Phosphorus as a mitigator of the effects of water stress on the growth and photosynthetic capacity of tropical C$_4$ grasses

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ABSTRACT. Water deficiency during the dry seasons influences the relationship between water and gas exchange in tropical grasses, reducing their productive potential. In addition, the phosphorus (P) deficiency Brazilian soils adds to the set of factors limiting crop production. In this context, the objective of this study was to evaluate the responses of different tropical forage species to phosphorus supplementation as mitigating the damage caused by water stress. Seeds of *Urochloa brizantha* cv. MG-4, *Urochloa decumbens* cv. Basilisk, *Panicum maximum* cv. Áries, *Panicum maximum* cv. Tanzânia and *Paspalum atratum* cv. Pojuca were germinated in pots containing 10 liters of red-yellow Acrisol type soil. Experiments were conducted by combining levels of phosphorus, 8,0 and 100,0 mg of P dm^{-3}, with two irrigation regimes, 100 and 40% replacement of transpired water. The biometric parameters, photosynthetic capacity, leaf water potential and soil chemical characteristics were evaluated, and the data was submitted to analysis of variance (ANOVA, $p < 0.05$), and subsequently the means were compared using a Tukey test ($p < 0.05$). The results showed for tropical grasses grown under water stress, there is a clear mitigating effect of phosphorus supplementation, especially on the maintenance of biomass growth.

Keywords: pasture, leaf gas exchanges, fertilization, water deficit.

Fósforo como atenuador dos efeitos da deficiência hídrica no crescimento e capacidade fotossintética de gramíneas tropical C$_4$

RESUMO. A deficiência hídrica provocada pela seca estacional influencia a relação entre a água e as trocas gasosas nas gramíneas forrageiras tropicais, reduzindo seu potencial produtivo. Complementando, a deficiência de fósforo (P) dos solos Brasileiros soma-se aos fatores limitantes da produção vegetal. Nesse contexto, o objetivo deste trabalho foi avaliar as respostas da suplementação de fósforo como mitigador dos danos causados pela deficiência hídrica. Sementes de *Urochloa brizantha* cv. MG-4, *Urochloa decumbens* cv. Basilisk, *Panicum maximum* cv. Áries, *Panicum maximum* cv. Tanzânia e *Paspalum atratum* cv. Pojuca foram germinadas em vasos de 10 litros contendo solo tipo Argissolo Vermelho-Amarelo distrófico. Os tratamentos foram realizados combinando-se duas concentrações de fósforo, 8 mg P dm^{-3} e 100 mg P dm^{-3}, com dois regimes de irrigação, 100 e 40% de reposição da água evapotranspirada. Parâmetros biométricos, capacidade fotossintética, avaliação do potencial de água foliar e análises químicas de solo foram avaliados e submetidos a analise de variância (ANOVA, $p < 0,05$), e posteriormente as médias comparadas pelo teste de Tukey ($p < 0,05$). Os resultados mostraram que as plantas cultivadas sob deficiência hídrica, torna-se claro o efeito mitigador da suplementação de P, principalmente na manutenção do crescimento.

Palavras-chave: pastagem, trocas gasosas foliares, adubação, restrição hídrica.

Introduction

The high production potential of annual grass species of C4 photosynthetic cycles has been attributed to high water use efficiency in tropical regions from low latitudes, high insolation and elevated temperatures (Rodrigues & Rodrigues, 1994). However, these grasses are also restricted by various environmental factors; thus, the presence of water and phosphorus in ideal conditions is crucial for the plants to reach their productive potential in tropical environments (Rao, 2001).

The water deficiency caused by seasonal drought affects the relationship between water and leaf gas exchanges in tropical grasses, leading to a reduction in yield (Novais & Smyth, 1999). According to Kaiser (1987), the water stress can cause severe inhibition of photosynthesis, even in

C$_4$ plants, primarily because of high diffusive resistance of the CO$_2$ entrance. This occurs because the water stress causes reduced turgor of the guard cells of the stomata, which is followed by closing of the stomatal pore (Silva, Soares, Oliveira, & Magalhães, 2001). In environments where plants are subject to water stress, whether by low availability of water in the soil or by high atmospheric demands caused by high temperatures and low atmospheric water vapor concentration, the plant closes the stomata to retain water, reducing loss by transpiration, which can limit the CO$_2$ available for photosynthesis (Peak, West, Messinger, & Mott, 2004). In addition to the climate variations, crop production around the world also is subject to difficulties related to the low availability of nutrients in the soil, and in particular, low levels of inorganic phosphorus (Pi). Phosphorus (P) is an important macronutrient, constituting approximately 0.2% of plant mass, a structural component of macromolecules such as nucleic acids, phospholipids and adenosine triphosphate (ATP) as well as a fundamental element of several biochemical reactions and metabolic cycles, including glycolysis and numerous stages of C$_3$ and C$_4$ cycles. After nitrogen, P is the macronutrient that most limits plant growth (Holford, 1997).

