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RESEARCH ARTICLE

Beneficial bacteria mitigate combined water and phosphorus deficit effects on upland rice

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Abstract

Background and aims Limited water and phosphorus availability are major challenges in upland rice production. Plant–microbe interactions, especially with beneficial bacteria, have shown promise in mitigating these stresses. This study investigated the influence of microbial inoculants with hydration-promoting and phosphorus-solubilizing abilities on upland rice yield under drought and phosphorus deficiency.

Methods Upland rice (BRS Esmeralda) plants were grown in a greenhouse with different water availability conditions (well-watered and drought), phosphorus levels (normal 200 mg dm^{-3} and low 20 mg dm^{-3}), and microbial treatments (no-microorganisms and single isolates, *Serratia marcescens* strains BRM 32114 and BRM 63523, and combined isolates *Bacillus toyonensis* BRM 32110 + BRM 32114 and BRM 63523 + BRM 32114). Root and shoot traits, as well as production components, were analyzed.

Results While the microbial treatments affected the roots, the larger effects were seen in the shoot rice plants. When both water and phosphorus were limited, grain yield decreased significantly. However, plants inoculated with beneficial bacteria showed a substantial increase in grain yield (average of 39.5% in 2019/2020 and 18.8% in 2020/2021) compared to uninoculated plants under combined stresses. This increase was especially pronounced in plants treated with BRM 63523 (strain) alone or combined with BRM 32114 (strain). These inoculated plants also showed improved photosynthetic activity (average increase of 24.6%), which may have contributed to the higher grain yield.

Conclusions Inoculating upland rice with specific *Serratia* strains effectively increased shoot and root traits under combined water and phosphorous stresses. These findings highlight the potential of plant–microbe interactions for sustainable upland rice production.

Keywords *Oryza sativa* L. · Root system · Shoot · Multifunctional microorganisms · Rainfed agriculture · Sustainability

Introduction

Statistics from 71 countries across Asia, sub-Saharan Africa, and Latin America indicate that lowland and upland rice (*Oryza sativa* L.) constitute 92% and 8%, respectively, of the total rice cultivation area (Saito et al. 2018). In Brazil, the upland rice cropping system is predominant in the Cerrado region (Brazilian savannas), which represents the country's main upland rice production area and the largest rainfed rice cultivation area in Latin America (Heinemann et al. 2019). Cultivation of upland rice would provide many benefits for the sustainability of agriculture in this vast biome where, for the most part, succession cropping is performed mainly with soybean in the summer and maize in the second-season (Furtini et al. 2022). Additionally, upland rice plays a crucial role in supplying grains to the Brazilian population, thereby promoting food security for a significant proportion of both farming and non-farming communities, while also stabilizing the domestic market (Martínez et al. 2014; Heinemann et al. 2019). However, challenges such as abiotic stresses need to be addressed to enhance the sustainability of upland rice cultivation.

Over the past three decades in Brazil, upland rice grain yield has more than doubled (from 1.197 kg ha^{-1} in 1986 to 2.404 kg ha^{-1} in 2020). However, despite this increase, the planting area and production have significantly decreased (from 4,498,300 ha and 5,383,800 tons in 1986 to 381,924 ha and 918,078 tons in 2020, respectively) (Embrapa Rice and Beans 2022). Upland rice cultivation requires substantial amounts of nitrogen (N), phosphorus (P), and potassium (K) (Deng et al. 2020). Its yield potential has declined by 70% over the last two decades, partly due to the high risk of drought (Heinemann et al. 2019). The Cerrado soils are generally acid and feature low-natural fertility with low-available P, K, and cation exchange capacity (CEC), and high-aluminum saturation (Petter et al. 2012). Therefore, phosphorus is a major limiting nutrient for plant productivity, mainly due to its high reactivity with cations such as aluminum (Al) and iron (Fe) in acidic soils (López-Arredondo et al. 2014). In agricultural settings, low P availability necessitates its replenishment with large amounts of P fertilizer derived from phosphate rock, a finite resource (Maathuis 2009). Besides low P availability, other abiotic factors such as drought, one of the most severe abiotic stresses, can limit rice yield in rainfed agriculture (Wu and Cheng 2014). It is crucial to define management practices to sustainably increase upland rice production to meet demand amid stagnant productive potential, increasing yield gaps, and climate change-induced yield reduction (Challinor et al. 2016; Zhao and Kumar 2016).

The limited availability of water and phosphorus (P) has led to the evolution of various plant and microorganism associations to meet these requirements. These include mycorrhizal symbioses, which occur in over 90% of plant species (Maathuis 2009), and interactions with a wide array of microbes, particularly plant growth-promoting bacteria (PGPB) (Pascale et al. 2020). These microbes enhance plant growth by increasing the supply or availability of primary nutrients such as P to the host plant when applied to the seed, plant surface, or soil (Lopes et al. 2021). They also enhance drought tolerance through mechanisms such as modification of phytohormonal levels, including abscisic acid (ABA), gibberellic acid, cytokinins, and indole-3-acetic acid (IAA); osmotic adjustment; enhancement of antioxidant enzymes; biofilm (exopolysaccharide) production; synthesis of ACC deaminase (ACCd) to reduce root ethylene levels; and increased water and nutrient uptake, gas exchange, and water use efficiency (Poudel et al. 2021).

