Original Research Article

Photosynthetic and Production of *Urochloa ruziziensis* Inoculated With *Azospirillum brasilense* Under Drought

ABSTRACT

Aims: Present study had as objective to evaluate the photosynthetic and production of *Urochloa ruziziensis* when inoculated with *Azospirillum brasilense* in the presence and absence of drought.

Study design: Randomized block design and factorial 2x2.

Methodology: The first factor is the presence or absence of seed inoculation with *A. brasilense* strains AbV5 + AbV6; the second factor is the presence or absence of drought. The evaluations consisted of relative water content (RWC), soil gravimetric moisture, net assimilation rate of CO_2 response in function of active photon flux density, apparent quantum efficiency, light compensation point, absolute integrity of membrane, damage to membranes, dry mass aborted leaves and total dry mass of aerial part.

Results: Results demonstrate that plants maintained in drought present a reduction in all evaluated variables. Under conditions of drought the use of *A. brasilense* promoted smaller variations in RWC, net assimilation rate of CO_2, apparent quantum efficiency, light compensation point, absolute integrity of membrane, damage to membranes, no variations were observed for dry mass aborted leaves and total dry mass of aerial part.

Conclusion: The inoculation of the seeds of *U. ruziziensis* with *A. brasilense* mitigates drought damage in plant physiology, but it is not able to mitigate leaf losses and plant productivity.

Keywords: gas exchange, plant growth promoting bacterium, membrane damage, dry mass

Comment [A1]: Minimum key words should be five.

1. INTRODUCTION

Water is a resource essential for plant development, the abiotic factor that restricts crop productivity. Drought conditions are characterized by periods when plants can't replace the water lost to the atmosphere [1], resulting in lower turgor, and deficit in biochemical and physiology functions, such as photosynthesis [2].

Perennial crops are most affected by drought over the years. Grassland, besides being cultivated perennially, presents the aggravation of being grown on degraded soils with low fertility and without mineral supplementation [3]. Among the various genres of pasture, *Urochloa* is distinguished by high dry matter production [4], satisfactory levels of protein and fiber, and high adaptability to various soils and climates. Among the climatic factors, the drought demands quickly and momentary adaptation by plants [5].

Genetic improvement is the main tool used for expression of drought tolerance, however it is a slow and expensive process, restricting the higher value-added crops such as corn and soybeans. However, reports indicate that management practices may increase the tolerance of plants to drought, stimulating soil water maintenance [6], higher land capacity use [7], and better nutrition and hormonal balance of plants [8].

Based on the above, the use of growth promoting bacteria is widely studied, mainly the genus *Azospirillum*, which stimulates the action of active mechanisms in tolerance to drought [9,10]. Inoculation with *A. brasilense* promotes increases in the plant root system [11], production of dry matter [12], crop production [11], gas exchanges [13] and hormone regulation. Recent literary indicate that this bacterial species also maximizes plants ability to tolerate saline environments [14].

Another aggravating point in drought conditions is the high incident light, leading to increased leaf temperature accompanied by stomatal closure, causing degradation of chlorophyll and reducing the saturation point of photosynthetic activity [15].

Thus, the present study had as objective to evaluate the photosynthetic and production of *Urochloa ruziziensis* when inoculated with *Azospirillum brasilense* in the presence and absence of drought.

2. MATERIAL AND METHODS

The experiment was conducted in a greenhouse in pots with a nominal capacity of 8.7 L, using as soil substrate from horizon A Hapludox eutrophic soil, under a randomized block design and factorial 2x2 with five replications. The first factor is the presence or absence of seed inoculation with *A. brasilense* strains AbV5 + AbV6; the second factor is the presence or absence of drought.

The inoculation was performed with 1 mL inoculum ($2x10^8$ CFU mL^{-1}) to 1000 seeds of *U. ruziziensis*, or $2x10^5$ CFU per seed. The seeds were homogenized by agitating with the inoculant, kept in the shade for 30 minutes and sowing. After they emerged, two plants *U. ruziziensis* were kept in each pot, with daily replacement of water until field capacity. The plants were constantly monitored to ensure adequate development, and that mineral supplementation was unnecessary.

