

Original Article

Nitrogen metabolism in maize plants submitted to drought, brassinosteroids and *azospirillum*

Metabolismo de nitrogênio em plantas de milho submetidas à deficiência hídrica, brassinosteróides e *azospirillum*

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Abstract

The water deficit in particular, reduces the productivity of vegetable crops. To minimize these harmful effects on agriculture, several agronomic and physiological practices are being studied, such as the use of bacteria and water stress attenuators, such as brassinosteroids. Considering the socioeconomic relevance of corn culture and its sensitivity when exposed to water deficit, the objective of the present study was to evaluate the action of brassinosteroids and *azospirillum* on nitrogen metabolism in corn plants subjected to water stress conditions. The experiment was carried out in a greenhouse, in a period of 47 days, with corn plants, using the hybrid K9606 VIP3. The design was completely randomized, in a 2x2x3 factorial scheme, with six replications. The first factor corresponds to two water regimes (presence and absence of water deficit). The second corresponds to inoculation via seed of *Azospirillum brasiliense* and absence of inoculation. And the third corresponds to the application of three concentrations of brassinosteroids (0, 0.3 and 0.6 μM). Were determined Nitrate; nitrate reductase; free ammonium; total soluble aminoacids; soluble proteins; proline; glycine betaine and glutamine synthetase. The lack of water in plants provided a reduction in the protein and nitrate reductase contents, in leaves and roots. For ammonium, plants with water deficit inoculated at a concentration of 0.3 μM, obtained an increase of 7.16 (70.26%) and 13.89 (77.04%) mmol NH₄⁺.Kg⁻¹. DM (Dry mass) on the leaf and root respectively. The water deficit in the soil provided significant increases in the concentrations of glycine betaine, nitrate, proline and aminoacids, both in the leaves and in the roots of the corn plants. On the other hand, the contents of glutamine synthetase had a reduction in both leaves and roots.

Keywords: hormone, bacterium, *Zea mays*.

Resumo

O déficit hídrico, em particular, reduz a produtividade das hortaliças. Para minimizar esses efeitos nocivos à agricultura, diversas práticas agrônômicas e fisiológicas estão sendo estudadas, como o uso de bactérias e atenuadores de estresse hídrico, como os brassinosteróides. Considerando a relevância socioeconômica da cultura do milho e sua sensibilidade quando expostos ao déficit hídrico, o objetivo do presente trabalho foi avaliar a ação de brassinosteróides e *azospirillum* sobre o metabolismo de nitrogênio em plantas de milho submetidas a condições de estresse hídrico. O experimento foi conduzido em casa de vegetação, no período de 47 dias, com plantas de milho, utilizando-se o híbrido K9606 VIP3. O delineamento experimental utilizado foi o inteiramente casualizado, em esquema fatorial 2x2x3, com seis repetições. O primeiro fator corresponde a dois regimes hídricos (presença e ausência de déficit hídrico). A segunda corresponde à inoculação via semente de *Azospirillum brasiliense* e ausência de inoculação. E a terceira corresponde à aplicação de três concentrações de brassinosteróides (0, 0,3 e 0,6 μM). Foram determinados Nitrato; nitrato redutase; amônio livre; aminoácidos solúveis totais; proteínas solúveis; prolina; glicina, betaína e glutamina sintetase. A falta de água nas plantas proporcionou redução nos teores de proteína e nitrato redutase, nas folhas e raízes. Para o amônio, plantas com déficit hídrico, inoculadas na concentração de 0,3 μM, obtiveram aumento de 7,16 (70,26%) e 13,89 (77,04%) mmol NH₄⁺.Kg⁻¹. MS (Massa seca) na folha e na raiz, respectivamente. O déficit hídrico no solo proporcionou aumentos significativos nos teores de glicina, betaína, nitrato, prolina e aminoácidos, tanto nas folhas quanto nas raízes das plantas de milho. Por outro lado, os teores de glutamina sintetase apresentaram redução tanto nas folhas quanto nas raízes.

Palavras-chave: hormônio, bactéria, *Zea mays*.