There is an increasing need for better-adapted upland rice cultivars that combine improved yield potential with lower sensitivity to drought and phosphorus deficit (Lafitte et al. 2006). In this study, we hypothesize that microbial inoculants with pro-hydration and P-solubilizing activities hold great potential for stabilizing upland rice grain yield under suboptimal environmental conditions. Therefore, this study aimed to examine the impact of beneficial microorganisms on upland rice's response to combined drought and phosphorus deficit. By enhancing the crop's nutrient and water uptake, we aim to optimize key traits crucial for maximizing rice grain production. This approach not only promotes sustainable practices but also protects soil and water resources, ultimately ensuring food security for the population.

Material and methods

Upland rice and beneficial bacteria

Cultivar improved BRS Esmeralda belongs to GAB (Germoplasm Active Bank) of the Embrapa Rice and Beans, Goiás, Brazil. The upland rice-breeding program of Embrapa Rice and Beans was released in 2014 (Castro et al. 2014).

Bacillus toyonensis (BRM 32110) and *Serratia marcescens* (BRM 32114 and BRM 63523) were obtained from the rhizospheres and phyllospheres of healthy rice plants in a commercial field (Filippi et al. 2011; Faria et al. 2023) 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.

Experimental design and treatments

The experiments were carried out under a greenhouse condition at the Integrated System for Drought-Induced Treatment (Portuguese acronym SITIS) plant phenotyping platform facility, at Embrapa Rice and Beans (16°28′00″ S, 49°17′00″ W, with an altitude of 823 m, from November 2019 to March 2020, and from December 2020 to April 2021. All treatments were performed in soil columns of diameter 25 cm and height 100 cm, placed on a digital scale to monitor the water amount in each column (Figure S1). The physicochemical characteristics of the Oxisol soil were determined according to Donagema et al. (2011) (Table S1). It was used a randomized block design following a triple factorial scheme with four replicates: two water conditions—well-watered, *WW* and droughted, *DR*. Four microbial treatments—*Serratia marcescens* (BRM 32114) and *S. marcescens* (BRM 63523) in 2019/2020; *Bacillus toyonensis* (BRM 32110) + *S. marcescens* (BRM 32114) and *S. marcescens* (BRM 63523) + *S. marcescens* (BRM 32114) in 2020/2021, and no-microorganism as control in both years of experimentation. As in the first year both bacteria proved to be effective, we decided that microbial treatments, in the second year, would consist of a mixture of bacteria to promote the increase of their beneficial actions. The use of co-inoculation in 2020/2021 had the objective of potentiating the action of the multifunctional microorganisms. The BRM 32110 strain was inserted into the second experiment since it had already been previously characterized as a multifunctional and beneficial microorganism for irrigated rice (Nascente et al. 2017). Before combining the isolates in a single treatment, a compatibility test was performed between the isolates, thus ensuring that one isolate would not be negatively influencing the other. The combination between compatible isolates goes beyond the sum of the individual effects, because there can be a synergism between the isolates to the benefit of the host plant. It is also important to highlight that the isolates used in this study were previously characterized regarding the production of lytic enzymes, solubilizes, biofilm and hormones (Martins et al. 2021; Faria et al. 2023). Two phosphorus (P) rates, as triple superphosphate: control treatment (200 mg dm^{-3}, normal phosphorus—*NP*), and stressed treatment (20 mg dm^{-3}, low phosphorus, *LP*), for soil with 42.1% clay in 2019 and 50.3% in 2020/2021. Fertilization with Si was performed in all treatments, using Agrosilício®, a commercial calcium and magnesium silicate ($CaSiO_3.MgSiO_3$), containing 10.5% Si, 27% Ca, and 6% Mg. Its incorporation in the soil occurred thirty days before planting (Souza et al. 2021), to a dose of two-ton ha^{-1}. Furthermore, on the planting day, the soil was enriched with other minerals purpose of the upland rice plants' nutritional suitability (Silva et al. 2021) (Table S1).

Water condition

The hydric treatments consisted of combinations of two water conditions: well-watered (*WW*) as control treatment and droughted (*DR*) as stressed treatment. In *WW* the amount of soil water was initially established to 80–85% of field capacity (FC) and kept throughout the crop cycle. For the *DR* treatment, irrigation was performed as in the control treatment until the plants reached the reproductive stage (R2 – collar formation on flag leaf/R3 – panicle exsertion), followed by the suspension of irrigation for five days. After this period, 50% of the volume of evapotranspired water of *WW* columns was replaced at the plate of the respective *DR* columns, for eight days in 2019 and twenty-two days in 2020. The amount of evapotranspirated water was estimated based on the water quantity required to keep the soil at FC 80–85% in the control treatment. After the *DR* 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 control columns, the evapotranspiration rate was determined daily (the 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. Each column contained four plants. The quantity of the evapotranspirated water and the average daily transpiration rate, during the water stress, in all columns and meteorological conditions of the SITIS phenotyping platform (monitored by a datalogger Hobo® U12-12, Onset Computer Corp. Ltd, Cape. Cod, MA, USA), in 2019 and 2020, are shown in the Table S2.