After 45 days sowing, drought was started. Therefore, all pots were previously irrigated to field capacity, and the treatments with drought had their irrigation suspended. Drought was maintained until the inhibition of photosynthetic activity at least one treatment, that occurred on the sixth day after the beginning of drought.

The relative water content (RWC) and gravimetric soil moisture were evaluated. Known leaf area segments were collected, and the RWC was determined by difference from the wet mass, full content of water mass after 6 hours at 25 ° C, and dry mass after 48 hours at 65 °C [16]. Substrate samples were collected, and gravimetric soil moisture was determined by difference from the mass at the time and the dry mass after 24 hours at 105 ºC.

The net assimilation rate of CO_2 (*A*) as a function of the light level was determined using an IRGA (Infra-Red Gas Analyzer) model LI-6400XT (Licor Inc. Lincoln, NE). The readings were performed in the morning using a concentration of CO_2 of 380 μmol mol^{-1}, a flow rate in the chamber of 500 μmol s^{-1}, and a block temperature of 25 °C. Evaluations were performed on fully developed leaves, photosynthetically active and with no injuries, located in the middle canopy, which were chosen randomly before drought imposition and marked, so that the same leaves would always be evaluated, at the following photosynthetic photon flux density values (PPFD – Q) 0; 20; 40; 80; 150; 300; 400; 700; 900; 1200; 1600; 2000 and 2400 μmol $m^{-2} s^{-1}$, using the variable net CO_2 assimilation rate.

To calculate the apparent quantum efficiency (Φ [μmol CO_2/μmol photons]) the following concentrations were used: 0; 20; 40; 80 and 150μmol $m^{-2} s^{-1}$ photon adjusting the equation ($A = a + \Phi Q$) where *a* and Φ are coefficients, and Q represents the PPFD, where Φ is the inverse of the angular coefficient of the line. At the intersection of the line in the X-axis, the value of the light compensation point [Γ (μmol $m^{-2} s^{-1}$)] was established. The response curve of *A* as a function of PPFD was adjusted by the rectangular hyperbolic function ($A = Amax Q/\text{“}a\text{”} + Q$), where *Amax* is the maximum assimilation rate of CO_2, *"a"* is an adjustment coefficient of the equation, and Q represents the PPFD.

To determine the percentage of absolute integrity of membrane and damage to membranes. For this leaf segments of 1.5 cm² is collection and washed with water deionized, after leaf segments were immersed in 50 mL of distilled and deionized water, and then conditioned in B.O.D. at 25 ° C for 24 hours, and determined the electric conductivity. After were sealed with aluminum foil and taken to a water bath at 100 ° C for 1 hour, allowed to cool at room temperature to 25 ° C, a new electrical conductivity reading [17].

The dry mass evaluation of aborted leaves and total dry mass of aerial part of plants were determined at the end of the evaluations on the sixth day being the dry mass of leaves aborted determined by the collection of all the leaves that presented less than 30% of green area located in the middle and lower third of the plants, in the end the rest of the part of the plants were collected to determine the total dry mass of aerial part. The samples were oven dried in forced air circulation at 65 ° C for 72 hours.

The RWC, soil moisture, absolute membrane integrity, membrane damage, dry mass of aborted leaves and total dry mass of aerial part were submitted to analysis of variance by the F test at 5% probability and when a significant difference was found, the data were compared using the Tukey test at 5% probability.

3. RESULTS AND DISCUSSION

This result showed an interaction between inoculation and drought for relative water content (RWC) and soil moisture. For the RWC the presence of drought led to a reduction of 39.9%, while the application of *A. brasilense* increased the RWC by 8.9%. For the RWC the presence of drought led to a reduction of 39.9%, while the application of *A. brasilense* increased the RWC by 8.9%. Soil moisture was reduced in treatments with drought in 56%, and among the treatments not observed significant differences (Table 1).

