UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

INOCULAÇÃO DE BACTÉRIAS PROMOTORAS DO CRESCIMENTO DE PLANTAS EM *Brachiaria Brizantha* cv. BRS Paiaguás E *Brachiaria ruziziensis* EM DÉFICIT HÍDRICO

Autor: Divaney Mamédio dos Santos
Orientador: Prof. Dr. Ulysses Cecato

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Estado do Paraná
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Autor: Divaney Mamédio dos Santos
Orientador: Prof. Dr. Ulysses Cecato

TITULAÇÃO: Doutor em Zootecnia - Área de Concentração Produção Animal

APROVADO em 25 de março de 2020.

Prof. Dr.Cecilio Viega Soares Filho

Profª Drª Sandra Galbeiro

Prof. Dr. Ossival Lolato Ribeiro

Drª Mariangela Hungria

Prof. Dr. Ulysses Cecato
Orientador

“Se eu pudesse deixar algum presente,

deixaria o acesso ao sentimento de amor à vida dos seres humanos.

A consciência de aprender tudo o que nos foi ensinado pelo tempo afora,

lembraria dos erros que foram cometidos,

com os sinais para que não mais se repetissem.

A capacidade de escolher novos rumos,

deixaria para você se pudesse,

o respeito à aquilo que é indispensável:

Além do pão, o trabalho, a ação.

E quando tudo mais faltasse para você,

eu deixaria se pudesse, um segredo:

o de buscar no interior de si mesmo

o respeito e a força interior para encontrar a saída.”

(Mahatma Ghandi)

Aos meus pais,

Cecília Santana Mamédio dos Santos e **Humberto Mamédio dos Santos**

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Com muito carinho

DEDICO

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BIOGRAFIA

DIVANEY MAMÉDIO DOS SANTOS, filho de Humberto Mamédio dos Santos e Cecília Santana Mamédio dos Santos, nasceu em 05 de outubro de 1989, em Gandu, Bahia.

Em dezembro de 2008, concluiu o curso Técnico em Agropecuária na Escola Média de Agricultura Regional da CEPLAC (EMARC) no campus de Valença-BA.

Em janeiro de 2014, concluiu o curso de Zootecnia na Universidade Federal do Recôncavo da Bahia.

Em agosto de 2015, matriculou-se no Programa de Pós-Graduação em Ciência Animal, em nível de Mestrado, área de concentração Nutrição e Alimentação Animal, na Universidade Federal do Recôncavo da Bahia.

No dia 10 de fevereiro de 2017, submeteu-se à banca para defesa da Dissertação intitulada ‘‘Influência do método de plantio e espaçamento na implantação de pasto de *Cynodon* com *Arachis*’’, tendo sido APROVADO.

Em março de 2017, matriculou-se no Programa de Pós-Graduação em Zootecnia, em nível de Doutorado, área de concentração Produção Animal, na Universidade Estadual de Maringá, realizando estudos na área de Produção e Manejo de Plantas Forrageiras, sob orientação da Professor Dr. Ulysses Cecato.

No dia 04 de outubro de 2019, foi aprovado no Exame Geral de Qualificação.

No dia 25 de março de 2020, submeteu-se à Defesa de Tese junto ao Programa de Pós-graduação em Zootecnia da Universidade Estadual de Maringá.

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RESUMO

O uso de bactérias promotoras do crescimento de plantas (BPCP) em pastagens é uma alternativa sustentável ao meio de produção e pode incrementar a produção de massa de forragem, além de ser uma tecnologia inovadora capaz de mitigar os efeitos da seca. O objetivo geral deste estudo foi avaliar o efeito da inoculação de sementes com BPCP sobre os parâmetros morfológicos e fisiológicos da *Urochloa brizantha* cv. BRS Paiaguás e *Urochloa ruziziensis* em déficit hídrico. O experimento foi desenvolvido em estufa agrícola da Universidade Estadual de Maringá, Maringá-PR, Brasil, em dois ciclos experimentais: (ensaio I - novembro de 2017 a julho 2018 e ensaio II - setembro de 2018 a maio de 2019). As bactérias inoculadas foram *Azospirillum brasilense* Ab-V5, *Pantoea ananatis* AMG 521 e *Pseudomonas fluorescens* CCTB 03, mais o tratamento controle (não inoculada) e quatro níveis de déficit hídrico (80, 60, 40 e 20%). O delineamento experimental foi em blocos casualizados, em esquema fatorial 4x4, com quatro repetições, totalizando 128 vasos (12 dm³ de capacidade) em cada ensaio. Foi realizada a correção da acidez do solo com elevação da saturação de bases para 55%, com incorporação de calcário dolomítico. Os vasos receberam adubações fosfatada e potássica, segundo análise de solo, e o equivalente a 20 kg de N ha^{-1}. Os níveis de déficit hídrico foram determinados fazendo uso dos valores de água disponível, encontrados a partir dos valores de capacidade de campo e do ponto de murcha permanente dos solos dos vasos. As sementes das gramíneas foram inoculadas na concentração final de 10^8 células mL^{-1}. Foram misturados 15 mL de inóculo para cada quilo de sementes. Foram semeadas cerca de 16 sementes por vaso. Aos 21 dias após a semeadura, foi realizado o primeiro desbaste, deixando-se oito plantas mais vigorosas por vaso, e o segundo desbaste com 30 dias após a semeadura, deixando cinco plantas. Aos 35 dias após a semeadura, foi realizado o corte de uniformização das plantas a 15 cm de altura de resíduo. Durante este período a umidade do solo foi mantida próxima da capacidade de campo e, somente após, foi feita a imposição do déficit hídrico. O nível de déficit hídrico em cada tratamento foi mantido por meio da pesagem dos vasos com balança digital, em intervalos de dois dias. Os cortes de massa realizados ao logo do período experimental, ocorreram quando as plantas atingiam entre 35 e 40 cm de altura, com 15 cm de altura de resíduo. O objetivo do **Artigo I** foi avaliar o efeito da inoculação com bactérias promotoras do crescimento de plantas sobre as características morfogênicas e estruturais da *Urochloa brizantha* cv. BRS Paiaguás e *Urochloa ruziziensis* submetidos ao estresse por seca. As bactérias inoculadas foram *Azospirillum brasilense* Ab-V5, *Pantoea ananatis* AMG 521 e *Pseudomonas fluorescens* CCTB 03, mais o tratamento controle (sem bactéria) e quatro níveis de déficit hídrico (80, 60, 40 e 20%). O delineamento experimental foi em blocos casualizados, em esquema fatorial 4x4, com quatro repetições, totalizando 128 vasos preenchidos com 9 dm³ de solo em cada ensaio experimental. A morfogênese foi realizada entre os meses de fevereiro e maio de 2018 e 2019, correspondendo às estações de verão e outono. Não houve efeito de interação entre os fatores estudados. Para o capim Paiaguás (verão) as inoculações das estirpes Ab-V5 e AMG 521 proporcionaram aumento no aparecimento foliar. A redução do déficit hídrico diminuiu o alongamento de colmo e senescência foliar, e aumentou de aparecimento foliar. No outono, a estirpe CCTB 03 e tratamento sem bactérias ocasionou a diminuição da senescência foliar, e o menor déficit proporcionou maior alongamento de folha e colmo e número de folhas vivas, e diminuiu a senescência foliar. Para o capim Ruziziensis (verão), o tratamento com a estirpe CCTB 03 e sem bactérias promoveu aumento na senescência foliar. No outono, a estirpe AMG 521 proporcionou maior filocrono. Nesta estação não foi observado efeito do déficit hídrico. As estirpes foram mais eficientes em contribuir para a menor taxa de senescência foliar nas gramíneas. A imposição de maiores níveis de déficit hídrico contribuiu para a redução do aparecimento de novo tecidos e aumento da senescência foliar. No **Artigo II** o objetivo foi avaliar o efeito da inoculação de bactérias promotoras do crescimento de plantas (BPCP) sobre as respostas fisiológicas da *Urochloa brizantha* cv. BRS Paiaguás e *Urochloa ruziziensis* submetidas ao estresse por seca. As bactérias inoculadas foram *Azospirillum brasilense* Ab-V5, *Pseudomonas fluorescens* CCTB03 e *Pantoea ananatis* AMG521, mais o tratamento controle (sem bactéria) e quatro níveis de déficit hídrico (80, 60, 40 e 20%). O delineamento experimental foi em blocos casualizados, em esquema fatorial 4x4, com quatro repetições, totalizando 128 vasos preenchidos com 9 dm³ de solo em cada ensaio experimental. A inoculação das BPCP demonstrou efeito sobre a digestibilidade *in vitro* da matéria seca e índice de SPAD dos capins Paiaguás e Ruziziensis, sendo as respostas semelhantes ao tratamento sem bactérias. A maior imposição de déficit hídrico (80%) revelou influência sobre a gramíneas, com redução dos teores de matéria seca, fibra em detergente neutro e ácido e lignina, e aumento da proteína bruta, digestibilidade *in vitro* da matéria seca, acúmulo de nitrogênio total, carboidratos solúveis e índice de SPAD. As BPCP não foram eficientes em proporcionar a melhoria dos parâmetros fisiológicos dos capins Paiaguás e Ruziziensis em condição de estresse por seca.

Palavras-chave: *Azospirillum*, déficit hídrico, estresse por seca, *Pantoea ananatis, Pseudomonas fluorescens,* bactéria

ABSTRACT

The use of plant growth promoting bacteria (PGPB) in pastures is a sustainable alternative to increment forage production. Besides, it is an innovative technology that can mitigate the effects of water deficit. The aim of this study was to evaluate the effect of seed inoculation with PGPB on the morphological and physiological parameters of *Urochloa brizantha* cv BRS Paiaguás and *Urochloa ruziziensis* under water deficit. The experiment was performed in a greenhouse at the State University of Maringá, Maringá-PR, Brazil, in two experimental cycles (Test I - November 2017 to July 2018, and Test II - September 2018 to May 2019). The inoculated bacteria strains were *Azospirillum brasilense* Ab-V5, *Pantoea ananatis* AMG 521 and *Pseudomonas fluorescens* CCTB 03, plus non-inoculated control and four water deficit levels (80, 60, 40 and 20%). The experiment was performed in a complete randomized block design, in a 4x4 factorial scheme with four replicates, totaling 128 pots (12 dm³ capacity) in each test. Soil acidity was corrected by increasing the base saturation to 55% by incorporating dolomitic limestone. All pots received phosphate and potassium fertilizations according to soil analysis and the equivalent of 20 kg N ha^{-1}. Water deficit levels were determined using the available water values, found from the values of field capacity and the permanent wilting point of the soil pots. Grass seeds were inoculated at a final concentration of 10^8 cells mL^{-1}. 15 mL of inoculum were mixed per kilogram of seed. About 16 seeds were sown per pot. On the 21st day after sowing, the first thinning was carried out, leaving eight most vigorous plants per pot, and the second thinning at 30 days after sowing, leaving five plants. At 35 days after sowing, the uniformity cut plants was performed at 15 cm of residue. During this period, soil moisture remained close to field capacity, only afterwards, the water deficit was imposed. The level of water deficit in each treatment was maintained by weighing the pots with digital scale at intervals of two days. Forage cuts made during the experimental period occurred when the plants reached 35 to 40 cm, with 15 cm of residue. The aim of **Article I**, was to evaluate the effects of inoculation with plant growth promoting bacteria (PGPB) on the morphogenic and structural characteristics of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* under drought stress. The inoculated bacteria strains were *Azospirillum brasilense* Ab-V5, *Pantoea ananantis* AMG 521 and *Pseudomonas fluorescens* CCTB 03, in addition to the non-inoculated control, all submitted to four water deficit levels (80, 60, 40 and 20%). The experimental design was a completely randomized block design, in 4x4 factorial scheme with four replicates, totaling 128 pots filled with 9 dm³ of soil. Morphogenesis was evaluated during summer and autumn seasons (February to May of 2018/2019). No interaction effects between the factors were observed. For Paiaguás grass (summer), the inoculation with the

Ab-V5 and AMG 521 strains increased leaf appearance rate. Reducing the water deficit decreased stem elongation and leaf senescence, and increased leaf appearance in Paiaguás grass. In autumn, the CCTB 03 strain and the non-inoculated control resulted in decreased leaf senescence, and the smallest water deficit provided greater leaf and stem elongation and the number of live leaves, and decreased leaf senescence. For Ruziziensis grass (summer), inoculation with strain CCTB03 and non-inoculated control, increased leaf senescence. In autumn, strain AMG521 provided higher phyllochron. In the summer season, there was no drought effect. PGPB, in general, were not efficient to improve the morphogenesis of Paiaguás and Ruziziensis grasses. The imposition of higher water deficit levels reduced the appearance of new tissues and increased leaf senescence rate. In **Article II**, the aim was to evaluate the effect of the inoculation of plant-growth promoting bacteria (PGPB) on the physiological responses of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* under drought stress. The inoculated bacteria strains were *Azospirillum brasilense* Ab-V5, *Pantoea ananantis* AMG 521 and *Pseudomonas fluorescens* CCTB 03, in addition to the non-inoculated control, all submitted to four water deficit levels (80, 60, 40 and 20%). Experimental delineation was done in random blocks, in 4x4 factorial scheme, with four repetitions, totaling 128 vases filled with 9 dm^3 of soil in each experimental trial. The inoculation of PGPB had an effect on the *in vitro* digestibility of the dry matter and the SPAD index of Paiaguás and Ruziziensis grasses, with responses that were similar to those of the control treatment. The greatest imposition of water deficit (80%) revealed influence over the grasses, with a reduction in the levels of dry mass, neutral and acid detergent fiber and lignin, and an increase in crude protein, *in vitro* digestibility of the dry matter, accumulation of total nitrogen, soluble carbohydrates and the SPAD index. The PGPB were not efficient in promoting improvement of the physiological parameters of Paiaguás and Ruziziensis grasses under drought stress.

Keywords: *Azospirillum*, drought stress, *Pantoea ananatis*, *Pseudomonas fluorescens*, rhizobacteria, water deficit

I - INTRODUCTION[1]

Plants' growth and development in agriculture are influenced by some environmental stresses and, depending on their severity and incidence, production systems may be seriously restricted, which leads to a poor performance.

Water stress, be it due to lack or excess of water, is one of the main stressor agents responsible for a negative impact on agricultural production (Dar et al., 2018). It causes hormonal imbalance, followed by physiological disorders with consequent senescence, abscission of parts of the plant's organs and increased susceptibility to diseases (Nadeem et al., 2010).

Water deficit (WD) can modify the operation and morphology of plants. It can even cause irreversible alterations (Staniak & Kocoń, 2015) in case of long and high-intensity exposure that exceeds the plant's predetermined genetic resistance, which can, in extreme cases, cause its death (Chaves & Oliveira, 2004).

Moderate WD reduces the growth and speed of foliar cell division due to the decrease in water content. As for longer WD, there can be metabolic changes, especially regarding the photosynthetic machinery of the plant, reducing its activity, possibly due to an increase in stomatal conductance and activities of the RuBisCo enzyme (Hura et al., 2007).

Adverse environmental conditions result in a great impact on the production and performance of grasses, which are highly responsive to good hydric conditions of the soil.

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[1] Revisão publicada na Research, Society and Development.

Mamédio, D., Cecato, U., Sanches, R., Silva, S. M. S., Silva, D. R., Rodrigues, V. O., Galbeiro, S., Barreiros, A. R. D., & Vicente, J. V. R. (2020). Do plant-growth promoting bacteria contribute to greater persistence of tropical pastures in water deficit? - A Review. *Research, Society and Development* 9, e523985756. doi: http://dx.doi.org/10.33448/rsd-v9i8.XX

Plants have a mechanism of tolerance to WD. Yet, it varies according to each species (Lisar et al., 2012). Therefore, it is evidently necessary to carry out studies with technologies that are capable of making plants more resistant to WD in a way that does not hinder their development and productivity. The use of technologies, such as the inoculation of plant-growth promoting bacteria (PGPB) can enhance the development of grasses under stressful conditions.

PGPB are microorganisms commonly found in rhizospheric environments with little or no stress. However, in hostile environments, some PGPB strains are not able to survive and compete for resources (Dar et al., 2018). Some others are not only efficient in resisting WD, but also capable of promoting the growth of host plants from mechanisms such as the biosynthesis of phytohormones (Upadhyay et al., 2011), mineralization and decomposition of organic matter, and enhancement of bioavailability of minerals, such as phosphorus (Kumar & Verma, 2018).

