, Brazil; <sup>3</sup>Federal University of Alfenas– UNIFAL, Institute of Nature Sciences - ICN, Alfenas, Minas Gerais, Brazil; <sup>4</sup>University José do Rosário Vellano– UNIFENAS, Section of Agricultural Sciences, Alfenas, Minas Gerais, Brazil.

Editors-in-Chief: Paulo Vitor Farago Associate Editor: Adriel Ferreira da Fonseca

Received: 2019.09.17; Accepted: 2020.12.18.

\*Correspondence: thiago.souza@unifal-mg.edu.br; Tel.: +553 532 991 419 (T.C.S).

# HIGHLIGHTS

- Azospirillum brasilense stimulates root growth in maize under water deficit.
- Maize inoculated with A. brasilense shows greater photosynthesis under drought conditions.
- Under water deficit, maize plants inoculated with A. brasilense showed greater water use efficiency
  - (WUE).

**Abstract:** The objective of this study was to evaluate the gas exchange, root morphology and nutrient concentration in maize plants inoculated with *A. brasilense* under two water conditions. The experiments were carried out in a greenhouse, one under irrigation and the other under water deficit. The treatments consisted of four *A. brasilense* inoculants (control (without inoculation), Az1 (CMS 7 + 26), Az2 (CMS 11 + 26) and Az3 (CMS 26 +42). At the V6 plant stage, water stress was imposed on maize plants for 15 days. The phytotechnical characteristics, gas exchange, root morphology, root dry matter and macronutrient analysis were evaluated after 15 days of water deficit imposition. The water deficit caused a reduction in the development of maize plants. The presence of *A. brasilense* Az1 under the same condition yielded higher photosynthesis, carboxylation efficiency, water use efficiency, and greater soil exploration with increased length, surface area and root volume of plants. Inoculation by *A. brasilense* increased root system volume by an average of 40 and 47% under irrigation and water deficit, respectively, when compared to non-inoculated plants. The inoculant Az1 attenuated the deleterious effects caused by drought and yielded the best growth of the root system, resulting in the tolerance of maize plants to water deficit.



## Keywords: Zea mays L., Photosynthesis; Water use efficiency; Water deficit; WinRhizo.

## INTRODUCTION

Plant growth and development are constantly influenced by environmental stress, a factor that most reduces the yield of cultivated areas in the world. Water deficit is the abiotic stress that most impact the yield of cultivated plants and has been increasing its intensity in the last decades, both in Brazil as well as in the world [1,2]. Maize plants can suffer drastic effects due to water deficit at various stages of their development. Stress in the vegetative stage (V6 - V8) can totally compromise the yield potential of this crop [3].

Plant tolerance to drought needs to be improved in order to meet food demands in areas with limited availability of water resources [4]. Several studies have been carried out aiming at the interaction of plants with microorganisms (biostimulants/biofertilizers) to mitigate these deleterious effects resulting from drought [5,6,7]. Plant growth promoting rhizobacteria (PGPR) have the ability to overcome adverse effects of drought [5] and influences growth and yield through their metabolic activities and the multiple mechanisms that are generated from this interaction with plants [9]. The most commonly observed effects of PGPR are the reduction in primary root growth rate and the increase in the number and length of lateral roots and root trichomes [10].

The genus *Azospirillum* is able to colonize a large number of species of cultivated plants, improving growth, development and yield [6,2]. Plant growth by *Azospirillum brasilense* occurs as a consequence of the combination of several physiological, biochemical and morphological mechanisms [11], possibly acting in an additive form or as a cascade effect. *A. brasilense* can produce and metabolize phytohormones, such as indole-3-acetic acid (AIA), gibberellins and cytokinins, besides other plant growth regulating molecules [12,11,13,10]. Moreover, it is possible to perform biological nitrogen fixation (BNF) [14], to improve root growth and to increase the absorption of water and ions [10].

The study by *A. brasilense* in plants can aid in the search for sustainable agricultural practices and possibly reveal the use of these bacteria as a strategy to mitigate the effects of biotic and abiotic stress on agricultural productivity [8]. However, the responses of the interaction of the bacteria with water stress depend on the intensity and duration of the stress, species and stage of plant growth [15]. The knowledge of the interaction between inoculations of *A. brasilense* strains with drought sensitive maize genotype (BRS 1040) in different water conditions is still little explored. Thus, it is important to consider that *A. brasilense* strains may differ in their properties of tolerance to drought, which justifies the selection of more effective strains [16]. In this context, the hypothesis of this study can be stated as the inoculation by *A. brasilense* reduces the harmful effects of water deficit in maize plants. The objective of this study was to evaluate the gas exchange, root morphology and nutrients content in maize plants inoculated by *A. brasilense* under two water conditions.