Bacteria inoculation

The bacteria were applied in three moments during the rice cycle, as a cell suspension: 1. Seed microbiolization; 2. Spray pulverization in the soil at 30 days after sowing (DAS); 3. Spray pulverization at rice shoots stage at 63 – 66 DAS. In 2020, the consortia *B. toyonensis* (BRM 32110) + *S. marcescens* (BRM 32114) and *S. marcescens* (BRM 63523) + *S. marcescens* (BRM 32114) were used in seed microbiolization and soil pulverization; while only *B. toyonensis* (BRM 32110) isolate and *S. marcescens* (BRM 63523) isolate were used in spray pulverization at rice shoots. The bacteria suspension was prepared in liquid medium 523 (nutrient broth) (Kado and Heskett 1970), in a shaking incubator, for 24 h at 28 °C. The suspension concentration of each bacteria was adjusted in a spectrophotometer to A540 = 0.5, which corresponds to 1×10^8 colony-forming units (CFU) per mL.

Before microbiolization, the seeds were disinfected with immersion in 70% alcohol for one minute and 0.05% sodium hypochlorite for the same period, followed by drying at room temperature at 29 °C. Microbiolization occurred with the immersion of the seeds in each bacteria suspension and the control treatment, by immersing the seeds in water, for 24 h at a temperature of 25 °C, under constant agitation, according to the methodology proposed by Filippi et al. (2011). At 30 DAS, 100 mL of the suspension (10^8 CFU) of each bacteria or distilled water (control) was applied to the soil. At 63 – 66 DAS, 100 mL of bacteria suspension (10^8 CFU) or distilled water (control) was applied to the BRS Esmeralda plants. A manual sprayer with a conical-type nozzle (TXVS2) pressured with CO_2, was used for the application of the bacteria.

Agronomic and morphophysiological measurements

Agronomic traits Grain yield (*GY*, g grain plant^{-1}, which means the total mass of grains, in grams, obtained for one plant per column) and its components, such as 100-grain mass (*100GM*, g) and spikelet sterility (*SS*, %). This was estimated as $SS = (NEG \times 100)\ TG^{-1}$, where NEG = number of empty grains, and TG = total number of grains. In addition, shoot dry matter biomass (*SDMB*, g plant^{-1}), through drying samples at 65 °C until a constant weight was achieved, was determined during the harvest period.

Gas exchange Photosynthetic rate (*A*, μmol CO_2 m^{-2} s^{-1}), transpiration rate (*E*, mmol H_2O m^{-2} s^{-1}), stomatal conductance (*gs*, mol H_2O m^{-2} s^{-1}) and internal CO_2 concentration (*Ci*, μmol mol^{-1}) were measured using a portable gas exchange analyzer in the infrared region (LCpro +) and taken on the last day of the drought period. The instantaneous water use efficiency (*WUE*, μmol CO_2 mmol^{-1} H_2O) was calculated as the ratio between *A* and *E* (Rosales et al. 2012), intrinsic water use efficiency (*iWUE*, μmol CO_2 mol^{-1} H_2O) as the ratio between *A* and *gs,* and carboxylation efficiency (*CE*) ratio A and Ci.

Water status Leaf water potential (*LWP*) was evaluated between 05h00 and 06h00 a.m. using a Scholander pressure chamber (Scholander et al. 1965). The reading was determined on two flag leaves of the primary tiller of two upland rice plants at the end of the water restriction period. Pressure was applied until exudation from the cut made in the leaf collar.

Phosphorus content Shoot and grain samples were digested using a 4:1 $HNO_3/HClO_4$ (nitric/perchloric) mixture at 105 °C for 90 min, 140 °C for 90 min, followed by 20 min at 170 °C to dehydrate any silica in the digest. The digests were diluted with MiliQ water 50 mL, and filtered through No. 6 Advantec filter paper. Tissue P content was determined by the inductively coupled plasma optical emission spectrometer (ICP-OES) (Ultima 2, Horiba Scientific). Phosphorus uptake in SDMB or grain, internal phosphorus efficiency (*IPE*), and phosphorus harvest index (*PHI*) were calculated using the following equations:

$$\text{P uptake in SDMB}\left(\text{mg plant}^{-1}\right) = \text{P content in SDMB}\left(\text{g kg}^{-1}\right) \times \text{SDMB}\left(\text{g plant}^{-1}\right)$$

$$\text{P uptake in grain}\left(\text{mg plant}^{-1}\right) = \text{P content in grain}\left(\text{g kg}^{-1}\right) \times \text{Grain Yield}\left(\text{g plant}^{-1}\right)$$

$$IPE\left(gg^{-1}\right) = \frac{\text{Grain Yield x 1000}}{\text{P uptake in SDMB} + \text{P uptake in grain}}$$

$$PHI\left(gg^{-1}\right) = \frac{\text{P uptake in grain}}{\text{P uptake in SDMB} + \text{P uptake in grain}}$$

P uptake in SDMB, P uptake in grain, *IP*E, and *PHI* were obtained at harvesting time in 2020/2021.

Root traits This system was evaluated according to the methodology described by Lanna et al. (2016). Briefly, to carry out the root system capture, acrylic tubes were installed inside the columns and four rice plants were planted around the tube. Root images were taken at a depth of 0 to 40 cm at the end of the drought period, given that the BRS Esmeralda is a shallow-rooted plant (Lanna et al. 2021). The root system traits were assessed by measuring root length density (*RLD*; cm), root surface area (*RSA*; cm^2), and root volume (*RV*; cm^3). Furthermore, they were divided into two diameter classes: (1) fine roots (*FR*) with a diameter ≤ 0.5 mm; and (2) thick roots (*TR*) with 1.0 mm ≤ diameter ≤ 2.5 mm (Guimarães et al. 2020). It was calculated the length, surface area, and volume of each root type (fine and thick roots respectively): fine root length (*FRL*; cm); thick root length (*TRL*; cm); fine root surface area (*FRSA*, cm^2); thick root surface area (*TRSA*, cm^2); fine root volume (*FRV*, cm^3) and thick root volume (*TRV*; cm^3). Thick roots were considered primary roots and fine roots as secondary or tertiary roots, which permitted to analysis of the capacity of soil exploration (laterally and vertically). The images were taken with a CI–600 Cano Scan scanner (CID Bio-Science, Version 3.1.19), followed by automatic image processing with WinRhizo software (Regent Instruments Inc, Version 2016). Root length density (*RLD*, cm cm^{-3}) was calculated as the ratio between *RL* and *RV* (Dusserre et al. 2012).

