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Plant growth-promoting rhizobacteria alleviate drought-stress in upland rice

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Abstract

Drought poses a substantial risk to global agriculture and food security. Plant growth-promoting rhizobacteria (PGPR) offer a promising biotechnological alternative to enhance crop resilience to drought. This study investigated the morphophysiological and agronomic responses of Samambaia Branco, a drought-sensitive upland rice landrace, inoculated with *Bacillus toyonensis* (BRM 32110), *Burholderia* sp. (BRM 32111), and *Serratia marcescens* (BRM 63523), under drought stress in a phenotyping platform. Drought significantly impaired overall crop performance, reducing grain yield by 27.7% and increasing spikelet sterility up to 18.4% in non-inoculated plants. However, PGPR-inoculated plants underwent robust belowground architectural restructuring, showing average increase in root length (29.4%), surface area (34.1%), volume (36.6%) and diameter (8.7%). This enhanced root system provided a mechanistic basis for superior water status. PGPR-inoculated plants sustained higher leaf water potential under drought stress (-0.67 MPa on average) than non-inoculated plants (-1.3 MPa). Hydraulic preservation was coupled with a profound shift in non-structural carbohydrate partitioning, characterized by depletion of starch reserves (26.5%) and total soluble carbohydrates (16.7%), alongside accumulation of low-molecular-weight compatible solutes (glucose, fructose, and sucrose) osmotic adjustment. During drought and recovery, PGPR-inoculated plants, particularly BRM 32110 and BRM 63523, optimized the tradeoff between carbon gain and transpirational loss, achieving a 12.4% increase in water use efficiency and partially lowering the physiological threshold for stomatal reopening. Multivariate principal component analysis confirmed clear treatments separation, highlighting distinct microbe-mediated metabolic pathways for drought tolerance. Overall, bioagent-based technologies can bolster the climate resilience of drought-sensitive landraces, improving crop performance.

Introduction

Drought remains a preeminent threat to global food security, severely limiting crop yields (Hu et al. 2025). As climate change intensifies, the projected increase of the frequency and intensity of summer droughts to drive sharp a significant crop output deficiency (Huang et al. 2024). This vulnerability is particularly pronounced in regions like the Brazilian Cerrado, where intermittent dry spells (veranicos) are common during the rainy season, imposing significant constraints on growth, development, and grain yield of the crops (Porto et al. 2026).

The decline in crop yield under water deficit is driven by a cascade of physiological and biochemical alterations (Hu et al. 2025). Water scarcity destabilizes core metabolic frameworks, including photosynthetic efficiency, respiratory flux, photoassimilates translocation, and nutrient uptake and flux (Caferri and Bassi 2022). These perturbations are primarily orchestrated by dwindling soil water potential, which triggers systemic adaptive responses (Seleiman et al. 2021). While plants possess endogenous mechanisms to attenuate these stressors (Zhang et al. 2023), interspecific association with plant growth-promoting rhizobacteria (PGPR) have emerged as a transformative biotechnological frontier for enhancing abiotic stress resilience (Fadiji et al. 2022).

PGPR bolster plant resilience by synthesizing bioactive compounds and optimizing nutrient acquisition (Braga et al. 2022). These microorganisms facilitate iron sequestration via siderophores, produce essential phytohormones such as indole-3-acetic acid (IAA), and solubilize recalcitrant minerals like phosphorus, thereby modulating plant growth through hormonal regulation (Braga et al. 2022). Under water-deficit conditions, PGPR further support osmotic adjustment by releasing osmoprotectants (e.g., glycine betaine) and stimulating the accumulation of proline, starch, and total soluble carbohydrates (Al-Turki et al. 2023). Additionally, they enhance drought tolerance by producing exopolysaccharides (biofilm), and synthesizing 1-aminocyclopropane carboxylic acid (ACC) deaminase, an enzyme that reduces ethylene production (Chandra et al. 2019; Huang et al. 2014; Glick et al. 2007; Wang et al. 2012). Notably, the efficacy of these microbial interventions is most pronounced under severe environmental constraints (Okorie 2026; Yuwono et al. 2005).

Considering the vast phenotypic diversity in upland rice for drought tolerance (Lanna et al. 2021), this study investigated how PGPR inoculation modulates the drought-responsive profile of the drought-sensitive upland rice landrace 'Samambaia Branco'. The objective was to evaluate the effects of different plant growth-promoting rhizobacteria (PGPR) on the morphophysiological and agronomic responses of this landrace under drought stress. We hypothesized that microbial inoculants, through their adaptive potential to drought, would improve the performance of this variety by enhancing physiological acclimation and agronomic performance under water deficit conditions. This approach not only promotes sustainable agricultural practices but also contributes to ensuring food security.

Material and Methods

Location of the experimental area

The experiments were carried out under a greenhouse condition at the Integrated System for Drought-Induced Treatment (Portuguese acronym SITIS) plant phenotyping platform facility, from October 2019 to March 2020. The SITIS is located at Embrapa Rice and Beans, Santo Antônio de Goiás, Goiás, Brazil (16°28’00” S, 49°17’00” W, with an altitude of 823 m). All treatments were performed in soil columns of diameter 25 cm and height 100 cm, placed over digital scales to monitor the water amount in each column containing four plants. The chemical characteristics of the Oxisol soil were determined according to Donagema et al. (2011) and are shown in Table 1.

Table 1
Physicochemical characteristics of soil placed in the columns at SITIS phenotyping platform in 2019/2020

pH_{H2O}	Ca^{+2}	Mg^{+2}	$H^{+} + Al^{+3}$	P	K^{+}	Cu^{+2}	Zn^{+2}	Fe^{+2}	Mn^{+2}
5.8									
	$mmol_c\ dm^{-3}$			$mg\ dm^{-3}$					
	26.7	11.2	17.0	1.6	100.0	1.1	1.8	11.3	27.2
OM* ($g\ kg^{-1}$)	EC**			Clay		Silt		Sand	
42.7	135.8			$g\ kg^{-1}$					
				421		212		367	
*OM = organic matter									
**EC = Eletric conductivity									

Upland rice and PGPR

A Brazilian upland rice landrace variety named Samambaia Branco was used for all experiments in this study. It is an accession of Embrapa's GAB (Germoplasm Active Bank) and its geographical origin is Mantenópolis, Espírito Santo state, Brazil. Its phenotypic characteristics are as follows: plant cycle of 120–140 days, intermediate grain quality and moderate cooking quality (Embrapa Alelo System). This genotype was specifically chosen due to its sensitivity to water deficit (Costa et al. 2023) so the bioagents pretreatment applied would show any visible effects.

Bacillus toyonensis (BRM 32110), *Burkholderia* sp. (BRM 32111), and *Serratia marcescens* (BRM 63523) were obtained from the rhizospheres and phyllospheres of healthy rice plants in a commercial field (Faria et al. 2023; Filippi et al. 2011) and their biochemical characteristics and taxonomic classification are available in Martins et al. (2021) and Faria et al. (2023). They are currently stored and preserved in the Multifunction Microorganisms Collection of Embrapa Rice and Beans.

Bacterial inoculation

PGPR suspensions were prepared in liquid medium 523 (nutrient broth) (Kado and Heskett 1970) under constant agitation for 24 hours at 28°C. Bacterial concentrations were standardized using a spectrophotometer to A540 = 0.5, corresponding to $1x10^8$ colony-forming units (CFU) per mL. PGPR were applied at three distinct stages of the rice life cycle: 1. Seed Microbiolization (before treatment, seeds were surface-disinfected by immersion in 70% ethanol, 1 min, and 0.05% sodium hypochlorite, 1 min, followed by air-drying). Microbiolization was performed by immersing seeds in the respective

bacterial suspensions) for 24 h at 25°C under constant agitation (Filippi et al. 2011). 2. Soil Application: At 30 days after planting (DAP), 100 mL of bacterial suspension 1x10^8 CFU mL^{-1} was applied directly to the soil. 3. Foliar Application: At 78 DAP, 100 mL of the respective treatments were applied to the rice shoots using a manual sprayer. Non-inoculated plants were used as a control treatment.