In the literature, there have been positive responses regarding the interaction between grasses and PGPB, proving the capacity of such organisms to alter the physiology of plants and make them more resistant to abiotic stressor agents. The inoculation of PGPB can lead to morphophysiological and productive improvements in *Urochloa* sp. (syn. *Brachiaria*).

Several studies have demonstrated to be inconclusive in their results, which points to the need for more research. Thus, in this review, we are presenting an overview of the causes, effects and responses of the inoculation of PGPB in grasses exposed to water deficit.

1. LITERATURE REVIEW

1.1. Physiological Mechanisms in Response to Water Deficit

The systems of livestock production are constantly subjected to several types of environmental stress throughout their productive cycles. They are exposed to toxicity by elements in the soil, high salinity of the soil, extreme temperatures and water deficit.

These systems have been facing frequent and long drought periods, especially due to the incidence of dry periods during the rainy season, which, depending on the region, can happen in different periods of the year, regardless of the season. The occurrence of WD in areas of animal production has been affecting its system and, consequently, its productivity.

WD is defined as an external factor capable of promoting some type of disadvantageous influence over plant species, leading to responses such as the capacity to tolerate stress, which simply is the ability to face different conditions of WD through a higher performance in the use of water resources available (Taiz & Zeiger, 2009). Maintenance of the hydrated plant cells and efficiency in water usage are mechanisms that plants use to survive (Odokonyero et al., 2017).

WD has been occurring in a faster way due to climate changes, and it has been affecting many regions in the world causing severe damage to primary production sectors, especially those related to farming. By 2050, a great part of the arable land on the planet will have been affected by WD with negative impacts on plants' growth and development (Kasim et al., 2012), considerable losses in cultures performance (Kaushal & Wani, 2016) and seasonality in production (Bonfim-Silva et al., 2011), as it is the case of some grasses. Drought is one of the main stressor agents that compromise productivity of pastures and cultures, especially in arid and semiarid regions (Odokonyero et al., 2017).

The stress caused by WD jeopardizes the relations plant-water, and unleashes a series of morphophysiological and biochemical responses in plants (Rahdari & Hoseini, 2012). Plants subject to WD have their germination and seeds vigor compromised, a reduction of the stomatal opening as a mechanism to avoid tissues dehydration through transpiration, and reduction of the enzymatic and photosynthetic activity (Lisar et al., 2012). Other mechanisms used by plants are leafroll, accumulation of solutes, delayed flowering and some hormonal signals (Hadiarto & Tran, 2011), besides other physiologic and metabolic processes.

When exposed to WD, plants have an immediate response by reducing their osmotic potential inside the cells (Zafari et al., 2017) and their roots' water potential. This happens in order to keep a positive water balance, ensuring water absorption from the soil or a decrease in transpiration (Guimarães et al., 2011).

In plants under stress, there is the inhibition of leaf elongation (Farooq et al., 2009), a decrease in the emission of new tissues (Borrell et al., 2000a) and a reduction of cell division and growth (Anjum et al., 2011) due to a loss of turgidity of the wall cell (Kaushal e Wani, 2016), and a reduction of tillering, compromising the structure of the canopy, not to mention the acceleration of leaf senescence (Inman-Bamber, 2004). That contributes to a smaller leaf area, with direct impact on light interception (Zafari et al., 2017), degradation of photosynthetic pigments (Streit et al., 2005) and a decrease in photosynthesis efficiency (Zafari et al., 2017).

In case of WD, the concentration of chlorophyll pigments and carotenoids can be used as an indicator for evaluating the sanity and integrity of the photosynthetic apparatus (Rong-Hua et al., 2006), thus, indicating if the plant is tolerant to WD (Jabeen et al., 2008).

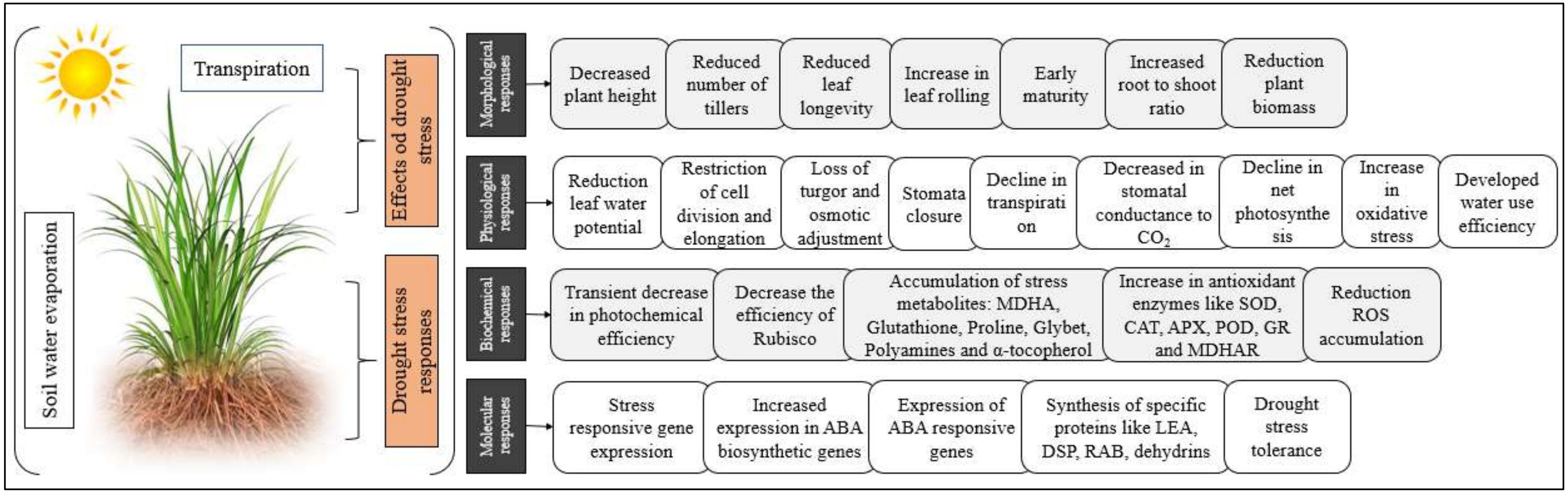
In order to protect themselves from WD, plants activate mechanisms of osmotic adjustment (Kaushal & Wani, 2016) and accumulate metabolites called osmoprotectors or compatible solutes such as proline (Staniak & Kocón, 2015), glycine (Souza et al., 2013) trehalose (Rodríguez-Salazar et al., 2009), glucose, sucrose and fructose (Urano et al., 2010).

Proline, for instance, is found in small amounts in plants, and one of its functions is to help with the osmotic adjustment of plants under drought stress, converting them into cells that are osmoprotected from the deleterious effects of dehydration caused by the constant loss of water through transpiration. It also prevents the denaturation of proteins, preserves the structure of the enzymes and acts like a buffer to stabilize the cellular redox potential. For that reason, this amino acid is considered an important parameter for selecting plants that are tolerant to WD (Nogueira et al., 2001).

In studies with sorghum (*Sorghum bicolor* L.), low availability of water in the soil gradually reduced leaf expansion and the emission of new tissues, with an impact on the production of biomass (Borrell et al., 2000a; 2000b). In sugarcane (*Saccharum officinarum* L.), there was the emission of new tillers and an increase in leaf senescence, thus compromising the canopy structure (Inman-Bamber, 2004).

The literature has shown several problems related to WD which result in stress, thus, jeopardizing grass species such as corn (*Zea mays* L.; Almeida et al., 2017), barley (*Hordeum vulgare* L.; Sanches et al., 2015), wheat (*Triticum aestivum* L.; Raheem et al., 2017), rice (*Oryza sativa* L.; Wei et al., 2017) and *Urochloa* sp. (Odokonyero et al., 2017).

As previously stated, plants make use of several mechanisms to ensure their survival. Figure 1 presents a summary of some effects of WD on plants and their consequences.



Carbon dioxide (CO_2), monodehydroascorbate (MDHA), monodehydroascorbate reductase (MDHAR), superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), peroxidases (POD), glutathione reductase (GR), reactive oxygen species (ROS), abscisic acid (ABA), late embryogenesis abundant (LEA) and dual-specificity phosphatase (DSP).

Figure 0-1. Morphological, physiological, biochemical and molecular responses of tolerance to water deficit stress in plants. Adapted from Shao et al. (2008), Ullah et al. (2017) and Oladosu et al. (2019).

When plants are exposed to WD conditions, they make use of tolerance mechanisms such as morphological, physiological and metabolic adjustments that allow them to overcome the stressor agent. However, depending on severity and duration, plants are not able to survive the external environmental stress by themselves, even if they make use of the aforementioned mechanisms.

Therefore, it is clearly necessary to carry out studies with new technologies that are capable of making forage species more resistant to WD, in a way that does not jeopardize their development and productivity.

1.2. Production of Phytohormones in Response to Drought Stress and its Effects on Plants

Hormones are substances produced by plants and microorganisms. They act by modifying the way specific cells function, and are responsible for promoting their growth and development.

The literature presents a huge amount of research describing the main classes of hormones produced by PGPB, such as auxins, cytokinins, gibberellins, abscisic acid (ABA) and ethylene, as shown in Figure 2.

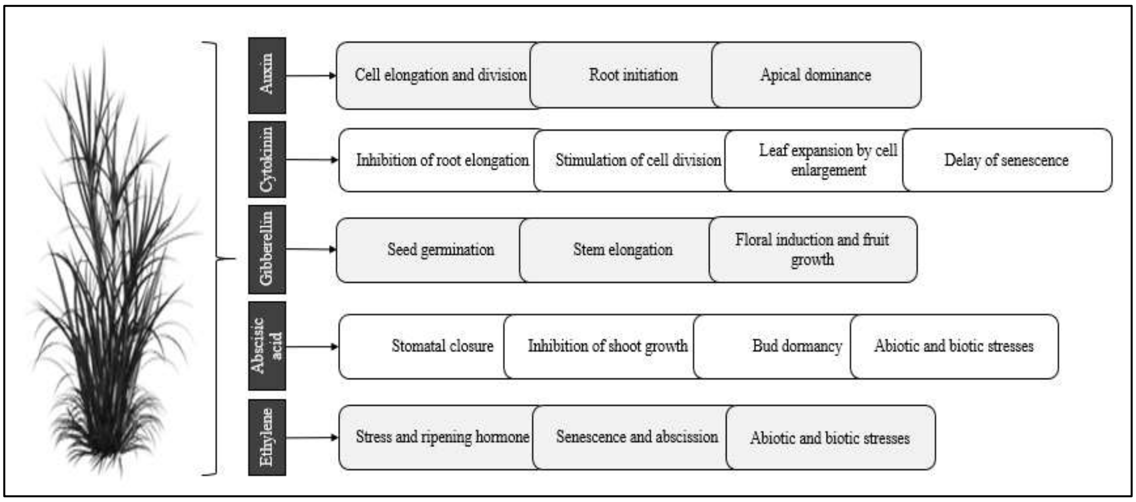


Figure 2. Descriptive summary of the main classes of phytohormones produced by plants growth promoting bacteria and its effects on the plant. Adapted from Spaepen (2015, p. 249).

Bacteria use hormones to interact with plants, stimulating them and, thus, starting the colonization process avoiding the activation of basal defense mechanisms of the plant (Pérez-Montaño et al., 2014).

Bacteria used in studies up to now have demonstrated specificity of results regarding the interaction between PGPB and grasses. Therefore, it is necessary to better understand the mechanisms used by plants under WD conditions.

Although it is not clear how growth stimulus occurs in plants associated with PGPB, it is known that the responses are different due to the distinct compounds (and their concentrations) produced by the microorganisms (Dimkpa et al., 2009), and this set of mechanisms promotes stress relief (Rolli et al., 2014).

Studies have evidenced that indole-3-acetic acid (IAA) is the main auxin produced by plants and PGPB (Kavamura et al., 2013a). The synthesizing of IAA can stimulate the proliferation and/or elongation of plant cells, due to the loosening of the plant's wall cell (Glick, 2014), besides promoting root growth and stimulating the differentiation of meristematic tissues (Souza et al., 2017).

The auxin produced by most bacteria has brought benefits to the growth of plants associated with PGPB, especially with an increment of roots production, due to the greater development of the secondary branching zone and the piliferous zone (Long et al., 2008). That way, there is an increase in the capacity of absorbing water (Kasim et al., 2012) and nutrients (Dimkpa et al., 2009), and greater extension of root exudation (Glick, 2014).

Many species of bacteria are recognized as synthesizers of IAA, such as the species *Azospirillum* sp., *Pantoea* sp. and *Pseudomonas* sp, used in the study of Mamédio (2020). In studies involving *Urochloa* sp., it was observed that 91% of the 81 inoculated strains synthetized IAA (Figueiredo et al., 2010). The synthesis of IAA by *Azospirillum* is one of the most relevant advantages for the growth of grasses (Fukami et al., 2017), and it ensures that the plant will be more tolerant to WD (Dimkpa et al., 2009). Similar responses were also found in the association between *Azospirillum*-*T. aestivum* L. (Arzanesh et al., 2011; Pereyra et al., 2012).

With the inoculation of *Pseudomonas* sp., researchers have observed that the production of IAA led to higher tolerance to WD, with the best survival index of the plants (Marulanda et al., 2009). In studies with the isolation of bacterial species associated with Cactaceae from the Brazilian semiarid region, it was observed that *Pantoea* sp. was one of the species that synthesized the greatest amount of IAA (Kavamura et al., 2013a).

Another mechanism that is beneficial to plants affected by WD, which is inherent in a group of bacteria, is the deaminase activity and the regulation of the enzyme 1-

aminocyclopropane-1-carboxylate (ACC) (Saleem et al., 2007). PGPB that contain the ACC deaminase enzyme can cause a decrease in the level of ethylene of the plant (Long et al., 2008), thus, reducing stress, since ACC is the precursor of this hormone (Saleem et al., 2007). It favors an increase in growth of the root and the aerial part (Glick, 2014).

The synthesis of ethylene is increased as a response to the stressor agent (Glick, 2005). This hormone acts as an important modulator of plant tissues growth and the normal development of the plant because, when its synthesis occurs at high levels, it unleashes the initial processes of chlorosis, senescence and leaf abscission (Glick, 2014). That is why the interaction between PGPB-grasses are important.

In bacteria that contain ACC deaminase, there is a reduction of the WD effect on the growth of roots and the aerial part (Dimkpa et al., 2009). In studies with *T. aestivum* L. cultivated in semiarid climate conditions and inoculated with bacteria that synthesize this enzyme, there was an increase in length, number and root mass when compared to the control treatment. That favored greater absorption of water and nutrients, resulting in a better growth and productivity, even under WD conditions (Shakir et al., 2012).

Another hormone that can be synthesized in response to cellular dehydration due to water deficiency in the soil is the abscisic acid (ABA) (Kaushal e Wani, 2015). It is an important compound synthesized by the root system in WD (Perlikowski et al., 2019). This acid is responsible for inducing stomatal closure, thus, avoiding loss of water by the cell, inhibition of seed germination and leaf senescence. It also stimulates the transcription of genes involved in the protection against dehydration and osmotic stress, with consequent production of proteins of osmotic stabilization and detox enzymes of ROS, such as CAT, SOD and the ascorbate-glutathione cycle (Prakash et al., 2019).

Studies with the inoculation of *A. lipoferum* in *Z. mays* L. evidenced a positive effect of this association for the mitigation of WD negative impacts, and attributed this result to the production of ABA (Cohen et al., 2009). The synthesis of this this phytohormone by the *A. brasilense* strain was also observed. It was found that its biosynthesis can hinder the cytokinins levels of the plant. Besides, under WD conditions, it can relieve the negative effects of stress (Spaepen, 2015).

Cytokinins, in their turn, are the phytohormones involved in cell division and the differentiation of the meristematic tissues of the aerial part and the roots of a plant (Spaepen, 2015), also in organs formation, leaf expansion and senescence delay (Davies, 2010). Bacterial cytokinins are noticed by the plant’s receptors and, for that reason, the presence of PGPB manages to potentialize the synthesis of this compound by the plant

(Spaepen, 2015).