The synthesis of the photoassimilates is dependent on the availability of Pi in the cytoplasm and is related to the transport of trioses from chloroplasts to the cytoplasm and the sucrose synthesis process (Hendrickson, Crow, & Furbank, 2004). During photosynthesis, chloroplasts receive Pi and release Triose-P to the cytoplasm. The flow between carbon and Pi is mediated by the Triose-P transporter, which transports the antiport system of Triose-P/Pi across the chloroplast membrane (Flügge, Häusler, Ludewig, & Fischer, 2003). In addition, the assimilated partition between starch and sucrose is dependent on a three-factor interaction: the concentration of cytoplasmic Pi, which regulates the export of Triose-P from the chloroplasts; the activity of the principal enzymes of sucrose synthesis (fructose-bisphosphate phosphatase, phosphate sucrose and phosphatase phosphate sucrose); and the regulation of ADP-glucose pyrophosphorylase activity (Massonneau, Martinoia, Dietz, & Mimura, 2000). Therefore, the shortage of Pi in the cytoplasm limits the ATP synthesis (Lawlor & Cornic, 2002) and the release of Triose-P in the chloroplast, which can restrict

of ribulose-1,5-bisphosphate synthesis (RuBP) and consequently lead to the limitation of photosynthesis.

Periods of water limitation, lasting approximately ten days, can drastically reduce the diffusive Pi flow from the soil to the plant, causing a significant loss of plant productivity (Novais & Smyth, 1999). The frequent threat of water limitation leads to the accumulation of Pi during periods with optimal water availability because the absorption of Pi is dependent on water availability in the soil. Therefore, after rehydration, absorption and Pi assimilation should be efficient as soon as the flow diffusion is reestablished (Novais & Smyth, 1999). According to Novais and Smyth (1999), before the loss of turgor and the folding of the leaves, both of which are common symptoms observed as a result of water deficiency in the field, there is a loss of productivity due to the absence of internal Pi accumulation capable of sustaining plant growth for long periods. In the literature, there is some evidence that the phosphorus supply is a potential mitigator of the damages caused by water stress on the growth and physiology of C$_3$ cycle plants (Firmano, Kuwahara, & Souza, 2009) and C$_4$ cycle plants (Kuwahara & Souza, 2009).

In Brazil, the genus most frequently used as forage is the genus *Urochloa* sp. (Syn. *Brachiaria* sp.) (Zimme, Euclides, & Macedo, 1995). Until the 1990s *U. decumbens* Stapf. species was the most widely used species in Brazil for grass forage due to its adaptatability and capacity to produce good yields in poor and acidic soils as well as its ability to be cultivated by seeds (Usberti, 1990). Originally from Africa, *Panicum maximum* is currently the most cultivated grass forage in tropical America used in pastures because of its ability to adapt to both tropical and subtropical conditions, high production and forage quality, grazing resistance and animal acceptance (Previero, Martins, Fonseca, & Groth, 1996). Of all of the forage grass taxa in the subtropical grasslands of Brazil, *Paspalum* sp. is distinctive because of its numerous perennial and summer growth species (Valls, 1990).

Thus, the aims of the present study was to evaluate the responses to changes in the phosphorus supply in grass pasture species growing with and without water limitation, testing the hypothesis of the phosphorus as a potential mitigator of the effects of water stress on the growth and physiology of different species of grass forage.