Table 1. Relative leaf water content (RWC) and soil moisture with *Urochloa ruziziensis* inoculated with *Azospirillum brasilense* in the presence or absence of drought

	RWC (%)			Soil moisture (%)		
	With Drought	Without Drought	Means	With Drought	Without Drought	Means
Control	49.48 bB	92.58 aA	71.03 b	15.00 aB	36.52 aA	25.76 a
A. brasilense	62.37 aB	93.58 aA	77.98 a	17.10 aB	36.42 aA	26.78 a
Means	55.92 B	93.08 A		16.05 B	36.47 A	
C.V. (%)		7.20			7.58	

**Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Tukey test 5% probability.*

The present result demonstrates that the plants used the water available in the vessels in a similar way, thus, the variations obtained for the RWC are possibly linked to the better utilization of the water contained in the foliar tissue. These results can be explained as reported in the literature where plants inoculated with *A. brasilense* use the water contained in the foliar tissues better, in order to remain hydrated for a longer period [13,18,19], the qualifying as an alternative treatment to increase the tolerance of plants to drought, in relation the physiological variations. This condition is reported in the literature as a signaling effect as a result of inoculation with plant growth promoting bacteria, occurring via stimulation of production and release compounds that signal the lack of water among these compounds are distinguished plant hormones, particularly auxin and abscisic acid, and also compounds such osmolytes betaines, proline and aminoacid [20].

Although these effects are not completely elucidated several papers have been reporting such condition. In corn plants, inoculation of *A. lipoferium* increased the concentration of aminoacids, proteins, proline and sugars, and the use of plant growth promoting bacteria showed promising effects and may help the crop tolerate the lack of water [21].

Thus, as the plants inoculated with *A. brasilense* presented higher RWC in comparison with the control plants, this condition allowed these plants to maintain higher values of net assimilation rate CO_2 ('*A*') when compared to the control. The results of ('A') obtained in the dry control did not adjust to the proposed response curve, presenting an average net assimilation rate CO_2 of -0.035 μmol m^{-2} s^{-1}, that is, the plants were consuming photoassimilates to survive the condition of drought, being that the net assimilatory rate CO_2 (*Amax*) obtained in the different luminosities arrived a 0.21 μmol m^{-2} s^{-1} (Figure 1a). In turn, when used to inoculation with *A. brasilense* plants obtained with greater values *Amax* 4.57 μmol m^{-2} s^{-1} (Figure 1b), that is, the plant, although it shows a reduction in the net assimilation rate of CO_2 when compared to the irrigated treatments that presented for the control value of 27.36 μmol m^{-2} s^{-1} (Figure 1c) and for the inoculation of seeds value of 26.14 μmol m^{-2} s^{-1} (Figure 1d), it was possible to inoculate plants with *A. brasilense* under conditions of drought maintaining greater photosynthetic activity.

In drought conditions photosynthesis becomes limited due to the need to break the water molecule to electronic excitation, releasing electrons which are used as initial acceptors in the production of ATP [1]. Another condition that leads to the reduction of *Amax* is associated to the limitation of CO_2 in the substomatal chamber caused by stomatal closure. Therefore, plants with higher RCW can maintain greater diffusion of CO_2 [22].

The high photosynthetic rates obtained for the irrigated plants are related to the photosynthetic efficiency of *U. ruziziensis*, being a C_4 with high photosynthetic capacity and very responsive to the elevation of luminosity when under adequate conditions.

Obviously, plants grown under water limitation conditions reduce the photosynthetic rate, thus the use of inoculation of seeds with *A. brasilense* results in mitigation of the effect of drought, that control plants had a reduction of A_{max} in 99.23% and 99.18% compared to irrigated control and irrigated seed inoculation, respectively. In turn, the inoculation of the seeds in water deficit decreased by A_{max} in 83.3% and 82.5% compared to irrigated control and irrigated seed inoculation, respectively. When comparing the treatments kept in drought control presented A_{max} 95.4% less than seed inoculation with *A. brasilense*.