PGPB, according to the specificities of each genre, also have the capacity of both stimulating and inhibiting alterations in the architecture of roots. They promote the plant's development through the synthesizing of gibberellins (GAs) (Martínez et al., 2016; Nelson & Steber, 2016) and stimulate important processes, such as seed germination, stem elongation, and the reproductive part of the plant, such as inflorescence (Zaidi et al., 2015), can also improve photosynthetic performance and chlorophyll pigments (You et al., 2012; Khan et al., 2015).

There is not much genetic evidence of the efficiency of PGPB when it comes to synthesizing GAs (Spaepen, 2015). Yet, based on more detailed analyses of the *A. lipoferum* strain, it is possible to characterize the biosynthesis of different GAs (Cassán et al., 2014), as observed in studies carried out by Cohen et al. (2009) involving corn (*Z. mays* L.).

1.3. Effects of PGPB on Grasses Subjected to Water Deficit Stress

The association PGPB-grasses can result in several benefits, such as a contribution to the sustainability of productive systems, with lower probability of pastures degradation (Hungria et al., 2016) through the possibility of contributing to part of the nitrogen (N) supply required by grasses (Marques et al., 2017) and, finally, mitigation of the negative effects of WD (Vurukonda et al., 2016).

The presence of PGPB can initiate a greater production of genes related to WD and, that way, enable tolerance to stress conditions (Kasim et al., 2012). However, little is known about the effects of PGPB on grasses from tropical climates, such as Urochloa sp. (Acuña et al., 2016), especially because most studies evaluated only the effects on plants growth (Dimkpa et al., 2009).

Studies carried out in the Brazilian semiarid region have shown that the use of xerotolerant microorganisms associated with vegetable crops may represent an alternative for cultivation in areas affected by WD (Kavamura et al., 2013b). Such microorganisms develop mechanisms to survive dry environments, such as the production of exopolysaccharides (Nocker et al., 2012), the formation of biofilms (Chang et al., 2007) and the production of osmolytes to avoid loss of cell water (McNeil et al., 1999).

These microorganisms are also capable of protecting the plant against desiccation by promoting a humid environment that favors the development of the root system. They also provide nutrients and some hormones that promote the plant's growth (Kavamura et al., 2013a). The exopolysaccharides synthesized by these microorganisms are hydrated compounds with around 97% of water, and they are responsible for keeping the roots hydrated for longer, thus, avoiding dehydration.

In case of low water availability in the soil, plants depend on microorganisms that enable them to increase their metabolic activity in order to resist WD (Sandhya et al., 2017).

Studies with the inoculation of *P. fluorescens* AKM-P6 and *P. putida* AKM-P7 in sorghum (*S. bicolor* L.) and wheat (*T. aestivum* L.) show that there was an increase in tolerance to stress due to the synthesis of proteins of high molecular weight and an improvement in the levels of cellular metabolites (Ali et al., 2009 e 2011).

In the association between *Azospirillum*-wheat (*T. aestivum* L.) under WD conditions, there was a greater content of leaf water and an increase in root growth, thus allowing an increase in the absorption of water and nutrients (Arzanesh et al., 2011). Other studies using the same species of PGPB and grasses have evidenced an increment in grain production and an adjustment of the volumetric cell wall of the grain, which improved its water status (Creus et al., 2004), and greater survival of the plants after a few days under WD (Kasim et al., 2012).

The inoculation of *Pantoea* sp. in corn (*Z. mays* L.) under WD resulted in greater leaf area and stem length, and an increment in dry biomass (Kavamura et al., 2013a). The inoculation of *A. lipoferum* led to better corn growth rates, besides a greater accumulation of free amino acids and soluble sugars (Qudsaia et al., 2013). As for the association *A. brasilense*-corn, there were increments of 7.9 and 4.3% in the accumulation of dry biomass of the aerial part and number of grains, respectively, at harvest (Cassán e Diaz-Zorita, 2016). Another study presented an increment of 16% in root dry matter mass (Coelho et al., 2017).

The application of *P. ananatis* AMG 501 in *U. brizantha* pasture, via leaf and root, led to an increase in production of biomass of 10 to 60% (Megías et al., 2017). The inoculation of *A. brasilense* Ab-V5 and Ab-V6 led to an increment of 27% in root mass, 28% in the number of tillers, and reduced the daily accumulation of forage mass to only 7% in comparison with the 17% of the control treatment (Leite et al., 2018).

Taking into account the examples aforementioned, it is clear that PGPB play a relevant role in the mitigation of WD effects, ensuring the survival of grasses. It is also clear that the use of this technology not only allows us to understand the action of these bacteria in the biological responses of plants, but also helps us when it comes to decision-making along with efforts to modernize agricultural production systems and make them more profitable and efficient from the perspective of sustainability.

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II - OBJETIVOS GERAIS

Avaliar o efeito da inoculação de sementes com bactérias promotoras do crescimento de plantas sobre os parâmetros morfológicos e fisiológicos de *Urochloa brizantha* cv. BRS Paiaguás e *Urochloa ruziziensis* em déficit hídrico.

CAPÍTULO III

(Normas: Crop and Pasture Science)

III - EFFECT OF SEED INOCULATION ON THE MORPHOGENIC AND STRUCTURAL CHARACTERISTICS ON *UROCHLOA BRIZANTHA* CV. BRS PAIAGUÁS AND *UROCHLOA RUZIZIENSIS* TOLERANCE TO DROUGHT STRESS

Abstract - This study aimed to evaluate the effects of inoculation with plant growth promoting bacteria (PGPB) on the morphogenic and structural characteristics of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* under drought stress. The inoculated bacteria strains were *Azospirillum brasilense* Ab-V5, *Pantoea ananantis* AMG 521 and *Pseudomonas fluorescens* CCTB 03, in addition to the non-inoculated control, all submitted to four water deficit levels (80, 60, 40 and 20%). The experimental design was a completely randomized block design, in 4x4 factorial scheme with four replicates, totaling 128 pots filled with 9 dm³ of soil. Morphogenesis was evaluated during summer and autumn seasons (February to May of 2018/2019). No interaction effects between the factors were observed. For Paiaguás grass (summer), the inoculation with the Ab-V5 and AMG 521 strains increased leaf appearance rate. Reducing the water deficit decreased stem elongation and leaf senescence, and increased leaf appearance in Paiaguás grass. In autumn, the CCTB 03 strain and the non-inoculated control resulted in decreased leaf senescence, and the smallest water deficit provided greater leaf and stem elongation and the number of live leaves, and decreased leaf senescence. For Ruziziensis grass (summer), inoculation with strain CCTB03 and non-inoculated control, increased leaf senescence. In autumn, strain AMG521 provided higher phyllochron. In the summer season there was no drought effect. PGPB, in general, were not efficient to improve the morphogenesis of Paiaguás and Ruziziensis grasses. The imposition of higher water deficit levels reduced the appearance of new tissues and increased leaf senescence rate.

Keywords: water deficit, strain, forage, morphogenesis, bacteria

Introduction

Low water availability due to drought influences plant-water relationships and triggers a series of morpho-physiological responses in the plant (Rahdari and Hoseini 2012), affecting germination and seed vigor and reducing stomatal opening to avoid tissue dehydration due to transpiration (Lisar *et al.* 2012).

Through drought stress, the plants reduce the osmotic potential within plant cells (Zafari *et al.* 2017) and root water potential to maintain a positive water balance, with the guarantee of soil water absorption or reduced transpiration (Guimarães *et al.* 2011).

The most severe drought (WD = water-deficient stress) can negatively affect grass morphogenic and structural characteristics and cause premature death. According to Chapman and Lemaire (1993), morphogenesis can be expressed by the tissue appearance rate, the expansion of new plant organs, and senescence. However, low water absorption by the roots promotes decreased cell turgor, reflecting in a reduction of cell division and growth (Anjum *et al.* 2011), smaller leaf area, with decrease in light interception and photosynthetic activity and, therefore, lower dry mass accumulation (Zafari *et al.* 2017).

The appearance, elongation and lifespan of leaves are representative characteristics of grass in the vegetative stage (Barbosa *et al.* 2011). Although genetically determined (Lemaire and Chapman 1996), they can be influenced by environmental factors, such as temperature (Duru and Ducrocq 2000) and water availability (Caetano and Dias-Filho 2008).

Facing the need to minimize the negative effects of drought, the association of plant growth promoting bacteria (PGPB) with tropical grasses might be relevant, increasing plants stress tolerance.

Microorganisms more adapted to drier environments, such as those called xerotolerant, develop some survival mechanisms such as exopolysaccharide production (Nocker *et al.* 2012), the formation of wet biofilms (Chang *et al.* 2007), and osmolytes production to avoid cellular water loss (McNeil *et al.* 1999), in addition to providing nutrients and hormones that promote growth plant, such as auxins, cytokinins and gibberellins (Kavamura *et al.* 2013).

There are reports showing positive effects of PGPB in grasses under WD. In corn (*Zea mays* L.), leaf water potential loss was reduced, while root growth, aerial biomass and leaf area were increased (Casanovas *et al*. 2002). In wheat (*Triticum aestivum* L.), there was higher plant survival (Kasim *et al*. 2012) and, in alfalfa (*Medicago sativa* L.), there were better photosynthetic indices and water absorption (Zafari *et al.* 2017). However, little is known about PGPB effects on tropical grass pastures, such as the genus *Urochloa* (syn. *Brachiaria* sp.) (Odokonyero *et al*. 2016).

The use of innovative technologies such as PGPB can provide improvements in the morphogenesis of tropical grass pastures, even under water-deficient stress. Therefore, this study aimed to evaluate the effects of inoculation with PGPB on the morphogenic and structural characteristics of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* under drought stress.

Material e Métodos

Site Location e and experimental design

The experiments were performed in protected environments, in greenhouse, at the State University of Maringá, Maringá, PR, Brazil (23°24'S, 51°56'W; 542 m a.m.s.l.), in two experimental cycles (Test I: November 2017-July 2018 and Test II: September 2018-May 2019). Morphogenesis was performed during summer and autumn seasons (February to May of 2018/2019).

In both Tests I and II a Ferralsol soil (Santos *et al.* 2018) was used. The soil was collected at 0-0.2 m depth, and showed the following chemical attributes: potential for hydrogen (pH in H_2O) = 5.3 and 4.8, calcium (Ca^{2+}) = 0.78 and 1.09 $cmol_c dm^{-3}$, magnesium (Mg^{2+}) = 0.52 and 0.44 $cmol_c dm^{-3}$, aluminum (Al^{3+}) = 0.13 and 0.05 $cmol_c dm^{-3}$, potassium (K^+) = 0.15 and 0.12 $cmol_c dm^{-3}$, phosphorus (P, Mehlich) = 9.16 and 20.54 mg dm^{-3}; base saturation (V) = 26.03 and 33.88%, cation exchange capacity (CEC pH 7.0) = 5.57 and 4.87 $cmol_c dm^{-3}$, organic matter (OM) = 9.11 and 9.36 g dm^{-3};

sand = 740 and 880 g kg^{-1}, silt = 40 e 20 g kg^{-1} and clay = 220 and100 g kg^{-1}. Soil acidity correction was carried out with elevation of base saturation to 55%, with the incorporation of dolomitic limestone with RPNT = 126% (relative power of total neutralization), remaining incubated for a period of 25 days.

The pasture grass species used were *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* (Germain and Evrard). The experiment was performed in a complete randomized block design, in a 4x4 factorial scheme with four replicates, using 64 plastic plots filled with 9 dm^3 of soil for each grass species, totaling 128 plots in each experimental test (i.e. n = 128 per test).

The inoculated bacteria strains were *Azospirillum brasilense* Ab-V5 (=CNPSo 2083), *Pantoea ananantis* AMG 521 (=CNPSo 2798) and *Pseudomonas fluorescens* CCTB 03 (=CNPSo 2719), plus non-inoculated control and four water deficit levels (WD = 80, 60, 40 and 20%). All strains are deposited at the “Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja” (World Federation Culture Collection, WFCC #1213; World Data Centre for Microorganisms, WDCM #1054). The bacteria were derived as follows: *A. brasilense* Ab-V5 selected in Brazil, initially for maize and wheat (Hungria *et al.* 2010); *P. ananatis* AMG521 isolated at University of Seville, Spain (Megías *et al.* 2016) and *P. fluorescens* CCTB 03, isolated by Total Biotecnologia (Curitiba, PR, Brazil).

For preparation of inoculant, the strains were grown in DYGS medium (Fukami *et al.* 2018), and the concentrations were adjusted to 10^8 cells mL^{-1}, according to previously obtained growth curves correlated with optical density previously obtained for each strain. For inoculation, 15 mL of each inoculant was used per kg of seeds before sowing. Seeds were dried for approximately 30 min in a cool and sun-sheltered location, after which they were seeded at 15 seeds per pot.

The pots were filled with soil, heavy, saturated with water and drained by gravity and then reweighed. Thus, the upper limit of available water (AW) [pots field capacity (θ_{PFC})] was determined by gravimetry. The permanent wilting point (θ_{PWP}) of soil was determined (-15,000 hPa) using a WP4-T Dewpoint Potentia Meter, according to Klein *et al*. (2010). Approximately 0.1 kg of soil was weighed and oven-dried at 105 °C for 24 h in order to determine the air-dried soil mass. Based on the information AW (m^3m^{-3}), calculated by the difference the values of θ_{PFC} and θ_{PWP}, it was possible to impose WD levels.

Before sowing all pots received the equivalent of 42 kg P_2O_5 ha^{-1} (simple superphosphate 18% P_2O_5) in a single dose, 141 and 169 kg K_2O ha^{-1} (potassium chloride 60% K_2O) divided into two applications, where the first was incorporated into the soil at sowing, and the second, a week after the first cut of plants, together with N-fertilization (20 kg N ha^{-1}, urea 45% N). Both fertilizations were carried out in Tests I and II, respectively, except for the P-fertilization that was not carried out in test II because the amount of P in the soil was above the optimal level working in the experiments.

About 15 seeds were sown per pot. Two weeks after seedling emergence, they were thinned, leaving five uniform plants per pot. Three weeks after the emergence, plant cutting was performed leaving15 cm of residue. During this period, the soil moisture was kept close to field capacity, with subsequent imposition of WD.

During the experiment, temperature and relative humidity were monitored. The average maximum and minimum temperatures recorded in the greenhouse for test I and II, respectively, were 32 and 40, 18 and 19 °C, respectively, with average relative air humidity of 64 and 53% (Figure 1).The WD level in each treatment was maintained by weighing the pots every day, with a digital scale of 20 kg maximum capacity, with uncertainty of 0.05 kg, DST-30/P-DM model (Triunfo, São Paulo, Brazil). Replacing water was performed through polyvinyl chloride tubes (PVC, 12 cm), with perforated walls, (Beutler and Centurion 2004), installed in the center of the pot to avoid water loss by evaporation and ensure irrigation directly into the grass roots system.

Measurement of morphogenic and structural characteristics

When the plants reached, on average, 35-40 cm in height, shoots were cut to 15 cm. Height measurements were performed three times each week by using a ruler with 1-mm increments.

Morphogenic and structural characterization were evaluated twice a week on two tillers per pot, marked by colored wire, totaling 36 evaluations throughout the experimental period. The lengths of the green leaves and pseudostem were measured by means of a ruler with 1-mm increments. For expanding leaves, the measurement was from the ligule of the last expanded leaf as a reference. Expanded leaves were measured from the ligule to the tip of the green leaf. The length of the pseudostem was obtained as the distance from the ground to the ligule of the youngest completely expanded leaf. Lengths of expanded leaves, cut leaves and dead leaves were also recorded. These measures were used to determine the following rates according to Sbrissia and Silva (2008):

- Leaf appearance rate (LAR): ratio of number of leaves per tiller appearing in the evaluated period to number of days in the period.
- Phyllochron (Phyllo): number of days in which two leaves grow on the same tiller.
- Leaf elongation rate (LER): ratio of total elongation of all leaf blades (cm) to number of days in the evaluation period (i.e. (final length – initial length)/no. of days counted).
- Leaf senescence rate (LSR): mean variation in length of the senescent portion of the leaf, which was obtained as the product of length of the senescent leaf blade

and proportion of corresponding senescent tissue observed throughout the evaluation period.

- Number of live (non-senescent) leaves (NLL).
- Duration of life of leaves (DLL): number of live leaves x Phyllo.
- Stem elongation rate (SER), difference in the length of the pseudostem between the end and the beginning of the experimental period divided by the number of days (i.e. (final length – initial length)/no. of days counted).