Water regimes

The hydric treatments consisted of two water levels: well-watered (*WW*) and drought stress (*DS*), being that in the first (*WW*) the amount of soil water was initially established to 80–85% of field capacity (FC) and kept throughout the crop cycle. For the *DS* condition, irrigation was performed as in the *WW* condition until the plants reached the V11 vegetative stage (collar formation on leaf 11 on main stem), followed by the suspension of irrigation for six days. After this period, 50% of the volume of evapotranspired water of *WW* columns was replaced at the plate of the respective *DS* columns, for 10 days. The amount of evapotranspirated water was estimated based on the water quantity required to keep soil at FC 80–85% in the *WW* plants (Table 2).

Table 2
Evapotranspiration and transpiration rate of the Samambaia Branco landrace variety modulated by PGPR throughout water-deficit period

Total evapotranspiration (L)	***WW****	***DS***	**Reduction (%)**
	12.21	**5.52**	**54.8**
Evapotranspiration rate (mL H_2O day^{-1} $plant^{-1}$)	195.0	87.0	55.4
Transpiration rate (mmol H_2O m^{-1} s^{-1})	3.47	1.99	42.7
Water regimes: **WW* - well-watered condition and ***DS* – drought stress condition			

After the water-deficit period, irrigation was restored until the end of the crop cycle R8 (at least one grain on the main stem panicle with a brown hull)/R9 (all grains that reached R6 have brown hulls). In the *WW* columns, the evapotranspiration rate was determined daily (difference between the reference mass and the column/day mass) and restored through irrigation placed on the soil surface to achieve the initial mass (reference mass) again (Fig. 1A). Meteorological conditions within the SITIS phenotyping platform, monitored via a Hobo® U12-12 datalogger (Onset Computer Corp. Ltd, Cape. Cod, MA, USA), are shown in Fig. 1B.

Morphophysiological traits

Leaf gas exchange

Gas exchange parameters were measured on two fully expanded top leaves in each column. The following variables were determined: photosynthetic CO_2 assimilation (*A*, μmol CO_2 m^{-2} s^{-1}), transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), and stomatal conductance (*gs*, mol H_2O m^{-2} s^{-1}). Measurements were performed on the last day of the water-deficit period using a portable gas exchange analyzer in the infrared region (LI-COR LI-6400xt). During measurements, the atmospheric CO_2 concentration was maintained at 400 μmol mol^{-1}, and the photosynthetically active radiation (PAR) was set to 1200 of photons m^{-2} s^{-1}. Water use efficiency (*WUE*, μmol CO_2 mol^{-1} H_2O) was calculated as *A*/*E* ratio (Guerra et al. 2014) and carboxylation efficiency (*CE*, [(μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$]) was determined as the *A*/*Ci* ratio (Costa et al. 2025). Evaluations were conducted between 08:00 and 10:00 a.m. across four evaluation time throughout water-deficit period:

Phase I - six days after irrigation cut-off (87 DAP); Phase II - two days after the plants received 50% of water replenishment at the column base (91 DAP); Phase III - nine days after the plants received 50% of water replenishment at the column base (96 DAP); and Phase IV - one day post rehydration (98 DAP).

Non-structural carbohydrates

On the last day of water-deficit period (97 DAP), the second fully expanded leaf from the shoot apex of the main stem was collected for non-structural carbohydrates analysis. Immediately following collection, leaves were partitioned into small fragments and enzymatically inactivated using a microwave oven (Consul, model Facilite 32 L) at maximal power in 10-second intervals for a total of 1 min. The samples were subsequently oven-dried at 50°C for 24 h to reach a constant mass. Once dehydrated, the leaf tissue was cryogenically ground into a fine powder using a mortar and pestle with liquid nitrogen. Sample processing and the extraction of non-structural carbohydrates, specifically total soluble carbohydrates (*TSC*), starch, and reducing sugars (sucrose, fructose and glucose) were determined (Costa et al. 2021).

Status hydric

On the last day of water-deficit period (97 DAP), leaves on the upper part of the plant were used to assess the relative water content (*RWC*, %), and osmotic potential (Ψs, MPa), according to Dutra et al. (2010). For *RWC*, 1.2 cm diameter leaf disks (total of five) were weighed (fresh matter) and transferred to a container with distilled water, in which they were kept in the dark for approximately 24 hours at 4^{o}C for rehydration. After this period, the disks were weighed to obtain turgid mass and placed in a laboratory oven at 65^{o}C for 48 hours to obtain dry mass. The RWC was estimated according to the formula: *RWC = (FM – DM)/(TW – DM)* x 100, in which *FM* = fresh matter of five disks; *DM* = dry matter of five disks; *TW* = turgid mass of five disks. For Ψs, leaves were collected and immediately placed in previously identified syringes and stored in a freezer at – 20^{o}C for later extraction of cell sap and reading in a vapor pressure

osmometer (Wescor 5600). The osmolarity (c) of the collected sap was analyzed and converted from mosmol Kg^{-1} to MPa using the formula: Ψs (MPa) = - c (mosmol kg^{-1}) x 2.58 x 10^{-3}.

Leaf water potential (Ψw, MPa) was also evaluated using a Scholander pressure chamber (Soilmoisture Equipment, model 3005) (Scholander et al. 1965), at 05:00 and 06:00 a.m. The reading was determined at the extremity (tip) of two flag leaves of the primary tiller of two upland rice plants, and the pressure was applied until exudation from the cut made in the leaf collar.

Root phenotyping

On the first day after the plants received 50% of water replenishment at the column base (90 DAP), the root system was evaluated. Briefly, to carry out the root system capture acrylic tubes were installed inside the columns and four rice plants were planted around the tube. The root system was assessed by measuring the length (*RL*, cm), surface area (*RSA*, cm^2), volume (*RV*, cm^3) and diameter (*RD*, mm) of the roots through images generated on a root scanner CI – 600 Cano Scan (CID Bio-Science, Version 3.1.19), with quantification by the WinRhizo software (Regent Instruments Canada Inc., version 2008a 32 bits) (Lanna et al. 2016). Root images corresponding to depth of 5 to 45 cm.

Agronomic traits

Grain yield (*GY*, g of grain $plant^{-1}$), which means the total mass of grains, in grams, was obtained for one plant per column, and yield components such as 100-grain mass (*100GM*, g) and spikelet sterility (*SS*, %) were determined on the harvest period. *SS* was estimated as: (*NEG* × 100) TG^{-1}, where *SS* = spikelet sterility, *NEG* = number of empty grains, and *TG* = total number of grains.

Experimental layout and statistical analysis

The experiment was conducted in a randomized complete block design (RCBD) using a 2 x 4 factorial scheme with four replicates. The first factor consisted of two water regimes: well-watered (*WW)* and drought stress (DS). The second factor comprised three microbial treatments: inoculation with *Bacillus toyonensis* (BRM 32110), *Burkholderia* sp. (BRM 32111), *Serratia marcescens* (BRM 63523), and a control treatment (non-inoculated plants).

Data on the effect of treatments on gas exchange parameters were submitted to analysis of variance (ANOVA) in a split-plot design, with interaction between treatments and assessment times. The remaining data were analyzed by one-way ANOVA. Data normality was assessed by the Shapiro-Wilk test, homogeneity of variances by Levene test, and means were compared using Tukey's test at 5% significance. Principal component analysis was performed between treatments and variables that showed significant differences to understand the influence of water deficit on the variables studied. All statistical analyses were performed using R software version 4.5.2 (2025-10-31) (R Core Team, 2026) with the "ExpDes.pt", "ggplot2" and "factoextra" packages.

Results

Morphophysiological traits of Samambaia branco modulated by PGPR under drought stress

The water-deficit effects on the *A*, *E*, *gs*, *WUE* and *CE* in Samambaia branco is shown in Table 3. The magnitude of these responses was significantly modulated by microbial treatments as well as by four evaluation time throughout drought stress period ($p < 0.05$).