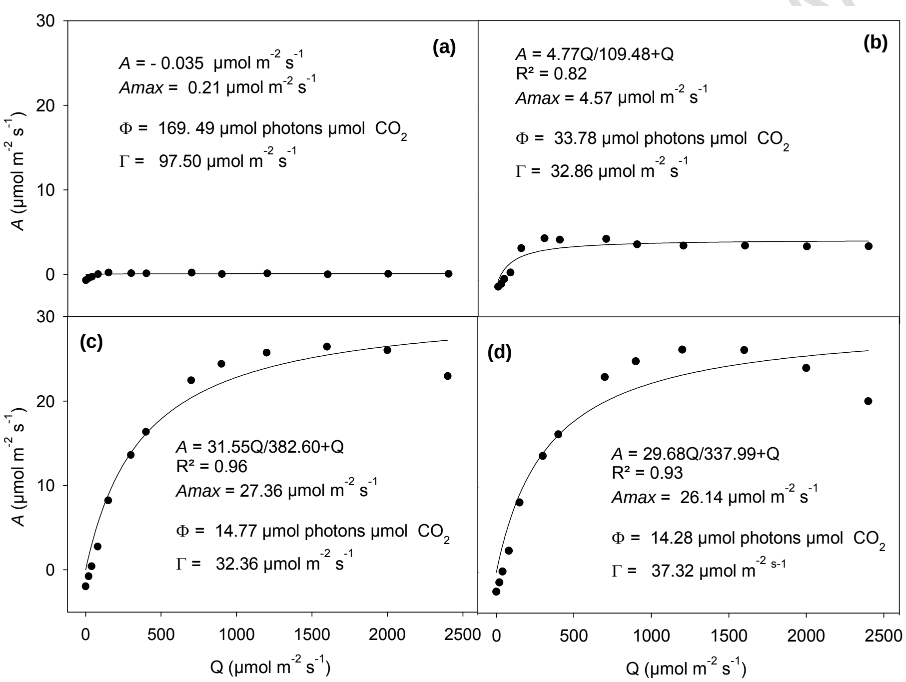

Fig. 1. Net assimilation rate of CO_2 *Urochloa ruziziensis* inoculated with *Azospirillum brasilense* in the presence or absence of drought. (a) control with drought; (b) seed inoculation *A. brasilense* with drought; (c) control without drought; (d) seed inoculation *A. brasilense* without drought.

Due to the changes promoted in 'A' the apparent quantum efficiency (Φ) in dry control was elevated 169.49 μmol photons μmol CO_2, while in seed inoculation this value was 37.78 μmol photons μmol CO_2, that is, a mitigation of 77.7%. When comparing Φ of the plants with and without drought, it was observed that the dry control had an elevation of 1147% and 1186% compared to irrigated control and irrigated seed inoculation, respectively, while the use of seed inoculation elevation 228.7% and 236.5% compared to irrigated control and irrigated seed inoculation, respectively.

The elevation of Φ in plants evidences that the photosynthetic apparatus is disordered, so that the efficiency in the use of ATP and NADPH in the Calvin cycle is impaired. Thus, [23], reports that the elevation of Φ represents also a deficiency in the use of light energy in the photochemical reactions of photosynthesis. The elevation of Φ is consequence of the limitation of CO_2 by stomatal closure [24], resulting in a lower efficiency in fixing the CO_2 with greater light energy expenditure to fix a molecule of CO_2, in result of the drought increase the photorespiration.

The evaluation of the light compensation point (Γ) confirms the demonstrated in '*A*', which indicated that dry control plants were consuming stored photoassimilates, thus [25] reported that the increase of Γ indicates plant respiration, that is, to overcome dry conditions of drought, control plants and seed inoculation with *A. brasilense* needed to use the accumulated photoassimilates, which explains why they did not differ in dry mass production per plant (Table 3). Thus, dry control plants showed Γ of 97,50 µmol m^{-2} s^{-1}, while those plants inoculated with *A. brasilense* had values similar to those of plants irrigated de 32.86 µmol m^{-2} s^{-1}, that is, reduction of 66.3%.