Statistical analyses

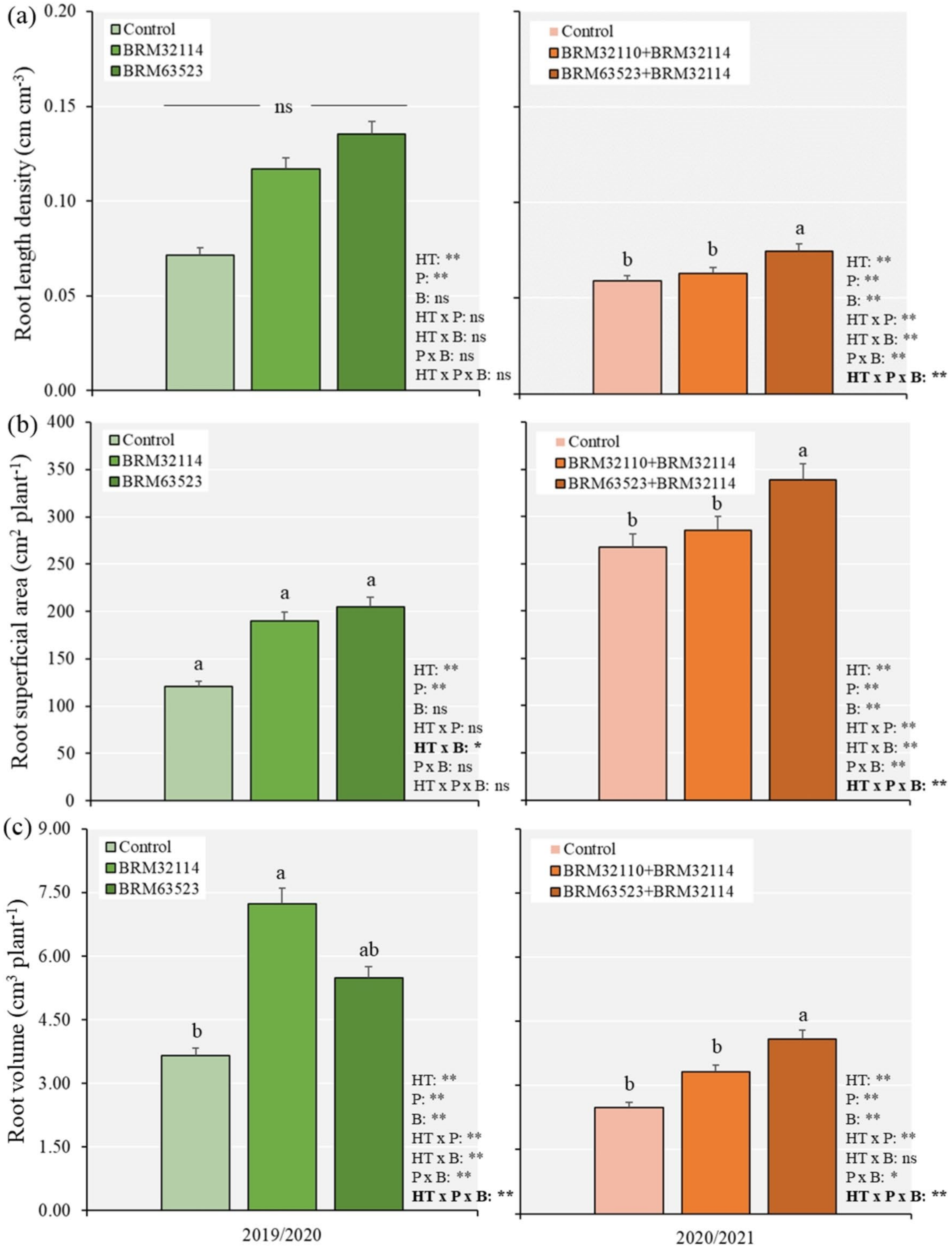
All data obtained were analyzed to normality by the Shapiro–Wilk test. The data were subjected to an analysis of variance (three-way ANOVA with all main factors evaluated as fixed factors), followed by Duncan's multiple range test for pairwise comparisons at a 5% level. The data were reported as means ± the standard error. Correlations between measured shoot and root traits of the BRS Esmeralda plants cultivated in 2020/2021 were determined with Pearson's correlation coefficient. Analyses were performed using the correlation procedure (Proc Corr) of the SAS software (SAS Institute Inc., Cary, NC, USA). The remaining statistical analyses were performed using the R version 4.3.2 (2023–10–31) with the package "ExpDes. pt".

