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Water use efficiency in soybean crop after inoculation with *Azospirillum brasiliense* in the Cerrado of Tocantins State, Brazil

Evandro Reina* and Joenes Mucci Peluzio

Biodiversity and Biotechnology Network of the Legal Amazon (BIONORTE), Federal University of Tocantins, Araguaína, Brazil.

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Crops in the Cerrado experience short drought stress periods that affect cultivation development, especially the water use efficiency (WUE), which can be modified by the use of *Azospirillum brasiliense* bacteria. In this sense, this study evaluated soybean plants' response to *A. brasiliense* inoculation, in relation to WUE, in the Cerrado of Tocantins State. During the growing season of 2015/2016, three experiments were conducted in Palmas municipality, with analyses in the phenological stages: R3 for the first planting and R2 for the second and third plantings. The experimental design used in each experiment was randomized block with ten treatments and four replications. The treatments were disposed in 2x5 factorial scheme, represented by two cultivars (M-9144RR and TMG 1288RR) and five different doses of *A. brasiliense* (0, 100, 200, 300 and 400 ml of the commercial product per hectare). They were applied through the leaf at V2 and R1 stages of the crops. WUE determination was done through photosynthesis measurement (IRGA), through which the photosynthesis and transpiration values were obtained. There was no WUE difference between the cultivars. *A. brasiliense* doses had effect in relation to WUE, on stressed crops. *A. brasiliense* inoculation did not show any significant change in WUE in crops with favorable conditions.

Key words: *Glycine max*, diazotrophics, photosynthesis, transpiration.

INTRODUCTION

The State of Tocantins, with an area of 227.720,567 km^2, is located at a region designated as Legal Amazon. It involves both the Amazon and the Cerrado biomes, in which territorial space is located in the largest hydrographic basin fully located inside the Brazilian territory (Tocantins-Araguaia River Basin). In this region, cultivation land is equivalent to 50% of the state territory. This, combined with logistic facilities for connecting to all parts of the country, has attracted the attention of farmers, national companies and international trading, who aim the internal and external market (EMBRAPA, 2016).

*Corresponding author. E-mail: evandroreina@uft.edu.br.

Table 1. Soil chemical analysis results from the experimental area in Palmas, growing season of 2015/2016.

pH	P	K	Al	H+Al	Ca+Mg	CTC	V	MO
(H_2O)	$mg.dm^{-3}$		$Cmol_c.\ dm^{-3}$				%	
5.65	17.28	40.0	0.0	1.5	3.3	5.5	61.82	1.8

PH: Hydrogen ionic potential; P: phosphorus; K: potassium; Al: aluminum; H + Al: potential acidity; Ca+Mg: calcium + magnesium; CTC: cationic exchange capacity; V: saturation by bases; MO: organic matter.

In the Cerrado, under low latitude conditions, due to drought periods and high temperatures, plants are subjected to stress periods, resulting in a reduction of the photosynthetic rate. This is caused by the decrease in plant metabolism that occurs due to increased diffusion resistance of atmospheric CO_2 to reach the carboxylation site caused by stomata closure (Cruz et al., 2016). Therefore, the utilization of parameters such as the water use efficiency (WUE) would allow effect fixing of carbon while transpiring (Ramos et al., 2014).

As the water use efficiency is related to the photosynthetic rate and transpiration of the plant, the application of this concept allows plants to adapt to water stress (dry), caused by the closure of the stomata, and makes plant to have osmotic adjustment, which is among other mechanisms used to adapt to the effective control of water use, even under low availability of water. It aims to stabilize production in the face of climatic changes. Thus, the adoption of cultivars with greater efficiency in the use of water, associated with the use of growth-stimulating microorganisms and increased photosynthetic rate, even under adverse conditions, could increase the tolerance of plants to water and thermal stress (Bulegon et al., 2016).

The use of plant growth promoter bacteria (PGPB) is a promising alternative to meet the growing food demand and also the necessity of natural resources conservation in the Cerrado, under favorable and unfavorable cultivating conditions (Cohen et al., 2015).

Revising the literature, there are several reports related to the beneficial effects of growth promoter bacteria (PGPB) under the following conditions: nitrogen biological fixation (Huergo et al., 2008), increased nitrate reductase activity when it develops as an endophytic in plants (Cassán et al., 2008), biological control of pathogens (Correa et al., 2008), enhanced plants' productivity (Rodrigues et al., 2014) and the production of hormones such as auxins, cytokinins, gibberellins, ethylene and a variety of other molecules (Bulegon et al., 2016).