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1. Introduction

Corn is a species belonging to the family of grasses, or Poaceae, having the second place as the most produced cereal in the country (Brasil, 2019).

In addition to the importance of maize cultivation in terms of production, the crop stands out for its diverse utility. In addition to acting directly on human and animal food, it is possible to produce a multitude of products, such as drinks, polymers, fuels and etc. (Miranda, 2018).

Throughout their development, plants in general can be exposed to a series of environmental stresses. The abiotic stress is one of them that most reduces crop productivity, negatively impacting agricultural sustainability is the water deficit caused by drought (Kerry et al., 2018). This adverse condition affects cereals such as corn, from germination, seedling performance and grain filling.

In this way, due the known high sensitivity of corn to the water stress (Welcker et al., 2006), research related to drought tolerance, showed an increase in the growth and crop yield in regions with water deficit (Li et al., 2009). Thus, to minimize the harmful effects of water deficit in the soil for crops, several agronomic and physiological practices are being applied, such as the use of bacteria and water stress attenuators such as brassinosteroids, that are growth regulators.

Brassinosteroids are a class of plant steroid hormones that act on cell elongation, allowing the growth of plants (Baghel et al., 2019). However, its effect depends on the growth stage of the plant (Freitas et al., 2015), the applied concentration (Freitas et al., 2014; Wei and Li, 2016) and the presence or absence of environmental stress (Tanveer et al., 2019). It is efficient in increasing resistance, especially at high temperatures, drought and salinity (Vardhini, 2012).

In addition to the application of hormones such as brassinosteroids, the use of *Azospirillum brasilense* bacteria presents itself as a promising alternative to improve the growth of grasses such as corn through nitrogen fixation. In several parts of the world, the use of bacteria in the formulation of inoculants, or biofertilizers, has been used and studied, and it is reported that these technologies can reduce production costs and environmental impact and increase crop productivity (Isawa et al., 2010; Bhattacharyya and Jha, 2012). The best known include members of the genus *Azospirillum*, *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Burkholderia*, *Serratia*, *Gluconacetobacter*, *Herbaspirillum*, *Azoarcus* and *Arthrobacter*, some of which are found on the root surface while others invade plant tissues, without cause apparent symptoms of disease, when they are known as endophytic (Sturz and Nowak, 2000; Rosenblueth and Martinez-Romero, 2006; Hardoim et al., 2008).

The mode of action of the bacteria *Azospirillum brasilense* when associated with grasses, disposes of phytohormone release, fixation of atmospheric nitrogen, reduction of nitrate and increase in the power of mineral absorption by plants (James, 2000). According to Spaepen, Vanderleyden and Okon (2009), *Azospirillum brasilenses* is capable of producing phytohormones (auxin, cytokinin and gibberellins) that can induce root growth and consequently improve the absorption of water and nutrients by plants.

In this context, there are few studies relating the effects of brassinosteroids and *Azospirillum brasilenses* on the corn crop subjected to drought. Thus, the objective of this work was to evaluate the influence of the application of brassinosteroid and *azospirillum* on the nitrogen metabolism in corn plants submitted to water stress conditions.

2. Material and Methods

2.1. Experiment

The experiment was developed in a greenhouse, which belongs to the Federal Rural University of the Amazon (UFRA), Belém City, Pará State (geographic coordinates 01° 27' 21" S, 48° 30' 16" W), from December 10th, 2019 to January 28th, 2020. In this region, the average temperature is around 26.5 °C, with significant air instability and average humidity around 84% (Bastos et al., 2002).

2.2. Experimental design and statistical analysis

To perform the experiment, corn seeds from the company KWS (Klein Wanzleben and Saat) were used, the hybrid (K9606 VIP3) developed by from Embrapa Amazônia Oriental. The experimental design used was completely randomized in a 2x2x3 factorial scheme, with six replications, totaling 72 experimental units. The first factor corresponds to two water regimes (presence of water deficiency and absence of water deficiency). The second factor corresponds to seed inoculation (at the moment of sowing) of *Azospirillum brasilense* and absence of inoculation. The third factor corresponds to the application of three concentrations of brassinosteroids (0.0 µM, 0.3 µM and 0.6 µM).