Comment [A2]: photo assimilates

Comment [A3]: photo assimilates

The occurrence of water deficiency associated with the reduction of the net assimilation rate of CO_2 leads to an inadequate functioning of the biochemical and physiological system resulting in the degradation of membranes. Thus, when absolute membrane integrity was assessed (AMI) were observed that the plants inoculated with *A. brasilense* independently of the presence or absence of drought promoted higher values, surpassing the control plants in 3.45%. When observing the treatments kept in water limitation, plants inoculated were higher than the control 6.9% (Table 2).

Similarly, to the AMI, membrane damage has shown that plants maintained in water deficit suffer greater damage than those absent from water limitation in 31%. When observing the treatments in drought, greater damages to the membrane were observed in the control, surpassing the inoculation in 22.8%. The control plants presented lower AMI and greater membrane damage in the presence of water deficiency, while those inoculated with *A. brasilense* did not differ among themselves in the presence or absence of drought.

Table 2. Absolute membrane integrity and membrane damage *Urochloa ruziziensis* inoculated with *Azospirillum brasilense* in the presence or absence of drought

	Absolute membrane integrity (%)			Membrane Damage (%)		
	With Drought	Without Drought	Means	With Drought	Without Drought	Means
Control	0.81 bB	0.86 aA	0.84 b	18.37 aA	13.52 aB	15.95 a
A. brasilense	0.87 aA	0.88 aA	0.87 a	14.96 bA	11.93 aA	13.44 a
Means	0.84 A	0.87 A		16.67 A	12.72 B	
C.V. (%)		3.10			26.29	

**Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Tukey test 5% probability.*

The degradation of membranes in plants occurs among other factors by the formation of free radicals, basically in the form of reactive oxygen species (ROS) which are formed in plants when exposed to stress, in the present study drought [1]. The production of ROS in the plant occurs steadily, and its inactivation, keeping the balance. When the plant is under drought, it loses the capacity of inactivation, due to the deregulation of the enzymatic activity, leading to the ROS degrading the membranes of the cell, with extravasation of the cellular content and consequently the cell death. The variations exposed in this study suggest that the use of *A. brasilense* can positively interfere with membrane inertness. This result is a reflection of the hormonal stimuli, since this characteristic in stressed plants is regulated by the activity of abscisic acid and cytokinin [8,26], cytokinin produced by *A. brasilense* [27,28], as well as abscisic acid [29], resulting in lower membrane degradation.

The variations observed in the physiological system and the damage caused to the membranes of the plants of U. ruziziensis result in responses to morphological levels in the form of abortion of plant organs and consequently reduction in the production of dry mass.

The plants kept in water restriction had a greater leaves abortion, being this value 41.2% superior compared to the irrigated plants. In a similar way the total dry mass of aerial part was superior without drought in 12.2%. When considering the interaction of the factors, no significant differences in the treatments were observed, and it was verified that, regardless of the treatment applied in the presence of drought, the leaves abortion was superior and total dry mass of aerial part was lower (Table 3).

Table 3. Dry mass of aborted leaves and total dry mass of aerial part *Urochloa ruziziensis* inoculated with *Azospirillum brasilense* in the presence or absence of drought

	Dry mass of aborted leaves			Total dry mass of aerial part		
	---------------------------------- (g plant^{-1}) ----------------------------------					
	With Drought	Without Drought	Means	With Drought	Without Drought	Means
Control	1.20 aA	0.81 aB	1.005 a	9.43 aB	10.03 aA	9.73 a
A. brasilense	1.20 aA	0.89 aB	1.045 a	8.71 aB	10.63 aA	9.67 a
Means	1.20 A	0.85 B		9.07 B	10.33 A	
C.V. (%)		18.09			7.98	

**Means followed by the same lowercase letter in the column and upper case in the row do not differ from each other by the Tukey test 5% probability.*

Although inoculationwit *A. brasilense* promoted beneficial effects on RWC, net assimilation rate of CO_2 and cell membrane preservation, such effects were not able to mitigate leaves abortion and reduction in dry plant mass.