Material and methods

The experiment was conducted under greenhouse conditions at the UNOESTE (University of West São Paulo) in Presidente Prudente, São Paulo State/Brazil. Seeds of *Urochloa brizantha* cv. MG-4, *U. decumbens* cv. Basilisk, *Panicum maximum* cv. Áries, *P. maximum* cv. Tanzânia and *Paspalum atratum* cv. Pojuca were germinated in pots of 10 L containing red-yellow Acrisol type soil (according to the Brazilian soil classification), with the folloowing original chemical characteristics: 4.0 pH CaCl$_2$, 27.0 mmol$_c$ dm^{-3} potential acidity (H+Al), 3.0 mmol$_c$ dm^{-3} aluminium (Al^{3+}), 4.0 g dm^{-3} organic matter (M.O.), 5.0 mmol$_c$ dm^{-3} calcium (Ca^{2+}), 1.0 mmol$_c$ dm^{-3} magnesium (Mg^{2+}), 0.8 mmol$_c$dm^{-3} potassium (K$^+$), 2.0 mg dm^{-3} phosphorus (P), 6.3 mg dm^{-3} sulfur (SO$^{-2}_4$), 7 mmol$_c$ dm^{-3} Sum of Bases (SB), 34 mmol$_c$dm^{-3} Cations Exchange Capacity (CTC). Fifty days before the sowing, the liming was performed using dolomitic lime with 95% PRNT (Relative Power of Total Neutralization), increasing the base saturation (V%) up to 70%.

The phosphorus treatments were performed with two concentrations of phosphorus, 8 mg dm^{-3} P (original concentration of soil after liming) and 100 mg dm^{-3} P achieved with 6.0 g pot^{-1} supplement of triple Superphosphate fertilizer CaH$_4$(PO$_4$)$_2$H$_2$O, where, according Kuwahara and Souza (2009), such amount of fertilizer has proved a great performance to *U. brizantha* under water deficit. The phosphorus was applied in a single dose at the time of sowing, with 15 seeds sown per pot. Thinning of seedlings was conducted twenty days after germination, leaving only one plant per pot. The plants were kept under constant irrigation for forty days, after which cutting was performed to standardize the plant height to 25 cm. At this point, the plants were subjected to two levels of irrigation based on the evaporation of Class A mini tank, with 100 and 40% of the evapotranspired water replaced.

The measurements in the Class A tank, located inside the greenhouse, were performed daily and, from these measurements, the irrigation water replacement rate was calculated based on the evaporation fractions of the Class A tank (ECA) (Doorenbos & Kassan, 1979):

$$hi = \frac{ECA \cdot kp \cdot kc \cdot pot\ area}{ef}$$

where: hi – irrigation water (L); ECA – Class A tank evaporation (mm); Kp – Coefficient of Class A tank (0.80); Kc – coefficient of culture; the pot area is 0.0573 m^2; and the application efficiency (ef) is 1 or 100%. The coefficient of culture was defined from 0.3 to 0.4 in the initial development stage and 1.1 to 1.4 in the final stage of development during a cycle of 36 days.

The experiment was randomly arranged using a triple factorial design with 5 (forage species) x 2 (irrigation levels) x 2 (P supply), totaling 20 treatments with 4 repetitions. Twice a week the pots were randomly rearranged to diminish the effect of environmental factors not considered in this study.

After 35 days of the plants development under water treatments, the measurements of pre-dawn leaf water potential were taken with a pressure chamber (model 1000, PMS Instruments, USA). Leaf photosynthetic capacity analyses were performed using a photosynthetic light response curve (A/PPFD, A = net CO$_2$ assimilation, PPFD = photosynthetic photons flux density), by evaluating the maximum assimilation of CO$_2$ (A$_{MAX}$); light compensation point (P$_{COMP}$); light saturation point (P$_{SAT}$), at which 90% of CO$_2$ absorption maximum is achieved; apparent quantum efficiency (AQE), corresponding to the slope of the initial linear region of A/PPFD curve; and leaf respiration (Rd) when PPFD = 0 µmol m^{-2} s^{-1}.

The light response curves (A-PPFD) utilized the following values of PPFD: 0, 25, 50, 100, 200, 400, 600, 800, 1000, 1200, 1400, and 1600 µmol photons m^{-2} s^{-1}. Each measured point was scored during 3 to 6 minutes, depending on the time required for stabilization of the measurements, for more the one day, randomly all the plants were evaluated, with the measures carried out during the 9am to 2pm of every day. The measurements were carried out with a gas exchange analyzer (IRGA LCA4 - ADC, Hodesddon, UK) connected to a leaf Chamber PLCN-4 (ADC), operating in an open system. While the measurements were being taken, the relative humidity of the chamber was maintained at values similar to the environment, but always less than 90%, with the CO$_2$ concentration at approximately 380 µmol L^{-1} and the leaf temperature adjusted to 30ºC. The A-PPFD curves were adjusted based on the equation of Prado and Morais (1997).