2.3. Experimental procedures

72 buckets with a capacity of 15 kg each were used, filled with a soil collected in the 0 to 20 cm (Samples of the superficial horizon of a Yellow Dystrophic Oxisol) depth layer at the Institute of Agricultural Sciences (ICA) at UFRA. After carrying out the soil collection, the samples were sieved to remove impurities, and after that, were homogenized.

The chemical analysis of the soil was carried out for fertility purposes, which was determined in the soil laboratory of the Brazilian Institute of Analysis (IBRA), according to the methods described by Raji et al (2001) and EMBRAPA (2009).

Before sowing, the corn seeds that were inoculated with *Azospirillum* were separated. Thus, 200 g of inoculant (*Azospirillum brasilense* - Strains AbV5 and AbV6, originated from Embrapa Amazônia Oriental) were mixed in 1 mL of distilled water and immediately afterwards the seeds (peat solid corn - *Zea mays*) were added and planted. Irrigation was carried out daily, adding water until reaching the field capacity of each bucket, which was observed through the drainage at the bottom of the buckets. The corn plants were obtained from the initial germination of five seeds per pot, with subsequent

maintenance of only one plant through thinning. At fifteen days after planting, the first application of brassinosteroids was performed using concentrations of 0.0 μM (Control plants, without application of brassinosteroids), 0.3 μM and 0.6 μM . The 0,3 and 0,6 μM Br (Sigma-Aldrich, USA) solutions were prepared by dissolving the solute in ethanol followed by dilution with Milli-Q water [ethanol:water (v/v) = 1:10,000] (Ahamed et al., 2013); the plants were in the V3 stage (three leaves completely expanded).

For each application, 72 mL of eponobrassinolide solution was used. Initially, 100 mL of eponobrassinolide at a concentration of 0.3 μM and 100 mL at a concentration of 0.6 μM was prepared. To the 100 mL of each concentration, 5 mL of Tween-20 was added as a surfactant. In all, two applications were made, the first at 15 days after the sowing, with plants in the V3 stage and the second at 30th day after the sowing, with plants in the V7 stage (Seven leaves completely expanded). The application was made by applying 3 mL of eponobrassinolide solution per plant, with a hand sprayer on the adaxial face (top) of the leaves of the middle third of the plants, always in the late afternoon (between 17h and 18h), in order to avoid losses by evaporation and drift.

Ten days after the last application of eponobrassinolide, severe water stress was applied to the plants that would receive the stress, with total water suspension (water availability at 0%). The other treatments continued to receive water until the experiment was withdrawn. Seven days after the suspension of irrigation, the plants were removed.

Plant collection was carried out at 47th day after sowing, at 04:30 a.m. Collected plants were separated into leaves, stems and roots (roots were washed in running water). They were stored in paper bags and placed in forced air ventilation oven at 65 °C, for 48 h. After the drying process was over, leaves and roots were weighed, ground in Wiley mill, properly stored in falcon tubes and subjected to biochemical analyses in the biodiversity studies on higher plants laboratory (EBPS).

2.4. Measurement of the variables

The following parameters were evaluated: nitrate (NO_3^-), based on the method recommended by Cataldo et al. (1975); nitrate reductase (NR) activity, based on the method *in vivo* recommended by Hageman and Hucklesby (1971); free ammonium (NH_4^+), based on the method by Weatherburn (1967); total soluble aminoacids, based on the method described by Peoples et al. (1989); soluble protein levels, based on the method described by Bradford (1976); proline, based on the method adopted by Bates et al. (1973), with adaptations made by Lobato and Ferreira; betaine glycine, based on the method recommended by Grieve and Grattan (1983); and glutamine synthetase activity, based on the method *in vitro* recommended by Kamachi et al. (1991).

The data were submitted to analysis of variance (ANOVA) using the F test ($p < 0.05$) and the averages obtained were submitted to the Tukey test ($p < 0.05$) using the software SISVAR version 5.4 (Ferreira, 2010).