Results

Synergistic effect of beneficial bacteria on the root system of the BRS Esmeralda plants cultivated under water and phosphorus deficit-affected soil

This study primarily focused on the analysis of the BRS Esmeralda root system. In 2019/2020, only the root volume (*RV*, *FRV*, and *TRV*, cm^3) was influenced by the triple interaction (hydric condition × phosphorus × beneficial bacteria) ($p < 0.01$). In 2020/2021, the root traits (*RLD*, *RSA*, and *RV*) were affected by combined stresses, and the triple interaction was observed (Table S3).

The application of beneficial bacteria did not significantly increase root traits under non-stress conditions (*WW* and *NP*) (Table S4). However, when exposed to combined water and phosphorus deficits, inoculated plants showed a significant increase in root traits, particularly in plants + BRM 63523 in 2019/2020, and plants + BRM 63523 + BRM 32114 in 2020/2021, compared to the corresponding uninoculated plants (Fig. 1 and Table S4). In 2019/2020, the root systems of plants + BRM 32114 and plants + BRM 63523 exhibited a root length density of 0.177 and 0.135 cm cm^{-3} plant^{-1}; surface area of 189 and 205 cm^2 plant^{-1}; and volume of 7.24 and 5.49 cm^3 plant^{-1}, respectively. In the control treatment (no-microorganism), these values were 0.072 cm cm^{-3} plant^{-1}, 121 cm^2 plant^{-1}, and



3.66 cm^3 plant^{-1} for length density, surface area, and volume, respectively. In 2020/2021, the root systems of plants + BRM 32110 + BRM 32114 and plants + BRM 63523 + BRM 32114 showed a root length density of 0.063 and 0.075 cm cm^{-3} plant^{-1}; surface area of 107 and 137 cm^2 plant^{-1}; and volume

◂**Fig. 1** Root length density (**a**), root superficial area (**b**) and root volume (**c**) of BRS Esmeralda plants cultivated under phosphorus and water deficit-affected soil ($n = 4$) in 2019/2020 and 2020/2021. The plants were submitted a combined drought and low phosphorus (20 mg dm.$^{-3}$) deficit and microbial treatments: no microorganism (control) and single isolates, BRM 32114 and BRM 63523, in 2019/2020, and combined isolates, BRM 32110 + BRM 32114 and BRM 63523 + BRM 32114, in 2020/2021. Root images were taken at a depth of 0 to 40 cm at the end of the drought period. Asterisks indicate statistically significant differences according to the F-test. * and **: Significant at 5 and 1% probability levels, respectively. Vertical bars denote mean ± SE. ns: non-significant. Different letters indicate significant differences among the treatments (Duncan's multiple range test at $p < 0.05$)

of 3.32 and 4.10 cm^3 plant^{-1}, respectively. In the control treatment (no-microorganism), these values were 0.059 cm cm^{-3} plant^{-1}, 89 cm^2 plant^{-1}, and 2.48 cm^3 plant^{-1} for length density, surface area, and volume, respectively.

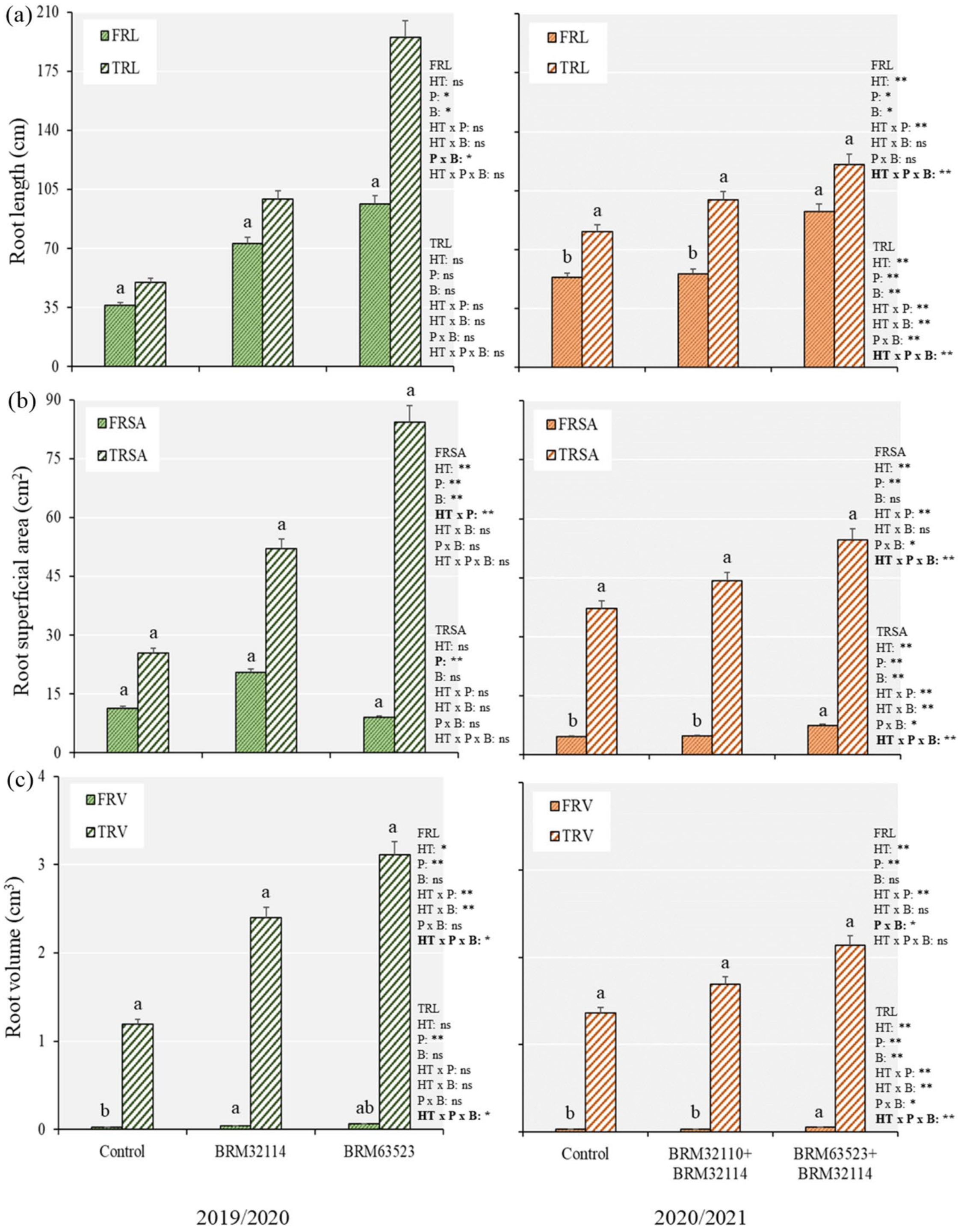
In detail, BRS Esmeralda plants pre-treated with beneficial bacteria under combined stresses significantly improved fine (diameter ≤ 0.5 mm) and thick roots (1.0 mm ≤ diameter ≤ 2.5 mm) compared to the corresponding uninoculated plants. In 2019/2020, the inoculated plants showed an average increase in fine root traits, such as fine root length (*FRL*), fine root surface area (*FRSA*), and fine root volume (*FRV*), of 134.5%, 40.8%, and 104.0%, respectively, compared to the corresponding uninoculated plants. Meanwhile, the thick root traits of inoculated plants, such as thick root length (TRL), thick root surface area (*TRSA*), and thick root volume (*TRV*), increased on average by 195.1%, 168.9%, and 131.5%, respectively, compared to the corresponding uninoculated plants. In 2020/2021, the average increase in fine root traits was 36.8%, 33.7%, and 33.3%, respectively, while the average increase in thick root traits was 36.4%, 31.4%, and 40.8%. Notably, the root system of BRS Esmeralda plants inoculated with BRM 63523 (2019/2020) and BRM 63523 + BRM 32114 (2020/2021) was significantly superior under combined stress conditions compared to the corresponding uninoculated plants (Fig. 2 and Table S4).