Hungria (2011) indicated that corn and wheat showed high yield when they were inoculated with *Azospirillum brasiliense* as compared to the control plants. *A. brasiliense* supplied nitrogen to the inoculated plants and produced phytohormones that stimulated root growth, due to the liberation of indole acetic acid (IAA), gibberellins and cytokinins. In addition, green corn plants in Marilia-SP exhibited higher chlorophyll content, stomatal conductance, hydric potential, cell wall extensibility, biomass production and high plant height after being inoculated with *A. brasiliense* compared to the control counterpart plants (Neto et al., 2013).

Several authors reported the use of *Bradyrhizobium* bacteria associated with *A. brasiliense* in different regions, covering different types of soils and climate. However, there are no reports of work in the state of Tocantins on the inoculation of this bacterium in association with *A. brasilienses* that influences the water use efficiency in soybean crop.

In this context, it is known that in legumes and/or grasses, the concomitant use of these bacteria has economic and environmental benefits. Due to the scarcity of information on the behavior of soybean plants inoculated with *A. brasilienses* regarding water efficiency use (WUE) in the Cerrado biome, the present study was carried out to evaluate the behavior of two soybean cultivars when inoculated with different doses of *A. brasiliense* in relation to WUE.

MATERIALS AND METHODS

In 2015-2016 growing season, three experiments were developed in the experimental area of the Tocantins Federal University (TFU) in the municipality of Palmas-TO (latitude 10°45' S, longitude 47°14' W, 220 m of altitude above sea level). Plantations were carried out in November 18th, December 2nd and December 17th 2015. The soil of this region is classified as Dystrophic Yellow Red Latosol (EMBRAPA, 2013); the region has plain relief, Aw climate, and is tropical with a dry season. Soil sample collection was performed at 0 to 20 and 20 to 40 cm depth layers according to the EMBRAPA methodology (2011). The soil chemical analysis was performed by a private soil analysis laboratory that meets the requirements of the Program of Quality of Analyzes of Laboratories of Fertility-PAQLF (EMBRAPA, 2011). The results are presented in Table 1.

The experiments were laid out in a randomized block design with ten treatments and four replications. The treatments were organized in factorial scheme 2x5, represented by two cultivars (M-9144RR and TMG 1288RR) and five doses of *A. brasiliense* (0, 100, 200, 300 and 400 ml of the commercial product per hectare). They were applied on the leaf surface: half of each dose was applied at V2 stage and the other half at R1 stage of the crops. The *A. brasiliense* strains used were AbV5 and AbV6, with the commercial concentration of 2×10^8 $Ufc.ml^{-1}$.

Meteorologic data (Figure 1) were obtained through the INMET, 2016 web site, based on the meteorologic station installed in the same municipality where the experiments were conducted.