## MATERIAL AND METHODS

## Experimental design, plant and microbiological material and growth conditions

The experiment was carried out in a greenhouse at Embrapa Milho e Sorgo, in Sete Lagoas - MG, Brazil, located at the geographical coordinates: 19°28' S, 44°15'08" W, and average altitude of 732 m. The temperature averages recorded during the evaluation period were maximum of 31.2°C and minimum of 12.91°C. Relative air humidity ranged from 30% to 72%. A simple BRS 1040 hybrid developed by Embrapa Milho e Sorgo breeding program was used. The inoculant used was obtained from the mixture of two *Azospirillum brasilense* homologous strains, at the ratio 1:1, belonging to the collection of diazotrophic bacteria of the Laboratory of Microbiology and Soil Biochemistry of Embrapa Milho e Sorgo. More information on the used strains of *A. brasilense* is found in Fonseca [17], Reis [18] and Ribeiro [19].

The experimental design was completely randomized (CRD), in a 4 x 2 factorial scheme, consisting of four inoculants of *A. brasilense*: control (without inoculation), Az1 (CMS 7 + 26), Az2 (CMS 11 + 26) and Az3 (CMS 26 + 42) and two contrasting water conditions (irrigation 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 optical density equal to 1.0 in absorbance at 500 nm, which is approximately  $10^8$  viable cells per mL. It was used 150 ml of cell suspension with  $10^8$  CFU / ml per 60,000 seeds. Seed inoculation was carried out using ground charcoal and cassava starch paste as a carrier. Ground activated charcoal was added to the bacterial cell suspension in a proportion of 1: 4 (w / v) and mixed with pre-moistened seeds with a solution

of cassava starch dissolved in 4% water. The pelleted and dried seeds were sown on the same day. The seeds without inoculation were sown without the carrier.

Sowing was accomplished in 20-Kg 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 considering the soil chemical analysis, applying the formulation 08-28-16 (10 g) NPK and micronutrients FTE BR12 (2.5 g) to 20 kg of soil at planting. The nitrogen topdressing was performed applying 4 g of urea per pot 30 days after planting.

The soil water content 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 with a screw thread at a depth of 20 cm.

All treatments were maintained at field capacity (FC) (soil water tension of -18 kPa) during the period prior to the imposition of stress. After 25 days of cultivation, maize plants at V6 growth stage were submitted to water deficit for a period of 15 days in the treatment that suffered the water restriction. In this treatment, the soil water tension was reduced to -138 KPa, which corresponds to 50% of the water available in the soil. The irrigated treatment did not receive alteration in the water condition, maintaining the field capacity. The water replenishment calculations were performed with the aid of a spreadsheet, made according to the water retention curve of the soil.

#### **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 and, from A and E, water use efficiency (WUE). An LI 6400 infrared gas analyzer (IRGA - LI-COR, Lincoln, NE, USA) was used, equipped with a fluorometer (LI-6400-40, LI-COR Inc.). Measurements were performed on a 1 cm<sup>2</sup> leaf area and the airflow in the chamber was at a CO<sub>2</sub> concentration of 380 mmol mol<sup>-1</sup>. A photon flux density (PPFD) of 1500 µmol m<sup>-2</sup> s<sup>-1</sup> was used with a red-blue LED light source and the chamber temperature was 28 °C.

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

At the end of the imposition of the 15 days of water stress, plant height was evaluated using a graduated ruler and the total leaf area of the plant (LA) was measured by a leaf area reader (LI-3100C, Licor, Nebraska, USA). The shoots were conditioned in paper bags and subjected to forced air drying at 65 °C for 72 hours to obtain the dry matter.

#### 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 and coauthors [20] 15 days after the plants were submitted to stress. The following characteristics were determined: root length (cm), root surface area (cm<sup>2</sup>), mean root diameter (mm) and root volume (cm<sup>3</sup>). The roots were then stored in paper bags and transported to a forced circulation oven at 65 °C until a constant mass was obtained.

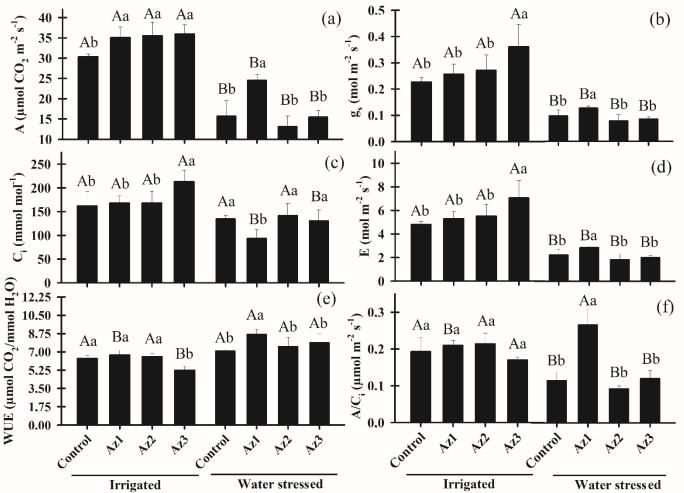
After shoot and root dry matter were determined, the samples were ground in a Willy mill. The ground material was used to determine the nutrient contents N, P, K, Ca, Mg and S in the dry matter, according to the methodology proposed by Silva [21].