Table 3
The significance levels (P-values) for rate CO_2 assimilation (*A*), transpiration rate (*E*), stomatal conductance (*gs*), water use efficiency (*WUE*), and carboxylation efficiency (*CE*) of the Samambaia Branco plants modulated by PGPR under *WW* and *DS* conditions

Source of variation	Treatments (T)	Day (D)	Interaction FC x D	CV 1 (T)	CV 2 (D)
A (μmol CO_2 m^{-2} s^{-1})	$1e^{-06}$ ***	2e-16 ***	$1e^{-06}$ ***	13.0	9.9
E (mmol H_2O m^{-2} s^{-1})	$2.2e^{-16}$ ***	$2.2e^{-16}$ ***	$2.2e^{-16}$ ***	14.0	14.1
gs (mol H_2O m^{-2} s^{-1})	$2e^{-16}$ ***	$2e^{-16}$ ***	$3e^{-16}$ ***	20.8	20.7
WUE (μmol CO_2 mol^{-1} H_2O)	0.838 ns	$2e^{-16}$ ***	$2e^{-06}$ ***	29.2	21.1
CE [(μmol m^{-2} s^{-1}) (μmol mol^{-1}) $^{-1}$]	$1.3e^{-05}$ ***	$2.2e^{-16}$ ***	0.0012 **	17.6	13.9

CV (%) = coefficient variation; ns = not significant; T = microbial treatments; D = four evaluation time throughout drought stress period; ** $p < 0.01$; *** $p < 0.001$

During the initial stress onset (Phase I; sixth day after irrigation cut-off), Samambaia branco (87 DAP) exhibited classic avoidance strategies, including leaf-rolling and a sharp decline in stomatal closure. At this phase, the *DS* condition reduced the *A* and *gs* by Samambaia branco plants compared to *WW* condition, regardless of PGPR application (Fig. 2A e 2C). Average values of *A* and *gs* ranged from 10.4 to 16.8 μmol CO_2 m^{-2} s^{-1} and 0.017 to 0.124 mol H_2O m^{-2} s^{-1} in *DS* and *WW* conditions, respectively. Interestingly, *E* remained stable at 4.0 mmol H_2O m^{-2} s^{-1} (Fig. 2B). As the stress progressed (Phases II and III), after plants received 50% of water replenishment at the column base for 1 and 10 days (91 and 96 DAP), respectively, low average values of *A* and *E* were observed in Samambaia branco under drought stress, regardless of PGPR application. *A* ranged from 7.25 to 12.36 μmol CO_2 m^{-2} s^{-1}, and *E* ranged from 3.62 to 6.78 mmol H_2O m^{-2} s^{-1} in *DS* and *WW* conditions, respectively. In Phase III, non-inoculated plants showed the lowest transpiration rate (2.71 mmol H_2O m^{-2} s^{-1}). On the contrary, Samambaia branco inoculated with PGPR partially recovered the *gs*, which ranged from 0.098 to 0.195 mol H_2O m^{-2}

s^{-1} in non-inoculated and PGPR-inoculated plants, respectively. In phase IV, one day post rehydration (98 DAP), Samambaia branco, regardless of PGPR application, restored the functioning of the photosynthetic machinery since the *DS* plants showed similar values of photosynthetic rate of the *WW* plants (average value 19.6 µmol CO_2 m^{-2} s^{-1}). By contrast, the recovery of *E* and *gs* in *DS* plants was only 80.8 and 69.6%, respectively, compared to *WW* plants.

The progressive soil water depletion triggered a strategic shift in the gas exchange dynamics of Samambaia branco. As *gs* declined more sharply than *A* throughout drought stress period, a significant upward trend in *WUE* was observed (Fig. 2D). In the last two evaluation time of gas exchange (Phases III and IV), PGPR-inoculated plants (particularly BRM 32110 and BRM 63523) exhibited superior water-spending strategies, achieving an average *WUE* of 3.65 µmol CO_2 mol^{-1} H_2O, a 12.4% increase over non-inoculated plants (average value 3.25 µmol CO_2 mol^{-1} H_2O). This suggests that these rhizobacteria optimized the tradeoff between carbon gain and transpirational loss, a critical trait for drought resilience. In addition to increased relative stomatal limitation, water-deficit also triggers metabolic limitations such as the reduction in maximum Rubisco carboxylation activity and electron transport. In our study, it was observed a carboxylation efficiency (*CE*) general reduction throughout the drought stress period compared to their *WW* plants, regardless of PGPR application (Fig. 2E). Average values of *CE* ranged from 0.031 (Phase I) to 0.034 (Phase II), and 0.023 [(µmol m^{-2} s^{-1}) (µmol mol^{-1})$^{-1}$] (Phase III). During this period, all the microbial treatment applied in Samambaia branco did not show the ability to help the plants to overcome the limitation in the diffusion of CO_2 by stomata and mesophyll and effective fixing of CO_2 (47.5% of *CE* reduction). At one day post hydration (Phase IV), Samambaia branco restored the activating of the Rubisco since the *DS* plants showed *CE* similar values of the *WW* plants, average value 0.057 [(µmol m^{-2} s^{-1}) (µmol mol^{-1})$^{-1}$].

To sustain physiological homeostasis under drought stress, Samambaia branco underwent a significant metabolic shift in carbohydrate partitioning. In this study, there was a significant difference ($p < 0.01$) for total soluble carbohydrate (*TSC*), starch, glucose, fructose, and sucrose for all variation sources (Fig. 3). The transition from Phase I to Phase III was characterized by a marked depletion of carbon reserves, with starch content declining by 26.5% (from 38.90 to 28.61 mg g^{-1} DM) and total soluble carbohydrates (*TSC*) decreasing from 5.04 to 4.20 mg g^{-1} DM. The decline of storage carbohydrates content was inversely coupled with a robust accumulation of low-molecular-weight sugars. Specifically, fructose levels surged nearly 3-fold (4.93 to 13.69 mg g^{-1} DM), highlight for BRM 63523-inoculated Samambaia branco, while sucrose and glucose concentrations increased by 6.2 and 16.4%, highlight for BRM 32110-inoculated Samambaia branco, respectively.

In relation to the hydric status, the drought stress effects showed a significant difference ($p < 0.05$) for Ψw, *RWC* and Ψs (Fig. 4). While non-inoculated Samambia branco exhibited a sharp decline in Ψw, reaching a value of -1.13 MPa, plants associated with PGPR maintainted significantly higher Ψw levels (Fig. 4B). Notably, BRM 32110-inoculated Samambaia branco followed BRM 32111 and BRM 63523-inoculated Samambaia branco maintained high water potential during drought stress period (-0.47, -0.73

and − 0.80 MPa, respectively), corresponding to 54.5, 36.4 and 27.3% higher than non-inoculated plants, respectively. Regarding *RWC*, a direct proxy for cellular hydration, the value ranged from 85.7% in the leaves of the *WW* plants to 77.2% in the leaves of the *DS* plants, regardless of PGPR application (Fig. 5A). However, the accumulation of compatible solutes (Ψs), although significant for all microbial treatments, it was numerically higher for the BRM 63523-inoculated Samambaia branco (-1.46 MPa) compared to the average of other treatments (-1.33 MPa) (Fig. 5C).

The analysis of root traits at 90 DAP revealed that microbial treatments significantly restructured the below-ground architecture, providing a mechanistic basis for the enhanced drought tolerance observed ($p \leq 0.05$; Fig. 5). Under drought stress, plants associated with PGPR exhibited a substantial expansion in their foraging apparatus compared to non-inoculated plants. The BRM 63523 stood out as the most potent modulator of root development, promoting an 57.7% increase in *RL* and a 44.2% expansion in *RSA*. Similarly, *RV* and *RD* were enhanced by 42.5% and 15.6%, respectively. BRM 32110 and BRM 32111 also showed significant, albeit more moderate, gains in these parameters, *RL* (10.9 and 19.7%), *RSA* (36.3 and 21.9%), *RV* (40.4 and 26.8%) and *RD* (2.1 and 8.3%), respectively.

Agronomic traits of Samambaia branco modulated by PGPR under drought stress

The effects of water regimes and rizhobacteria inoculation on the agronomic performance of Samambaia Branco were evaluated via grain yield (*GY*) and its components: 100-grain mass (*100GM*) and spikelet sterility (*SS*). Significant difference ($p \leq 0.05$) were observed across all variation sources (Fig. 6).