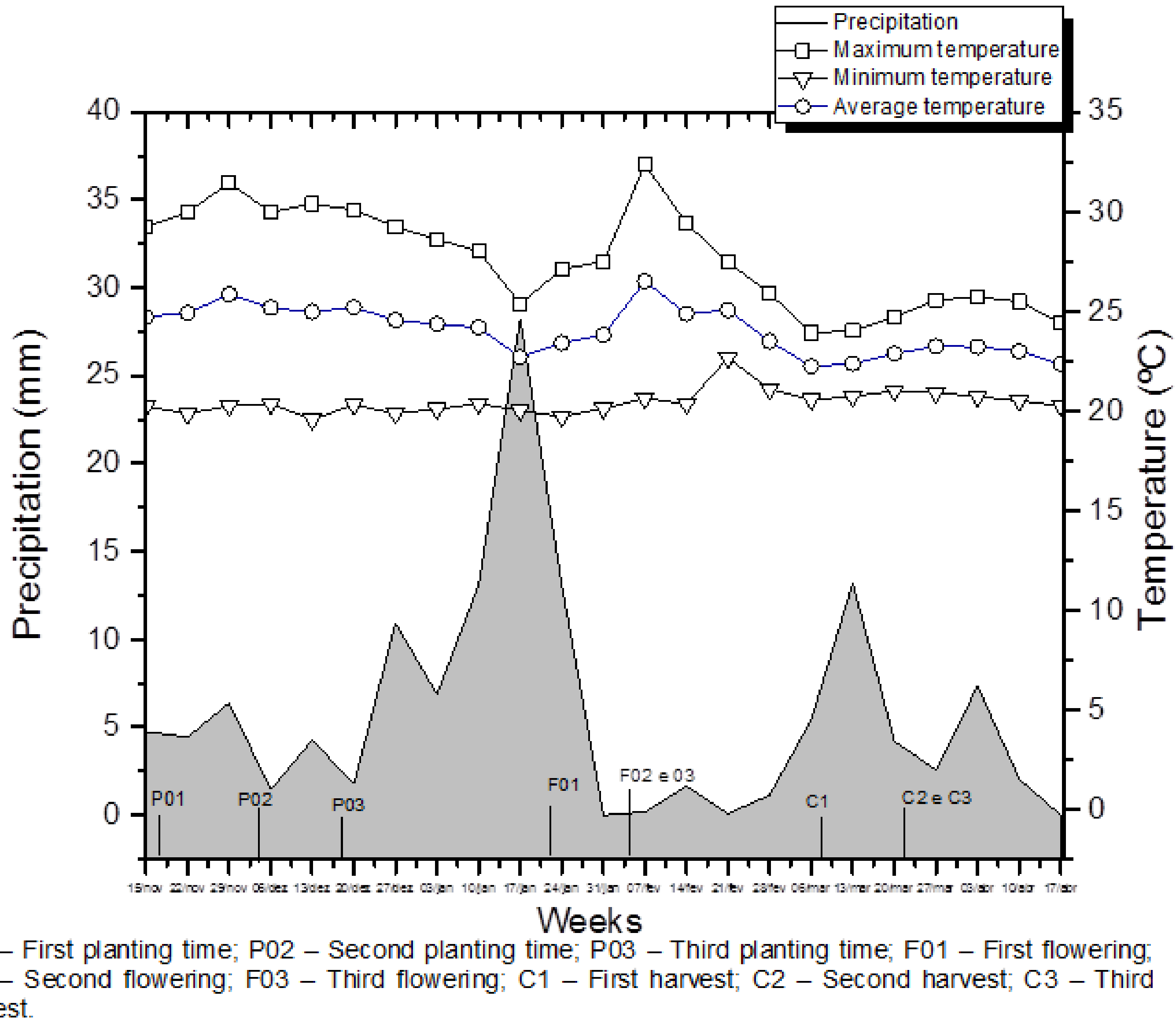

Figure 1. Weedkly climatic data from Palmas between Novemeber 15th, 2015 and April 17th, 2016.

Both cultivars M9144RR and TMG 1288RR are traditionally planted in the region, presenting determined growth habit and high fertility requirement. Based on maturity groups, cultivar M9144RR belongs to group 9.1 and TMG 1288RR to group 8.8.

Before planting, the soil was plowed and harrowed. The experimental plots had four rows of 5.0 m length, spaced by 0.5 m, totaling a plot area of 10 m^2 with 300 plants. When the inoculant was applied via the leaf, the sprayer jets were directed to the plants of the two central rows of the plot so that the lateral rows functioned as a border for avoiding the contamination of the other plots. 400 kg ha^{-1} of simple superphosphate corresponding to approximately 80 kg of P_2O_5 ha^{-1} was applied. Planting density was carried out in order to obtain 15 plants per linear meter, and 10 days after emergence, thinning was done when necessary. Final plant population was about 300.000 plants/ha^{-1}. The seeds were treated with fungicide (Carboxin + Thiram 200 SC -> 2.5 mL + 2.5 mL of water kg^{-1}). During planting, they were inoculated with *Bradyrhizobium japonicum* strains, using the product Biomax® Premium Peat- Soybean (Strains SEMIA 5079 + SEMIA 5080), at 60 g/50 kg dose of seed. At harvesting stage, only plants from the net plots (two center rows in each plot) were used. During the crop cycle, no irrigation was used, as water was available to the plant through precipitation.

The pests were controlled by applying the active principle Bifenthrin + Imidacloprid (50 + 100 gL^{-1}). Azoxystrobin (0.5 L ha^{-1}) was applied to control diseases and weeds where Glyphosate (360 gL^{-1}) was needed. Fertilization with potassium chloride at 166 kg ha^{-1} (approximately 100 kg ha^{-1} K_2O) was performed; half of the dose was applied at V2 stage and the other half at V4 stage.