#### Data analysis

For all characteristics evaluated, the means and the standard error (SE) were calculated. For statistical analysis of the results, it was used the analysis of variance (ANAVA) and the Skott-Knott test for comparing averages at (p≤0.05) significance, by using the Sisvar version 4.3 (Federal University of Lavras, Lavras, Brazil).

# RESULTS

Inoculation by *A. brasilense* increased leaf gas exchange in maize. On the first day of water stress, all the inoculants under irrigation increased A when compared to the control (Figure 1a); gs, Ci and E were higher with the presence of the inoculant Az3, when compared to the other treatments (Figure 1bcd). The WUE was lower with Az3 inoculation (Figure 1e). The ratio A/Ci did not show statistical differences in the treatments for the same water condition ( $p \le 0.05$ ) (Figure 1ef).

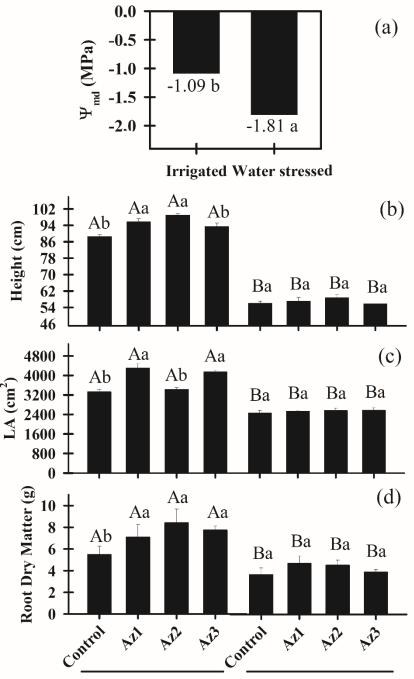


**Figure 1.** Leaf gas exchange on the first day of water deficit imposition on maize. (A) photosynthesis (a); (gs) stomatal conductance (b); (Ci) internal carbon (c); (E) transpiration (d); (WUE) water use efficiency (e); (A/Ci) carboxylation efficiency (f). Means followed by the same letter do not differ among themselves by the Scott-Knott test at 5% probability ( $p \le 0.05$ ). Each value indicates the treatment mean  $\pm$  SE. Lower case letters compare treatments within the same condition. Upper case letters compare the same treatments in the two water conditions.

In general, the water deficit reduced A, gs, Ci and E when compared to the irrigated condition ( $p \le 0.05$ ) (Figure 1). Under water deficit, the inoculant Az1 increased A, gs, E and water use efficiency (WUE), besides the ratio A/Ci in maize plants (Figure 1abdef). Plants with Az1 presented lower Ci in relation to the other treatments (Figure 1c).

On the last day of water stress imposition (15 days), irrigation was better for the same variables (A, gs, Ci, E, A/Ci and WUE) than the water deficit condition, with no statistical difference among the treatments with inoculation by *A. brasilense* (supplementary material).

The  $\Psi$ md was higher in the irrigated plants in 66% ( $\Psi$ md -1.09), when compared to plants under water deficit ( $\Psi$ md -1.82) (Figure 2a).