Synergistic effect of beneficial bacteria on shoot traits of the BRS Esmeralda plants cultivated under water and phosphorus deficit-affected soil

Shoot traits of BRS Esmeralda plants exhibited significant main effects from beneficial bacteria inoculation under combined stresses ($p \leq 0.05$) (Table S3). As expected, BRS Esmeralda shoot traits were superior under well-watered (*WW*) conditions compared to drought (*DR*) conditions at both phosphorus (P) rates evaluated. Moreover, data from the SITIS greenhouse over the two years of cultivation revealed distinct responses of the beneficial bacteria-treated plants to water conditions and P rates. In 2019/2020, leaf water potential (*LWP*) showed an interaction between water condition and phosphorus, with no response from bacteria. Under *DR* and low phosphorus (*LP*) conditions, plants showed a 66% reduction in *LWP* compared to *WW* plants. In 2020/2021, a triple interaction of factors was observed, with *LWP* reduction of 30% and 210% for BRM32110 + BRM32114 and BRM63523 + BRM32114, respectively (Fig. 3).

In 2019/2020, inoculated plants of BRS Esmeralda showed reduction of 38.7%, 35.1%, and 29.0% in photosynthesis, transpiration, and stomatal conductance, respectively, compared to corresponding uninoculated plants under combined stresses. In 2020/2021, the inoculated plants exhibited a 24.7% increase in the photosynthetic rate, alongside reduction of 4.1% in transpiration and 25.9% in stomatal conductance, compared to corresponding uninoculated plants (Table S5). Under combined stresses, the intrinsic water use efficiency (*iWUE*) of plants inoculated with BRM63523 was 31.4% higher than that of corresponding uninoculated plants. In plants inoculated with BRM32110 + BRM32114 and BRM63523 + BRM32114, *iWUE* was 115.0% and 38.8% higher, respectively, compared to uninoculated plants. An increase in carboxylation efficiency (*CE*) was observed in inoculated plants under drought (*DR*) and low phosphorus (*LP*) conditions in 2020/2021. Plants inoculated with BRM32110 + BRM32114 and BRM63523 + BRM32114 showed increases of 44.0% and 32.0%, respectively, compared to the control (uninoculated plants) (Fig. 3).

Regarding shoot dry biomass (*SDBM*), BRS Esmeralda plants responded to combined stress by slowing down their growth (Fig. 4). However, the introduction of beneficial bacteria helped mitigate this reduction in shoot growth. Under *LP* conditions, the *SDBM* of plants inoculated with BRM32114 and BRM63523 was 73.6% and 103.8% higher, respectively, than that of corresponding uninoculated plants. The SDBM of plants inoculated with BRM32110 + BRM32114 and BRM63523 + BRM32114 was 34.1% and 13.8%



higher, respectively, compared to uninoculated plants (Fig. 4 and Table S5).