Photosynthesis (A) and transpiration (E) were measured at R3 stage for the first planting time, and at R2 for the second and third planting time, using a portable photosynthesis measurement system (IRGA Infrared Gas Analyzer) model Li- 6400 (Li-Cor, Biosciences Inc., Nebraska, EUA). Measurements were performed at 9:00 am, when leaves were totally expanded and mature at the two central rows of each plot.

Table 2. Summary of the variance analysis of water use efficiency (WUE) due to planting time, cultivars and leaf *A. brasiliense* inoculation doses in soybean plants cultivated in Palmas-TO, during 2015/2016 growing season.

VS	DF	MS
Planting times	2	2.1501*
Cultivars	1	0.0804*
Leaf inoculation doses	4	0.0823*
Planting time x cultivars	2	0.1024*
Planting time x leaf inoculation doses	8	0.0642*
Cultivars x leaf inoculation doses	4	0.0637ns
Planting time x cultivars x leaf inoculation doses	8	0.0403*
Block/planting time	6	0.0060^{ns}
Error	54	0.0177
Total	89	
CV (%)	6.05	

*Significant p<0.05 and non-significant p<0.05, by the F test.

During measurements, photosynthetic active radiation (PAR), atmospheric CO_2 inside the leaf chamber and the chambers block temperature were kept constant, with values of 2000 µmol photons m^2 s^{-1}, 380 to 400 µmol CO_2 m^2 s^{-1} and 26 to 27°C, respectively. The reference air was collected and homogenized in a 5 L gallon before reaching the assimilation chamber. The net photosynthetic rate per unit leaf area (A) (µmol CO_2 $m^2.s^{-1}$) and the transpiration (E) (mol H_2O $m^2.s^{-1}$) were calculated using the variations of CO_2 and steam values inside the chamber, measured by the infrared gas analyzer of the portable photosynthesis system (Von Caemmerer and Farquhar, 1981). With the results of this system, WUE was calculated through the ratio between A (net photosynthetic rate) and E (transpiration).

The WUE data obtained in each planting time were subjected to analysis of individual variance and, in sequence, to an analysis of combined variance in which the smallest mean residual square did not differ more than seven times from the highest (Cruz and Regazzi, 2004). In the following step, the cultivars rates and planting time were compared by the Scott-Knott test, at 5% significance. For each *A. brasiliense* dose, in each cultivar and planting time, a polynomial regression model was adjusted. In the absence of functional relationship between doses and cultivars, the Scott-Knott test was applied, at 5% significance.

The statistical program used to analyze the data obtained was SISVAR 5.0 (Ferreira, 2011) and for the construction of the graphics, the software Origin Pro 8.0 was used.

RESULTS AND DISCUSSION

The analysis of variance for WUE (Table 2) revealed significant effect for all factors, except for the interaction of cultivars x leaf inoculant doses of *A. brasiliense.* The significance of the triple interaction indicates the cultivars' differential effect due to different doses and planting times. The latter is represented by variation in precipitation and temperature during the experimental period (Graphic 1). The coefficient of variation obtained was of 6.05%, indicating a good accuracy in conducting the experiments (Scapim et al., 1995). The statistical average for WUE, net values of photosynthesis rate (A) and transpiration (E), resulting from the triple interaction planting time x cultivar x *A. brasiliense* doses are presented in Table 3. As there was no functional relationship between *A. brasililiense* doses and the cultivars or/and planting times, a mean grouping test was used in the comparative study between the doses of A. *brasiliensis*. The WUE values obtained in this study were superior to the ones obtained by Nonato (2016), in soybean plantations subjected to different doses of *A. brasiliense* and *B. japonicum* inoculation.

In relation to the cultivars, for each dose and planting time, no significant differences were detected in the WUE, probably due to cultivars that had similarities in the number of days to complete their cycles, as they belonged to groups having very close maturation to each other. Procópio et al. (2004) studied WUE in soybean crops, beans and weed but did not find differences in WUE, as compared to plants with similar cycle. In addition, they observed that plants with long cycle tend to be more efficient in using water in water-limited environments. In this way, when selecting cultivars for regions with periods of drought, we must take into account not only the WUE, but also the cycle of cultivars to be used in order to maintain production. When *A. brasiliense* doses were compared in each cultivar and within the same planting time, no significant effects were detected in relation to WUE between the inoculant doses in the first planting time (Table 3). On the other hand, for the following planting times, there were significant differences between *A. brasiliense* doses to soybean cultivars. This might be related to the response of different cultivars to *A. brasiliense* doses used in this study.