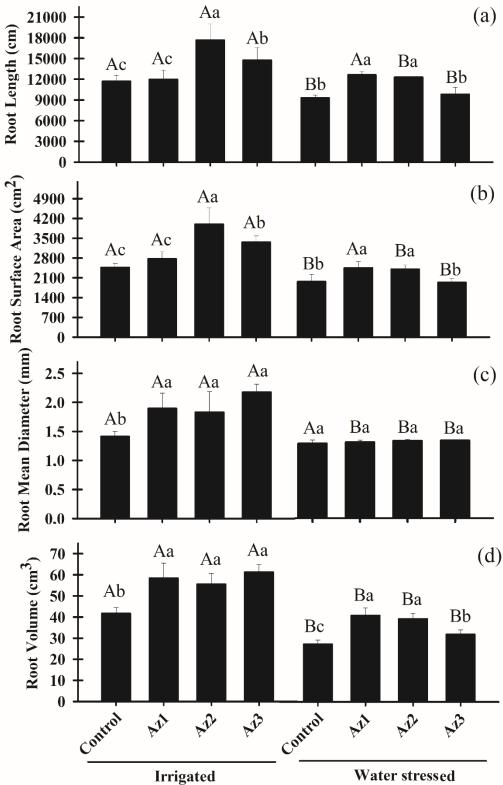


**Figure 2.** Water potential (a) and phytotechnical data - plant height (b); leaf area (LA) (c); root dry matter (d) of maize plants inoculated by *A. brasilense* under irrigation and water deficit conditions, after the fifteen days of stress. Means followed by the same letter do not differ among themselves by the Scott-Knott test at 5% probability ( $p \le 0.05$ ). Each value indicates the treatment mean  $\pm$  SE. Lower case letters compare treatments within the same condition. Upper case letters compare the same treatments in the two water conditions.

When inoculated with the inoculants Az1 and Az2 under irrigation, the maize plants increased height by 7% and 11%, respectively, when compared to the control (Figure 2b). The LA showed an increase of 29% and 25%, respectively, with the inoculants Az1 and Az3 when compared to the treatment without inoculation (Figure 2c). Root dry matter was increased by 29% by inoculant Az1, 53% by Az2 and 41% by Az3, when compared to the control under the same condition (Figure 2d). The water deficit reduced height, LA and root dry mass of maize plants when compared to the plants in the irrigated condition ( $p \le 0.05$ ) (Figure 2). Under water deficit, there was no difference between the inoculants for the same variables ( $p \le 0.05$ ) (Figure 2bcd).

In general, all inoculants favored the root development of maize plants under irrigation (Figure 3). Under this condition, the inoculants Az2 and Az3 yielded greater length, surface area, mean diameter and volume

of the root system, compared to non-inoculated plants (Figure 3a). In the presence of all inoculants, the mean diameter and root volume were higher, when compared to the control (Figure 3cd).



**Figure 3.** Root morphology of maize plants inoculated by *A. brasilense*. Length (a); surface area (b); mean diameter (c); root volume (d). Means followed by the same letter do not differ among themselves by the Scott-Knott test at 5% probability ( $p \le 0.05$ ). Each value indicates the treatment mean ± SE. Lower case letters compare treatments within the same condition. Upper case letters compare the same treatments in the two water conditions.

Under water deficit, the inoculants Az1 and Az2 yielded the best root growth, surface area and root volume, compared to non-inoculated plants (Figure 3abd). There was no difference in mean root diameter between treatments ( $p\leq0.05$ ) (Figure 3c).

Under irrigation, the presence of *A. brasilense* statistically changed the concentrations of K, Ca and S ( $p\leq0.05$ ) (Table 1). Whereas inoculation did not change the nutrients (N, P, K, Ca, S and Mg) for the water deficit condition ( $p\leq0.05$ ) (Table 1).

**Table 1.** Concentration of macronutrients (g plant<sup>-1</sup>) in the total dry biomass of maize plants inoculated with *A. brasilense* under irrigation and water deficit.

Conc.	Irrigated				Water stressed				CV
(g plant <sup>-1</sup> )	Control	Az1	Az2	Az3	Control	Az1	Az2	Az3	(%)
Ν	3.18 aA	3.81 aA	3.62 aA	3.82 aA	2.00 aB	2.28 aB	2.16 aB	2.13 aB	13.38
Р	0.22 aA	0.26 aA	0.26 aA	0.38 aA	0.16 aB	0.19 aB	0.17 aB	0.17 aB	18.94
К	2.74 bA	3.45 aA	2.93 bA	3.48 aA	1.48 aB	1.67 aB	1.71 aB	1.65 aB	16.10
Ca	0.63 bA	0.82 aA	0.79 aA	0.88 aA	0.45 aA	0.56 aB	0.51 aB	0.49 aB	19.64
S	0.28 bA	0.28 bA	0.27 bA	0.35 aA	0.16 aB	0.18 aB	0.17 aB	0.16 aB	17.31
Mg	0.37 aA	0.45 aA	0.42 aA	0.44 aA	0.19 aB	0.24 aB	0.21 aB	0.21 aB	18.60

Means followed by the same letter do not differ among themselves by the Scott-Knott test at 5% probability (p≤0.05). Lower case letters compare treatments within the same condition. Upper case letters compare the same treatments in the two water conditions.

#### DISCUSSION

Water deficit is responsible for causing modifications from the molecular to the morphophysiological levels, limiting plant growth and development. In this study, it was shown that both root morphology and gas exchange of BRS 1040 maize plants were strongly affected in a few days of drought. It was also noted that bacterial inoculation by *A. brasilense* improved maize root system growth and leaf gas exchange under both conditions. As a consequence, increased soil exploration by the roots for greater uptake of water and nutrients.