Statistical analyses

The statistical analyses were performed with the PROC GLIMMIX of the statistical package SAS version 9.2 (SAS Institute, Cary, NC, USA). Analyzes were performed after the grouping of averages of Tests I and II. Data for each grass were analyzed separately. In the analysis, morphogenic and structural parameters were considered as fixed effects, whereas blocks and tests were considered as random effects. Data were tested for residues normality (Shapiro-Wilk test, Shapiro and Wilk, 1965) and variances homogeneity (Bartlett test, Bartlett, 1950). The linear and quadratic effects of water deficit levels were evaluated by orthogonal contrasts, and when observed significant effect of inoculation, the means of each bacterium were compared using the LSMeans Pdiff command from SAS software ($P \leq 0.1$).

Results

No interaction effects were observed between the PGPB inoculations and WD for any of the morphogenic parameters evaluated in Paiaguás and Ruziziensis grasses, in the summer and autumn seasons.

The results of the morphological and structural characteristics of the Paiaguás grass in the summer (Table 1) showed that the leaf appearance rate (LAR, $p = 0.0830$) varied with PGPB inoculation, with the highest value recorded for Ab-V5 and AMG 521 strains (0.13 and 0.13 no. of leaves day^{-1}, respectively), although not statistically different from the non-inoculated control.

In this same season, the stem elongation rate (SER, $p = 0.0902$ cm day^{-1}), leaf appearance rate (LAR, $p = 0.0698$ no. of leaves day^{-1}) and leaf senescence rate (LSR, $p = 0.0450$ cm day^{-1}) were affected by WD (Table 1). These variables were adjusted to the linear regression model, revealing the influence of the severity of AW in the morphogenic parameters of Paiaguás. For the SER, a linear decrease with WD (80 to 20%) was observed, similar to the LSR. For the LAR, the highest WD (80%) resulted in a delay in the appearance of the leaf band of the tiller and decreased size.

For the morphogenesis of Paiaguás in autumn (Table 2), PGPB affected the leaf senescence rate (LSR, $p = 0.0943$), with the highest value recorded for Ab-V5 and AMG 521 (0.29 and 0.18 cm day^{-1}, respectively). A lower LSR was observed in treatments with CCTB 03 and in the non-inoculated control, 0.12 and 0.12 cm day^{-1}, respectively.

Regarding the effect of WD on the morphogenic and structural characteristics of Paiaguás in autumn (Table 2), effects were observed on the leaf elongation rate (LER, $p = 0.0764$ cm day^{-1}), stem elongation rate (SER, $p = 0.0025$ cm day^{-1}), number of live leaves (NLL, $p = 0.0621$ no. of live leaves tiller^{-1}) and the leaf senescence rate (LSR, $p = 0.0563$ cm day-1). These variables were adjusted to the linear regression model, revealing the effect of WD on this grass. Increasing linear behavior was observed for the LER, SER and NLL, with an increase in the AW for grasses. The highest WD resulted in a reduction in the NLL and an increase in the LSR.

The results of the morphological and structural characteristics of the Ruziziensis in the summer (Table 3) showed that the leaf senescence rate (LSR, p = 0.0696) varied with PGPB inoculation, with the highest value recorded for the treatments with the CCTB 03 strain and the non-inoculated control, 0.08 and 0.13 cm day^{-1}, respectively.

For the data collected in the autumn, we observed an effect of bacterial inoculation on the phyllochron result (Phyllo, p = 0.0844) with the highest value recorded for AMG 521 (18.76 no. of days leaf^{-1}), therefore, with longer time interval to show two consecutive leaves (Table 4).

Our results indicated that the WD level imposed on Ruziziensis grass in the summer (Table 3) and autumn (Table 4) seasons did not affect morphogenesis.

Discussion

In this study, effects of inoculation of PGPB verified in the LAR (Table 1), the LSR (Tables 2 and 3) and Phyllo (Table 4), were observed, and could probably be attributed to an increased production of phytohormones, such as auxins, cytokinins and gibberellins.

The results confirm benefits of PGPB inoculation reported in tropical grasses such as *U. brizantha* cv. Xaraés, *U. brizantha* cv. BRS Paiaguás and *U. ruziziensis* (Duarte *et al.* 2020), maize (Cohen *et al*. 2009; Rodríguez-Salazar *et al.* 2009; Zucarelli *et al*. 2011; Fukami *et al.* 2017), *U brizantha* cv. Marandu and *U. ruziziensis* (Hungary *et al.* 2016), *Pennisetum purpureum* Schum (Pereira *et al*. 2015).

In the literature, it is also reported that PGPB may enhance the plant hormones synthesis, contributing to increased plant growth and, according to Taiz and Zeiger (2013), they can change cell wall expansion, the LER and the useful life of these organs.

The morphogenic and structural characteristics of grasses are highly influenced by the soil AW, since water, temperature and solar radiation are factors that, in appropriate conditions, are responsible for the growth of grasses. According to Ludlow and Ng (1977), leaf expansion, for example, is one of the most sensitive physiological processes to WD, because cell division and growth are severely affected by cell turgor, compromising the LER, with the interruption of these processes before beginning stages of photosynthesis. WD also shows a negative effect on the SER, LAR, DLL and LSR (Ferro *et al.* 2015).

The LER is an important indication of the dynamics of forage accumulation, demonstrating a high positive correlation with forage biomass (Horst *et al.* 1978). For Silveira *et al.* (2010), grasses with a low potential for leaf accumulation due to a low LER, generally have a higher LAR, indicating a smaller period of leaf elongation and a higher turnover of the leaf tissue.

The LAR is the morphogenic characteristic that deserves more emphasis, as it directly affects leaf size (Horst *et al.* 1978), showing an inverse relationship, with the lower appearance rate of larger leaves, due to the higher path inside the sheath from the emergence to full leaf expansion Mesquita and Neres (2008). Grasses with reduced leaf size, can also mean leaves with a lower amount of fiber and, consequently, higher nutritional value (Waghorn and Clark 2004). However, the nutritional value of grass has greater relation with the management rather than with the size of the leaf blade, since well managed grasses tend to be more nutritious, according to studies reported by Fulkerson and Donaghy (2001), especially due to the maintenance of the greater leaf:stem ratio (Silveira *et al.* 2015). Depending on the structure of the canopy, grass availability and quality, the animal intake can be affected.

In our study, the decrease in NLL with the highest WD, probably associated with limited water extraction, caused loss of turgor of plant cells, reduction in DLL and, as a result, earlier senescence (Table 3). Each grass species has an NLL that remains relatively constant throughout the plant cycle, because this is a predetermined genetic characteristic, especially when the plant is harvested respecting its physiological limit of defoliation tolerance (Lemaire and Chapman 1996).

In our study, the highest LSR related to the WD in Paiaguás grass occurred in the autumn season and remained constant, regardless of the WD (Table 3), possibly due to lower leaf renewal resulting from low temperatures and irradiance. According to Fagundes *et al.* (2006), leaf senescence is a mechanism to anticipate the death of tissue established by the plant, to reduce the area of the breathable leaf and, therefore, tolerance to WD longer. However, this mechanism used by plants compromises pasture production, reducing herbage allowance and its nutritional value.

In our study, the highest WD resulted in an increase in the SER in Paiaguás in the summer season (Table 2). These results can be attributed to different temperature conditions (Figure 1) verified throughout the experiments. For tropical grasses, temperature is a crucial and necessary factor for growth of morphological components, as mentioned by Durant *et al.* (1991). Possibly, the lowest LAR observed during periods of extreme temperature inside the greenhouse, combined with less water availability for grasses, perhaps contributed to a greater SER.

Stem production in pasture, provided it is well managed, under grazing conditions or manual harvesting, can contribute to the maintenance of pastures and serve as a component to accumulate reserves. Pereira *et al.* (2015) reported that, particularly in tropical grasses, the stem may become an important reservoir of photoassimilates in relation to leaf elongation.

Stem elongation has a great expressiveness in the canopy structure, and according to the amount produced, it can compromise grazing efficiency, causing a reduction in the leaf:stem ratio, and negatively affect animal performance.

Silveira *et al.* (2010) reported that the LER and SER are morphogenic variables showing high correlation with forage accumulation, and contributes to the identification of grasses with biomass production potential regardless of seasonality and with similar growth patterns.

Understanding the interaction between the environment and morphophysiological mechanisms of grasses is the key to achieve better pasture establishment and longevity (Chapman and Lemaire 1993). Based on this information, strategies can be planned to impact the forage grass morphogenesis and modify the dynamics of biomass accumulation through changes in canopy structure, such as leaf, stem and tiller size (Martuscello *et al.* 2011).

Conclusions

Plant growth promoting bacteria, in general, were not efficient to improve morphogenic and structural parameters of *Urochloa brizantha* cv. BRS Paiaguás e *Urochloa ruziziensis* under drought stress. The PGPB strains were more effective in contributing to the lower leaf senescence rate in grasses. The highest water deficit level reduced the leaf appearance rate, leaf and stem elongation rate, number of live leaves, and increased in leaf senescence.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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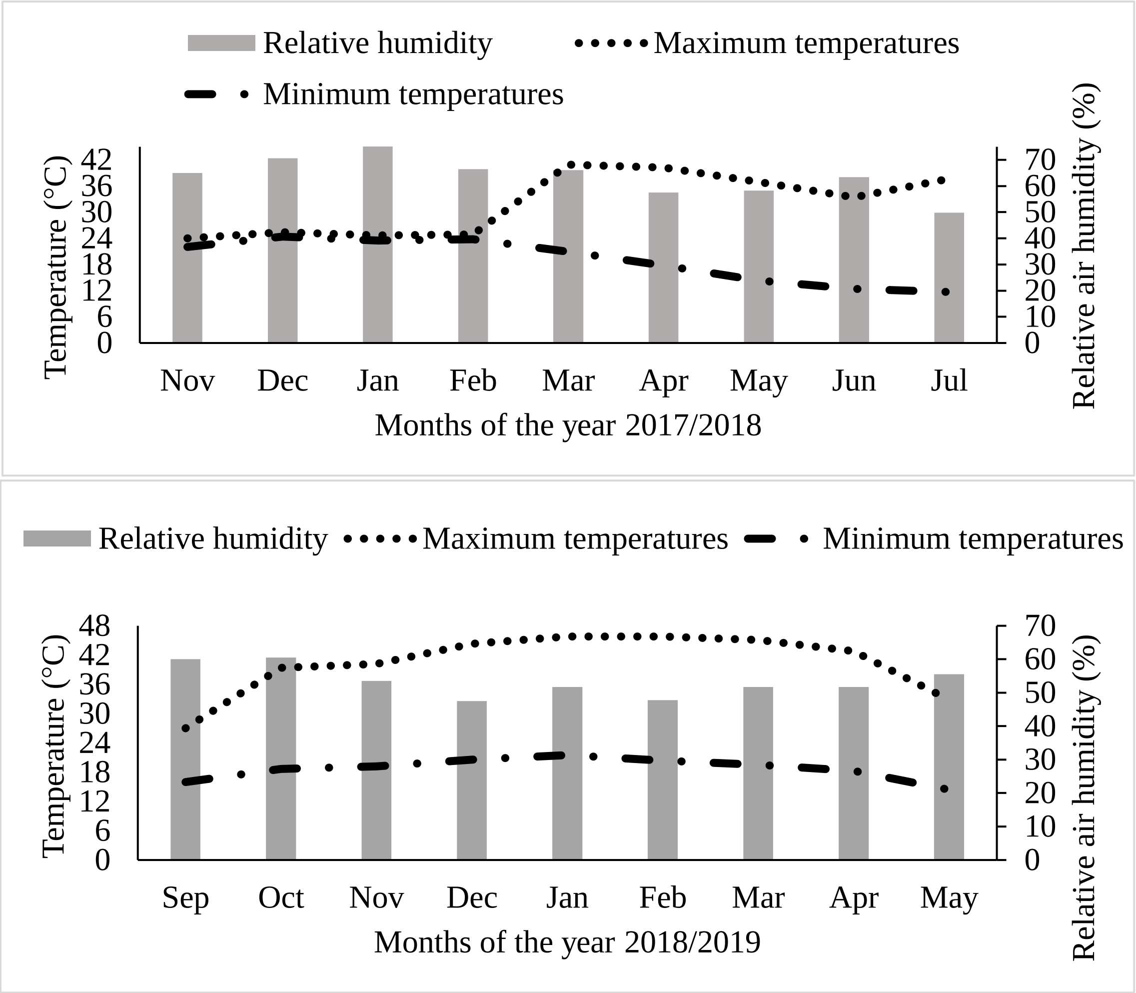

Fig. 1. Climatic conditions (relative humidity, maximum and minimum temperatures) registered with the digital Thermo-Higrometer apparatus at greenhouse, State University of Maringá, Maringá, PR, Brazil, during the experimental periods.

Table 1. Leaf elongation rate (LER), stem elongation rate (SER), leaf appearance rate (LAR), phyllochron (Phyllo), duration of life of leaves (DLL), number of live leaves (NLL) and leaf senescence rate (LSR) of *Urochloa brizantha* cv. BRS Paiaguás inoculated with plant growth promoting bacteria under drought stress in summer season.

Azospirillum brasilense Ab-v5; *Pantoea ananantis* AMG 521; *Pseudomonas fluorescens* CCTB03; Regression (L = linear and Q = quadratic). Date are means ± SEM = standard error of mean (n = 4). Means followed by different lowercase letters in each line are significantly different (P < 0.1, LSMeans test).

Parameter	Plant Growth Promoting Bacteria				P value
	Ab-V5	AMG521	CCTB03	non-inoculated	
LER (cm day-^{1})	1.10±0.24	1.06±0.24	1.04±0.24	1.07±0.24	0.9833
SER (cm day^{-1})	1.10±0.51	1.07±0.51	1.16±0.51	1.11±0.51	0.5508
LAR (no. of leaves day^{-1})	0.13±0.03a	0.13±0.03a	0.11±0.03b	0.13±0.03ab	0.0830
Phyllo (no. of days leaf^{-1})	9.06±2.09	9.11±2.09	9.31±2.09	9.28±2.09	0.9888
DLL (days)	37.23±7.37	41.14±7.37	37.68±7.37	41.04±7.37	0.7983
NLL (no. of live leaves tiller^{-1})	4.44±0.40	5.16±0.40	4.02±0.40	4.46±0.40	0.1325
LSR (cm day^{-1})	0.22±0.18	0.10±0.18	0.12±0.18	0.30±0.18	0.3153

Parameter	Water Deficit Level (%)				P value	
	80	60	40	20	L	Q
LER (cm day^{-1})	1.12±0.24	1.05±0.24	0.98±0.24	1.14±0.24	0.9898	0.3050
SER (cm day^{-1})	1.19±0.51	1.11±0.51	1.06±0.51	1.09±0.51	0.0902	0.2250
LAR (no. of leaves day^{-1})	0.12±0.03	0.12±0.03	0.13±0.03	0.13±0.02	0.0698	0.8278
Phyllo (no. of days $leaf^{-1}$)	9.46±2.09	8.79±2.09	9.44±2.09	9.07±2.09	0.8443	0.8115
DLL (days)	37.81±7.37	36.00±7.37	40.31±7.37	40.31±7.37	0.2228	0.5361
NLL (no. of live leaves $tiller^{-1}$)	4.25±0.40	4.33±0.40	4.84±0.40	4.67±0.40	0.2607	0.7148
LSR (cm day^{-1})	0.31±0.18	0.18±0.18	0.20±0.18	0.05±0.18	0.0450	0.9094

Table 2. Leaf elongation rate (LER), stem elongation rate (SER), leaf appearance rate (LAR), phyllochron (Phyllo), duration of life of leaves (DLL), number of live leaves (NLL) and leaf senescence rate (LSR) of *Urochloa brizantha* cv. BRS Paiaguás inoculated with plant growth promoting bacteria under drought stress in autumn season.

Azospirillum brasilense Ab-v5; *Pantoea ananantis* AMG 521; *Pseudomonas fluorescens* CCTB03; Regression (L = linear and Q = quadratic). Date are means ± SEM = standard error of mean (n = 4). Means followed by different lowercase letters in each line are significantly different ($P < 0.1$, LSMeans test).