At the first planting time (11/18th), there were more favorable conditions of precipitation and temperature (Figure 1) for the plant development in relation to the second (12/02nd) and third (12/17th), when some hydric

Table 3. Deployment averages between planting time x cultivar x dose to water use efficiency in soybean crops, in Palmas – TO, during the growing season of 2015/2016.

Planting times	Dose 0		Dose 100		Dose 200		Dose 300		Dose 400	
	9144	1288	9144	1288	9144	1288	9144	1288	9144	1288
18/11	2.50^{Aa1}	2.46^{Aa1}	2.61^{Aa1}	2.44^{Aa1}	2.71^{Aa1}	2.48^{Aa1}	2.44^{Aa1}	2.36^{Aa1}	2.48^{Aa1}	2.32^{Aa1}
02/12	2.21^{Ba1}	2.07^{Ba2}	2.08^{Ba2}	2.19^{Ba1}	2.55^{Aa1}	2.39^{Aa1}	2.13^{Ba1}	2.04^{Ba2}	2.27^{Aa1}	2.10^{Aa1}
17/12	2.02^{Ba1}	2.06^{Ba1}	1.81^{Cb2}	1.98^{Ba1}	1.78^{Ba2}	1.97^{Ba1}	1.90^{Ca2}	2.02^{Ba1}	1.99^{Ba1}	1.70^{Ba2}
A/E (1º)	19.6/9.3	18.35/7.4	21.6/8.7	17.8/7.1	16.1/6.8	17.0/7.4	20.2/6.6	16.2/7.7	25.2/7.5	14.6/5.8
A/E (2º)	18.1/8.5	17.1/7.6	16.2/7.7	15.2/6.8	16.6/6.3	14.3/6.4	15.7/6.1	14.8/6.6	15.2/7.9	15.1/6.4
A/E (3º)	17.1/7.6	14.9/7.5	13.9/8.2	14.8/8.4	16.6/9.1	11.64/7.2	16.6/7.8	14.1/7.5	14.2/6.8	10.2/4.6

Means between planting times, considering the same cultivar and dose (ml / hectare), followed by the same capital letter in a column, do not differ from each other by the Scott-Knott test with 5% of probability; Means between cultivars, considering the same planting time and dose, followed by the same lowercase letter in line, do not differ from each other by the Scott-Knott test with 5% of probability. Means between doses, considering the same cultivar and the same planting time, followed by the same number, do not differ from each other by the Scott-Knott test with 5% of probability. A/E– statement of photosynthesis rates and transpiration, considering cultivars, doses and planting times.

deficit and higher temperatures occurred during flowering and grain filling periods (Figure 1). This differential result for the USA in relation to the seasons and cultivars demonstrates the efficiency of *A. brasiliense* in adverse conditions. This suggests its use in crops in the Cerrado as a palliative to the damages caused to the production due to drought.

In plants subjected to hydric deficit and high temperatures during the reproductive stages, some physiological change such as stomatal closure was observed in this study. In addition, we also observed a reduction in atmospheric CO_2 availability at the carboxylation site, promoting reduction in photosynthetic activity and transpiration (Flexas et al., 2008; Hatfield et al., 2011; Ku et al., 2013). This resulted in winding and falling of leaves, flowers and pods abortion, leading to low productivity (Hatfield et al., 2011; Ku et al., 2013).

Studies have shown that reduction in stomatal conductance under adverse conditions may restrict CO_2 fixating rate, decrease the concentration in the substomata cavity and intercellular spaces, which would result in low photosynthetic rate (Soares et al., 2013; Lauteri et al., 2014). The photosynthetic rate reduced in this work. This confirms the above in relation to the cultures of the second and third seasons, due to drought periods that cause the decrease of the US values when comparing the three growing seasons.

According to Serraj and Sinclair (2002), WUE for water stress (dry) may be associated with osmotic adjustment. It contributes to water absorption and cellular turgor maintenance in a way that physiological processes, such as photosynthesis and cell enlargement, are not interrupted. In plants subjected to different water level availability on soil, it was found that WUE index increased, resulting in stomata closure as plants responded to hydric stress, seeking to reduce transpiration (Mencuccini et al., 2000; Tatagiba et al., 2008; Maes et al., 2009; Roza, 2010). These variations are also important for the maintenance of production in regions that have drought periods during the crop cycle. Examples are crops in the Brazilian Cerrado, that serve as reference in the selection of cultivars that present a better response to drought.