Inoculation by *A. brasilense* under irrigation yielded an increase in the photosynthetic rate (A), which may have resulted in higher height and LA. In addition, there was an increase in all variables of the root system, with an increase in root biomass. Consequently, these roots could exploit the soil profile. These results can confirm the benefits brought by *A. brasilense* and suggest the use of these inoculants to optimize maize crop growth. However, the presence of *A. brasilense* did not alter the concentration of nutrients under irrigation. Quadros and coauthors [22] reported similar results with increased maize height (R1), dry matter and N content in treatments with *A. brasilense* inoculation. In addition, Fukami and coauthors [23] described that *Azospirillum* leads to the development of plants by helping acquire important resources such as water, nitrogen, phosphorus and other minerals or by moderating hormonal levels in plants. Besides, in the production of maize in high technology and with irrigation, the use of these inoculants based on *A. brasilense* is indicated and can intensify the development of this crop.

The exposure of maize to water deficit decreased leaf water potential ( $\Psi$ md), but there was no difference between treatments with the presence of *A. brasilense*. However, different results were found by Arzanesh and coauthors [24], who described that strains of *Azospirillum* sp. decreased water potential and increased relative leaf water content in wheat plants subjected to drought.

Regarding gas exchange, one of the first responses of plants to water deficit is the stomatal limitation [25,26], reducing gs and E as an evolutionary mechanism that reduce the loss of water to the atmosphere [27]. Stomatal closure under water deficit conditions reduces CO<sub>2</sub> absorption by the plants, reducing carbon in the mesophyll of the leaves, thus reducing the photosynthetic rate and plant development [28,2]. It is possible that, as a consequence of this decrease in leaf gas exchange during the plant exposure period to this condition, mainly in A (C fixation), the effect of reducing height, LA and dry matter of maize roots was observed, when compared to plants under irrigation.

However, for the conditions of this study, the presence of *A. brasilense* CMS 7 + 26 (Az1) under water deficit increased A, gs, E and carboxylation efficiency and decreased internal carbon values. This result may indicate that, even with similar amounts of carbon in the leaf mesophyll, the carboxylation activity (Rubisco) was higher and, consequently, there was more  $CO_2$  consumption. This result suggests that the presence of Az1 inhibited the non-stomatal limitation of the biochemical metabolism and the photochemical reactions (photosynthetic dissipation efficiency or quenchings) that the water deficit can cause in the plants [29,25,30]. In addition, Az1 yielded increased stomatal opening (gs), assisting leaf biophysical processes, which are directly related to the gas exchange of plants with the environment (gs and E). However, it is noteworthy to

mention that in field conditions there may be environmental factors that can increase or decrease the photosynthetic rates in inoculated plants.

Inoculation by Az1 also yielded greater water use efficiency (WUE) in drought. This increase in WUE by plants under this adverse condition (drought) may be related to *A. brasilense* being able the osmotic adjustments in apoplastic and/or symplastic space [31]. Thus, this process is a response of plants to the water deficit that mitigate dehydration in their tissues. Plants with better water status can maintain the photosynthetic rate (A and A/Ci), and provide better leaf cooling by transpiration [32]. Therefore, within the global scenario with a view to global warming, plants with higher WUE capacity may contribute to decrease the deleterious effects of drought and increase agricultural productivity [33].

However, it is worth mentioning that, at the end of the water stress imposition (15 days), the bacteria did not alter leaf gas exchange and the water potential under both conditions (data not shown). The exposure time and the amount of water (intensity) under water deficit may have possibly inhibited *A. brasilense* growth and metabolic responses in maize plants. Under unfavorable or stressful conditions, *A. brasilense* presents several protective mechanisms, such as the formation of cysts [34]. As a consequence, inactivation of the metabolism of the bacteria may occur.

Under irrigation, it is suggested that the non-difference between the treatments for gas exchange with the inoculants is due to the PGPR having greater influence in the first vegetative stages. Lin and coauthors [35] found better responses in the initial growth of maize plants between V4 and V6 stages with the use of PGPR, when compared to the VT stage. The same authors justified this fact due to the phytohormones and plant growth regulators secreted by PGPR [8] stimulating seedling development in the early stages when the nutrient requirement is still low. In addition, the recommended use of N may mask the influence of PGPR on maize growth when the plants reached their later stages of vegetative growth [35].