$$A = A_{max} [1 - e^{-k(DFFF - PCL)}]$$

where, A$_{max}$ is the net rate of maximum photosynthesis, and *e* is the base of natural logarithm

(2.718), k the proportionality constant associated with the curve concavity (ranges from 0.001 to 0.009) and PCL is the light compensation point. The apparent quantum efficiency (AQE) was obtained from the following equation derived from the inclination of the straight line in the first linear phase of the curve obtained from equation (Prado & Morais, 1997), calculated as:

$$AQE = k\,A_{max}\,(e^{\,k\,PCL})$$

At the end of experiment, plant growth was evaluated on the basis of: I) leaf area measurement with a planimeter (model LI-3000A, Li-Cor, USA), II) the number of tillers, and III) the dry mass of the shoot and root system. The determinations of the dry mass were performed by drying the root and shoot at 60°C in a forced aeration oven until a constant mass was achieved.

The analysis of the chemical composition of the soil at the end of the experiment was performed with composite soil samples from each treatment, and they were analyzed according to the method described by Raij, Andrade, Cantarella, and Quaggio (2001).

The data were subjected to analysis of variance (ANOVA, $p < 0.05$), and the averages were compared using the by Tukey test ($p = 0.05$) with the Statistical Analysis Systems (SAS, 2003).

Results and discussion

In general, the grasses that were subjected to water stress showed significantly affected growth and plants of the genus Brachiaria were particularly affected. On the other hand, the signs of stress mitigation caused by phosphorus supplementation were evident in plants in the water deficient group.

Water deficiency imposes limitations on the plant production. In our study, these limitations were primarily observed in the plants subjected to water deficiency that were not supplemented with P (Figures 1 and 2). On the other hand, P supplementation of plants subjected to water deficiency resulted in plants with production values (leaf area and dry weight of shoot, root and tiller) significantly greater than those from the treatments without additional P (Figure 1 and 2), indicating the positive contribution of P to species development under water stress.

The high concentration of Pi in plants promotes improved synthesis of starch and/or

sucrose in the cytoplasm, as a result of more efficient carbon assimilation (Dickson, 1991). The starch stored can be subsequently metabolized (hydrolyzed to glucose) when photosynthesis is no longer able to meet the demand for energy during the vegetative growth or reproductive stage of plants. This partition of reserves as well as the regulation of photosynthetic activity is essential for plant growth and development in both perennial and annual species (Goldschmidt & Koch, 1996).
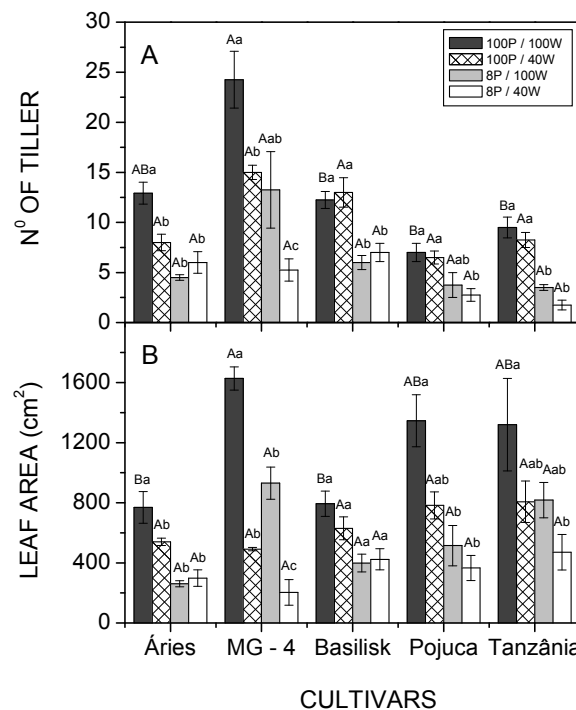

Figure 1. Number of tiller (A) and Leaf area (cm², B) after water restriction treatment. The bars indicate standard error, the capital letters indicate significant difference in treatments among different cultivars, and the lowercase letters indicate significant differences among treatments inside the cultivars. The means were compared by the Tukey test ($p < 0.05$).