Under *WW* condition, no significant differences in *GY* were detected among microbial treatments ($p < 0.05$), with an overall mean of 24.80 g grain plant^{-1}. In contrast, *DS* condition significantly impaired agronomic performance, regardless of the PGPR inoculation, resulting in a mean *GY* of 17.92 g grain plant^{-1}, a 27.7% reduction compared to the *WW* condition. However, it was observed that PGPR-inoculated Samambaia branco under drought stress, showed grain yield higher than non-inoculated plants (Fig. 6A). Regarding yield components, *DS* condition nearly doubled spikelet sterility, increasing from a mean of 9.0% in *WW* to 16.8% in *DS* conditions. The highest sterility rate was observed in non-inoculated plants under drought stress (18.4%) (Fig. 6B). Conversely, the *100GM* remained stable across water regimes, maintaining an average of approximately 3.55 g (Fig. 6C).

Multivariate analysis

Principal component analysis (PCA) revealed that the first two principal components accounted for 81.2% of the total variance, with PC1 explaining 62.8% and PC2 explaining 18.4% (Fig. 7). This high cumulative variance indicates that the two-dimensional projection adequately represented the multivariate structure of the dataset. PC1 was primarily driven by water status-related variables, highlighting water availability as the main source of physiological variation among treatments. Notably,

sucrose, fructose and glucose contents, together with spikelet sterility, were projected in the opposite direction to the remaining variables, indicating negative correlations with traits associated with plant water status and physiological performance.

The PCA scores showed a clear separation between water regimes, mainly along PC1. Well-watered (WW) plants clustered closely with leaf water potential (Ψw), relative water content (RWC) and osmotic potential (Ψs), reflecting the strong positive association among these variables and the maintenance of plant water status under non-stress conditions. In contrast, drought-stressed (DS) plants were positioned closer to spikelet sterility, reinforcing the detrimental effect of water deficit on reproductive performance. Among microbial treatments, Samambaia Branco plants inoculated with BRM 32110 were strongly associated with glucose and sucrose accumulation, whereas those inoculated with BRM 32111 and BRM 63523 were more closely related to fructose accumulation. These distinct associations suggest strain-specific modulation of carbohydrate partitioning, indicating that different PGPR promoted drought acclimation through complementary metabolic strategies associated with osmotic adjustment.

Discussion

Understanding the mechanisms governing crop drought responses, particularly through interactions with PGPR, is crucial and urgent for developing resilient agricultural systems (Sharma et al. 2025). This study integrated agronomic traits with the morphophysiological adaptative mechanisms of Samambaia branco inoculated with rhizobacteria. Key among these adaptations were shifts in gas exchange dynamics, which serve as high-fidelity indicators of drought severity and phenotypic plasticity (Huang et al. 2024).

During Phase I (early-stage drought), Samambaia branco exhibited a sharp 86.3% decline in stomatal conductance (*gs*) without significant changes in transpiration rate (*E*) across both inoculated and non-inoculated treatments. This suggest that early-stage stomatal control in this genotype prioritizes the maintenance of leaf turgor over evaporative colling. Furthermore, the synergistic interplay between leaf-rolling and stomatal closure likely attenuated solar radiation incidence and excessive water loss, thereby delaying the progression of drought stress (Wang et al. 2023). Under the sustained drought of Phases II and III (50% water at the base of the column), photosynthetic rate (*A*) and *E* remained markedly suppressed. However, PGPR-inoculated plants maintained higher *gs* (reflecting greater stomatal density and activity) than non-inoculated plants. This implies that plant-microbe interactions may lower the physiological threshold for stomatal reopening even under water-limited conditions. Upon rehydration (Phase IV), *A* was rapidly restored, whereas *E* e *gs* exhibited incomplete recovery. This legacy effect indicates that Samambaia branco maintains a conservative hydraulic strategy despite the restoration of soil moisture. When drought stress is temporarily imposed, the associated soil microbial communities can retain a structural or functional "legacy effect" that continues to modulate plant health and growth post-stress (Zhao et al. 2026).

The capacity of Samambaia branco to perceive environmental stimuli and modulate its hydraulic status was directly reflected in its water-use efficiency (*WUE*). Inoculation with isolates BRM 32110 and BRM

63523 significantly enhanced *WUE*, driven by optimized stomatal regulation; these plants maintained approximately 78.0% of their photosynthetic capacity despite a 48.8% reduction in *gs*. This optimized *WUE* facilitates leaf water retention amidst CO_2 diffusion limitations, shielding the photosynthetic apparatus from desiccation (Abou Jaoudé et al. 2024). Conversely, drought-induced declines in carboxylation efficiency (*CE*), a physiological proxy for the in vivo activity and functional capacity of the enzyme Rubisco (Cui et al. 2025), were observed across all treatments. However, the rapid recovery of *CE* within 24 hours of rehydration indicates that these declines stemmed from metabolic down-regulation rather than irreversible photo-oxidative damage to the photosystems.

Drought-induced leaf senescence and reserve mobilization are core strategies for stress mitigation, involving the translocation of stored sugars and starch from older leaves or stems (source tissues) to developing tissues (sinks) (Ghate et al. 2017; Tan et al. 2023). Both strategies are fundamental for maintaining root growth, enabling osmotic adjustment, and protecting against oxidative damage during the vegetative stage. Our results revealed a strategic shift in carbohydrate profiles: a reduction in starch and total soluble carbohydrates was coupled with a significant accumulation of hexoses and disaccharides, particularly in plants inoculated with BRM 32110 and BRM 63523. The conversion of insoluble reserves into soluble sugar is a vital osmotic adjustment mechanism. This biochemical adaptation increases sap solute concentration, effectively lowering osmotic potential (Ψs), which allows the plant to maintain water uptake and protect cellular integrity during drought stress (Chen et al. 2024).

In this study, both the accumulation of compatible solutes (reflected in decreased Ψs) and a moderate reduction in relative water content (*RWC*) was observed in all drought stress Samambaia branco plants during the vegetative stage. The increase of sap solute concentration indicates a robust capacity for osmotic adjustment, maintaining a sustained pressure gradient for water influx as soil moisture declined. Concurrently, the controlled 10% reduction of *RWC* suggest a high capacity to conserve water and modulate photosynthetic capacity to tolerate fluctuating moisture availability (Da Mata et al. 2023; Wang et al. 2022). Notably, PGPR-inoculated plants maintained a higher leaf water potential throughout drought stress period than non-inoculated plants. This "hydraulic lifeline", mediated primarily by the BRM 32110 and BRM 63523 isolates, refined the plant's osmotic machinery and prevent cellular desiccation.

Roots play a critical role in sensing drought and exhibit diverse adapted responses tailored to prevailing soil moisture deficit (Kalra et al. 2023). These structural adaptations are critical for maximizing water and nutrient extraction, escaping soil-borne diseases, and outcompeting weeds under stress (Holz et al. 2024; Kamoshita et al. 2004; Kato et al. 2007; Siopongco et al. 2006). Roots are also synthesize chemical signals in response to progressive drought, which retrogradely regulate leaf stomatal conductance, transpiration and shoot growth (Burridge et al. 2022). In this study, inoculation with PGPR, most notably BRM 32110 and BRM 63523, promoted significant increases in length and surface area, maximizing the soil-root interface. Functionally, this represents an enhanced hydraulic foraging capacity, allowing the root system to explore deeper soil profiles and sustain water uptake as the upper soil layers dry out. This shift toward a more robust root system architecture explains the superior leaf water

potential recorded during Phase III in PGPR-inoculated plants, establishing a direct link between microbe-induced morphological plasticity and whole-plant physiological resilience.

Ultimately, this direct link between bacterial inoculants and physiological resilience culminated in greater yield stability. Under drought stress, PGPR-inoculated 'Samambaia Branco' experienced a yield reduction of only 25.9%, compared to a 32.0% loss in non-inoculated plants. These results corroborate previous findings demonstrating that bioagents enhance gas exchange and biomass accumulation, which directly translates into superior grain yield under stress (Costa et al. 2023; De Oliveira et al. 2025; Nascente et al. 2017).