3. Results and Discussion

3.1. Nitrogen in corn plant leaves as a function of water management, inoculation with concentrations of *azospirillum* and brassinosteroids

The corn plants with water deficiency, inoculated with *azospirillum*, in concentrations of 0 and 0.3 μM of brassinosteroids, presented higher values of ammonium in the leaves (9.09 and 10.19 $\text{mmol NH}_4^+ \cdot \text{Kg}^{-1} \cdot \text{DM}$) respectively, compared to the irrigated treatments (5.81 and 5.56 $\text{mmol NH}_4^+ \cdot \text{Kg}^{-1} \cdot \text{DM}$) in the same study condition, corresponding to an increase of 36.08% and 45.44% respectively (Table 1). In addition, when comparing the treatments with water deficit, inoculated and without inoculation, in the concentration 0.3 μM of brassinosteroids, the inoculated plants obtained an increase of 7.16 $\text{mmol NH}_4^+ \cdot \text{Kg}^{-1} \cdot \text{DM}$ (70.26%) of ammonium.

It is thus observed that there was an increase in the ammonium content in the stressed plants. This increase may be linked to the glutamate dehydrogenase (GDH) route, since it acts on NADH dependence, deactivating the glutamate and generating ammonium (NH_4^+) and 2-Oxyglutarate to form it, promoting an accumulation of ammonium through of a mismatch between its production and the reduction of the activity of the enzyme glutamine synthetase (Cruz et al., 2008).

The treatments with water deficiency and inoculated with *azospirillum*, in the highest concentration of brassinosteroids, presented lower ammonium value in the leaves compared to the other dosages.

For the protein parameter, plants with water deficiency, inoculated or not and in the lowest concentration of brassinosteroids, presented lower values of proteins in the leaves compared to the irrigated treatments (Table 1). This result demonstrates that the lower the water potential of the soil, the greater the activity of the proteolytic enzymes that are responsible for the degradation of the reserve of proteins in plants, reducing their contents in the leaves, in addition to reducing their synthesis during the lack of water (Freitas, 2014).

While, when comparing the plants with water deficiency, inoculated and without inoculation and in the concentration of 0 μM , the inoculated plants presented an increase of 46.39% of protein. In addition, the plants with water deficiency, inoculated and in concentrations 0.3 and 0.6 μM obtained increases in the values of proteins in relation to the concentration 0 μM .

The irrigated plants, inoculated or not and in all concentrations of brassinosteroids, presented higher values of nitrate reductase in the leaves in comparison with the plants with water deficiency. These results show that water deficiency in plants reduces the total amount of functional activity of the nitrate reductase enzyme, where the lack of water in the soil decreases the activity of this enzyme due to the reduction of the water flow through the transpiratory current and with that also the flow of nitrate to the leaves, since this enzyme is highly dependent on its substrate (Shaner and Boyer, 1976).

While plants with deficiency, inoculated and without inoculation and in concentrations 0.3 and 0.6 μM obtained

increases in nitrate reductase values in relation to the concentration of 0 μM (Table 1).

3.2. Biochemical responses in corn plant leaves as a function of water management, inoculation with concentrations of azospirillum and brassinosteroids

When observing Table 2, in the inoculated water deficit plants, and in all concentrations of brassinosteroids, presented higher values of glycine betaine in the leaves compared to the irrigated and inoculated plants. Proline, glycine-betaine and carbohydrates, among organic compounds, are the solutes most commonly accumulated in plants when under stress conditions (Ashraf et al., 2011). In addition to osmotic adjustment, these solutes play a role in stabilizing enzymes/proteins and in protecting membrane integrity (Bohnert and Jensen, 1996). The increase in glycine-betaine levels is associated with the function of being an excellent osmotic adjuster in plant species, where there is a particular signaling and consequently an increase in the synthesis of this amino acid (Silva et al., 2009). In young sugarcane plants (*Saccharum* spp.), Carlin and Santos (2009) obtained similar results, where an increase in glycine-betaine was observed under water deficit, but it was not considered osmotic protection.

