



**Full Length Article**

## Water Availability and Microbiological Inoculation Alters Development and Yield of Buffel Grass (*Cenchrus ciliaris*)

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### Abstract

This study examined the effect of inoculation with growth-promoting microorganisms and different levels of water availability on productive and morphogenetic traits of buffel grass (*Cenchrus ciliaris* L. cv. Aridus). The experiment was laid out in a randomized block design with a 4 × 2 factorial arrangement. The factors tested were microbial inoculation with *Azospirillum brasilense* + *Rhizophagus intraradices* or no inoculation (control) and four levels of water availability (20, 40, 60 and 80% of pot capacity). Five replicates were used, totaling 40 plastic pots. The water availability of 80% positively affected the morphogenetic traits, providing a 28% higher leaf appearance rate and a 50% higher leaf elongation rate; a 3-day shorter leaf lifespan; 19% larger leaves; and a 40% higher leaf area index compared with greater water restriction. Shoot, leaf, and root DM yields increased by 45, 49 and 61%, respectively, with 80% of water availability. Inoculation with plant growth-promoting microbes increased leaf elongation rate (by 25%), shoot DM yield (14%), and root dry matter (DM) yield (20%). Therefore, water stress affects the morphogenesis and productive development of buffel grass cv. Aridus. Inoculation with plant growth-promoting microbes can strengthen the root system and shoots of buffel grass, as well as increasing its resilience to adverse climatic conditions.

**Keywords:** *Cenchrus ciliaris*; Drought tolerance; Inoculation; Mycorrhizal fungus; Water restriction

### Introduction

The pastoral environment must be regarded as a complex ecosystem where the main factors (animal, plant, climate, and soil) interact with and influence one another through the transfer of different levels of energy. The climate factor directly impacts plant and animal production in the tropics, especially in semi-arid regions, where rainfall occurs with irregular distribution as well as at low frequency and intensity (Gaur and Squires 2018).

These situations warrant the use of species capable of tolerating low water availability, such as buffel grass (*Cenchrus ciliaris* L.), a species originating in East Africa and Southeast Asia that was introduced in Brazil in the 1950 (Oliveira 1993). Buffel grass was established in semi-arid regions for ruminant feeding due to characteristics such as easy implementation, good nutritional value for these arid

regions (6–9% crude protein and 50% digestibility), and its well-known tolerance to drought conditions (Carrizo *et al.* 2021). However, the sparse rainfall in these regions is one of the main abiotic factors that cause serious losses in the yield, persistency, and nutritional value of the species (Tommasino *et al.* 2018; Maranhão *et al.* 2019).

The soil in these regions harbors enormous macro- and microbiological diversity, and in the zone of contact with plant roots (rhizosphere), microorganisms act through different processes of synergistic and antagonistic interaction according to the relationship between plant and microorganism and these interactions have been explored in recent years with the use of biological products based on plant growth-promoting bacteria and arbuscular mycorrhizal fungi (AMF) (Santoyo *et al.* 2021).

The synergism between these microorganisms is attributed to the production of compounds by some bacteria

that increase cell permeability and, therefore, the rate of root exudation, which stimulates hyphal growth and facilitates root penetration by the fungus (Jeffries *et al.* 2003). In addition, these bacteria produce phytohormones that stimulate root growth and increase mycorrhizal colonization (Villarreal *et al.* 2016).

Mycorrhizal fungi, on the other hand, provide a niche and/or habitat for bacteria, which can use their structures as intermediaries to reach the root tissue epidermis (Villarreal *et al.* 2016). Additionally, they provide nutrients for the bacteria that colonize the surfaces or interior of the spores, protecting against drying, radiation, predation, and salinity (Levy *et al.* 2009).

Nonetheless, several cases involve data heterogeneity and a certain degree of specificity between plants, bacterial species, and AMF isolates. For this reason, it is essential to investigate efficiency of the combined use of these inoculants in tropical forage plants, under water deficit conditions, to allow the elucidation and adoption of this biotechnology by rural producers. The objective of this study was to examine whether co-inoculation with *A. brasilense* and *R. intraradices* improves the morphogenetic and productive development of buffel grass plants under water stress.