Parameter	Plant Growth Promoting Bacteria				P value
	Ab-V5	AMG521	CCTB03	non-inoculated	
LER (cm day^{-1})	1.42±0.27	0.82±0.27	1.00±0.27	0.80±0.27	0.3224
SER (cm day^{-1})	0.30±0.10	0.28±0.10	0.33±0.10	0.28±0.10	0.3795
LAR (no. of leaves day^{-1})	0.07±0.41	0.88±0.41	0.08±0.41	0.07±0.42	0.4057
Phyllo (no. of days leaf^{-1})	16.72±2.05	15.66±2.05	15.64±2.05	14.27±2.06	0.2824
DLL (days)	60.63±12.84	57.92±12.54	59.74±12.84	53.42±12.87	0.6781
NLL (no. of live leaves tiller^{-1})	3.72±0.43	5.70±0.43	3.92±0.43	3.83±0.44	0.8745
LSR (cm day^{-1})	0.29±0.12a	0.18±0.12ab	0.12±0.12b	0.12±0.12b	0.0943

Parameter	Water Deficit Level (%)				P value	
	80	60	40	20	L	Q
LER (cm day^{-1})	0.79±0.27	0.86±0.27	0.94±0.27	1.46±0.27	0.0764	0.3889
SER (cm day^{-1})	0.26±0.10	0.26±0.10	0.31±0.10	0.36±0.10	0.0025	0.2564
LAR (no. of leaves day^{-1})	0.06±0.41	0.07±0.41	0.07±0.42	0.89±0.41	0.1796	0.3279
Phyllo (no. of days leaf^{-1})	15.39±2.05	15.69±2.05	15.86±2.06	15.35±2.05	0.9294	0.6445
DLL (days)	56.06±12.84	58.97±12.84	54.89±12.87	61.79±12.84	0.5119	0.6561
NLL (no. of live leaves tiller^{-1})	3.48±0.43	3.84±0.43	3.73±0.44	4.13±0.43	0.0621	0.9200
LSR (cm day^{-1})	0.21±0.12	0.23±0.12	0.21±0.12	0.06±0.12	0.0563	0.1193

Table 3. Leaf elongation rate (LER), stem elongation rate (SER), leaf appearance rate (LAR), phyllochron (Phyllo), duration of life of leaves (DLL), number of live leaves (NLL) and leaf senescence rate (LSR) of *Urochloa ruziziensis* inoculated with plant growth promoting bacteria under drought stress in summer season.

Azospirillum brasilense Ab-v5; *Pantoea ananantis* AMG521; *Pseudomonas fluorescens* CCTB03; Regression (L = linear and Q = quadratic). Date are means ± SEM = standard error of mean (n = 4). Means followed by different lowercase letters in each line are significantly different (P < 0.1, LSMeans test).

Parameter	Plant Growth Promoting Bacteria				P value
	Ab-V5	AMG521	CCTB03	non-inoculated	
LER (cm day^{-1})	0.79±0.23	0.73±0.22	0.75±0.22	0.80±0.22	0.8704
SER (cm day^{-1})	1.10±0.57	1.04±0.57	1.02±0.57	1.00±0.57	0.4190
LAR (no. of leaves day^{-1})	0.11±0.20	0.11±0.20	0.09±0.20	0.48±0.20	0.4019
Phyllo (no. of days leaf^{-1})	9.59±1.27	9.53±1.25	10.82±1.25	10.90±1.25	0.7242
DLL (days)	43.96±3.98	40.14±3.90	41.26±3.90	46.84±3.90	0.3714
NLL (no. of live leaves tiller^{-1})	4.65±0.72	4.56±0.72	4.17±0.72	4.47±0.72	0.4790
LSR (cm day^{-1})	0.04±0.08b	0.04±0.08b	0.08±0.08ab	0.13±0.08a	0.0696

Parameter	Water Deficit Level (%)				P value	
	80	60	40	20	L	Q
LER (cm day^{-1})	0.76±0.23	0.70±0.23	0.76±0.22	0.86±0.22	0.2881	0.2904
SER (cm day^{-1})	1.06±0.57	1.00±0.57	1.04±0.57	1.08±0.57	0.5930	0.2775
LAR (no. of leaves day^{-1})	0.46±0.20	0.11±0.20	0.10±0.20	0.11±0.20	0.2130	0.3392
Phyllo (no. of days leaf^{-1})	10.61±1.26	10.15±1.26	9.63±1.25	10.45±1.25	0.7242	0.3141
DLL (days)	42.07±3.94	44.78±3.94	41.83±3.90	43.53±3.90	0.9143	0.8640
NLL (no. of live leaves tiller^{-1})	4.06±0.72	4.73±0.72	4.44±0.72	4.63±0.72	0.1743	0.2975
LSR (cm day^{-1})	0.07±0.08	0.08±0.08	0.07±0.08	0.07±0.08	0.8604	0.8780

Table 4. Leaf elongation rate (LER), stem elongation rate (SER), leaf appearance rate (LAR), phyllochron (Phyllo), duration of life of leaves (DLL), number of live leaves (NLL) and leaf senescence rate (LSR) of *Urochloa ruziziensis* inoculated with plant growth promoting bacteria under drought stress in autumn season.

Azospirillum brasilense Ab-v5; *Pantoea ananantis* AMG 521; *Pseudomonas fluorescens* CCTB03; Regression (L = linear and Q = quadratic). Date are means ± SEM = standard error of mean (n = 4). Means followed by different lowercase letters in each line are significantly different ($P < 0.1$, LSMeans test).

Parameter	Plant Growth Promoting Bacteria				P value
	Ab-V5	AMG521	CCTB03	non-inoculated	
LER (cm day^{-1})	0.64±0.10	0.65±0.10	0.70±0.10	0.67±0.10	0.7092
SER (cm day^{-1})	0.20±0.12	0.19±0.12	0.24±0.12	0.22±0.12	0.2205
LAR (no. of leaves day^{-1})	0.88±0.43	0.06±0.41	0.07±0.42	0.07±0.41	0.4368
Phyllo (no. of days leaf^{-1})	16.59±2.93b	18.76±2.92a	16.07±2.93b	16.23±2.92b	0.0844
DLL (days)	72.00±16.34	77.62±16.33	70.54±16.35	66.99±16.33	0.2784
NLL (no. of live leaves tiller^{-1})	4.37±0.32	4.38±0.31	4.41±0.31	4.19±0.31	0.8420
LSR (cm day^{-1})	0.04±0.06	0.06±0.06	0.10±0.06	0.06±0.06	0.3162

Parameter	Water Deficit Level (%)				P value	
	80	60	40	20	L	Q
LER (cm day^{-1})	0.62±0.10	0.66±0.10	0.65±0.10	0.72±0.09	0.1356	0.7124
SER (cm day^{-1})	0.20±0.12	0.21±0.12	0.20±0.12	0.24±0.12	0.1818	0.5581
LAR (no. of leaves day^{-1})	0.06±0.43	0.07±0.42	0.06±0.41	0.88±0.41	0.1950	0.3343
Phyllo (no. of days $leaf^{-1}$)	17.47±2.93	14.83±2.93	17.07±2.92	16.28±2.92	0.3798	0.9270
DLL (days)	72.05±16.37	75.03±16.35	73.27±16.33	66.80±16.33	0.3224	0.2319
NLL (no. of live leaves $tiller^{-1}$)	4.19±0.32	4.49±0.31	4.35±0.31	4.31±0.31	0.7863	0.3710
LSR (cm day^{-1})	0.06±0.06	0.07±0.06	0.05±0.06	0.07±0.06	0.8787	0.6393

CAPÍTULO IV

(Normas: Frontiers in Plant Science)

IV - PHYSIOLOGICAL RESPONSES OF PAIAGUÁS AND RUZIZIENSIS GRASSES INOCULATED WITH PLANT-GROWTH PROMOTING BACTERIA UNDER DROUGHT STRESS

ABSTRACT

The aim of this study is evaluating the effect of the inoculation of plant-growth promoting bacteria (PGPB) on the physiological responses of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* under drought stress. The inoculated bacteria strains were *Azospirillum brasilense* Ab-V5, *Pantoea ananantis* AMG 521 and *Pseudomonas fluorescens* CCTB 03, in addition to the non-inoculated control, all submitted to four water deficit levels (80, 60, 40 and 20%). Experimental delineation was done in random blocks, in 4x4 factorial scheme, with four repetitions, totaling 128 vases filled with 9 dm^3 of soil in each experimental trial. The inoculation of PGPB had an effect on the *in vitro* digestibility of the dry matter and the SPAD index of Paiaguás and Ruziziensis grasses, with responses that were similar to those of the control treatment. The greatest imposition of water deficit (80%) revealed influence over the grasses, with a reduction in the levels of dry mass, neutral and acid detergent fiber and lignin, and an increase in crude protein, *in vitro* digestibility of the dry matter, accumulation of total nitrogen, soluble carbohydrates and the SPAD index. The PGPB were not efficient in promoting improvement of the physiological parameters of Paiaguás and Ruziziensis grasses under drought stress.

KEYWORDS: ***Azospirillum*****, bacteria, grass, inoculation,** ***Pantoea ananatis, Pseudomonas fluorescens,*** **water deficit**

INTRODUCTION

Drought stress is one of the factors that mostly contribute to compromising the development of pastures (Odokonyero et al., 2017) and, thus, the reduction of their productive potential. According to Glick (2012), plants usually go through cycles with different intensities of growth inhibition.

High temperatures and drought are the most important types of stress with huge impact on plants' growth, development and productivity (Fahad et al., 2017), forcing them to adjust their metabolism and causing different morphophysiological and biochemical responses (Rahdari e Hoseini, 2012; Lisar et al., 2012).

Plants under stress are affected by inhibition of leaf elongation (Farooq et al., 2009), cell division and growth (Anjum et al., 2011), reduction of dry mass in the aerial part (Bonfim-Silva et al., 2011), greater accumulation of voluble sugars and smaller foliar area with direct impacts on luminous interception (Zafari et al., 2017), degradation of photosynthetic pigments (Streit et al., 2005) and a decrease in photosynthetic efficiency (Zafari et al., 2017), which results in losses in terms of nutritional value of the forage.

In light of the foregoing, there is an urgent need for aligning productive means to sustainable management practices of production (Sá et al., 2019), such as the use of plants-growth promoting bacteria (PGPB) (Perez et al., 2016). The association of these microorganisms with forage plants is an innovative and promising approach. It can contribute to the sustainability of productive systems, to a decrease in the probability of pastures degradation (Hungria et al., 2016) by contributing to part of nitrogen (N) supplies required by grasses (Marques et al., 2017), and to the mitigation of the negative impacts of drought stress (Vurukonda et al., 2016).

The presence of bacteria can lead the plant to produce more genes related to stress by water deficit (WD) and, thus, allow tolerance to this condition (Kasim et al., 2012). Yet, little is known about the effects of diazotrophic microorganisms on forage of tropical climate, such as *Urochloa* spp., (Odokonyero et al., 2016), especially because most of the studies analyzed only evaluated the effects on plants' growth (Dimkpa et al., 2009).

Some studies have demonstrated the positive effects of the inoculation of PGPB in forage plants subjected to WD, through the maintenance of leaves' water potential. These effects comprise better root growth, greater accumulation of forage mass, a more efficient photosynthetic performance and plants surviving for longer (Kasim et al., 2012; Zafari et al., 2017).

The pivotal role of PGPB is evident, since they are capable of improving physiological parameters of tropical grasses under drought stress. Therefore, the aim of this study is evaluating the effect of the inoculation of plant-growth promoting bacteria

(PGPB) on the physiological responses of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* under drought stress.

MATERIAL AND METHODS

Local site and experimental design

The experiments was performed in protected environments, in greenhouse, at the State University of Maringá, Maringá, PR, Brazil (23°24'S, 51°56'W; 542 m a.m.s.l.), in two experimental cycles (Test I: November 2017-July 2018 and Test II: September 2018-May 2019).

In both Tests I and II a Ferralsol soil (Santos *et al.* 2018) was used. The soil was collected at 0-0.2 m depth, and showed the following chemical attributes: potential for hydrogen (pH in H_2O) = 5.3 and 4.8, calcium (Ca^{2+}) = 0.78 and 1.09 $cmol_c dm^{-3}$, magnesium (Mg^{2+}) = 0.52 and 0.44 $cmol_c dm^{-3}$, aluminum (Al^{3+}) = 0.13 and 0.05 $cmol_c dm^{-3}$, potassium (K^+) = 0.15 and 0.12 $cmol_c dm^{-3}$, phosphorus (P, Mehlich) = 9.16 and 20.54 mg dm^{-3}; base saturation (V) = 26.03 and 33.88%, cation exchange capacity (CEC pH 7.0) = 5.57 and 4.87 $cmol_c dm^{-3}$, organic matter (OM) = 9.11 and 9.36 g dm^{-3}; sand = 740 and 880 g kg^{-1}, silt = 40 e 20 g kg^{-1} and clay = 220 and 100 g kg^{-1}. Soil acidity correction was carried out with elevation of base saturation to 55%, with the incorporation of dolomitic limestone with RPNT = 126% (relative power of total neutralization), remaining incubated for a period of 25 days.

The pasture grass species used were *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* (Germain and Evrard). The experiment was performed in a complete randomized block design, in a 4x4 factorial scheme with four replicates, using 64 plastic plots filled with 9 dm^3 of soil for each grass species, totaling 128 plots in each experimental test (i.e. n = 128 per test).

The inoculated bacteria strains were *Azospirillum brasilense* Ab-V5 (=CNPSo 2083), *Pantoea ananantis* AMG 521 (=CNPSo 2798) and *Pseudomonas fluorescens* CCTB 03 (=CNPSo 2719), plus non-inoculated control and four water deficit levels (WD = 80, 60, 40 and 20%). All strains are deposited at the "Diazotrophic and Plant Growth-Promoting Bacteria Culture Collection of Embrapa Soja" (World Federation Culture Collection, WFCC #1213; World Data Centre for Microorganisms, WDCM #1054). The bacteria were derived as follows: *A. brasilense* Ab-V5 selected in Brazil, initially for maize and wheat (Hungria *et al.* 2010); *P. ananatis* AMG521 isolated at University of

Seville, Spain (Megías *et al.* 2016) and *P. fluorescens* CCTB 03, isolated by Total Biotecnologia (Curitiba, PR, Brazil).

For preparation of inoculant, the strains were grown in DYGS medium (Fukami *et al.* 2018), and the concentrations were adjusted to 10^8 cells mL^{-1}, according to growth curves previously obtained correlated with optical density previously obtained for each strain. For inoculation, 15 mL of each inoculant was used per kg of seeds before sowing. Seeds were dried for approximately 30 min in a cool and sun-sheltered location, after which they were seeded at 15 seeds per pot.

The pots were filled with soil, heavy, saturated with water and drained by gravity and then reweighed. Thus, the upper limit of available water (AW) [pots field capacity (θ_{PFC})] was determined by gravimetry. The permanent wilting point (θ_{PWP}) of soil was determined (-15,000 hPa) using a WP4-T Dewpoint Potentia Meter, according to Klein *et al.* (2010). Approximately 0.1 kg of soil was weighed and oven-dried at 105 °C for 24 h in order to determine the air-dried soil mass. Based on the information AW (m^3m^{-3}), calculated by the difference the values of θ_{PFC} and θ_{PWP}, it was possible to impose WD levels.

Before sowing all pots received the equivalent of 42 kg P_2O_5 ha^{-1} (simple superphosphate 18% P_2O_5) in a single dose, 141 and 169 kg K_2O ha^{-1} (potassium chloride 60% K_2O) divided into two applications, where the first was incorporated into the soil at sowing, and the second, a week after the first cut of plants, together with N-fertilization (20 kg N ha^{-1}, urea 45% N). Both fertilizations were carried out in Tests I and II, respectively, except for the P-fertilization that was not carried out in test II because the amount of P in the soil was above the optimal level working in the experiments.

About 15 seeds were sown per pot. Two weeks after seedlings emergence, they were thinned, leaving five uniform plants per pot. Three weeks after the emergence, plant cutting was performed leaving15 cm of residue. During this period, the soil moisture was kept close to the field capacity, with the subsequent imposition of WD.

During the experiment, temperature and relative humidity were monitored. The average maximum and minimum temperatures recorded in the greenhouse for the test I and II, respectively, were 32 and 40, 18 and 19 °C, respectively, with average relative air humidity of 64 and 53% (Figure 1).