Treatments with water deficit inoculated or not and in the lowest concentration of brassinosteroids, obtained the highest levels of betaine glycine in the leaves in comparison

with the doses 0.3 and 0.6 μM , demonstrating that neither the bacteria nor the hormone promoted increments in this parameter but the reduction of the water potential of the soil, thus promoting the accumulation of solutes in the tissues of the plants in order to maintain the osmotic adjustment (Table 2).

Glutamine synthetase in leaves (Table 2) showed higher values in irrigated and inoculated plants and in all concentrations of brassinosteroids compared to plants with water deficiency. These results demonstrate that when in conditions of water deficiency, the enzyme becomes less effective due to the low concentrations of ATPs in the cellular environment, reducing the sugar available for the production of energy and reducing powers, since this enzyme is dependent of energy from photosynthetic activity in the leaves and the respiratory process in the roots (Swarbreck et al., 2011; Tercé-Laforgue et al., 2013).

While nitrate, proline and amino acids in the leaves of corn plants, were higher in treatments with water deficiency. In addition, when comparing the treatments with deficient inoculated and without inoculation, the presence of the bacteria did not increase the levels of nitrate, proline and amino acids. However, the nitrate in the leaves, in the inoculated plants with water deficiency and in the highest concentration of brassinosteroids, showed an increase of 18% in comparison with the 0.3 μM concentration and 9.85% with the lowest concentration

Table 1. Ammonium, protein and nitrate reductase contents in maize plant leaves, based on interaction among water management, inoculation with azospirillum, and brassinosteroid concentrations.

Irrigation	Inoculation	Brassinosteroids ($\mu\text{M Br}$)		
		0	0.3	0.6
Ammonium ($\text{mmol NH}_4^+ \cdot \text{Kg}^{-1} \cdot \text{DM}$)				
Irrigated (I)	Presence	5.81Ba α^*	5.56Ba α	4.21Aa α
Irrigated (I)	Absence	6.70Ba α	3.71Ab α	2.82Ab α
Water deficit (WD)	Presence	9.09Aa β	10.19Aa α	3.50Ab α
Water deficit (WD)	Absence	12.43Aa α	3.03Ab β	2.27Ab α
CV (%)	22.61			
Protein (mg of protein/g DM)				
Irrigated (I)	Presence	20.84Aa α	26.34Aa α	22.88Aa β
Irrigated (I)	Absence	22.10Ab α	23.66Aab α	28.69Aa α
Water deficit (WD)	Presence	14.83Bb α	23.36Aa α	24.07Aa α
Water deficit (WD)	Absence	7.95Bb β	20.26Aa α	24.17Aa α
CV (%)	15.9			
Nitrate reductase ($\mu\text{moles of NO}_2\text{-g}^{-1} \cdot \text{DM}$)				
Irrigated (I)	Presence	1.69Ab α	1.83Ab β	2.39Aa α
Irrigated (I)	Absence	1.75Ab α	2.24Aa α	2.10Aab α
Water deficit (WD)	Presence	0.32Bb α	1.03Ba β	0.83Ba α
Water deficit (WD)	Absence	0.09Bc α	1.57Ba α	1.01Bb α
CV (%)	16.04			

*Means followed by the same letter (uppercase letters for water regimes, lowercase letters for brassinosteroid doses and Greek letters for inoculation with *Azospirillum brasilense*) did not differ from each other in the Tukey test, at 5% probability level. CV: coefficient of variation; DM: Dry matter.

Table 2. Betaine glycine, glutamine synthetase, nitrate, proline and amino acids in leaves of maize plants, based on the interaction among water management, inoculation with *azospirillum*, and brassinosteroid concentrations.