## Materials and Methods

### Experimental details

The experiment was carried out on buffel grass (*Cenchrus ciliaris* L. cv. Aridus) from October to December 2020 in a greenhouse in the Forage Crops and Pasture section of the State University of Southwest Bahia, Juvino Oliveira campus, located at the coordinates: 15°38'46" of south latitude, 40°15'24" of west longitude and average altitude of 280 m, in the municipality of Itapetinga, Brazil. The minimum, maximum, and mean temperatures inside the greenhouse were recorded throughout the experimental period (Fig. 1).

A sandy-loam soil was used, according to a soil analysis protocol collected on the campus of UESB. The soil was collected from the arable layer (0 to 20 cm), crushed, passed through a 4 mm mesh sieve, and dried in air. Soil chemical analysis was carried out at the Department of Agricultural and Soil Engineering at UESB (Table 1).

According to the recommendations of the Soil Fertility Commission of Minas Gerais State (Alvarez *et al.* 1999), liming was not necessary, since the base saturation value in the collected soil layer was 79%. Only phosphorus (P) and nitrogen (N) correction was necessary. Therefore, after the uniformity cut, the area was top-dressed with 50 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> in the form of single superphosphate (18% P<sub>2</sub>O<sub>5</sub>), corresponding to 1.39 g pot<sup>-1</sup>; and 50 kg ha<sup>-1</sup> N in the form of urea (44% N), corresponding to 0.57 g pot<sup>-1</sup>.

### Evaluated treatments

The experiment was laid out in a 4 × 2 factorial arrangement

with four levels of water availability (20, 40, 60 and 80% of pot capacity) and two inoculation conditions (inoculation with *A. brasilense* and *R. intraradices*, or no inoculation), in a randomized block design. Five replicates were used, totaling 40 plastic pots with a capacity of 12 L and 706.5 cm<sup>2</sup> of area, which were filled with 10 dm<sup>-3</sup> of soil.

To determine pot capacity (PC), the pot with dried soil were weighed, soaked, fully drained, and weighed again. The maximum soil water-holding capacity (25%) was determined as the difference between wet (after draining) and dry weights, following the procedure described by Souza *et al.* (2000). The amount of water needed to restore each PC was calculated relative to this difference. To maintain the soil close to PC at the different levels of water availability, all pots were weighed twice daily, at 08:00 and 16:00 h.

### Sowing and inoculation

Prior to planting, buffel grass, the seeds were inoculated following the instructions of the commercial product Azototal® (100 mL 50 kg<sup>-1</sup> seeds, which provides 2 × 10<sup>-8</sup> CFU mL<sup>-1</sup> of the AbV5 and AbV6 strains). The seeds were then homogenized and kept in the shade for 30 min.

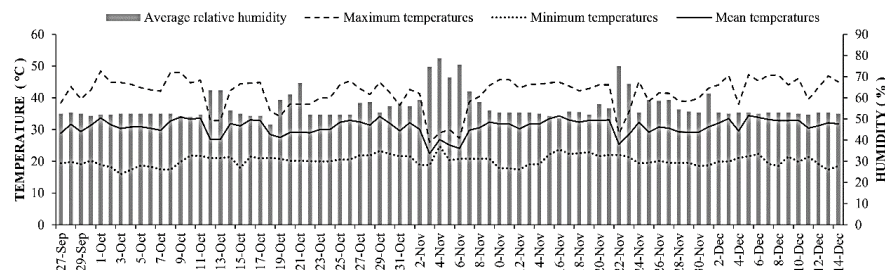
The seeds were planted in October 2020. Simultaneously to this stage, inoculation was carried out with the mycorrhizal fungus *R. intraradices* in a central location of the pot, following the instructions of the commercial product Rootella BR® of 120 g ha<sup>-1</sup>, with 20,800 propagules g<sup>-1</sup> and taking into account the proportions of soil volume of the pot.