In the present study, the amount of extracted nutrient soil in each P treatment can be inferred by the percentage of the nutrient maintained in the soil after the growth period of each of the plants tested under different conditions (Table 1). For plants that did not receive P supplementation, which had original P levels of approximately 8 mg day^{-3} after the first cut, the percentages of P maintained in the soil were similar between the treatments with and without water restriction, except for Tanzânia grass (*P. maximum*), which tended to extract more P under water deficiency.
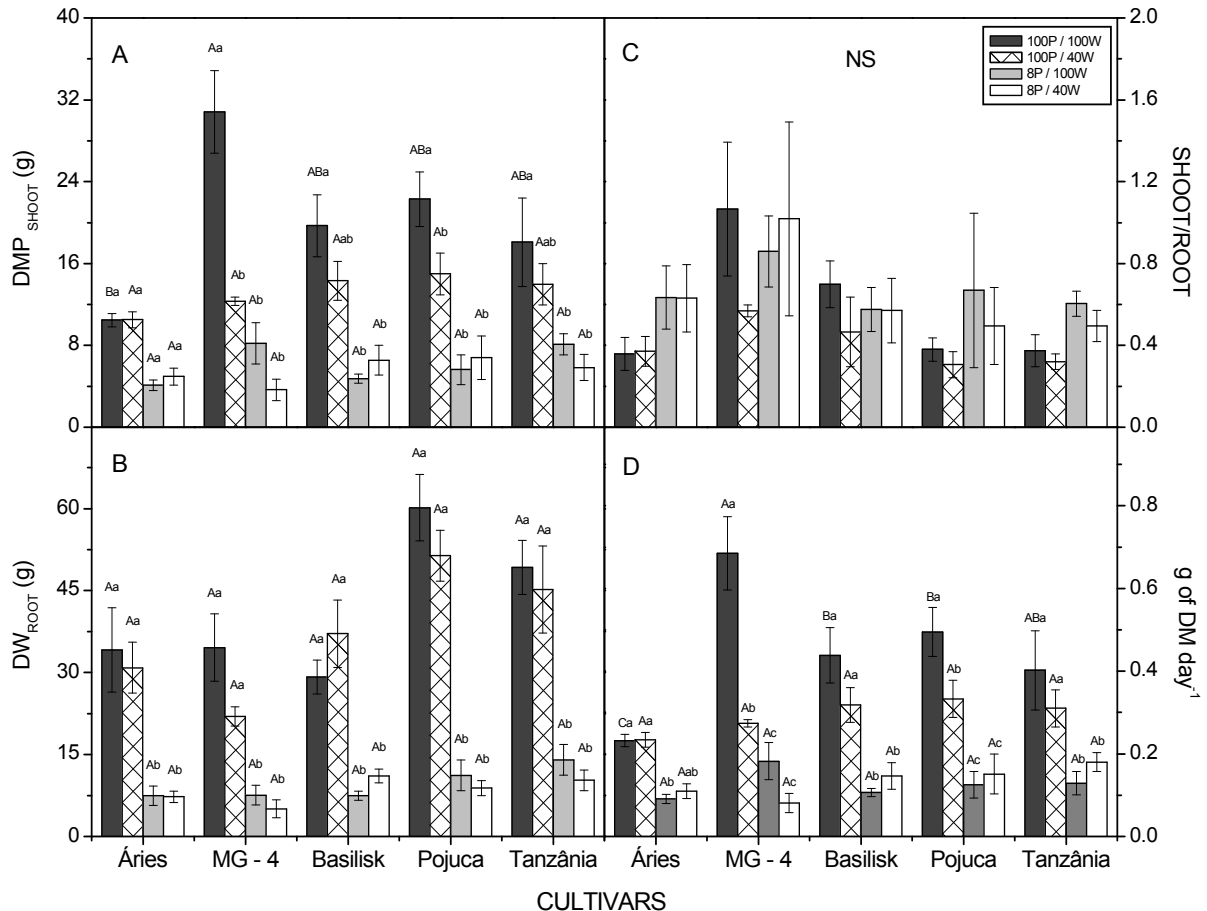
Figure 2. Shoot dry matter production (DMP$_{SHOOT}$, g, A), Root dry weight (DW$_{ROOT}$, g, B), the ratio between plant shoots and root biomass (SHOOT/ROOT, C) and gain of dry matter per day (g of DM g^{-1}, D) after water restriction treatment. The bars indicate standard errors, the capital letters indicate significant differences in treatment among cultivars and the lowercase letters indicate significant differences among treatments inside the cultivars. The means were compared by the Tukey test (p < 0.05).