Conclusion

This study demonstrated that inoculation with plant growth-promoting rhizobacteria effectively modulated the morphophysiological, biochemical, and agronomic responses of the drought-sensitive upland rice landrace 'Samambaia Branco', thereby improving its performance under water deficit. PGPR-mediated drought mitigation was primarily associated with enhanced root system plasticity, which promoted better plant water status, optimized gas exchange, and induced distinct patterns of carbohydrate partitioning that contributed to osmotic adjustment. These responses collectively improved plant acclimation to drought and reinforced the potential of bioagent-based technologies as a sustainable strategy to increase the resilience of drought-sensitive upland rice.

Although these greenhouse results are promising, validating the performance of these microbial inoculants under field conditions remains an essential next step, as the complex interactions among environmental factors and native soil microbiota may influence the consistency and magnitude of the observed benefits.

Moreover, the strain-specific metabolic responses observed among PGPR treatments indicate that different bacterial isolates employ complementary mechanisms to enhance drought acclimation, reinforcing the importance of selecting microbial inoculants according to their functional traits.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

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No funding was received to assist with the preparation of this manuscript.

Author Contribution

ACL, MGM, APC, MCCF, RS conceived the idea and designed the experiment; NBC, GOPF, GAB, ACL conducted the analyzes and collections at SITIS. ACL and NBC analyzed the data and led the writing of the manuscript. MRSAL contributed the draft and reviewed the final version. All authors contributed to the drafts and gave final approval.

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Data availability

The data are contained within the article.

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Figures

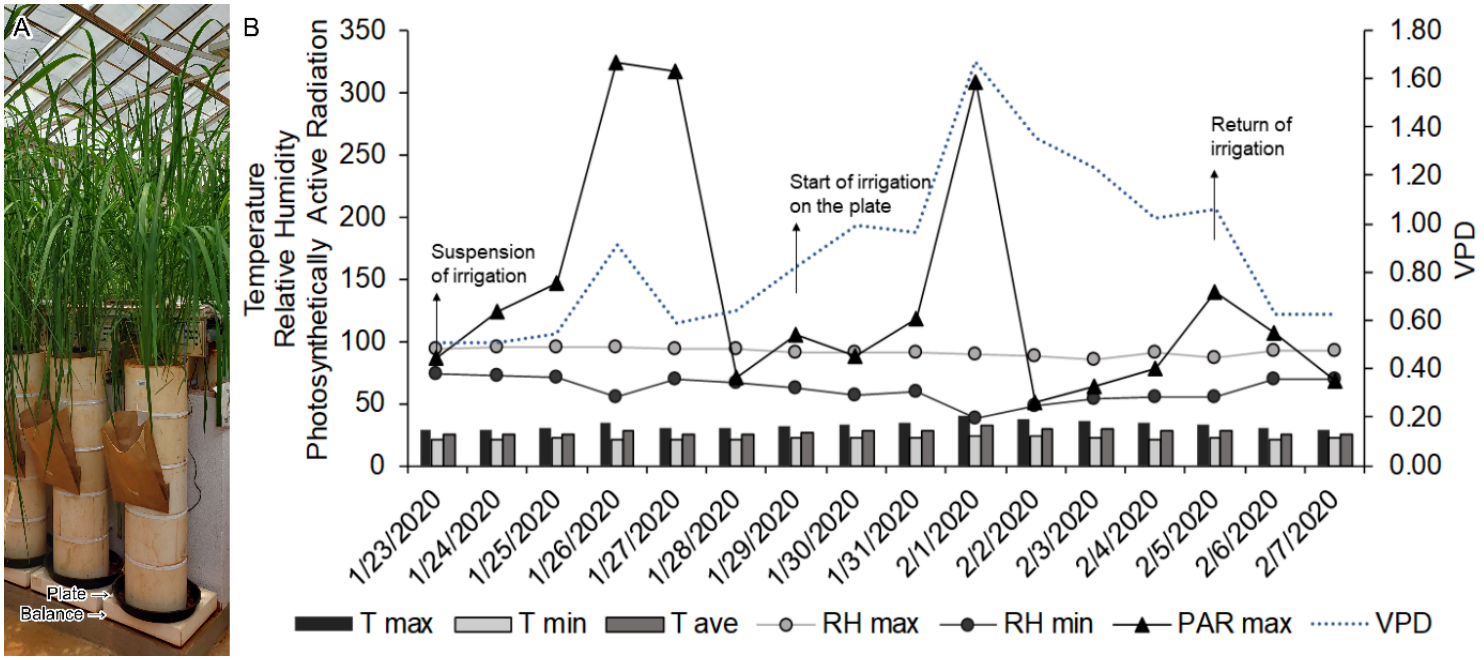


Figure 1

A Growing column, detail of the plate and digital scales for monitoring the reference mass. **B** Data climate collected during water-deficit period (jan – fev/2020). Temperature maximum (T max, ºC), average (T ave, ºC), and minimum (T min, ºC); Relative Humidity maximum (RH max, %) and minimum (RH min, %); Photosynthetically active radiation (PAR max, µmol m^{-2} s^{-1}); Vapor pressure deficit (VPD, MPa)