### Experimental evaluations

**Plant growth details:** Upon showing approximately two fully expanded leaves, the plants were thinned to four plants per pot. During the initial establishment phase (23 days after germination), the soil in the pots was kept close to PC. After this initial period, the plants were cut for uniformity at a height of 10 cm, the nitrogen rate was applied, and re-inoculation was performed using the same rate of the bacterial inoculant.

These water conditions were maintained for 10 days and, later, the plants were subjected to water regimes of 20, 40, 60 and 80% of soil PC, in which they remained for 24 days. The end of the water restriction period was determined by the observation of curling and senescence of the leaves under 20% PC regime. At the end of the stress period, the plants were harvested, the pots were dismantled, and the evaluations were performed.

**Morphogenetic and structural traits:** Once the levels of water availability were implemented, two tillers per pot were marked with colored ribbons (80 tillers in total) and evaluated every three days throughout the experimental period. The following variables were evaluated in each marked tiller: appearance of the leaf apex; leaf length, which was measured according to their stage of development. In



**Fig. 1:** Average relative humidity (%) and maximum, minimum, and mean temperatures (°C) during the experimental period

**Table 1:** Chemical characteristics of cultivated soil

Soil characteristics	Values
pH	6.30
Organic matter	7.00 g kg <sup>-1</sup>
Phosphorus	15.00 mg kg <sup>-1</sup>
Potassium	0.97 cmol kg <sup>-1</sup>
Calcium	1.50 cmol kg <sup>-1</sup>
Magnesium	1.60 cmol kg <sup>-1</sup>
Sum of bases	4.10 cmol kg <sup>-1</sup>
Cation exchange capacity	4.10 cmol kg <sup>-1</sup>
Base saturation	79.00 %

the fully expanded leaves, the length from the tip to the ligule was measured. In the case of expanding leaves, the same procedure was performed, only considering the ligule of the last fully expanded leaf as a measurement reference. For the senescent leaves, the difference between the final senescent length (yellowing and blackening) and the initial senescent length was observed. Pseudostem length was considered to be the distance from the ground to the last ligule. These data were then used to calculate the leaf appearance rate (leaves day<sup>-1</sup>), phyllochron (days leaf<sup>-1</sup>), leaf elongation rate (cm day<sup>-1</sup>), stem elongation rate (cm), number of live leaves, leaf senescence rate, final leaf length (cm), and leaf lifespan (LLS = number of live leaves × phyllochron) (Lemaire and Chapman 1996). Plant height (cm) was measured on the day of dismantling, at the end of the period of water stress and rehydration, moments before the harvest for evaluations. This measurement was obtained with a graduated ruler, without compressing the forage and considering the height of the curvature of the leaves around the ruler as the upper limit. Tiller density was determined after measuring plant height, at the end of the water stress and rehydration period, by counting the number of tillers per pot. Vegetative tillers were considered those whose flower primordia was not externalized, whereas reproductive tillers were those that showed this characteristic.

**Biomass production:** At the end of the water stress period, two clumps were cut at 10 cm above ground level and the collected material was identified and separated into leaves, pseudostem (stem + sheaths), and dead material. To determine the pre-dried weight, the desiccated material was weighed fresh and after pre-drying in a forced-air oven at 65°C for 72 h. Another two clumps of the pots were used for the analysis of leaf area, which involved separating and

then scanning the leaves. The digitized images were evaluated in ImageJ software, which determines the area of the image covered by the leaves by contrast with the uncovered area. Leaf area was calculated as the sum of the area of the images referring to the two plants in each replicate. The values were summed to calculate total leaf area per pot (cm<sup>2</sup> pot<sup>-1</sup>). Leaf area index (LAI) was determined by dividing the total leaf area by the total area of the pot filled with soil.

**Root evaluation:** The roots collected after the harvest were initially used for the measurement of length (cm). This variable was obtained using a graduated ruler fixed to a flat surface on which the root was placed. Subsequently, root volume (mL) was determined using a volumetric beaker containing a certain amount of water, where the fresh root was introduced and the root volume of each pot was calculated by difference. Next, the roots were weighed and dried in a forced-air oven at 65°C for 72 h to determine DM yield.