Irrigation	Inoculation	Brassinosteroids ($\mu\text{M Br}$)		
		0	0.3	0.6
Betaine glycine (μg of glycine betaine/g DM)				
Irrigated (I)	Presence	9.00Ba β^*	8.96Ba α	9.60Ba α
Irrigated (I)	Absence	9.94Ba α	9.25Ba α	10.03Ba α
Water deficit (WD)	Presence	26.11Aa β	18.29Ac β	22.37Ab β
Water deficit (WD)	Absence	30.21Aa α	22.70Ac α	23.98Ab α
CV (%)	3.85			
Glutamine synthetase (mmoles of γ -glutamylhydroxyte/kg DM of tissue/hour)				
Irrigated (I)	Presence	19.78Ab α	21.43Aa α	18.77Ab β
Irrigated (I)	Absence	18.94Aa α	19.58Aa β	19.91Aa α
Water deficit (WD)	Presence	9.17Bb α	12.35Ba α	12.84Ba α
Water deficit (WD)	Absence	8.39Bb α	11.89Ba α	12.20Ba α
CV (%)	4.61			
Nitrate (μmoles of $\text{NO}_3^-/\text{g DM}$)				
Irrigated (I)	Presence	1.23Bb β	0.86Bc β	1.71Ba β
Irrigated (I)	Absence	2.44Ba α	1.77Bb α	2.05Bb α
Water deficit (WD)	Presence	5.58Ab β	5.05Ac β	6.19Aa β
Water deficit (WD)	Absence	7.13Aa α	5.95Ac α	6.72Ab α
CV (%)	4.61			
Proline ($\mu\text{moles Pro/g DM}$)				
Irrigated (I)	Presence	3.09Bb α	4.03Ba α	3.46Bb α
Irrigated (I)	Absence	3.20Ba α	3.17Ba β	3.31Ba α
Water deficit (WD)	Presence	9.17Aa β	5.22Ac β	7.78Ab β
Water deficit (WD)	Absence	12.90Aa α	6.93Ac α	8.73Ab α
CV (%)	5.39			
Amino acids (μmoles of A.A. g^{-1}DM)				
Irrigated (I)	Presence	95.34Bb α	104.37Ba α	99.25Bab α
Irrigated (I)	Absence	96.07Ba α	101.26Ba α	98.58Ba α
Water deficit (WD)	Presence	139.97Aa β	128.33Ab β	134.15Aab α
Water deficit (WD)	Absence	150.50Aa α	135.84Ab α	131.66Ab α
CV (%)	3.38			

*Means followed by the same letter (uppercase letters for water regimes, lowercase letters for brassinosteroid doses and Greek letters for inoculation with *Azospirillum brasilense*) did not differ from each other in the Tukey test, at 5% probability level. CV: coefficient of variation; DM: Dry matter.

(Table 2). Studies show that a criterion for drought tolerance studies is the accumulation of free proline, being this osmoprotective used as a biochemical-physiological indicator of water stress (Dias, 2014; Costa et al., 2015).

3.3. Nitrogen in corn plant roots as a function of water management, inoculation with concentrations of *azospirillum* and brassinosteroids

The ammonium in the roots showed an increase of 61.29% in the inoculated plants with water deficiency

and in the concentration 0.3 μM in comparison with the irrigated plants in the same condition of study. This result is probably linked to the fact that the bacteria of the genus *Azospirillum*, can act on the growth of plants by reducing the nitrate that is in the root, to ammonium. Ferreira et al. (1987) observed, for example, that bacteria of this genus can act on plant growth by reducing NO_3^- in plant roots. Thus, plants would not spend energy to reduce nitrate to ammonia, and this energy can be directed to other vital processes of plant metabolism (Reis Júnior et al., 2008).

However, at the 0.6 μM concentration, the irrigated and inoculated plants obtained higher values in relation to those with water deficiency also inoculated (Table 3).

It is also possible to observe that, for irrigated plants, the presence of the bacterium provided increments of 49.34%, 36.45% and 57.65%, in the levels of ammonium in the roots of the plants in concentrations 0; 0.3 and 0.6 μM respectively, compared to plants irrigated without inoculation. Whereas the plants with deficient inoculated and in the concentration 0.3 μM , both the bacteria and the brassinosteroids provided an increase (77.04%) in the contents of ammonium in the roots in comparison with the deficient plants without inoculation (Table 3).