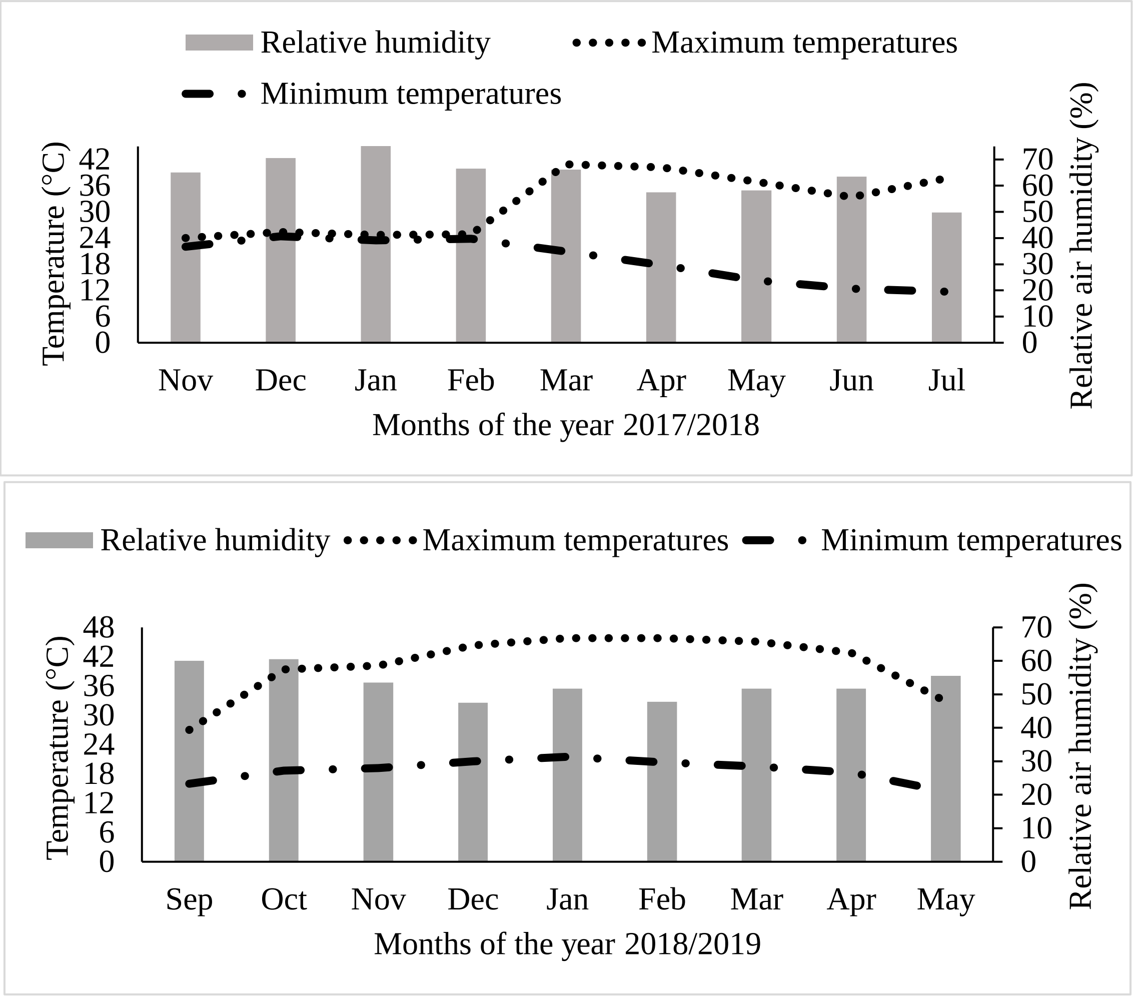

Figure 1 Climatic conditions (relative humidity, maximum and minimum temperatures) registered with the digital Thermo-Higrometer apparatus at greenhouse, State University of Maringá, Maringá, PR, Brazil, during the experimental periods.

The WD level in each treatment was maintained by weighing the pots every day, with a digital scale of 20 kg maximum capacity, with uncertainty of 0.05 kg, DST-30/P-DM model (Triunfo, São Paulo, Brazil). Replacing water was performed through polyvinyl chloride tubes (PVC, 12 cm), with perforated walls, (Beutler and Centurion 2004), installed in the center of the pot to avoid water loss by evaporation and ensure irrigation directly into the grass roots system.

Measuring the studies parameters

When the plants reached, on average, 35-40 cm in height, shoots were cut to 15 cm. Height measurements were performed three times each week by using a ruler with 1-mm increments.

Before each cut, we scanned the SPAD (Soil Plant Analysis Development) index for determining *a*, *b* and total chlorophyll. The SPAD index was determined with the last leaf, completely expanded, with a portable meter (Clorofilômetro clorofiLOG®), model CFL1030 (Falker, Rio Grande do Sul, Brasil). Each scan of the SPAD index is equivalent to the average result obtained by the measuring in leaves of three tillers from each vase.

After cutting, the material was identified and morphological components were separated (leaf blade, stem + sheath), weighed and dried in a stove with forced-air circulation, at 55 °C for 72 hours. Then, it was all weighed again for determination of dry mass and then grinded in a stationary grinder "Thomas Wiley", adapted with a 2 mm sieve.

The dry matter (DM), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (LIG), *in vitro* digestibility of the dry matter (IVDDM), nitrogen (N), crude protein (CP) and soluble carbohydrates (SCH) were quantified in the foliar blade through near-infrared reflectance spectroscopy (NIRS) (Foss NIRSystems, XDS Rapid Content Analyzer, Denmark).

For the NIRS scanning, we constructed a calibration curve based on the laboratorial analytical data of 110 samples, which were analyzed for DM and N by using the micro-Kjeldahl method (Tecator, Sewedan), and converted into protein using the 6.25 factor, according to the methodology described by the AOAC (1990).

The NDF and ADF were determined according to Van Soest et al. (1991), and LIG according to Goering and Van Soest (1970). The IVDDM was obtained through the techniques (traditional methodology) described by Tilley and Terry (1963) and Holden (1999), with the use of an artificial rumen DAISY^{II} (ANKOM™ Technology Corp., Fairport, NY). As for the SCH, it was obtained according to Hall’s (2000) adapted methodology.

For elaborating the calibration curves, the spectra of the samples were scanned with the software ISIScan and exported into the software WinISI III Project Manager 1.50e (Infrasoft International, LLC, 2000, Port Matilda, PA, USA). The reflectance data were stored with log 1/R in intervals of 2 nm between 700 and 2500 nm.

The principal component analysis (PCA) was done before the calibration curves were elaborated, by using the regression model of the partial least squares (PLS). Cross validation was performed with the software confronting the laboratorial analysis data, and those estimated by the calibration curve of the NIRS. After the elimination of the outliers, we used 72 samples for DM, 79 for NDF, 79 for ADF, 73 for LIG, 71 for

IVDDM, 79 for N, 77 for CP and 80 for SCH. For the DM, NDF, ADF, LIG, IVDDM, N, CP and SCH, respectively, we obtained the following parameters for validating of the curve: coefficient of correlation for calibration (R^2 = 0.88, 0.92, 0.94, 0.79, 0.86, 0.99, 0.99 and 0.90), standard error of cross-validation (SECV = 0.38, 1.41, 0.66, 0.17, 4.67, 0.07, 0.48 and 0.28%), R^2 coefficient of determination for cross validation (1-VR = 0.87, 0.87, 0.90, 0.60, 0.73, 0.99, 0.99 and 0.82), error of prediction (SEP = 0.45, 1.05, 0.48, 0.15, 3.37, 0.10, 0.35 and 0.26) and mean±standard deviation of values measured (X±SD = 90.63±1.04, 51.49±3.93, 24.69±2.12, 1.74±0.27, 68.54±8.68, 1.94±0.69, 12.15±4.33 and 3.52±0.66).

The determination of accumulation of total nitrogen (ATN g.kg) in the aerial part was obtained by multiplying the nitrogen concentration (g.kg) by dry mass (g) collected in each pot.

Statistical analyses

The statistical analyses were performed with the PROC GLIMMIX of the statistical package SAS version 9.2 (SAS Institute, Cary, NC, USA). Analyzes were performed after the grouping of averages of Tests I and II. Data for each grass were analyzed separately. In the analysis, physiological parameters were considered as fixed effects, whereas blocks and tests were considered as random effects. The cuts within each Test was analyzed as a repeated measurement, and the covariance structure was selected based on the smallest Akaike Information Criterion value (Littell et al., 1998). Data were tested for residues normality (Shapiro-Wilk test, Shapiro and Wilk, 1965) and variances homogeneity (Bartlett test, Bartlett, 1950). The linear and quadratic effects of water deficit levels were evaluated by orthogonal contrasts, and when observed significant effect of inoculation, the means of each bacterium were compared using the LSMeans Pdiff command from SAS software ($P \leq 0.1$).

RESULTS

The percentages of dry matter of Paiaguás and Ruziziensis grasses were not influenced by the inoculation of PGPB (91.33 and 91.45% on average, respectively). There was influence of WD on the DM of Paiaguás (cut 2, p = 0.0533) and Ruziziensis (cuts 2 and 3, p = 0.0024 and 0.0258, respectively). For Paiaguás and Ruziziensis, the average levels of DM were 91,33 and 91, 44%, respectively.

The average percentages of neutral detergent fiber (NDF % in the DM) and acid detergent fiber (ADF % in the DM), lignin (LIG % in the DM) and crude protein (CP % in the DM) are presented in Table 1. There were no effects of the inoculation of PGPB on NDF, ADF and LIG results in both grasses.

In the NDF, we verified an effect of the WD on Paiaguás (cuts 1, 2, 3 and 5; p = 0.0652, 0.0038, 0.0458 and 0.0076, respectively) and in Ruziziensis (cuts 1, 3 and 4; p = 0.0717, 0.0324 and 0.0286, respectively).

As for the ADF, the WD had influence on Paiaguás (cuts 2, 3 and 5; p = <. 0001, 0.0007 and 0.0715, respectively) and Ruziziensis (cuts 1, 2, 3 and 4; p = 0.0007, 0.0327, 0.0024 and <.0001, respectively). For LIG, the WD had effects only on Ruziziensis (cuts 1 and 4; p = 0.0166 and 0.0650, respectively).

There was an adjustment of the aforementioned variables to the regression model, revealing the effect of the WD over both grasses. Regarding the NDF, ADF and LIG, there was an increasing linear behavior, which made more water available to the grasses.

The inoculation of PGPB had no effects on the CP of both grasses (Table 1). For WD imposition, we observed the influence on the levels of CP in Paiaguás (cuts 3 and 5; p = 0.0110 and 0.0101, respectively) and Ruziziensis (cuts 3 and 4; p = 0.0165 and 0.0056, respectively). The effect of the WD on CP adjusted to the linear regression model, thus, demonstrating a decreasing behavior with a reduction of the WD and, consequently, an increase in water availability.

Table 1 Neutral detergent fiber (NDF % in the DM), acid detergent fiber (ADF % in the DM), lignin (LIG % in the DM) and crude protein (CP % in the DM) of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* inoculated with plant growth promoting bacteria (PGPB) under water deficit (WD%).

		Urochloa brizantha cv. BRS Paiaguás					*Urochloa ruziziensis*			
		Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Cut 1	Cut 2	Cut 3	Cut 4
---------Neutral Detergent Fiber (% in the DM)---------										
PGPB	Ab-V5	55.30±0.57	56.94±0.76	57.44±1.46	61.13±1.69	64.88±1.89	57.42±1.42	59.54±0.96	63.01±0.47	63.61±0.62
	AMG 521	55.06±0.57	56.41±0.76	59.07±1.46	60.88±1.69	64.33±1.89	58.35±1.42	59.38±0.96	63.54±0.47	64.21±0.62
	CCTB 03	54.91±0.57	56.50±0.76	58.95±1.46	61.41±1.69	64.32±1.89	58.27±1.42	59.91±0.96	62.99±0.47	64.16±0.62
	non-inoculated	54.57±0.57	56.44±0.76	58.94±1.46	60.69±1.69	64.67±1.89	58.54±1.42	59.38±0.96	63.15±0.47	63.80±0.62
	P value	0.8353	0.9430	0.8740	0.9861	0.9724	0.9462	0.9700	0.8226	0.8429
WD (%)	80	54.04±0.57	55.25±0.76	57.25±1.46	59.10±1.69	62.63±1.89	55.94±1.42	58.67±0.96	62.51±0.47	63.37±0.62
	60	54.79±0.57	55.91±0.76	57.60±1.46	61.01±1.69	63.89±1.89	57.77±1.42	59.23±0.96	63.21±0.47	63.45±0.62
	40	55.36±0.57	57.14±0.76	59.78±1.46	61.98±1.69	65.49±1.89	59.22±1.42	59.86±0.96	63.80±0.47	63.74±0.62
	20	55.66±0.57	57.97±0.76	60.19±1.46	62.01±1.69	66.19±1.89	59.66±1.42	60.40±0.96	64.17±0.47	65.21±0.62
P value	L	0.0652	0.0038	0.0458	0.1383	0.0076	0.0717	0.1444	0.0324	0.0286
	Q	0.3614	0.8970	0.9809	0.5191	0.7762	0.4294	0.9670	0.4760	0.1584
--------- Acid Detergent Fiber (% in the DM)---------										
PGPB	Ab-V5	26.26±1.30	25.86±0.40	28.34±0.82	28.02±0.85	29.10±0.88	26.85±1.20	26.10±0.82	25.74±0.55	26.98±0.30
	AMG 521	27.13±1.30	25.68±0.40	28.71±0.82	27.41±0.85	29.01±0.88	27.87±1.20	26.21±0.82	25.55±0.55	27.46±0.30
	CCTB 03	26.83±1.30	25.07±0.40	28.91±0.82	28.06±0.85	28.69±0.88	27.58±1.20	26.16±0.82	25.81±0.55	26.90±0.30
	non-inoculated	26.68±1.30	25.71±0.40	29.17±0.82	27.62±0.85	29.09±0.88	27.77±1.20	26.23±0.82	25.74±0.55	26.59±0.30
	P value	0.9490	0.1841	0.6185	0.8360	0.9326	0.6676	0.9995	0.9886	0.2428
WD (%)	80	25.93±1.30	24.71±0.40	27.70±0.82	27.08±0.85	28.19±0.88	25.50±1.20	24.70±0.82	24.40±0.55	26.23±0.30
	60	26.76±1.30	25.54±0.40	28.24±0.82	27.84±0.85	28.97±0.88	27.38±1.20	26.03±0.82	25.47±0.55	26.65±0.30
	40	27.40±1.30	25.71±0.40	29.42±0.82	28.21±0.85	29.22±0.88	28.50±1.20	26.73±0.82	26.15±0.55	27.00±0.30
	20	27.21±1.30	26.37±0.40	29.76±0.82	27.97±0.85	29.50±0.88	28.69±1.20	27.20±0.82	26.81±0.55	28.06±0.30