### Statistical analysis

The data were subjected to analysis of variance (ANOVA) adopting the following sources of variation: soil water availability, microbiological inoculation and the interactions among these factors. The mean values of the microbiological inoculation were compared by the Fisher's least significant difference using the and the significant variables were compared by the Tukey's HSD test at 1 and 5% significance level. Regression analysis was applied to the soil water availability, and equations were selected based on the coefficient of determination and the significance of the parameters using the SAS statistical package (2002).

## Results

### Morphogenetic and structural traits

The interaction between water availability and microbial inoculation of buffel grass did not affect its leaf appearance rate (LAR), phyllochron (PHY), leaf elongation rate (LER), pseudostem elongation rate (PER), or leaf senescence rate (LSR) (Table 2). Inoculation with plant growth-promoting microbes increased the rate of the appearance of successive leaves (LAR) by 11%. Additionally, it reduced the time

**Table 2:** Morphogenetic traits of buffel grass grown under different levels of water availability, with or without microbial inoculation

Variable	Water availability				Inoculation		P-value			CV (%)
	20	40	60	80	With	W/o	W×I	Water	Inoc	
LAR	0.18	0.19	0.22	0.23	0.21	0.19	0.49	0.01*	0.01*	10.67
PHY	5.73	5.24	4.47	4.43	4.67	5.32	0.41	0.01*	0.03	17.65
LER	1.68	2.06	2.42	2.52	2.41	1.93	0.36	0.01*	0.01*	11.53
PER	0.13	0.23	0.28	0.35	0.25	0.24	0.12	0.01*	0.85	26.80
LLS	29.20	30.58	26.52	25.99	26.77	29.36	0.56	0.013	0.02	11.91
FLL	15.92	17.96	17.91	18.94	17.75	17.63	0.27	0.01*	0.10	4.54
LSR	1.55	1.13	1.10	1.24	1.37	1.13	0.11	0.01*	0.01*	16.80
NLL	5.21	5.87	5.83	5.88	5.54	5.76	0.49	0.016	0.50	8.91
NRT	1.90	2.70	5.40	2.50	3.00	3.25	0.54	0.01*	0.69	11.55
NVT	50.80	48.50	50.20	47.00	45.70	52.55	0.01*	-	-	5.03
PH	19.50	20.40	25.90	24.90	23.65	21.70	0.06	0.01	0.01*	10.59
LAI	1.24	1.48	1.80	1.73	1.60	1.52	0.05	0.01*	0.50	24.24

Note: F values and significance for the variables inoculation (Inoc), water available (Water) and W×I interaction by Tukey's HSD test at 5% probability. LAR: leaf appearance rate (leaves tiller<sup>-1</sup> day<sup>-1</sup>); PHY: phyllochron (days leaf<sup>-1</sup> tiller<sup>-1</sup>); LER: leaf elongation rate (cm tiller<sup>-1</sup> day<sup>-1</sup>); PER: pseudostem elongation rate (cm tiller<sup>-1</sup> day<sup>-1</sup>); LLS: leaf lifespan (days); FLL: final leaf length (cm); LSR: leaf senescence rate (cm day<sup>-1</sup>); NLL: number of live leaves; NRT: number of reproductive tillers; NVT: number of vegetative tillers; PH: plant height; LAI: leaf area index; \* Values < 0.01

**Table 3:** Number of vegetative tillers of buffel grass grown under different levels of water availability, with or without inoculation with plant growth-promoting microorganisms

Inoculation	Water availability (%)				CV	Equation	R <sup>2</sup>
	20	40	60	80			
With	42.2B	46.6B	47.0B	47.0A	5.12	1	0.96
Without	59.4A	50.4A	53.4A	48.0A	6.78	2	0.72

Means followed by the same lowercase letter in the column do not differ by Tukey's HSD test at 5% probability. Equation 1:  $y = -0.00275X^2 + 0.349X + 36.5$ ; Equation 2:  $y = 0.00163X^2 - 0.3335X + 64.35$

interval between the appearance of two consecutive leaves (phyllochron) by 14% and provided a 25% higher LER than control treatment (Table 2).