Comment [A4]: inoculation with

This condition is possibly due to the aggressiveness of the effects of drought on plant physiology. The incidence of drought leads to a morphological response in the plants that culminate when in advanced stages in abortion of plant organs, this way reduction in the production of dry mass of aerial part and increase in the mass of drought of aborted leaves. In this sense it is demonstrated in a study with forage plants that the incidence of water deficiency leads to a reduction in dry mass productivity due to leaf abortion and lower physiological plant activity, which results in a reduction of green leaf blades [4], condition also observed in the present study.

In this way, the inoculation of the seeds of *U. ruziziensis* is shown as an option to mitigate the damages caused by the occurrence of drought on the physiological system, however, not alleviating the losses in dry mass production of aerial part.

4. CONCLUSION

The inoculation of the seeds of *Urochloa ruziziensis* with *Azospirillum brasilense* raises the relative water content, with smaller reduction in the net assimilation rate of CO_2 and reduction of the cellular membranes damage due to the incidence of water deficit. However, it is not able to reduce the abscission of plant organs and increase the production of dry mass of aerial part.

REFERENCES

1. Taiz L, Zeiger E. Plant Physiology. Porto Alegre: Artemed; 2013. 918 p.
2. Chastain DR, Snider JL, Collins GD, Perry CD, Whitaker J, Byrd SA. Water deficit in field-grown Gossypium hirsutum primarily limits net photosynthesis by decreasing stomatal conductance, increasing photorespiration, and increasing the ratio of dark respiration to gross photosynthesis. J Plant Physiol. 2014; 171 (17): 1576-85.
3. Barcellos AO, Ramos AKB, Vilela L, Martha Junior GB. Sustainability of animal production based on intercropping pastures and use of exclusive legumes, in the form of a protein bank, in the Brazilian tropics. Rev Bras Zootec. 2008; 37 (Special Supplement): 51-67.
4. Mattos JLS, Gomide JA, Huaman CAM. Effect of water deficit on the growth of Brachiaria species in greenhouse. Rev Bras Zootec. 2005; 34 (3): 746-54.
5. Souza FHD, Matta FP, Fávero AP. Construction of grasshopper ideotypes for various uses. 1st ed. Brasilia: Embrapa; 2014. 381 p.
6. Silva RAB, Lima JRDS, Antonino ACD, Gondim PSDS, de Souza ES, Barros G. Water balance in a regolithic soil cultivated with Brachiaria decumbens Stapf. Rev Bras Ciênc Solo. 2014; 38 (1): 147-57.
7. Ferreira EP of B, Knupp AM, Didonet CCGM. Growth of rice cultivars (Oryza sativa L.) influenced by inoculation with plant growth promoting bacteria. Biosci J 2014; 30 (3): 655-65.
8. Fioreze SL, Rodrigues JD, Carneiro JPC, Silva ADA, Lima MB. Physiology and production of soybean treated with kinetin and calcium under water deficit and shading. Pesqui Agropec Bras. 2013; 48 (11): 1432-9. THE
9. Perrig D, Boiero ML, Masciarelli OA, Penna C, Ruiz OA, Cassan FD, Luma MV. Plant-growth-promoting compounds produced by two agronomically important strains of Azospirillum brasilense, and implications for inoculant formulation. Appl Microbiol Biotechno. 2007; 75 (5): 1143-50.
10. Gitti DDC, Arf O, Portugal JR, Corsini DCDC, Rodrigues RAF, Kaneko FH. Plant coverages, nitrogen doses and seed inoculation with Azospirillum brasilense on upland rice in the no - tillage system. Bragantia. 2012; 71 (4): 509-17.
11. Influence of seed treatment on initial development of maize and wheat seedlings inoculated with Azospirillum brasilense. Sci Agrar Parana. 2013; 12 (3): 175-81.
12. Rodrigues LFOS, Guimarães VF, Silva MB, Junior ASP, Klein J, Costa ACPR. Agronomic characteristics of wheat as a function of Azospirillum brasilense, humic acids and nitrogen under greenhouse conditions. Rev Bras Agricultural Eng and Ambient. Campina Grande; 2014; 18 (1): 31-7.
13. Inagaki AM, Guimarães VF, Lana MC, Klein J, Costa ACPR, Rodrigues LFOS, Rampim, L. Maize initial growth with the inoculation of plant growth-promoting bacteria (PGPB) under different soil acidity levels. Aust J Crop Sci. 2015; 9 (4): 271-80.