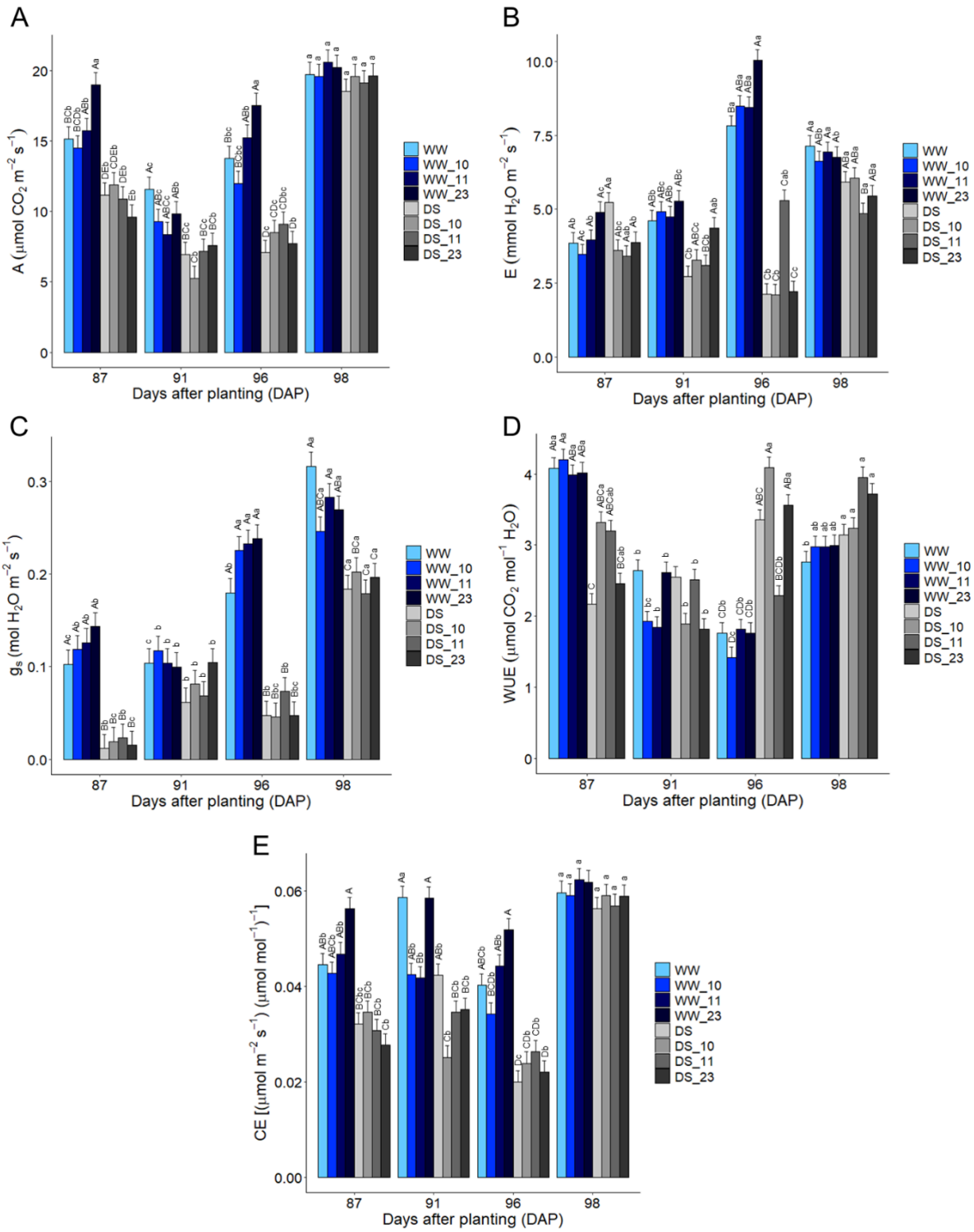


Figure 2

Dynamics of leaf gas exchange in Samambaia Branco modulated by PGPR under *WW* and *DS* conditions (n = 4). **A** Photosynthetic CO_2 assimilation (*A*), **B** Transpiration rate (*E*), **C** Stomatal conductance (*gs*), **D** Water use efficiency (*WUE*), and **E** Carboxylation efficiency (*CE*). Vertical bars denote mean ± SE. The means following same letters has no significant difference at $p \leq 0.05$ (Tukey's test). Uppercase letters represent the difference between microbial treatments within the same DAP (days after planting).

Lowercase letters represent the difference between DAP for the same microbial treatment. Water regimes: well-watered (*WW*) and drought stress (*DS*). Microbial treatments: 10: Samambaia branco inoculated with *Bacillus toyonensis*, 11: Samambaia branco inoculated with *Burkholderia* sp., 23: Samambaia branco inoculated with *Serratia marcescens*. Non-inoculated plants as control treatment. The timeline encompasses: Phase I - six days after irrigation cut-off (87 DAP); Phase II - two days after the plants received 50% of water replenishment at the column base (91 DAP); Phase III - nine days after the plants received 50% of water replenishment at the column base (96 DAP); and Phase IV - one day post rehydration (98 DAP)

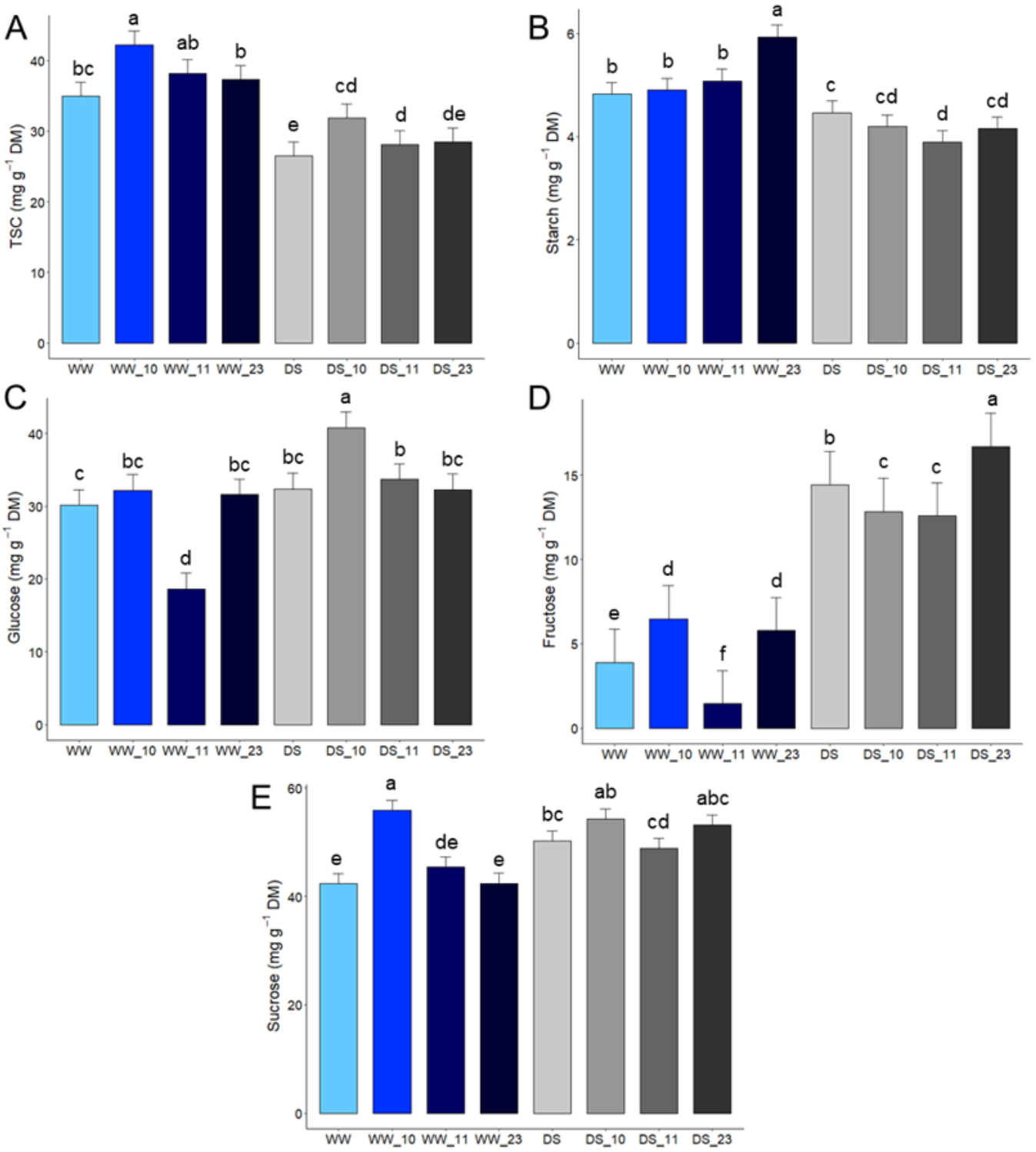


Figure 3

Non-structural carbohydrates content of Samambaia Branco modulated by PGPR under *WW*and *DS* conditions (n = 4). **A** total soluble carbohydrate (*TSC*), **B** starch, **C** glucose, **D** fructose, and **E** sucrose. Means followed by the same letter do not differ according to Tukey's test ($p < 0.05$). Vertical bars denote mean ± SE. Water regimes: well-watered (*WW*) and drought stress (*DS*). Microbial treatments: 10: Samambaia branco inoculated with *Bacillus toyonensis*, 11: Samambaia branco inoculated with

Burkholderia sp., 23: Samambaia branco inoculated with *Serratia marcescens*. Non-inoculated plants as control treatment