Higher soil water availability showed a positive linear response in LAR (Fig. 2a), LER (Fig. 2c), and PER (Fig. 2d), which increased by 28, 50 and 169%, respectively, and reduced phyllochron (Fig. 2b) by 1.3 days and LLS by three days (Fig. 2e). With the gradual increase in water availability decreased LSR (Fig. 2f) to a minimum estimated value of 1.06 cm day<sup>-1</sup> at 56.87% of PC, after which point it increased. Final leaf length responded linearly to the increase in water availability (Fig. 3a), increasing by 19% (P<0.01), whereas number of live leaves (NLL) showed a quadratic response (P<0.05), with a maximum estimated value of 5.95 leaves at 62.65% of PC (Fig. 3b).

There was a significant interaction effect between inoculation with plant growth-promoting microbes and water availability on the number of vegetative tillers (NVT) (Table 2). The inoculated treatment exhibited a quadratic polynomial curve with an estimated maximum value of 47 tillers at 63.45% of PC and an increase of 11%. Control treatment, however, showed a decreasing quadratic response to the increase in soil water availability, with a minimum number of 48 tillers at 80% of PC, showing a 24% decrease (Table 3).

Reproductive tillers began to appear at 21 days of regrowth. There was no significant interaction effect between water availability and microbial inoculation on the number of reproductive tillers of buffel grass (P>0.05). This variable showed a significant response only to the water availability factor in isolation (P<0.01; Fig. 3c), in a

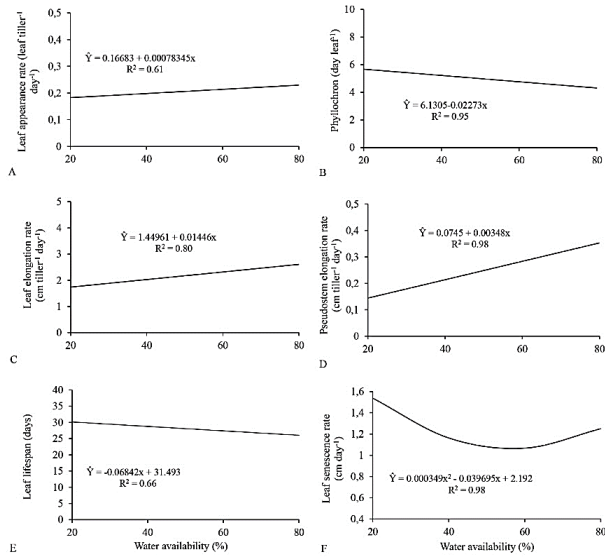
quadratic function whose estimated maximum was 4.34 tillers at 54.92% of PC. The LAI of buffel grass fitted a quadratic model in response to increasing water availability (P<0.01), with a maximum estimated index of 1.76 at 73% of PC, which represents a 40% growth (Fig. 3d). Plant height, in turn, had a positive linear response (P<0.01), increasing 5.4 cm (Fig. 3e).

### Biomass production

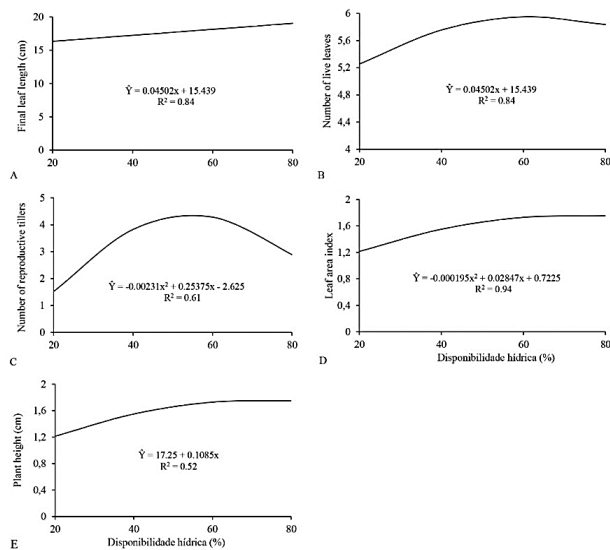
The interaction between inoculation with plant growth-promoting microbes and water availability was non-significant for the DM yield of the leaves, pseudostems, dead material, or shoots of buffel grass (P>0.05). However, the isolated factors had a significant effect (P<0.01) on all variables, except dead material. Inoculation with microbes influenced (P<0.05) the DM yields of leaves, pseudostems, and shoots, which were 16, 18 and 14% higher, respectively than those obtained with the control treatment (Fig. 4).