Bulegon et al. (2016) reported that transpiration tends to be lower in non-co-inoculated plants and photosynthesis tends to be stable with the use of *A. brasiliense*, influencing the water use efficiency of soybean. In this work the obtained results present higher values for transpiration and photosynthesis in plants that did not receive *A. brasiliense* as compared to the plants that received different doses of the inoculant (Table 3).

Antony and Singandhupe (2004) studied the irrigation influence on WUE in sweet pepper crops and verified a negative linear relationship with net photosynthesis. In other words, photosynthesis rate increases are associated with a WUE reduction, due to the stomatal opening. Hungria, (2011) reported increase in plant tolerance to abiotic stresses due to the production of phytohomones when they were associated with *Azospirillum* bacteria genera. Rodrigues and Fioreze (2015) also reported similar effects with further discovery that these bacteria identify signs emitted by plants under stressful conditions; they trigger responses along with plant, resulting in increased tolerance to different stresses.

Different authors did not obtain an increase in productivity of beans and soybeans using *A. brasiliense* (Gitti et al., 2012; Bassani et al., 2015; Zuffos et al., 2016). Despite this, an increase in yield has been observed when the plants were associated with *A. brasiliense* as N source for soybean crop (Hungria, 2011), and when corn plantations were associated with *A. brasiliense* together with manure as a nitrogen source (Muller et al., 2016). The comparative study including planting time, different doses and within the same cultivar revealed a higher WUE value at the first planting time ($11/18^{th}$), for both cultivars and all *A. brasiliense* doses; yet, do not differ significantly from the second planting time (12/02nd) in the 200 and 400 ml doses of the commercial product ha^{-1}. However, smaller WUE value was obtained at the third planting time (12/17th).

Under water conditions adequate for the development of the plants such as those found in the first planting season (Figure 1), the WUE values were derived from a greater increase in the liquid photosynthetic rate (A) in relation to the transpiration (E) due to the availability of water for the plants (Table 3). On the other hand, in the second and third planting seasons, where the availability of water to the plants was less favorable to their development, lower WUE values were obtained as a result of a greater decrease of A in relation to E. The variation between the values found from A and E demonstrates the plants' response to water stress caused by periods of drought during the plants' development. Considering that the ratio between these parameters indicates the WUE, the decrease of A and increase of E to the detriment of the water conditions of the plant cause the WUE to be smaller in the second and third planting seasons.

The smallest WUE values in the second (12/02nd) and third (12/17th) planting times, of all different *A. brasiliense* doses, occurred probably as a result of a greater abscisic acid (ABA) accumulation in the crops planted in December as compared to the first planting time (11/18th). This is due to ABA synthesis produced by the plant itself (promoted by the unfavorable environments) and also, to the *Azospirillum* ABA synthesis, which promoted greater reduction in the photosynthetic activity (A) to the detriment of the transpiration (E).

Under hydric stress, plants produce substances like proline, which is regulated by abscisic acid (Sharma and Verslues, 2010) and inorganic ions. These stabilize membrane proteins and lipids structure, preserving enzymes functions while making the water adhere to these molecules structure in dehydrated cells (Cohen et al, 2009; Sharma and Verslues, 2010; Khan et al., 2013; Cohen et al., 2015).

Cohen et al. (2015) evaluated the response of *Arabidopsis thaliana* plants associated with *A. brasiliense* under drought conditions and found that abscisic acid (ABA) produced by the bacterium increased the root system and stomatal closure due to proline synthesis. Besides, the bacteria provided three times more ABA to the inoculated plants than the control plants under similar experimental conditions.

Conclusion

The cultivars show the same behavior in relation to the WUE, showing that they have the same mechanism of response to water stress. The action of *A. brasiliense* on the WUE becomes perceptible under conditions of water stress for the plant due to the effect of the hormones available to the plant that responds to water stress. The doses of inoculant containing *A. brasiliense* present different results due to the bacterial concentration applied in the plant. With the use of *A. brasiliense* there is a greater reduction in the A and E values under water stress as compared to the absence of the bacterium being recommended to crops in places with periods of drought.

In the selection of cultivars that present the best WUE indices, the crop cycle must also be observed. Inoculation with *A. brasiliense* did not promote significant changes in the WUE in crops under favorable conditions. The concept of WUE can be used in genetic improvement through the selection of cultivars that present better responses to the dry period, aiming at maintaining production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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