The most pronounced effects on grain yield (*GY*) were observed in uninoculated plants subjected to combined stresses (*DR* and *LP*), in which they experienced reduction of 13.9% in 2019/2020 and 47.3% in 2020/2021. In contrast, the average *GY* reduction in inoculated plants under combined stresses was 15.4%,

◂**Fig. 2** Length (**a**), superficial area (**b**), and volume (**c**) of the fine (diameter ≤ 0.5 mm) and thick roots (1.0 mm ≤ diameter ≤ 2.5 mm) of the BRS Esmeralda plants cultivated under low phosphorus (20 mg dm.$^{-3}$) and drought deficit-affected soil ($n = 4$) in 2019/2020 and 2020/2021. Moreover, microbial treatments: no microorganism (control) and single isolates, BRM 32114 and BRM 63523, in 2019/2020, and combined isolates, BRM 32110 + BRM 32114 and BRM 63523 + BRM 32114, in 2020/2021. Root images were taken at a depth of 0 to 40 cm at the end of the drought period. Asterisks indicate statistically significant differences according to the F-test. * and **: Significant at 5 and 1% probability levels, respectively. Vertical bars denote mean ± SE. ns: non-significant. Different letters indicate significant differences among the treatments (Duncan's multiple range test at $p < 0.05$)

5.88%, 26.7%, and 29.3% for plants inoculated with BRM32114, BRM63523, BRM32110 + BRM63523, and BRM63523 + BRM32114, respectively. *GY* of plants inoculated with BRM32114, BRM63523, BRM32110 + BRM63523, and BRM63523 + BRM32114 was 24.2%, 54.8%, 13.1%, and 24.5% higher, respectively, compared to uninoculated plants under combined stresses. This suggests that plants associated with beneficial bacteria showed greater yield stability.

Uninoculated plants consistently displayed a higher percentage of spikelet sterility (*SS*) compared to inoculated plants in 2020/2021 (Fig. 4 and Table S5). However, a triple interaction of factors was observed for SS, with a reduction in the SS percentage of 54.0% and 26.9% in plants inoculated with BRM32110 + BRM32114 and BRM63523 + BRM32114, respectively (Fig. 4).

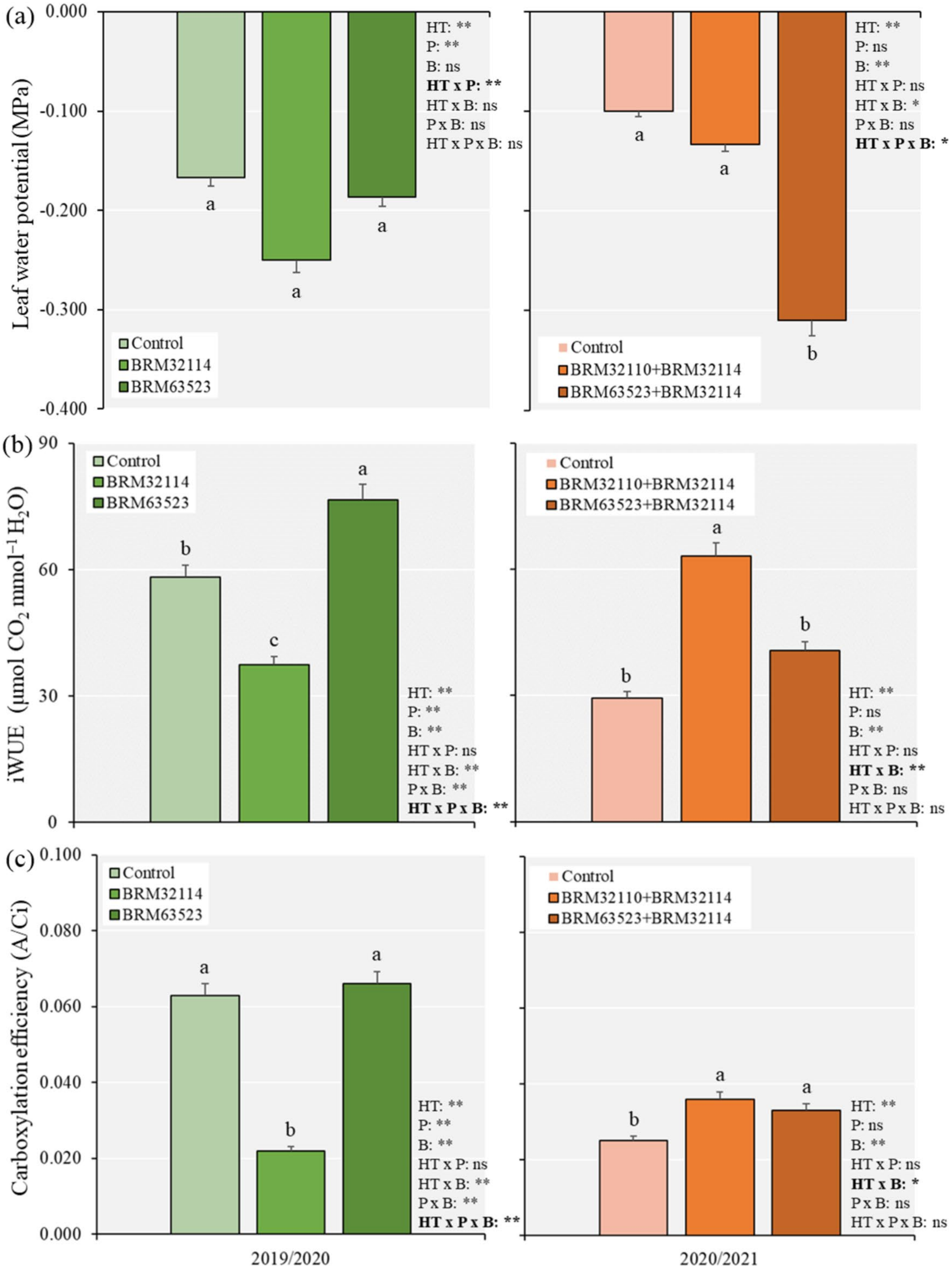
Regarding the characterization of phosphorus (P) uptake by BRS Esmeralda plants, P uptake in *SDMB* of plants inoculated with BRM32110 + BRM32114 and BRM63523 + BRM32114 was 40.0% and 26.5% higher than that of uninoculated plants under combined stresses. Additionally, P uptake in *SDMB* of plants inoculated with BRM32110 + BRM32114 and BRM63523 + BRM32114 increased by 43.4% and 31.9%, respectively, compared to their *WW* plants under *LP* stress. Conversely, uninoculated plants showed a 30.3% reduction in P uptake in *SDMB* compared to their *WW* plants under *LP* stress (Fig. 5).