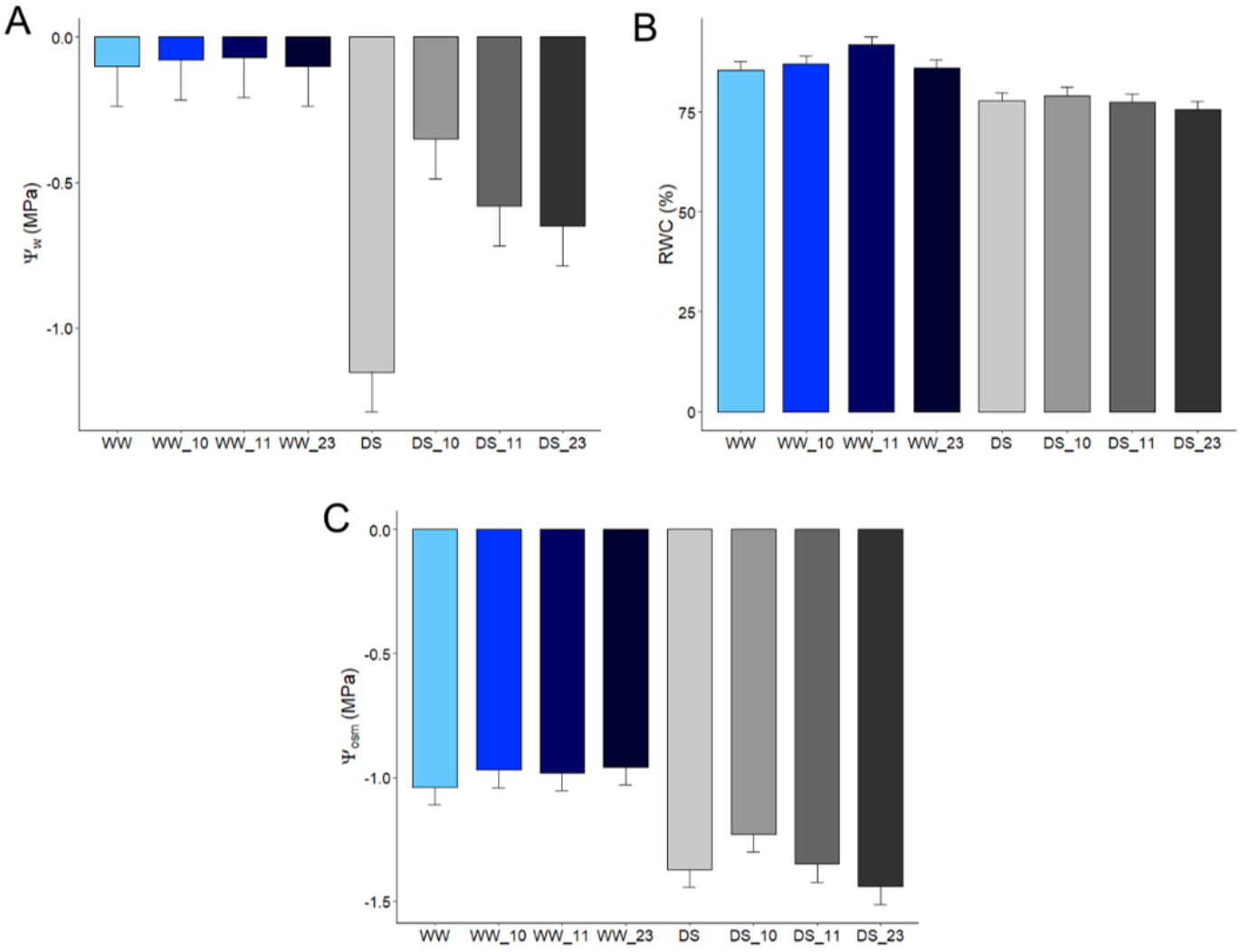


Figure 4

Water status of Samambaia Branco plants modulated by PGPR under *WW* and *DS* conditions (n = 4). **A** relative content water (*RCW*), **B** water potential (Ψw), **C** osmotic potential (Ψs). Means followed by the same letter do not differ according to Tukey's test ($p < 0.05$). Vertical bars denote mean ± SE. Water regimes: well-watered (*WW*) and drought stress (*DS*). Microbial treatments: 10: Samambaia branco inoculated with *Bacillus toyonensis*, 11: Samambaia branco inoculated with *Burkholderia* sp., 23: Samambaia branco inoculated with *Serratia marcescens*. Non-inoculated plants as control treatment.

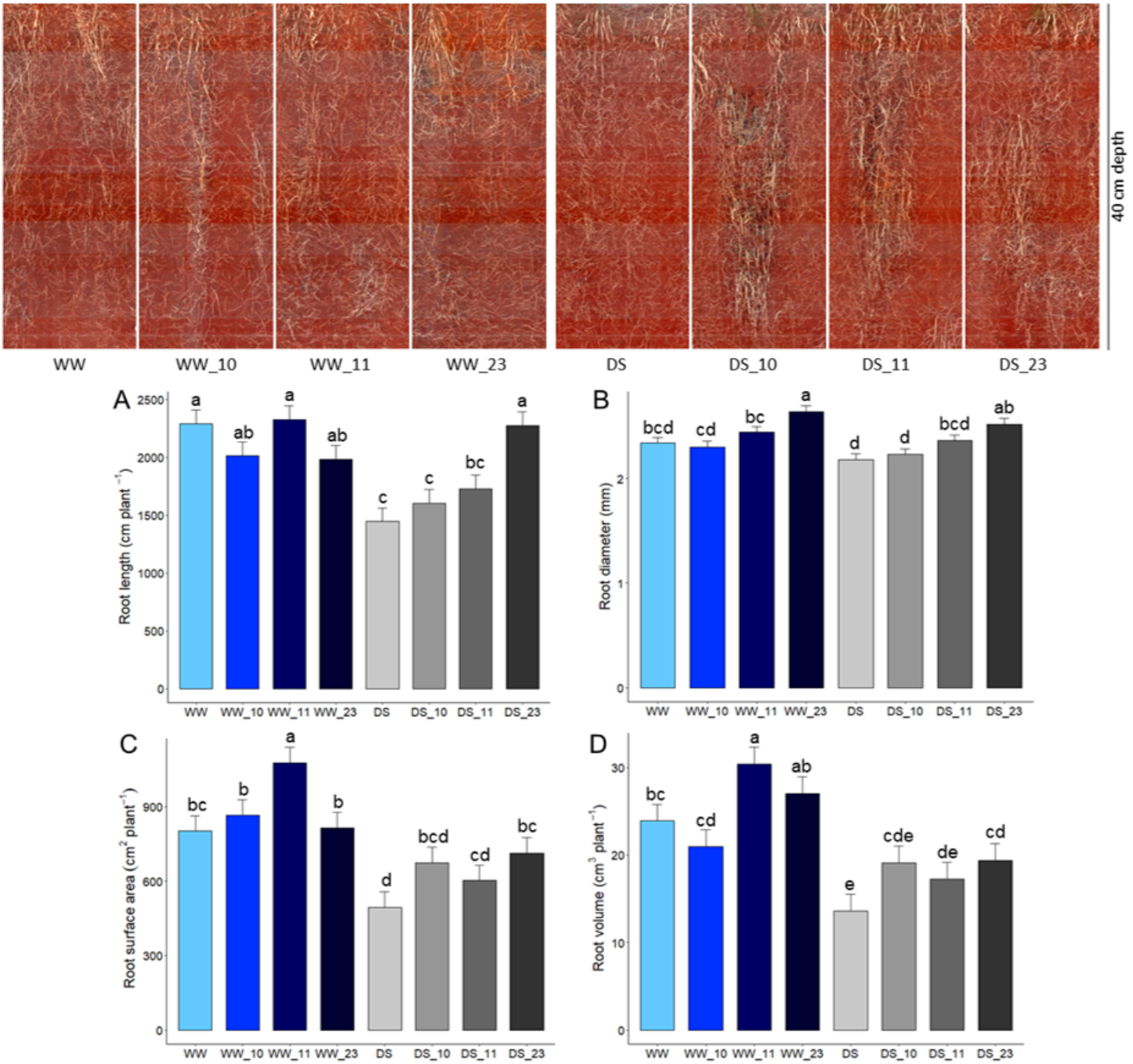


Figure 5

Root system images of the Samambaia Branco modulated by PGPR under *WW* and *DS* condition. Root length, *RL*(A), Root diameter, *RD* (B), Root surface area, *RSA* (C) and Root volume, *RV* (D) at depth (0-40 cm) of the soil at 90 DAP (n = 4). Means followed by the same letter do not differ according to Tukey's test ($p < 0.05$). Vertical bars denote mean ± SE. Water regimes: well-watered (*WW*) and drought stress (*DS*). Microbial treatments: 10: Samambaia branco inoculated with *Bacillus toyonensis*, 11: Samambaia branco inoculated with *Burkholderia* sp., 23: Samambaia branco inoculated with *Serratia marcescens*. Non-inoculated plants as control treatment.