### Root evaluation

Shoot and leaf DM yields responded quadratically to increasing water availability (Fig. 5), with estimated maximum values of 11.80 g pot<sup>-1</sup> (shoots) at the water level of 75.21% of PC, and 5.56 g pot<sup>-1</sup> (leaves) at 68.34% of PC, which represents an increase of 49%. Pseudostem DM yield showed a positive linear response with a 65.5% higher value (Fig. 5). The interaction between water availability and microbial inoculation did not influence (P>0.05) root DM yield, volume or length. However, these variables showed a



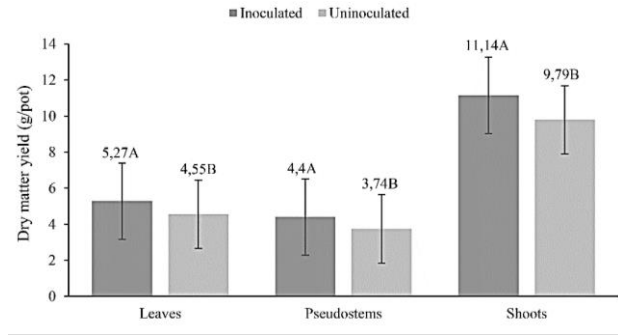
**Fig. 2:** Leaf appearance rate (A), phyllochron (B), leaf elongation rate (C), pseudostem elongation rate (D), leaf lifespan (E), and leaf senescence rate (F) of buffel grass plants under different levels of water availability



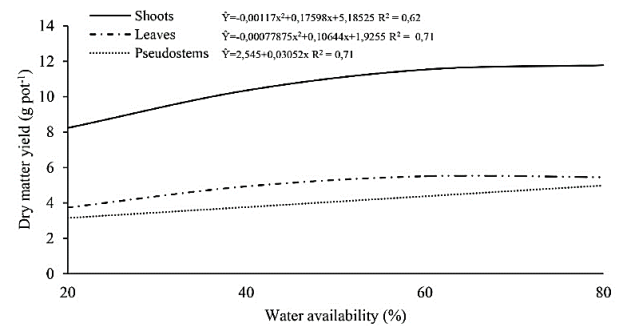
**Fig. 3:** Final leaf length (A), number of live leaves (B), number of reproductive tillers (C), leaf area index (D), and plant height (E) of buffel grass under different levels of water availability

significant response to the factors in isolation. Inoculating the buffel grass plants with *A. brasilense* and *R. intraradices* provided a 20% higher DM ( $P < 0.01$ ) and 15% larger roots ( $P < 0.01$ ) compared with the control set.

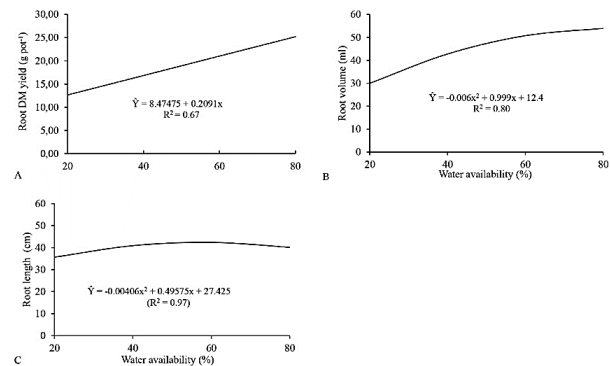
Root DM yield increased linearly, to a maximum of 61%, with increasing water availability (Fig. 6a). Root volume and length, on the other hand, showed a quadratic response (Fig. 6b, c), with estimated maximum values of 54 mL at 80% of PC and 42.55 cm at 61.01% of PC.