P value	L	0.1145	<.0001	0.0007	0.2514	0.0715	0.0007	0.0327	0.0024	<.0001
	Q	0.4181	0.7564	0.8236	0.3991	0.6246	0.1114	0.6205	0.7177	0.2953
--Lignin (% in the DM)--										
PGPB	Ab-V5	2.06±0.07	1.80±0.06	1.80±0.07	1.64±0.05	1.55±0.03	2.10±0.09	1.66±0.04	1.59±0.02	1.62±0.01
	AMG 521	2.12±0.07	1.80±0.06	1.77±0.07	1.66±0.05	1.54±0.03	2.22±0.09	1.67±0.04	1.60±0.02	1.63±0.01
	CCTB 03	2.09±0.07	1.76±0.06	1.77±0.07	1.66±0.05	1.56±0.03	2.21±0.09	1.68±0.04	1.60±0.02	1.62±0.01
	non-inoculated	2.04±0.07	1.81±0.06	1.79±0.07	1.61±0.05	1.52±0.03	2.20±0.09	1.76±0.04	1.60±0.02	1.61±0.01
	P value	0.8393	0.9280	0.9885	0.9081	0.9170	0.5992	0.1693	0.9947	0.8887
WD (%)	80	1.99±0.07	1.78±0.06	1.77±0.07	1.63±0.05	1.51±0.03	1.99±0.09	1.67±0.04	1.59±0.02	1.55±0.01
	60	2.07±0.07	1.80±0.06	1.78±0.07	1.65±0.05	1.53±0.03	2.22±0.09	1.71±0.04	1.59±0.02	1.57±0.01
	40	2.14±0.07	1.80±0.06	1.79±0.07	1.64±0.05	1.55±0.03	2.27±0.09	1.71±0.04	1.60±0.02	1.59±0.01
	20	2.12±0.07	1.79±0.06	1.81±0.07	1.66±0.05	1.58±0.03	2.29±0.09	1.68±0.04	1.61±0.02	1.63±0.01
P value	L	0.1322	0.9169	0.6931	0.6673	0.1618	0.0166	0.8124	0.4725	0.0650
	Q	0.4549	0.8388	0.9507	0.9773	0.9639	0.1082	0.3343	0.6909	0.1069
---Crude Protein (% in the DM)---										
PGPB	Ab-V5	20.31±1.69	15.98±1.40	12.77±1.47	11.03±1.50	8.15±1.61	14.80±2.36	11.69±1.16	9.54±0.47	7.11±0.69
	AMG 521	20.06±1.69	16.86±1.40	11.93±1.47	11.36±1.50	8.61±1.61	14.01±2.36	11.16±1.16	9.78±0.47	6.47±0.69
	CCTB 03	20.08±1.69	17.97±1.40	11.72±1.47	10.36±1.50	8.31±1.61	14.37±2.36	10.85±1.16	9.58±0.47	6.54±0.69
	non-inoculated	20.06±1.69	16.90±1.40	12.21±1.47	11.69±1.50	8.78±1.61	14.12±2.36	11.75±1.16	9.54±0.47	6.62±0.69
	P value	0.9933	0.5442	0.9075	0.8057	0.9330	0.9856	0.9132	0.9811	0.8036
WD %)	80	20.27±1.69	17.86±1.40	13.76±1.47	12.78±1.50	10.04±1.61	15.48±2.36	11.65±1.16	10.45±0.47	7.65±0.69
	60	20.17±1.69	17.10±1.40	13.53±1.47	11.01±1.50	8.69±1.61	14.79±2.36	11.63±1.16	9.66±0.47	6.81±0.69
	40	19.74±1.69	16.61±1.40	10.85±1.47	9.97±1.50	7.78±1.61	13.70±2.36	11.38±1.16	9.62±0.47	6.80±0.69
	20	20.34±1.69	16.15±1.40	10.47±1.47	10.68±1.50	7.34±1.61	13.32±2.36	10.79±1.16	8.72±0.47	5.47±0.69
P value	L	0.9454	0.1942	0.0110	0.1074	0.0101	0.2911	0.5370	0.0165	0.0056
	Q	0.6365	0.8747	0.9350	0.2191	0.5472	0.9200	0.7843	0.9077	0.6299

DM = dry matter; *Azospirillum brasilense* Ab-V5; *Pantoea ananantis* AMG 521; *Pseudomonas fluorescens* CCTB 03; Regression (L = linear and Q = quadratic). Date are means ± SEM = standard error of mean (n = 4). Means followed by different lowercase letters in each line are significantly different (P < 0.1, LSMeans test).

The average results of *in vitro* digestibility of dry matter (IVDDM % in the DM) are shown in Table 2. The inoculation of PGPB had an effect on Paiaguás (cut 4; p = 0.0789), with greater percentages of IVDDM with the inoculation of the strains Ab-V5 and AMG521, and for the control treatment, when compared to the results of CCTB03.

Table 2 *In vitro* digestibility of dry matter (IVDDM % in the DM) of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* inoculated with plant growth promoting bacteria (PGPB) under water deficit (WD%).

-----------------------------*Urochloa brizantha* cv. BRS Paiaguás----------------------------

		Cut 1	Cut 2	Cut 3	Cut 4	Cut 5
PGPB	Ab-V5	72.45±2.84	71.87±8.46	74.98±1.68	74.44±1.80ab	78.29±2.63
	AMG 521	70.87±2.84	72.29±8.46	75.01±1.68	74.55±1.80ab	79.85±2.63
	CCTB 03	73.54±2.84	74.36±8.46	74.34±1.68	72.51±1.80b	77.42±2.63
	non-inoculated	74.37±2.84	73.52±8.46	74.16±1.68	77.58±1.80a	79.52±2.63
	P value	0.7967	0.9596	0.9742	0.0789	0.8157
WD (%)	80	76.14±2.84	74.62±8.46	77.44±1.68	79.13±1.80	86.01±2.63
	60	73.94±2.84	73.18±8.46	76.77±1.68	74.58±1.80	81.90±2.63
	40	70.23±2.84	73.06±8.46	73.05±1.68	74.30±1.80	75.00±2.63
	20	70.92±2.84	71.19±8.46	71.22±1.68	71.07±1.80	72.17±2.63
P value	L	0.1019	0.9309	0.0033	0.0002	<.0001
	Q	0.5784	0.9922	0.7237	0.6267	0.7527

-----------------------------------*Urochloa ruziziensis*---------------------------------------

		Cut 1	Cut 2	Cut 3	Cut 4
PGPB	Ab-V5	73.31±2.14	69.19±2.58	62.10±2.23	53.38±1.34
	AMG 521	71.26±2.14	69.92±2.58	61.70±2.23	52.48±1.34
	CCTB 03	69.76±2.14	68.03±2.58	62.21±2.23	53.51±1.34
	non-inoculated	70.05±2.14	67.16±2.58	64.59±2.23	54.13±1.34
	P value	0.6407	0.6304	0.7922	0.8570
WD (%)	80	77.65±2.14	69.94±2.58	64.51±2.23	56.16±1.34
	60	70.67±2.14	68.18±2.58	63.26±2.23	53.47±1.34
	40	68.91±2.14	68.84±2.58	61.82±2.23	53.16±1.34
	20	67.16±2.14	67.33±2.58	61.01±2.23	50.70±1.34
P value	L	0.0021	0.3216	0.2360	0.0077
	Q	0.1082	0.9398	0.9217	0.9332

DM = dry matter; *Azospirillum brasilense* Ab-V5; *Pantoea ananantis* AMG 521; *Pseudomonas fluorescens* CCTB 03; Regression (L = linear and Q = quadratic). Date are means ± SEM = standard error of mean (n = 4). Means followed by different lowercase letters in each line are significantly different (P < 0.1, LSMeans test).

The WD influenced the percentage of IVDDM in Paiaguás (cuts 3, 4 and 5; p = 0.0033, 0.0002 and <.0001, respectively) and in Ruziziensis (cuts 1 and 4; p = 0.0021 and 0.0077, respectively). There was an adjustment to the regression model, revealing the effect of the WD over both grasses. It was verified that, for the IVDDM, there was a decreasing linear behavior with a reduction of the WD (80% to 20%).

The average results of the accumulation of total nitrogen (ATN g.kg) in the aerial part of the grasses are presented in Table 3. The inoculation of PGPB had no effects on the results of the ATN in both grasses.

Table 3 Accumulation of total nitrogen (ATN g.kg) in the aerial part of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* inoculated with plant growth promoting bacteria (PGPB) under water deficit (WD%).

----------------------------*Urochloa brizantha* cv. BRS Paiaguás----------------------------

		Cut 1	Cut 2	Cut 3	Cut 4	Cut 5
PGPB	Ab-V5	0.49±0.06	0.47±0.04	0.40±0.04	0.30±0.05	0.23±0.05
	AMG 521	0.49±0.06	0.52±0.04	0.38±0.04	0.29±0.05	0.24±0.05
	CCTB 03	0.48±0.06	0.52±0.04	0.36±0.04	0.28±0.05	0.24±0.05
	non-inoculated	0.48±0.06	0.52±0.04	0.39±0.04	0.30±0.05	0.24±0.05
	P value	0.9081	0.6791	0.8123	0.9537	0.9959
WD (%)	80	0.47±0.06	0.47±0.04	0.41±0.04	0.33±0.05	0.26±0.05
	60	0.49±0.06	0.50±0.04	0.44±0.04	0.29±0.05	0.24±0.05
	40	0.49±0.06	0.54±0.04	0.35±0.04	0.27±0.05	0.23±0.05
	20	0.49±0.06	0.52±0.04	0.34±0.04	0.27±0.05	0.22±0.05
P value	L	0.3324	0.2133	0.0654	0.0980	0.2217
	Q	0.4006	0.4169	0.5620	0.4596	0.7384

-------------------------------------*Urochloa ruziziensis*-------------------------------------

		Cut 1	Cut 2	Cut 3	Cut 4
PGPB	Ab-V5	0.40±0.05	0.32±0.04	0.22±0.02	0.15±0.01
	AMG 521	0.37±0.05	0.31±0.04	0.23±0.02	0.14±0.01
	CCTB 03	0.39±0.05	0.32±0.04	0.23±0.02	0.14±0.01
	non-inoculated	0.39±0.05	0.33±0.04	0.23±0.02	0.14±0.01
	P value	0.9509	0.9895	0.9914	0.8951
WD (%)	80	0.41±0.05	0.30±0.04	0.24±0.02	0.17±0.01
	60	0.39±0.05	0.33±0.04	0.23±0.02	0.14±0.01
	40	0.38±0.05	0.33±0.04	0.23±0.02	0.14±0.01
	20	0.37±0.05	0.32±0.04	0.21±0.02	0.13±0.01
P value	L	0.2714	0.8197	0.1676	0.0475
	Q	0.9326	0.6566	0.8985	0.8276

Azospirillum brasilense Ab-V5; *Pantoea ananantis* AMG 521; *Pseudomonas fluorescens* CCTB 03; Regression (L = linear and Q = quadratic). Date are means ± SEM = standard error of mean (n = 4). Means followed by different lowercase letters in each line are significantly different (P < 0.1, LSMeans test).

The WD had influence on the concentration of the ATN in Paiaguás (cuts 3 and 4; p = 0.0654 and 0.0980, respectively) and Ruziziensis (cut 4; p = 0.0475). There were effects of the WD on the ATN, with an adjustment to the linear regression model, demonstrating a decreasing behavior with an increase in water availability.

The average percentages of soluble carbohydrates (SCH % in the DM) are presented in Table 4. The inoculation of PGPB had no effects on the levels of SCH in Paiaguás and Ruziziensis.

Table 4 Soluble carbohydrates (SCH % in the DM) of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* inoculated with plant growth promoting bacteria (PGPB) under water deficit (WD%).

------------------------------*Urochloa brizantha* cv. BRS Paiaguás--------------------------						
		Cut 1	Cut 2	Cut 3	Cut 4	Cut 5
PGPB	Ab-V5	3.37±0.12	3.91±0.12	3.94±0.16	4.11±0.17	4.41±0.12
	AMG521	3.26±0.12	3.89±0.12	3.87±0.16	4.04±0.17	4.44±0.12
	CCTB03	3.29±0.12	4.00±0.12	3.84±0.16	3.99±0.17	4.44±0.12
	non-inoculated	3.39±0.12	3.80±0.12	3.76±0.16	4.07±0.17	4.40±0.12
	P value	0.7101	0.6831	0.8827	0.9485	0.9828
WD (%)	80	3.43±0.12	4.03±0.12	4.04±0.16	4.36±0.17	4.58±0.12
	60	3.33±0.12	3.97±0.12	4.01±0.16	4.00±0.17	4.48±0.12
	40	3.28±0.12	3.78±0.12	3.79±0.16	3.98±0.17	4.41±0.12
	20	3.27±0.12	3.83±0.12	3.55±0.16	3.88±0.17	4.21±0.12
P value	L	0.2351	0.1012	0.0245	0.0373	0.0055
	Q	0.6345	0.6376	0.5146	0.4063	0.5876

-----------------------------------*Urochloa ruziziensis*---------------------------------------					
		Cut 1	Cut 2	Cut 3	Cut 4
PGPB	Ab-V5	3.45±0.12	4.30±0.12	4.40±0.13	5.50±0.09
	AMG521	3.21±0.12	4.20±0.12	4.28±0.13	5.37±0.09
	CCTB03	3.26±0.12	4.12±0.12	4.37±0.13	5.47±0.09
	non-inoculated	3.29±0.12	4.05±0.12	4.34±0.13	5.42±0.09
	P value	0.3850	0.3879	0.9174	0.6302
WD (%)	80	3.47±0.12	4.60±0.12	4.52±0.13	5.31±0.09
	60	3.27±0.12	4.10±0.12	4.39±0.13	5.50±0.09
	40	3.26±0.12	4.07±0.12	4.31±0.13	5.45±0.09
	20	3.20±0.12	3.99±0.12	4.17±0.13	5.50±0.09
P value	L	0.0856	0.0088	0.0560	0.1269
	Q	0.4845	0.1043	0.9747	0.3470

DM = dry matter; *Azospirillum brasilense* Ab-V5; *Pantoea ananantis* AMG 521; *Pseudomonas fluorescens* CCTB 03; Regression (L = linear and Q = quadratic). Date are means ± SEM = standard error of mean (n = 4). Means followed by different lowercase letters in each line are significantly different (P < 0.1, LSMeans test).

There were effects of the WD on the SCH in Paiaguás (cuts 3, 4 and 5; p = 0.0245, 0.0373 and 0.0055, respectively) and Ruziziensis (cuts 1, 2 and 3; p = 0.0856, 0.0088 and 0.0560, respectively). For this variable, there was an adjustment to the regression model with a decreasing linear behavior, with a reduction of the WD.

The average values registered for the relative content of nitrogen (SPAD index) represented by chlorophyll (Chl), are shown in Table 5. There were effects of the inoculation of PGPB on the SPAD index, with influence on Chl *a* – SPAD (cut 1; p = 0.0312, 0.0569) of Paiaguás and Ruziziensis, respectively, Chl b – SPAD (cut 1; p = 0.0004) of Paiaguás and total Chl (cut 1; p = 0.0774) of Ruziziensis (Table 5).

For the Chl of Paiaguás grass, the greatest value was found in the control treatment (33.7) compared to the inoculated treatments (an average of 32.2). In Ruziziensis, the smallest value occurred with the inoculation of the strain Ab-V5 (22.1). In Chl *b*, the greatest value was found in the inoculation of strain Ab-V5 and the control treatment (12.8 and 12.7, respectively). For the total Chl, the smallest value was registered for strain Ab-V5 (31.0).

There were effects of the WD on Chl *a* – SPAD (cut 1; p = <.0001) in Ruziziensis. In Chl *b* – SPAD (cuts 1 and 2; p = 0.0085 and 0.0327, respectively) of Paiaguás and (cuts 1 and 4; p = <.0001 and 0.0923, respectively) of Ruziziensis in the total Chl (cut 1; p = <.0001) of Ruziziensis. All the results observed in the SPAD index demonstrated adjustment to the regression model with decreasing linear behavior as the WD decreased (80 to 20%), thus revealing the influence of water quality in the soil for the development of the grasses under study.

There was interaction (p = 0.0148) between the PGPB and the WD for total Chl – SPAD evaluated in the leaves of Paiaguás (Table 6). The greatest value of total Chl – SPAD observed at the highest level of restriction in terms of water availability (80%) was found in strain Ab-V5. For the WD level of 60%, strain AMG 251 and the control treatment contributed to a greater value of total Chl – SPAD, whereas at the WD level of 40%, there were greater values for the inoculation of strains AMG521 and CCTB03. For the WD level of 20%, the greatest value of total Chl – SPAD was found in the control treatment.

Table 5 SPAD index (chlorophyll = Chl *a*, *b* and total) quantified in the foliar blade of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* inoculated with plant growth promoting bacteria (PGPB) under water deficit (WD%).