Table 1. Percentage of retained phosphorus remaining in the soil after the water restriction period. Sampling was carried out on all repetitions of each treatment and homogenized. The experimental conditions were 100 and 8 P (with and without phosphorus supply), 40 and 100 W (with and without water restriction).

Cultivars	Percentage of Phosphorus in the soil (%)			
	100 P / 100 W	100 P / 40 W	8 P / 100 W	8 P / 40 W
Áries	70,83	75,00	80,00	90,00
MG – 4	41,94	67,74	44,44	46,67
Basilisk	79,34	30,58	55,56	44,44
Pojuca	21,05	51,13	50,00	47,50
Tanzânia	46,79	37,61	83,33	66,67

Water deficiency tended to reduce the Ψ_{WATER} values of all treatments (Figure 3A and B), especially in MG-4, Pojuca and Tanzânia cultivars with supplementation of P (Figure 3A and B). For Áries and Basilisk cultivars, the reduction in water potential was less expressive, in the treatment with water deficit compared to others cultivars, in the Áries cultivar noted that both treatments with addition of P, values of DMP$_{shoot}$ were similar, though larger than the treatments without P addition, this result can be explained by the fact that this cultivar be more demanding of high fertility

soils (Previero et al., 1996), where, for the conditions of the experiment, production and the level of water restriction does not imply in significant reduction of productivity.

Reductions in leaf water availability can result in a restriction of CO_2 diffusion, by the stomatal limitation (Figure 3C), transpiration (Figure 3D), consequently reducing leaf photosynthetic activity (Flexas, Gulias, Jonassom, Medrano, & Mus, 2001). However, according to the results from the light response curves, particularly the maximum photosynthesis (A$_{MAX}$) value, there were no significant differences between plants hydrated

without P supply and plants under water deficiency with P supply (Figures 4 and 5). This trend of reduced Ψ_{WATER} may have been caused by osmotic adjustment induced by P (Al-Karaki, Clark, & Sullivan, 1996).

The tendency for plants to have more highly developed root systems under water deficiency, was observed in the present study (Figure 5B), supporting the preference for radicular phosphorus interception under controlled irrigation, and, as a consequence, higher phosphorus extraction among plants. Diffusive flux, which is the predominant mechanism of P transport in the soil, can limit the P supply of water deficient plants; however, this limitation can be compensated for by increasing the surface area of the root system. Thus, changes in the root system architecture can contribute to higher P acquisition, this stronger relationship between root biomass and the number of plant shoots is a response to the low availability of P, thus

providing a greater absorption surface in relation to the total plant biomass and improving the acquisition of the nutrient (Wissuwa, 2003). However, these changes in the plant shoot - root ratio were not observed in the present study (Figure 2C), which could be a side effect of the potted cultivation conditions.