In Table 3, it is also possible to observe that irrigated plants, inoculated or not and at a concentration of 0 μM , presented higher values of protein and nitrate reductase in the roots compared to plants with water deficiency. For Maraghni et al. (2011) protein metabolism is significantly affected by the lack of water in plants, after the perception of stress the protein biosynthesis process is one of the first paralyzed metabolic events, thus initiating proteolysis, which promotes an increase in the content of soluble amino acids, with a consequent reduction in protein concentration (Brito et al., 2008). In addition, plants under stressed conditions promoted a reduction in nitrate reductase activity. The assimilation of nitrogen is affected by water deficit, with drastic reductions in

the activity of the nitrate reductase enzyme, from small decreases in water potential in soil macro and micropores (Botrel et al., 2000).

Whereas, the plants with water deficiency, inoculated or not, presented protein increments and nitrate reductase in concentrations 0.3 and 0.6 μM , in comparison with the concentration 0 μM . This demonstrates that for plants in conditions of water deficiency in the soil, *azospirillum*, in the presence or not of the hormone, was not as efficient, while brassinosteroids, in the presence or not of the bacterium, attenuated the effects of stress.

3.4. Biochemical responses in corn plant roots as a function of water management, inoculation with concentrations of *azospirillum* and brassinosteroids

Regarding the content of glycine betaine in the roots of corn plants (Table 4), higher values were observed in plants with water deficiency, inoculated or not, and in all concentrations of brassinosteroids compared to irrigated plants. In addition, when comparing plants with water deficiency and in all concentrations of brassinosteroids (0; 0.3 and 0.6 μM), the presence of the bacterium provided a decrease of 12.84%, 20.31% and 6.27% respectively, in the content of glycine betaine compared to uninoculated plants. The increase in glycine-betaine concentrations in plants under water deficiency is probably associated with

Table 3. Ammonium, protein and nitrate reductase concentrations in maize plant roots, based on the interaction among water management, inoculation with *azospirillum* and brassinosteroid concentrations.

Irrigation	Inoculation	Brassinosteroids ($\mu\text{M Br}$)		
		0	0.3	0.6
Ammonium ($\text{mmol NH}_4^+ \cdot \text{Kg}^{-1} \cdot \text{DM}$)				
Irrigated	Presence	7.52Ba α^*	6.53Bab α	5.88Ab α
Irrigated	Absence	3.81Bab β	4.15Aa β	2.49Bb β
Water deficit	Presence	16.28Ab β	18.03Aa α	4.59Bc α
Water deficit	Absence	19.88Aa α	4.14Ab β	4.81Ab α
CV (%)	10.69			
Protein (mg of protein/ g DM)				
Irrigated	Presence	8.34Aa α	10.54Aa α	9.15Aa β
Irrigated	Absence	8.84Ab α	9.47Aab α	11.47Aa α
Water deficit	Presence	5.93Bb α	9.34Aa α	9.63Aa α
Water deficit	Absence	3.18Bb β	8.10Aa α	9.67Aa α
CV (%)	15.9			
Nitrate reductase ($\mu\text{moles of NO}_2\text{-g}^{-1} \cdot \text{DM}$)				
Irrigated	Presence	2.02Ab α	2.20Ab β	2.87Aa α
Irrigated	Absence	2.10Ab α	2.69Aa α	2.52Aab α
Water deficit	Presence	0.39Bb α	1.23Ba β	0.99Ba α
Water deficit	Absence	0.11Bc α	1.88Ba α	1.21Bb α
CV (%)	16.05			

*Means followed by the same letter (uppercase letters for water regimes, lowercase letters for brassinosteroid doses and Greek letters for inoculation with *Azospirillum brasilense*) did not differ from each other in the Tukey test, at 5% probability level. CV: coefficient of variation; DM: Dry matter.

Table 4. Betaine glycine, glutamine synthetase, nitrate, proline and amino acid concentrations in maize plant roots, based on the interaction among water management, inoculation with *azospirillum* and brassinosteroid concentrations.