		Urochloa brizantha cv. BRS Paiaguás					*Urochloa ruziziensis*			
		Cut 1	Cut 2	Cut 3	Cut 4	Cut 5	Cut 1	Cut 2	Cut 3	Cut 4
Chl *a* – SPAD										
PGPB	Ab-V5	32.60±0.64b	28.69±0.86	23.54±1.06	21.71±1.33	20.00±1.06	24.11±0.52b	20.63±0.85	17.90±0.89	16.95±0.41
	AMG 521	31.92±0.64b	27.17±0.85	21.57±1.06	21.74±1.33	19.39±1.06	24.97±0.52ab	20.35±0.83	18.18±0.89	16.57±0.40
	CCTB 03	31.98±0.64b	28.57±0.85	21.97±1.06	20.96±1.33	19.33±1.06	25.83±0.52a	20.92±0.83	17.95±0.89	17.02±0.40
	non-inoculated	33.73±0.64a	28.73±0.86	22.85±1.07	21.04±1.35	18.68±1.07	24.85±0.52ab	21.48±0.83	18.52±0.89	16.95±0.40
	P value	0.0312	0.2188	0.4147	0.9173	0.6245	0.0569	0.7314	0.8594	0.8563
WD (%)	80	32.50±0.64	28.52±0.85	22.71±1.06	22.61±1.33	19.87±1.06	27.81±0.52	20.96±0.83	18.42±0.89	16.52±0.41
	60	32.85±0.64	28.53±0.86	22.76±1.06	21.10±1.33	18.66±1.06	25.56±0.52	20.95±0.85	17.82±0.89	16.17±0.40
	40	32.58±0.64	28.59±0.86	21.79±1.07	20.52±1.35	19.77±1.07	23.29±0.52	20.65±0.83	17.85±0.89	17.02±0.40
	20	32.30±0.64	27.53±0.85	22.66±1.06	21.22±1.33	19.12±1.06	23.12±0.52	20.83±0.83	18.46±0.89	16.95±0.40
P value	L	0.6810	0.2934	0.7862	0.2973	0.7161	<.0001	0.8375	0.9500	0.1919
	Q	0.5115	0.3898	0.6502	0.2807	0.6898	0.1930	0.8937	0.2853	0.6978
Chl *b* – SPAD										
PGPB	Ab-V5	12.79±0.35a	9.83±0.56	6.67±0.50	5.77±0.66	4.64±0.49	6.16±0.34	5.23±0.43	3.73±0.35	3.84±0.11
	AMG 521	11.53±0.35b	9.34±0.56	5.67±0.50	5.82±0.66	4.35±0.49	6.24±0.34	4.94±0.43	3.99±0.35	3.72±0.10
	CCTB 03	11.31±0.35b	9.41±0.56	5.87±0.50	5.53±0.66	4.35±0.49	6.97±0.34	5.39±0.43	3.69±0.35	3.72±0.11
	non-inoculated	12.66±0.36a	9.43±0.56	6.23±0.51	5.41±0.66	4.05±0.49	6.39±0.34	5.71±0.43	4.12±0.35	3.68±0.10
	P value	0.0004	0.8596	0.4928	0.8979	0.5198	0.1979	0.6169	0.3705	0.6170
WD (%)	80	12.66±0.35	10.41±0.56	6.30±0.50	6.16±0.66	4.57±0.49	8.07±0.34	5.23±0.43	4.04±0.35	3.86±0.11
	60	12.24±0.35	9.73±0.56	6.14±0.50	5.61±0.66	4.05±0.49	6.75±0.34	5.41±0.43	3.73±0.35	3.81±0.11
	40	11.77±0.36	9.13±0.56	5.73±0.51	5.22±0.66	4.56±0.49	5.51±0.34	5.34±0.43	3.75±0.35	3.72±0.10

	20	11.62±0.35	8.74±0.56	6.27±0.50	5.53±0.66	4.20±0.49	5.44±0.34	5.31±0.43	4.03±0.35	3.57±0.10
P value	L	0.0085	0.0327	0.8191	0.2428	0.6366	<.0001	0.9206	0.9829	0.0923
	Q	0.6618	0.2252	0.4738	0.3274	0.7753	0.3410	0.7997	0.1614	0.2815
--Chl total – SPAD--										
PGPB	Ab-V5	-	38.52±1.32	30.20±1.53	27.48±1.98	24.64±1.55	31.04±0.81b	25.87±1.25	21.62±1.24	20.77±0.51
	AMG 521	-	36.51±1.30	27.24±1.53	27.55±1.98	23.74±1.55	31.98±0.81ab	25.29±1.23	22.18±1.24	20.29±0.50
	CCTB 03	-	37.98±1.30	27.84±1.53	26.49±1.98	23.69±1.55	33.54±0.81a	26.30±1.23	21.65±1.24	20.31±0.51
	non-inoculated	-	38.36±1.32	29.08±1.56	26.45±2.00	22.73±1.66	31.99±0.81ab	27.20±1.23	22.65±1.24	20.23±0.50
	P value	-	0.4437	0.4345	0.9128	0.5894	0.0774	0.6787	0.7396	0.8242
WD (%)	80	-	38.25±1.30	29.01±1.53	28.77±1.98	24.44±1.55	36.63±0.82	26.19±1.23	22.48±1.24	20.22±0.51
	60	-	38.94±1.32	28.90±1.53	26.72±1.98	22.71±1.55	33.06±0.82	26.35±1.25	21.55±1.24	19.74±0.51
	40	-	37.72±1.32	27.52±1.56	25.74±2.00	24.33±1.56	29.56±0.82	25.98±1.23	21.59±1.24	20.83±0.50
	20	-	36.47±1.30	28.94±1.53	26.75±1.98	23.32±1.55	29.32±0.82	26.14±1.23	22.48±1.24	20.82±0.50
P value	L	-	0.1321	0.7969	0.2759	0.6924	<.0001	0.9199	0.9877	0.1588
	Q	-	0.3204	0.5816	0.2901	0.7119	0.1530	0.9960	0.2351	0.5940

Azospirillum brasilense Ab-V5; *Pantoea ananantis* AMG 521; *Pseudomonas fluorescens* CCTB 03; Regression (L = linear and Q = quadratic). Date are means ± SEM = standard error of mean (n = 4). Means followed by different lowercase letters in each line are significantly different (P < 0.1, LSMeans test).

Table 6 Effect of interaction between plant-growth promoting bacteria (PGPB) and water deficit level (WD %) on the SPAD index (total chlorophyll) determined at the first evaluation (cut 1) of *Urochloa brizantha* cv. BRS Paiaguás.

PGPB	Water Deficit Level (%)				SEM
	80	60	40	20	
Ab-V5	48,87Aa	45,67Bb	44,40Ba	45,60Bab	1,400
AMG521	42,47Ab	45,15Ab	44,22Aa	44,95Ab	1,400
CCTB03	45,85ABa	42,82BCb	46,20Aa	41,27Cc	1,400
non-inoculated	46,40Ba	49,67Aa	45,75Ba	46,80ABa	1,400

Azospirillum brasilense Ab-v5; *Pantoea ananatis* AMG521; *Pseudomonas fluorescens* CCTB03. Nível de significância (P<0,1). Date are means ± SEM = standard error of mean (n = 4). Means followed by different lowercase letters in the column and uppercase letters in the line are significantly different (P < 0.1, LSMeans test).

DISCUSSION

The absence of effects of PGPB inoculation in the variables referring to the nutritious value in the grasses covered by the study (NDF, ADF, LIG Table 1; and SCH Table 4), with the exception of the IVDDM (Table 2) and Chl – SPAD (Table 5), can be due to several factors.

The non-influence of the association of the strains with Paiaguás and Ruziziensis grasses can be explained by the use of inadequate combinations of strain/grass, since it is known that these microorganisms do not respond to all grass species. Moreover, according to Taketani et al. (2017), the occurrence of WD can have a negative impact on the activities of some microorganisms, inactivating the groups that are more sensitive to such condition.

Another factor that must be taken into consideration is the use of the basal dose of N-fertilizer of 20kg ha^{-1} in this study. It may not have been enough to activate bacterial activity, reducing rhizosphere colonization, as reported by Marschner et al. (2006).

Although in our study there was no effect of the inoculation of PGPB on the percentage of DM in Paiaguás and Ruziziensis, Odokonyero et al. (2016) pointed that the association between grass and PGPB demonstrates a potential to increment the percentage of DM. Besides, it can be an important tool for production systems in semiarid zones.

The level of WD can lead to physiological consequences, such as a decrease in the percentage of DM, due to the limitation imposed by the WD on grasses. Díaz et al. (2004) emphasize that the percentage of foliar DM points to the strategy of resources used by the plant regarding the efficiency of nutrients assimilation and conservation.

According to Jongebloed et al. (2004), the percentage of foliar DM can be reduced by the percentage of non-structural carbohydrates, such as sugars and proteins. A similar response was found in this study, where CP (Table 1) and SCH (Table 4) demonstrate a behavior that was opposite to the level of DM, that is, the greater the DM percentage, the smallest the content of non-fibrous components.

The increase in sugar production can mean a decrease in the performance regarding forage mass, as observed by Zafari et al. (2017). Likewise, accumulation of sugar also means that the grass is using this adaptive mechanism as an osmotic adjustment, which will lead to more tolerance to draught stress (Kaushal and Wani, 2016).

Another factor that contributes to the alteration in nutritious value of the *Urochloas* sp. under study can be related not only to the greater increment of DM, but also to the increase in fibrous fractions of the grass (NDF and ADF; Table 1), allied to the maturity stage and the imposition of WD.

The accumulation of DM happens due to its deposition, especially in the cell wall, followed by the association of lignin to the fibrous structure of the cell wall (Farina, 2011). Due to the increase in components that are slightly digestible or non-digestible by animals, the nutritious value of the grass is reduced (Cano et al., 2004), as well the digestibility of the food, due to the strong negative correlation among these components (Mahyuddin, 2008), and that will certainly lead to less cattle productivity.

CP and ATN values in this study were higher in the first cuts in comparison to the subsequent ones. There was a variation in CP of 20.1 to 8.5 and 14.3 to 6.7% (Table 1), and the same applied to the ATN, with a variation of 0.54 to 0.22 and 0.41 to 0.13 g.kg (Table 3) for Paiaguás and Ruziziensis, respectively.

The highest content of N and, consequently, of CP found in the first cuts of Paiaguás and Ruziziensis is due to the fact that the foliar blades were possibly younger and, thus, they demonstrate higher concentrations of this nutrient. Yet, as the plant reaches foliar maturity, the amount of this nutrient tends to be reduced in the cellular content, along with a gradual increase in the cell wall and tissues lignification, mainly because of the advancement of leaf senescence with time.

The nitrogen reduction in the aerial part of the grasses was possibly due to the fact that N-fertilizer was not reapplied in every mass cut, plus the gradual decrease in organic matter found in the soil of the experimental vases. This may have stimulated the PGPB to compete with the grasses for N consumption.

Minson (1990) emphasizes that tropical grasses can often present CP percentages that are inferior to 10%. The author also points that this value may be related to the photosynthetic “C4” metabolism pathway, due to the greater presence of stem and vascular bundles in the foliar blade.

The stress caused by WD has influence on the metabolism of proteins (Vaseva et al., 2012), with effects on the increase in N content of the leaf (Valim et al., 2016). The data presented by this study corroborate this information, since we verified an increment in the percentage of N and CP as there was more WD imposition on the grasses. However, it is important to highlight that such increase is due to the fact that turgidity loss of the plant cells leads to a lower dilution of N inside the plant cell, thus, increasing its concentration. The same behavior is observed in other components of the cellular content.

According to Dechen and Nachtigall (2007), the percentage of N in the plant can range from 2 and 75 g kg^{-1} in the DM, with values that are considered appropriate for the plant’s growth between 20 and 50 g kg^{-1}. Plants that grow within low concentrations of N present light green pigmentation due to the generalized chlorosis in the leaf, especially the oldest ones (Cecato et al., 2011). Therefore, in conditions under which there is the amount of N that is necessary to sustain the growth potential, a plant achieves a better performance in terms of forage mass (Sandaña et al., 2019).

Studies report that N deficiency reduces a plant’s photosynthetic rate, thus, compromising the action of rubisco enzyme, which withholds most of the N that the leaf contains (Carelli et al., 1996), and compromising also several physiological processes, such as biosynthesis of proteins and chlorophyll (Taiz and Zeiger, 2013), cell multiplication and differentiation, with direct or indirect interference with the plant’s development (Malavolta et al., 1997).

Currently, the status of N in a plant has been deduced based on the level of chlorophyll (Guimarães et al., 2016), so much so that studies have been positively correlating the level of such pigment with the yield of some plants (Rocha et al., 2005). It is also used for determining a need for using N-fertilizer in agricultural crops (Reis Jr et al., 2008).

The absence of effect of the inoculation of PGPB in variables such as CP (Table 1), ATN (Table 3) and photosynthetic pigments (Table 5) is not a characteristic that applies only to this study, since Leite et al. (2018), when dealing with Marandu grass inoculated with PGPB evaluated in the dry season, did not verify any differences in terms of responses comparing the inoculated group with the control one. When evaluating the effect of the WD in isolation, we can notice a decrease in the photosynthetic rates, as reported by Zafari et al. (2017).

Cassán and Diaz-Zorita (2016) emphasize that *Azospirillum* bacteria present distinct responses that depend on the soil conditions and the type of crop. In addition, in case of severe drought during the establishment phase of the plant, the inoculation may not work. However, even in a WD situation, the positive effect of the inoculation is evident. The authors also report that the multiple actions of the bacteria that belong to the aforementioned group are known for enhancing the growth of the plants, as well as their productivity. These bacteria mitigate the impact of abiotic tensions, increasing the efficiency of the use of resources from the production environment (e.g. nutrients and water).

The increase in the concentration of chlorophyll, which are related to greater impositions of WD, can be due to the fact that the grasses have possibly activated their protection mechanism to the photosynthetic device, through the development of their chloroplasts, as an acclimation response to the stress agent, as reported by Tabot e Adams (2013). Besides its main role in the absorption and transference of electrons to the reaction center of photosynthesis, chlorophyll *a* is also a strong indicative of photosynthetic capacity (Zafari et al., 2017).

In general, when water is not abundantly available in the soil, the chlorophyll molecule goes through degradation. That results in a decrease in the chlorophyllic pigments as a way of dissipating the energy surplus in order to avoid photoinhibition and photoxidation, giving rise to oxidative stress (Carvalho et al, 2011; Carlin et al., 2012). Fiaz et al. (2014) highlight that water restrictions inhibits the actions of the protochlorophyllide reductase, an enzyme that is responsible for forming photosynthetic pigments, including carotenoids.

It is important to take into account that, when WD imposition on grasses is reduced, their foliar water potential should increase, once their leaves will have more turgidity. This condition has a positive correlation with the plant's photosynthetic performance and, consequently, with a greater production of photosynthetic pigments. Yet, in this study, the photosynthetic pigments had a behavior that was different from that presented by the literature, with higher SPAD values registered together with the highest levels of WD imposition on the grasses (Table 5).

Although there was no correlation among the factors for most of the parameters covered by the study, the literature has been showing that the most probable explanation for this type of result may be attached to the interference of PGPB in the plant's metabolism, because one of the benefits of these microorganisms is that, besides producing phytohormones, they also stimulate the production of these substances by the plants, and great part of what is produced is connected to genes that are related to drought stress, as shown by Kasim et al. (2012). According to Kavamura et al. (2013), these bacteria can protect the plant against desiccation by promoting a humid environment that favors the development of the root system, besides providing nutrients and some hormones that promote the plant's growth for example.

There is a lot of results incompatibility with respect to data found in the literature on the effects of inoculations in tropical grasses. For that reason, we highlight that it is necessary to carry out more studies and painstaking analysis in order to better understand the effects of PGPB inoculation in grasses subjected to WD.

CONCLUSION

The plant-growth promoting bacteria were not efficient in improving the physiological parameters of *Urochloa brizantha* cv. BRS Paiaguás and *Urochloa ruziziensis* under water deficit conditions.

The results point to the specificity of the effects of the bacteria strains in the different genotypes of *Urochloa*, once each strain had a different response for each parameter of each grass species in the study.

The higher imposition of water deficit led to an increase in the levels of fibers and lignin. At the same time, it contributed to an increase in cellular content.

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V – GENERAL CONCLUSIONS

The use of plant-growth promoting bacteria in tropical grasses is an alternative to the maintenance of pastures growth and development, even when the nutritional profile of the soil does not meet the needs of the grasses and environmental conditions are adverse.

The literature shows conflicting results regarding the effects of the interaction PGPB-grasses. Moreover, there are not many studies testing inoculants in tropical grasses under water deficit conditions. For that reason, we reaffirm the need for studies with the grass species that are mostly explored in animal production, as well as more detailed analyses of the efficiency of such technology, in order to better understand the effects of the interaction between PGPB and grasses in a context of water deficit.

The use of this technology shows great potential to become a reality in the formation and persistence of pastures, due to the great interest by livestock farmers, mainly because it is an advantageous alternative to livestock grazing, soil management and environmental quality, due to its low cost, and also for responding to society's that claims for more sustainable livestock production.

There is still a long way ahead of us regarding research done with the inoculation of tropical grasses, mainly when it comes to field tests in order to verify if the results are as promising as those found in controlled environments, especially if the responses in other grasses also comprise the genus *Urochloa*.

The results found in this type of research, as long as positive, may allow, in the future, the development and trading of products capable of contributing to a greater persistence of pastures in water deficit situations.