According to Guimarães, Mutton, Ferro, Ravaneli, and Silva (2008), phosphorus supply can increase the tissue levels of free proline, which is an important regulator of cellular osmotic potential. This induction of osmotic adjustment may, in some species, be caused by the slow development of water deficiency, resulting in the maintenance of cell turgor at low water potentials during dry conditions and allowing the retention of metabolic processes in the plant. In general, supplementation of P for plants under water deficiency can mitigate the deleterious effects of water stress on growth.
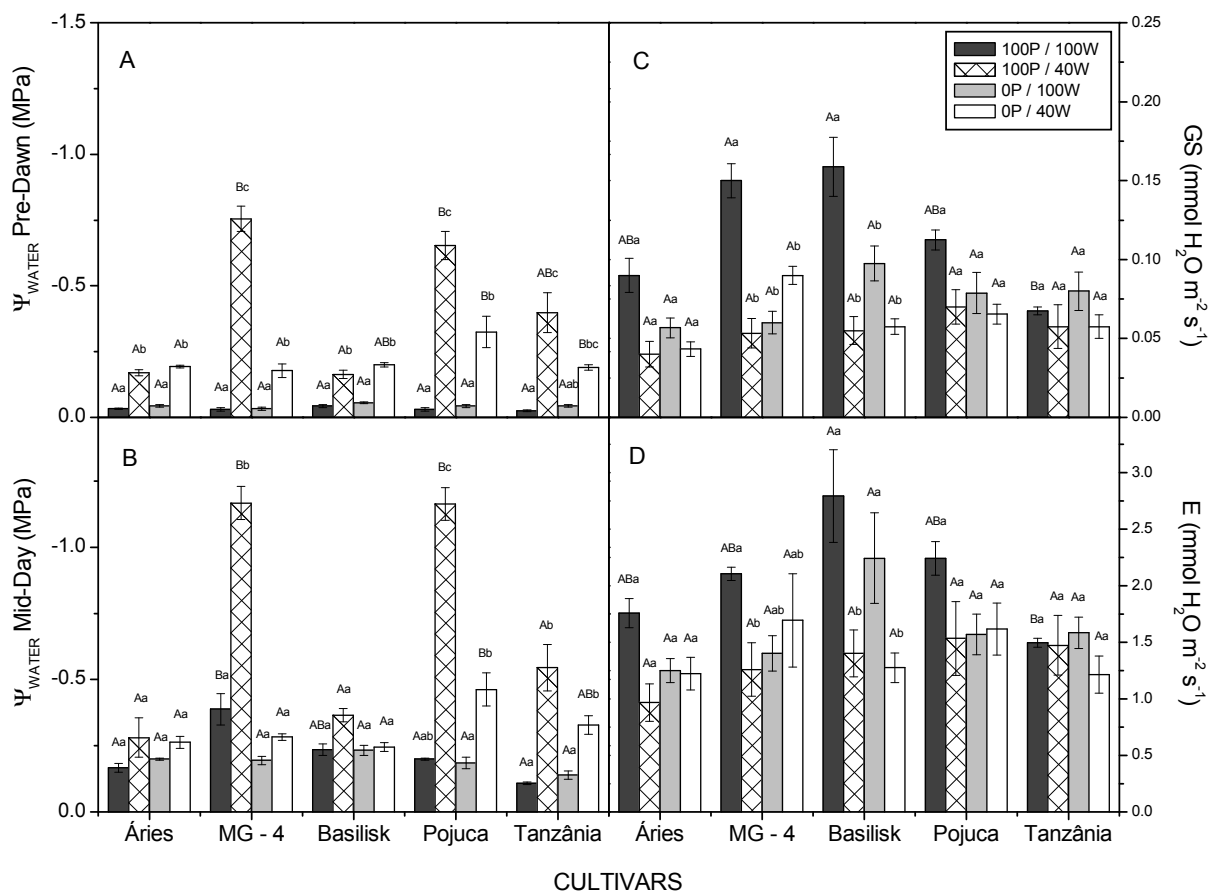

Figure 3. Water Potential Pre-dawn (Ψ_{WATER}, MPa, A), Water Potential Mid-Day (Ψ_{WATER}, MPa, B), Stomatal Conductance (GS, mmol H_2O m^{-2} s^{-1}, C) and Transpiration (E, mmol H_2O m^{-2} s^{-1}, D) of two irrigation levels (100 and 40%) for different doses of phosphorus (0.0 and 6.0 g vase^{-1}) in different cultivars of grass forage. The bars indicate standard errors, the capital letters indicate significant differences in treatment among cultivars and the lowercase letters indicate significant differences among treatments inside the cultivars. The means were compared using the Tukey test ($p < 0.05$).
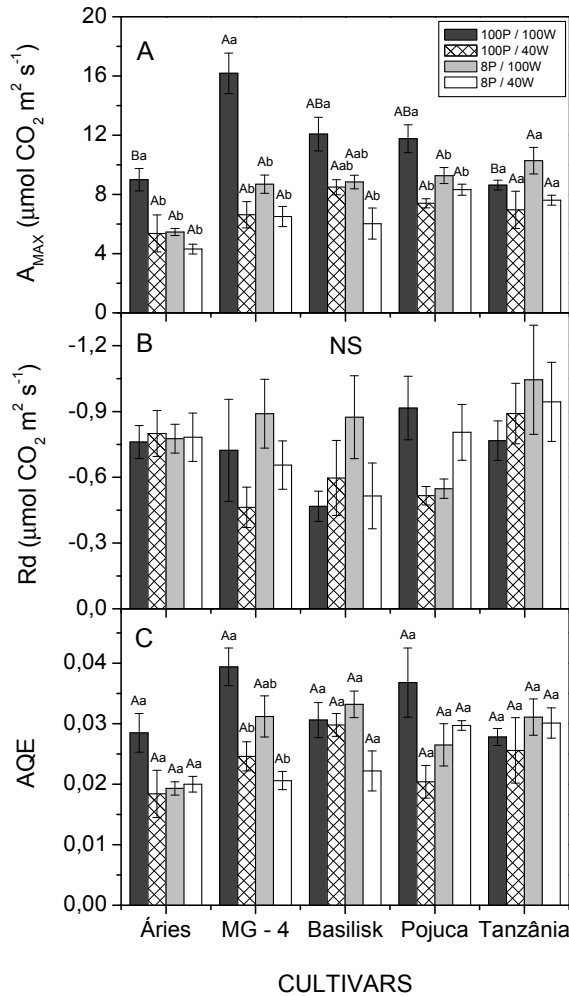
Figure 4. CO$_2$ maximum assimilation (A$_{MAX}$, µmol CO$_2$ m^{-2} s^{-1}, A), Respiration (Rd, µmol CO$_2$ m^{-2} s^{-1}, B) and Apparent quantum efficiency (AQE, C) values extracted from the light curves response (A-PPFD). The bars indicate the standard errors, the capital letters indicate significant differences in treatments among cultivars, and the lowercase letters indicate significant differences among treatments inside the cultivars. The means were compared using the Tukey test (p < 0.05).