14. Slama I, M'Rabet R, Ksouri R, Talbi O, Debez A, Abdelly C. Water deficit stress applied only or combined with salinity and physiological parameters and antioxidant capacity in Sesuvium portulacastrum. Flora - Morphol Distrib Funct Ecol Plants. 2015; 213 (1): 69-76.

15. Chavarria G, Durigon MR, Klein VA, Kleber H. Photosynthetic restraint of soybean plants under varying water availability. Ciên. Rural. 2015; 45 (8): 1387-93.

16. Slavik B. Methods of studying plant water relations. 1st ed. Berlin: Springer-Verlag Berlin Heidelberg; 1974. 452 p.

17. Blum A, Ebercon A. Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Science. 1981. p. 43–7.

18. Swarthout D, Harper E, Judd S, Gonthier D, Shyne R, Stowe T, et al. Measures of leaf-level water-use efficiency in drought stressed endophyte infected and non-infected tall fescue grasses. Environ Exp Bot. 2009;66(1):88–93.

19. Bulegon LG, Battistus AG, Guimarães VF, Inagaki AM, Offemann LC, Souza AKP, Oliveira PSR. Physiological responses of Urochloa ruziziensis inoculated with Azospirillum brasilense to severe drought and rehydration conditions. Aust J Crop Sci. 2017;11(10):1283–9.

20. 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.

21. Bano Q, Ilyas N, Bano A, Zafar N, Akram A, Hassan FUL. Effect of Azospirillum inoculation on maize (Zea mays L.) under drought stress. Pakistan J Bot. 2013;45(1):13–20.

22. Chaves MM, Oliveira MM. Mechanisms underlying plant resilience to water deficits: prospects for water-saving agriculture. J Exp Bot. 2004; 55 (407): 2365-84.

23. Ghannoum O. C4 photosynthesis and water stress. Ann Bot. 2009; 103 (1): 635-44.

24. Machado DFSP, Andrade Lagôa AMM, Ribeiro RV, Ribeiro Marchiori PE, Machado RS, Machado EC. Low nocturnal temperature and water deficit in the photosynthesis of sugarcane. Pesqui Agropec Bras. 2013; 48 (5): 487-95.

25. Larcher W. Physiological plant ecology. 4th ed. Berlin: Springer-Verlag Berlin Heidelberg; 2003. 514 p.

26. Rivero RM, Kojima M, Gepstein A, Sakakibara H, Mittler R, Gepstein S, et al. Delayed leaf senescence induces extreme drought tolerance in a flowering plant. Proc Natl Acad Sci. 2007; 104 (49): 19631-6.

27. Kuss AV, Kuss VV, Lovato T, Flôres ML. Nitrogen fixation and indoleacetic acid production in vitro by endophytic diazotrophic bacteria. Pesqui Agropec Bras. 2007; 42 (10): 1459-65.

28. Andreazza R, Kuss A V, Antoniolli ZI, Jacques RJS, Santos ML, König F, et al. Diazotrophic bacteria and vermicompost as alternative sources of n for irrigated rice. Science and Nat. 2013; 35 (2): 15-23.

29. Cohen AC, Bottini R, Piccoli PN. Azospirillum brasilense Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. Plant Growth Regul. 2008;54(2):97–103.