**Fig. 4:** Dry matter yield of leaves, pseudostems, and shoots in buffel grass inoculated or uninoculated with plant growth-promoting microorganisms. Means followed by the same letter do not differ from each other by Tukey's test at 5% probability



**Fig. 5:** Dry matter yields of shoots, leaves, and pseudostems in buffel grass under different levels of water availability



**Fig. 6:** Root DM yield (A), root volume (B), and root length (C) in buffel grass plants subjected to different levels of water availability

## Discussion

In our study, by comparing the decreases in LER and LAR (50 and 28%, respectively) under lower water availability, we confirm the assertion of Lemaire *et al.* (2008) who stated that LAR is the last element to be penalized by water deficits, unlike LER, which is dependent on turgor pressure for cell elongation. As stated by Coussemont *et al.* (2021), the decreased turgor pressure of leaves and stems of grasses

caused by water stress results in an inhibition of cell expansion and differentiation, thereby reducing elongation rate.

Higher water availability influenced an increase in tissue turnover dynamics, as seen by the higher LAR and LER and shorter phyllochron, which caused a direct reduction in LLS. The LLS represents an important morphogenetic trait in the determination of tissue turnover, as it indicates the maximum forage yield potential (maximum amount of live material per area). Additionally, it is a parameter for determining grazing intensity (in continuous grazing) or the frequency between grazing events (in rotational grazing), thus ensuring the maintenance of LAI at values close to those necessary for greater efficiency in intercepting incident radiation and for maximum growth rates (Costa *et al.* 2013).

Both water stress and greater water availability resulted in higher LSR (Fig. 2f), mainly because leaf senescence is a common effect in plants nearing the stage of maturity and can also occur in situations of severe water deficit (Brevedan and Egli 2003). As the water deficit is prolonged, there is an acceleration in leaf senescence and death of established tillers, inhibition of tillering and branching, and retardation of plant growth and development (Oliveira *et al.* 2017).

The applied moisture levels affected the structural traits of buffel grass, which is a reflection of the influence of soil water level on physiological and morphogenetic variables of the plant. Final leaf length decreased under lower levels of water availability. This is also a reflection of the effect of water stress on LER and LAR, since the growth period of a leaf is a constant fraction of the interval between the appearance of consecutive leaves. Additionally, while increases in LER are directly correlated with a larger final leaf size, smaller leaves are associated with higher LAR (Cruz *et al.* 2021).

Water stress also influenced the NLL of buffel grass, which is a structural trait, determined genetically by the species (Paciullo *et al.* 2017), and which can be influenced by characteristics of the environment where the plant is grown. Upon reaching a certain NLL, there is a balance between LAR and the senescence of leaves that have exceeded their lifespan. In this situation, the emergence of a new leaf implies the senescence of the leaf that preceded it, maintaining NLL relatively constant (Lemaire *et al.* 2011). Under low water availability, forage grasses make adaptations in an attempt to reduce water losses, e.g., by reducing leaf area, decreasing leaf cell division and expansion, altering leaf shapes, and triggering leaf senescence and abscission (Taiz *et al.* 2017).

The tillering dynamics of tropical forage grasses has a rearrangement capacity, depending on the conditions imposed on the forage plant (water stress, inoculations, intensity and frequency of harvesting or grazing, etc.). Furthermore, depending on the growth condition, the species may alter the proportion between tiller density and

weight/height, in addition to the basal, aerial, and reproductive characteristics. Hence, the leaf area is able to maximize light absorption and help the plant to withstand imposed water stress condition imposed as noted in the present case.

A decreased of number of reproductive tillers observed under greater water availability may imply that there was adequate growth condition, and buffel grass stopped prioritizing the tillers production rather invested its photoassimilates for foliar maintenance. It is important to note that factors such as the water regime can affect not only the vegetative development of forage grasses, but also the uniformity of inflorescence emission as well as seed filling, since this process requires a considerable amount of photoassimilates (Lima *et al.* 2020). Although the early flowering of buffel grass is known to be a strategy to perpetuate the species (Oliveira *et al.* 2023), its influence in reducing the nutritional value of the pasture makes this characteristic a target to be postponed in the area, which can be achieved mainly by shortening the grazing cycles. However, further investigations are needed to understand the behavior of the types of tillers that make up the pasture and how their combinations influence the structural arrangement of the available forage under water stress conditions and microbial inoculation.