As discussed, inoculation by *A. brasilense* modified the root morphology of maize plants under both conditions, with increase in length, surface area and root volume. The presence of *A. brasilense* in the plants under irrigation and water deficit yielded a mean increase of 40% and 47%, respectively, in root volume, when compared to plants without inoculation. For both conditions, the emphasis is mainly on the inoculants Az1 and Az2, which improved the development of the root system (irrigated and stressed), reflecting on a higher root dry matter (irrigated). This investment in the development of the root architecture of plants with Az1 is possibly related to a higher photosynthetic rate. These results corroborate with those found by Coelho and coauthors [36], who found an increase in the root dry matter in maize of 16% with inoculation by *A. brasilense*.

In Poaceae (maize), the lateral roots and the root trichomes are the regions preferentially colonized by PGPR (*Azospirillum*) [37]. Recent studies have shown that inoculation by *A. brasilense* can alter root morphology through the production of plant growth regulating substances, such as phytohormones [5], mainly AIA (Indol-3-acetic acid) [11]. These phytohormones stimulate the formation of new roots, thus providing greater root surface area [38,39,40,2]. In addition, Reis [18] researching strains of *Bacillus* and *Azospirillum* found that the production of AIA by *A. brasilense*, strains 1626 and 2142, was 65.78 and 52.15 µg mL<sup>-1</sup>, respectively. These strains are the same studied in this work, where 1626 corresponds to CMS 26 and 2142 to CMS 42, reaffirming the beneficial effects on the development of the root system.

The development of greater root surface area is directly related to the efficiency of the plant in acquiring nutrients and water from the soil. For this reason, this response in improving the development of the root system may also be related to *A. brasilense* being able to perform biological N<sub>2</sub> fixation [12,41].

Cassán and coauthors [6] emphasized the effect of *A. brasilense* on the roots, considering it a clear advantage for plants to improve plant nutrition, thus providing greater resistance and tolerance to environmental stresses. Under water deficit, root growth is preferably in depth as the soil dries. This is a strategy of acclimating plants to the demand for water, which may reflect greater crop yield. In addition, Souza and coauthors [30] reported that a well-developed and deeper root system contributes substantially to the tolerance of plants under water deficit.

However, the presence of the inoculants did not alter the biomass of the root system under water deficit. This may indicate that, with the presence of *A. brasilense*, there was a greater exploitation of the soil to obtain water with the same energy investment (photoassimilates) for the roots. Lynch and coauthors [1] described that a plant that is able to acquire a limiting soil resource such as water and nutrients at a reduced metabolic cost will have superior yield, since it will have more energy (metabolic resources) available for development and reproduction.

In the irrigated condition, the inoculation by *A. brasilense* (Az1, Az2 and Az3) provided an increase in the concentrations of K, Ca and S. This result corroborates with those found by Teixeira Filho and coauthors

[42] which reported that inoculation with *A. brasilense* in wheat resulted in increase in N and S concentrations in straw (physiological maturity), when compared to the treatment without inoculation.

Under water deficit, it is possible to suggest that the time of exposure to this stress limited the activity of *A. brasilense* and, consequently, the absorption of some nutrients by maize plants. Vurukonda and coauthors [7] mentioned that water stress, due to drought, reduced the availability and transport of nutrients from the soil to the plants, since the transportation of the nutrients to the roots is limited by water.

The results of the interaction of *A. brasilense* with the plants are quite dynamic. However, it is possible to verify the positive effect on maize growth with inoculation in both study conditions, justifying the use of these inoculants. It is worth mentioning that these results are promising, but suggest more experiments in different field trials.

## CONCLUSION

In high-technology maize and with irrigation, the use of *A. brasilense*-based inoculants (Az1, Az2 and Az3) is indicated and may intensify crop growth. In addition, under water deficit conditions, the inoculant Az1 attenuated the harmful effects caused by drought resulting in better growth of the root system and photosynthesis of maize plants. Thus, agricultural practices involving inoculation with diazotrophic bacteria of the genus *Azospirillum* can contribute to the mitigation of water deficit in maize under controlled conditions. These results indicate the beneficial effects on the morphophysiology of maize plants under water deficit, but suggest more experiments in different field trials.