Irrigation	Inoculation	Brassinosteroids ($\mu\text{M Br}$)		
		0	0.3	0.6
Betaine glycine (μg of glycine betaine/g DM)				
Irrigated	Presence	10.62Ba β^*	10.58Ba α	11.33Ba α
Irrigated	Absence	11.78Ba α	10.90Ba α	11.95Ba α
Water deficit	Presence	30.83Aa β	21.39Ac β	26.59Ab β
Water deficit	Absence	35.37Aa α	26.84Ac α	28.37Ab α
CV (%)	3.41			
Glutamine synthetase (mmols of γ -glutamyl hydroxide/kg tissue DM/hour)				
Irrigated	Presence	13.00Ab α	14.30Aa α	12.65Ab α
Irrigated	Absence	12.91Aa α	12.83Aa β	13.04Aa α
Water deficit	Presence	6.11Bb α	8.22Ba α	8.65Ba α
Water deficit	Absence	5.85Bb α	8.10Ba α	8.15Ba α
CV (%)	4.44			
Nitrate (μmols of $\text{NO}_3^-/\text{g DM}$)				
Irrigated	Presence	1.47Bb β	1.04Bc β	2.05Ba β
Irrigated	Absence	2.92Ba α	2.12Bb α	2.46Bb α
Water deficit	Presence	6.70Ab β	6.06Ac β	7.42Aa β
Water deficit	Absence	8.55Aa α	7.14Ac α	8.06Ab α
CV (%)	4.61			
Proline ($\mu\text{mols Pro/g DM}$)				
Irrigated	Presence	3.94Bc α	5.03Ba α	4.42Bb α
Irrigated	Absence	4.25Ba α	4.12Ba β	4.18Ba α
Water deficit	Presence	11.58Aa β	6.71Ac β	10.27Ab β
Water deficit	Absence	16.76Aa α	8.75Ac α	10.98Ab α
CV (%)	3.50			
Amino acids (μmols of A.A. g. ⁻¹ DM)				
Irrigated	Presence	115.39Bc α	126.37Ba α	120.11Bb α
Irrigated	Absence	117.30Bc α	122.83Ba β	119.53Bab α
Water deficit	Presence	169.31Aa β	155.10Ac β	161.83Ab β
Water deficit	Absence	181.77Aa α	163.90Ab α	164.98Ab α
CV (%)	1.38			

*Means followed by the same letter (uppercase letters for water regimes, lowercase letters for brassinosteroid doses and Greek letters for inoculation with *Azospirillum brasilense*) did not differ from each other in the Tukey test, at 5% probability level. CV: coefficient of variation; DM: Dry matter.

better absorption and transport of water from the soil to the aerial part through osmotic adjustment, in addition to greater protection of the cell membrane, as well as protection against the oxidative stress mechanism of plants (Ashraf and Harris, 2004).

For glutamine synthetase, both in the leaves and in the roots of corn plants, the highest values were observed in irrigated plants. We can also observe that plants with water deficiency inoculated or not with *azospirillum*, showed an increase in glutamine synthetase in concentrations 0.3 and 0.6 μM of brassinosteroids (Table 4).

While for nitrate, proline and amino acids in the roots, the highest values were found in plants with water deficiency. Furthermore, in plants with water deficiency in all concentrations of brassinosteroids, the presence of *azospirillum* promoted a decrease of 21.64%, 15.13% and 7.94%, respectively, in the nitrate content, of 30.91%, 23.31% and 6.47% in the content of proline and 6.85%, 5.37% and 1.91% in the amino acids in the roots of the corn plants. This result may be related to the fact that the bacteria of the genus *Azospirillum* reduces nitrate to ammonium, thus the concentration of nitrate decreases

in the presence of the bacterium and increases in the absence. For Kappes et al. (2017) there are interactions between N and diazotrophic bacteria in the assimilation and use of this nutrient by plants.

4. Conclusion

The application of *Azospirillum brasilense* in maize crops has affected nitrogen metabolism in leaves and roots of plants subjected to water deficit; the same behavior was observed in plants subjected to brassinosteroid application. Using the plant growth-promoting bacterium *Azospirillum brasilense* via seed, as well as brassinosteroid application, helped mitigating the negative effects of water deficit on nitrogen metabolism in the leaves and roots of maize plants.

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