The levels of water availability affected the plant height (Fig. 3e) and LAI (Fig. 3d) of buffel grass. The latter parameter constitutes the synthesis of the morphogenetic and structural traits of the grass and is a consequence of the balance between processes that determine the supply (photosynthesis) and demand (respiration and metabolism) of photoassimilates, which establish the pace of pasture growth (Pontes *et al.* 2010).

Inoculating the plants with the microorganisms *A. brasilense* and *R. intraradices* increased the biomass production of shoots, leaves, and pseudostems of buffel grass. Several studies have reported the effect of the mycorrhizal fungus *R. intraradices* in increasing biomass in several crops, e.g., *Eleusine coracana* (Tyagi *et al.* 2018), soybean (Jie *et al.* 2021) and maize (Stoffel *et al.* 2020). Its effects were correlated with greater nutrient uptake (mainly phosphorus), greater root system development, and improved photosynthetic efficiency in abiotic stress conditions.

Some studies describe the beneficial effects of co-inoculation between *A. brasilense* and AMF for both the inoculated plants and the microorganisms. Rouseaux *et al.* (2020) evaluated co-inoculation of *A. brasilense* and *Rhizoglossum irregulare* and N fertilization in *Brachiaria hibrida* cv. Mulato II and obtained similar values using a biofertilizer at a rate of 100 kg ha<sup>-1</sup>, with a 43% increase in DM yield in comparison to the control plants (without inoculation or fertilization). In addition, the authors reported that the levels of mycorrhizal colonization were the result of the effectiveness of the fungus *R. irregulare* and the contribution of *A. brasilense* to the improvement of the

levels of root occupation by the fungus.

Decline in leaf and pseudostem yields in buffel grass and other tropical forage grasses under water deficits is widely reported (Maranhão *et al.* 2019; Souza *et al.* 2020). This result is mainly due to the key importance of water for the processes of maintenance of cell turgor and expansion, which explains the response of shoot biomass production to the different levels of water availability studied in the present trial.

Microbial inoculation increased root growth, biomass production and length (Fig. 6). Smith and Read (2008) described that the mycelium network of AMF connected to the roots is able to increase the root volume of plants and consequently improve their water and nutrient uptake efficiency. On the other hand, the colonization of the rhizosphere and root tissues by *A. brasilense* produces phytohormones that stimulate root growth as well as nitrogen uptake through biological nitrogen fixation, making this association of microorganisms particularly beneficial for structuring the root system of forage grasses.

Hungria *et al.* (2021) examined the root morphology of *Urochloa brizantha* cv. Marandu inoculated with *A. brasilense* and *Pseudomonas fluorescens* and found a 93% higher root DM weight, a 12% denser root system, 83% longer roots, and 33% more radicular branches when *U. brizantha* was inoculated with *A. brasilense*, demonstrating the effect of inoculation in strengthening the root system of the species. The response of the buffel grass root system to the different levels of water availability denotes the impact of water deficiency on this plant species, as evidenced by the traits of root volume, length, and DM (Fig. 6).

## Conclusion

The ideal moisture range for the cultivation of buffel grass cv. Aridus is 55 to 70% of the soil's maximum water-holding capacity, as it maximizes the number of live leaves, leaf production, and leaf area index and minimizes leaf sensitivity and the number of fertile tillers. Inoculation improves the productive potential of shoots and root system of buffel grass, increasing its adaptability to semi-arid environments and making it more resilient to the current scenarios of climate change and global warming.

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## Author Contributions

EMP: Planned the experiments, investigation, performed the analysis and writing original draft. FAT, DDF, CAAOF and KSA: Supervision, validation and review the text. HSS, ERTC, JPS, JRSF, FMJ and TMV: Investigation, collected

samples and experimental evaluations. All the authors have read and agreed to the submitted version of the manuscript.

## Conflicts of Interest

All authors declare no conflict of interest.

## Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

## Ethics Approval

Not applicable to this paper.

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