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

# REFERENCES

- 1. Lynch JP, Chimungu JG, Brown KM. Root anatomical phenes associated with water acquisition from drying soil: targets for crop improvement. Environ Exp Bot. 2014;65(21):6155-66.
- 2. Dar ZM, Masood A, Mughal AH, Asif M, Malik MA. Review on Drought Tolerance in Plants Induced by Plant Growth Promoting Rhizobacteria. J Pharmacogn Phytochem. 2018; 7(3):2802-4.
- 3. Magalhães PC, Durães, FOM. Fisiologia da produção de milho. Embrapa Milho e Sorgo-Circular Técnica (INFOTECA-E), 2006.)
- 4. Ngumbi E, Kloepper J. Bacterial-mediated drought tolerance: current and future prospects. Appl Soil Eco. 2016;105:109-25.
- 5. Kaushal M. Portraying Rhizobacterial Mechanisms in Drought Tolerance: A Way Forward Toward Sustainable Agriculture. In: PGPR Amelioration in Sustainable Agriculture. Woodhead Publishing, 2019.195-216.
- Cassán F, Vanderleyden J, Spaepen S. Physiological and agronomical aspects of phytohormone production by model plant-growth-promoting rhizobacteria (PGPR) belonging to the genus <u>Azospirillum</u>. J Plant Growth Regul. 2014;33(2):440-59.
- 7. Vurukonda SSKP, Vardharajula S, Shrivastava M, SkZ A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res. 2016;184:13-24.
- 8. Fukami J, Cerezini P, Hungria M. *Azospirillum:* benefits that go far beyond biological nitrogen fixation. AMB Express. 2018;8(1):73.
- 9. Van Oosten MJ, Pepe O, Pascale S, Silletti S, Maggio A. The role of biostimulants and bioeffectors as alleviators of abiotic stress in crop plants. Chem Biol Technol Agric. 2017;4(1):1-12.
- 10. Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y, Muller D. et al. Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci. 2013;4(356):1-19.
- 11. Bashan Y, Bashan LE. How the plant growth-promoting bacterium *Azospirillum* promotes plant growth a critical assessment. Adv Agron. 2010;108(77):136.
- 12. Fibach-Paldi S, Burdman S, Okon Y. Key physiological properties contributing to rhizosphere adaptation and plant growth promotion abilities of *Azospirillum brasilense*. FEMS Microbiol Lett. 2012;326(2):99-108.
- 13. Romero AM, Vega D, Correa OS. *Azospirillum brasilense* mitigates water stress imposed by a vascular disease by increasing xylem vessel area and stem hydraulic conductivity in tomato. Appl Soil Ecol. 2014;82:38-43.
- Martins DC, Borges ID, Cruz JC, Netto DAM. Produtividade de duas cultivares de milho submetidas ao tratamento de sementes com bioestimulantes fertilizantes líquidos e *Azospirillum* sp. Rev Bras Milho Sorgo. 2016;15(2):217-28.
- 15. Naseem H, Ahsan M, Shahid MA, Khan N. Exopolysaccharides producing rhizobacteria and their role in plant growth and drought tolerance. J Basic Microbiol. 2018;1(14):1-14.