This mitigating effect of P has been previously observed in plants such as *B. brizantha* cv. MG-5 Vitória (Kuwahara & Souza, 2009), supporting the results obtained in this study. This mechanism of drought tolerance can enable the plant to continue with cell expansion and stomatal dynamics despite water stress, improving plant growth and photosynthesis (Kumar & Singh, 1998). In addition, as the exposure of the plants to water stress became longer (35 days), the photosynthetic apparatus likely acclimatized, restoring the homeostatic capacity of photosynthesis. Despite this trend of photosynthetic capacity maintenance in plants under water stress, the reductions in plant growth indicate that photoassimilates may

have been preferentially targeted for cellular repair and maintenance processes, reducing the energy available for the maintenance of biomass production (Amthor, 1994).
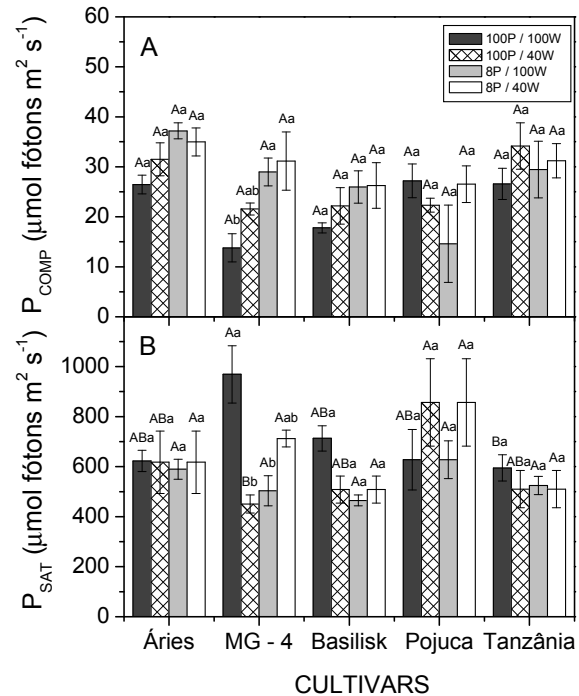

Figure 5. Light compensation point (P$_{COMP}$, µmol CO$_2$ m^{-2} s^{-1}, A) and Light saturation point (P$_{SAT}$, µmol CO$_2$ s^{-1}, B), values extracted from the light curves response (A-PPFD). The bars indicate the standard errors, the capital letters indicate significant differences in treatments among cultivars, and the lowercase letters indicate significant differences among treatments inside the cultivars. The means were compared using the Tukey test (p < 0.05).

Conclusion

The results of this study confirmed that tropical grasses could show different responses water stress and to different nutritional soil conditions, according to the species and cultivars evaluated. In conclusion, comparing the plants grown under water deficiency, support previous theories of the mitigating effect of P supplementation on plants under water stress, especially considering the effects on maintenance of root growth and the number of tillers.

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