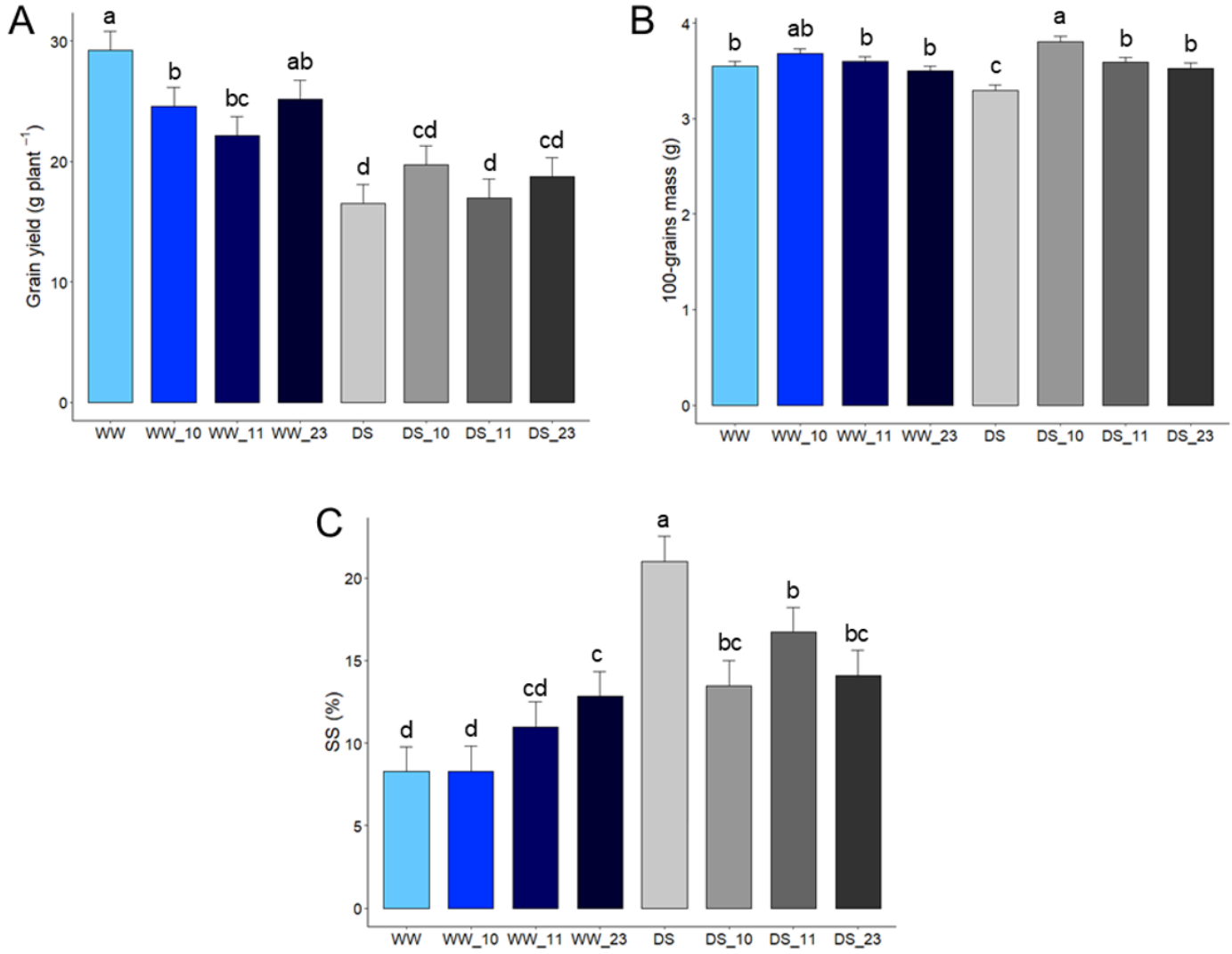


Figure 6

Agronomic performance of Samambaia Branco modulated by PGPR under *WW* and *DS* conditions. (**A**) Grain yield (*GY*), (**B)** 100-grains mass (*100GM*), (**C)** spikelet sterility (*SS*). Bars represent means ±SE (n = 4). Different letters indicate significant differences between microbial treatments within each water regime according to Tukey's test ($p < 0.05$). Water regimes: well-watered (*WW*) and drought stress (*DS*). Microbial treatments: 10: Samambaia branco inoculated with *Bacillus toyonensis*, 11: Samambaia branco inoculated with *Burkholderia* sp., 23: Samambaia branco inoculated with *Serratia marcescens*. Non-inoculated plants as control treatment.

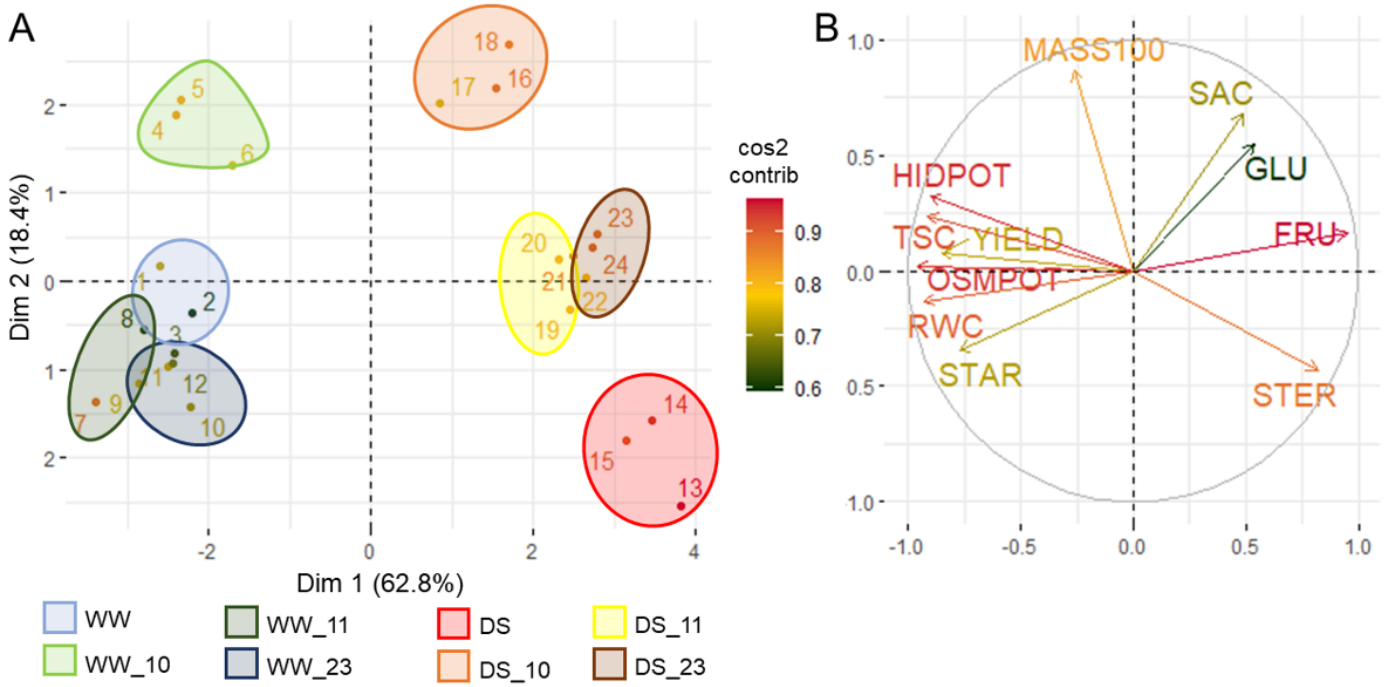


Figure 7

The principal component analysis (PCA) biplots showing of treatments (**A**) and variables (**B**) of non-structural sugars content, water status and agronomic traits. Water regimes: well-watered (*WW*) and drought stress (*DS*). Microbial treatments: 10: Samambaia branco inoculated with *Bacillus toyonensis*, 11: Samambaia branco inoculated with *Burkholderia* sp., 23: Samambaia branco inoculated with *Serratia marcescens*. Non-inoculated plants as control treatment. FRU: fructose, GLU: glucose, HIDPOT: water potential, OSMPOT: osmotic potential, MASS100: 100-grains mass, RWC: relative water content, SAC: sucrose, STAR: starch, STER: spikelet sterility, TSC: total soluble carbohydrates, and YIELD: grain yield