- 16. García JE, Maroniche G, Creus C, Suárez-Rodríguez R, Ramirez-Trujillo JA, Groppa MD. In vitro PGPR properties and osmotic tolerance of different *Azospirillum* native strains and their effects on growth of maize under drought stress. Microbiol Res. 2017;202:21-9.
- Fonseca LMF. Impacto da inoculação com estirpes de *Azospirilum* sp. sobre a produtividade de milho. 2014. Dissertação do Programa de Pós-Graduação em Ciências Agrárias da Universidade Federal de São João Del Rei. Sete Lagoas – MG. 2014.48p.
- 18. Reis DP. Produtividade de milho e ecologia microbiana da rizosfera de plantas sob diferentes métodos de inoculação e níveis de nitrogênio. Dissertação do Programa de Pós-Graduação em Bioengenharia da Universidade Federal de São João Del Rei. São João Del Rei MG. 2015,61p.
- 19. Ribeiro VP. 2018. Inoculação simples e mista com Azospirillum brasilense e Bacillus sp. em plantas de milho: desenvolvimento de tecnologias para sistemas agrícolas sustentáveis. Dissertação do Programa de Pós-Graduação em Bioengenharia da Universidade Federal de São João del Rei. São João Del Rei – MG. 2018,73p.
- 20. Souza TC, Castro EM, Magalhães PC, Alves ET, Pereira FJ. Early characterization of maize plants in selection cycles under soil flooding. Plant Breed. 2012;131(4):493-501.
- Silva FC. Manual de análises químicas de solos, plantas e fertilizantes. Rio de Janeiro: Embrapa Solos, 2009;1-370.
- 22. Quadros PD, Roesch LFW, Silva PRF, Vieira VM, Roehrs DD, Camargo FAO. Desempenho agronômico a campo de híbridos de milho inoculados com *Azospirillum*. Ceres. 2014;61(2):209-18.
- 23. Fukami J, Ollero FJ, Megías M, Hungria M. Phytohormones and induction of plant-stress tolerance and defense genes by seed and foliar inoculation with *Azospirillum brasilense* cells and metabolites promote maize growth. AMB Express. 2017;7(1):153.
- 24. Arzanesh MH, Alikhani HA, Khavazi K, Rahimian HA, Miransari M. Wheat (*Triticum aestivum L*.) growth enhancement by *Azospirillum sp.* under drought stress. World J Microbiol Biotechnol. 2011;27(2):197-205.
- 25. Souza TC, Castro EM, Magalhães PC, Lino LO, Alves ET, Albuquerque PEP. Morphophysiology, morphoanatomy, and grain yield under field conditions for two maize hybrids with contrasting response to drought stress. Acta Physiol Plant. 2013;35:3201-11.
- Reis CO, Magalhães PC, Avila RG, Almeida LG, Rabelo VM, Carvalho DT. et al. Action of N-Succinyl and N, O-Dicarboxymethyl Chitosan Derivatives on Chlorophyll Photosynthesis and Fluorescence in Drought-Sensitive Maize. J Plant Growth Regul.2018;1-12. https://doi.org/10.1007/s00344-018-9877-9.
- 27. Ávila RG, Magalhaes PC, Alvarenga AA, Lavinsky ADO, Campos CN, Souza TC, Gomes Júnior CC. 2017. Drought-tolerant maize genotypes invest in root system and maintain high harvest index during water stress. Rev Bras Milho Sorgo. 15(3):450-60.
- 28. Mutava RN, Prince SJK, Syed NH, Song L, Valliyodan B, Chen W, Nguyen HT. Understanding abiotic stress tolerance mechanisms in soybean: a comparative evaluation of soybean response to drought and flooding stress. Plant Physiol Biochem. 2015;86:109-20.
- 29. Demirevska K, Simova-Stoilova L, Vassileva V, Feller U. Rubisco and some chaperone protein responses to water stress and rewatering at early seedling growth of drought sensitive and tolerant wheat varieties. Plant Growth Regul. 2008;56(2):97.
- 30. Souza TC, Magalhães PC, Castro EM, Duarte VP, Lavinsky AO. Corn root morphoanatomy at different development stages and yield under water stress. Pesqui Agropecu Bras. 2016;51(4):330-9.
- 31. Kasim WA, Osman ME, Omar MN, El-Daim IAA, Bejai S, Meijer J. Control of drought stress in wheat using plantgrowth-promoting bacteria. J Plant Growth Regul. 2013;32(1):122-30.
- 32. Maurel C, Verdoucq L, Rodrigues O. Aquaporins and plant transpiration. Plant Cell Environ. 2016; 39(11):2580-7.
- Kørup K, Lærke PE, Baadsgaard H, Andersen MN, Kristensen K, Münnich C, et al. Biomass production and water use efficiency in perennial grasses during and after drought stress. Glob Change Biol Bioenergy. 2018;10(1):12-27.
- 34. Marchal K, Vanderleyden J. The "oxygen paradox" of dinitrogen-fixing bacteria. Biol Fertil Soils. 2000;30(5-6):363-73.
- 35. Lin Y, Watts DB, Kloepper JW, Torbert HA. Influence of Plant Growth-Promoting Rhizobacteria on Corn Growth Under Different Fertility Sources. Commun Soil Sci Plant Anal. 2018;49(10):1239-55.
- 36. Coelho AE, Tochetto C, Turek TL, Michelon LH, Fioreze SL. Inoculação de sementes com *Azospirillum brasilense* em plantas de milho submetidas à restrição hídrica. Sci Agrar. 2017;16(2):186-92.
- 37. Combes-Meynet E, Pothier JF, Moënne-Loccoz Y, Prigent-Combaret C. The *Pseudomonas* secondary metabolite 2,4-diacetylphloroglucinol is a signal inducing rhizoplane expression of *Azospirillum* genes involved in plant-growth promotion. Mol. Plant Microbe. Interact. 2017;24:271-84.

- 38. Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Fact. 2014;13(1):66.
- 39. Cassán F, Diaz-Zorita M. *Azospirillum* sp. in current agriculture: From the laboratory to the field. Soil Biol Biochem. 2016;103:117-30.
- 40. Calzavara AK, Paiva PHG, Gabriel LC, Oliveira ALM, Milani K, Oliveira HC, et al. Associative bacteria influence maize (Zea mays L.) growth, physiology and root anatomy under different nitrogen levels. Plant Biol. 2018;20(5):870-8.
- 41. Döbereiner J, Pedrosa FO. Nitrogen-fixing bacteria in non leguminous crop plants. Science Tech, Springer Verlag, Madison, USA. 1987;1-155.
- 42. Teixeira Filho MCM, Galindo FS, Buzetti S, Santini JMK. Inoculation with *Azospirillum brasilense* Improves Nutrition and Increases Wheat Yield in Association with Nitrogen Fertilization. In: Wheat Improvement, Management and Utilization. 2017;99-114.



© 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY NC) license (https://creativecommons.org/licenses/by-nc/4.0/).