Inoculation with BRM32110 + BRM32114 and BRM63523 + BRM32114 significantly increased phosphorus (P) uptake in grains by 9.3% and 30.0%, respectively, compared to uninoculated plants. However, under *LP* stress, P uptake in the grains of these inoculated plants decreased by 28.1% and 35.5%, respectively, compared to their *WW* plants. Importantly, uninoculated plants showed an even greater reduction (50.5%) in P uptake under *DR* and *LP* conditions compared to their *WW* plants under *LP* conditions. Furthermore, the application of combined stresses led to reduction of 46% in P content and 68% in P accumulation in the grains. Phosphorus content in grains decreased from 3.6 mg/g in the WW/NP treatment to 1.9 mg/g in the DR/LP treatment, with no difference between WW and DR plants under LP conditions. Phosphorus uptake in grains decreased from 20.0 mg/plant in the WW/NP treatment to 6.4 mg/plant in the DR/LP treatment, with reduction of 28% in LP and 49% in NP due to drought. No differences in internal phosphorus efficiency (*IPE*) and phosphorus harvest index (*PHI*) were observed between the microbial treatments and the control treatment (no microorganisms).

Discussion

In this study, we propose the establishment of a technological set consisting of a drought-tolerant cultivar associated with beneficial bacteria to stabilize upland rice production in environments with erratic rainfall and phosphorus (P) deficiency. The beneficial effects of bacteria have already been reported to enhance upland rice tolerance to combined biotic and abiotic stresses (Faria et al. 2023; Sousa et al. 2018; Souza et al. 2015; Souza et al. 2021; Sperandio et al. 2017). However, little attention has been given to plant responses to inoculation with beneficial bacteria under combined abiotic stresses such as drought and phosphorus deficit, which frequently occur in field conditions in the Brazilian Cerrado.

The bacterial strains used in our study, *Bacillus toyonensis* (BRM 32110) and *Serratia marcescens* (BRM 32114 and BRM 63523), can produce plant growth-promoting substances (PGPs) such as indoleacetic acid (IAA), salicylic acid, cellulases, phosphatases, and siderophores (particularly BRM 32114 and BRM 63523) (Martins et al. 2021; Sperandio et al. 2017). In addition, Costa et al. (2023) showed that the consortium BRM 63523 + BRM 32114, associated with silicon (Si), promotes greater drought tolerance in landrace upland rice. Silicon is


(a)
Leaf water potential (MPa)
0.000
-0.100
-0.200
-0.300
-0.400
a
a
a
HT: **
P: **
B: ns
HT x P: **
HT x B: ns
P x B: ns
HT x P x B: ns
Control
BRM32114
BRM63523
a
a
b
HT: **
P: ns
B: **
HT x P: ns
HT x B: *
P x B: ns
HT x P x B: *
Control
BRM32110+BRM32114
BRM63523+BRM32114
(b)
iWUE (µmol CO2 mmol-1 H2O)
90
60
30
0
Control
BRM32114
BRM63523
b
c
a
HT: **
P: **
B: **
HT x P: ns
HT x B: **
P x B: **
HT x P x B: **
Control
BRM32110+BRM32114
BRM63523+BRM32114
b
a
b
HT: **
P: ns
B: **
HT x P: ns
HT x B: **
P x B: ns
HT x P x B: ns
(c)
Carboxylation efficiency (A/Ci)
0.100
0.080
0.060
0.040
0.020
0.000
Control
BRM32114
BRM63523
a
b
a
HT: **
P: **
B: **
HT x P: ns
HT x B: **
P x B: **
HT x P x B: **
Control
BRM32110+BRM32114
BRM63523+BRM32114
b
a
a
HT: **
P: ns
B: **
HT x P: ns
HT x B: *
P x B: ns
HT x P x B: ns
2019/2020
2020/2021

◂**Fig. 3** Leaf water potential (*LWP*) (**a**), intrinsic water use efficiency (*iWUE*) (**b**), and carboxylation efficiency (*CE*) (**c**) of BRS Esmeralda plants cultivated under low phosphorus (20 mg dm.$^{-3}$) and drought deficit-affected soil ($n=4$). Moreover, microbial treatments: no microorganism (control) and single isolates, BRM 32114 and BRM 63523, in 2019/2020, and combined isolates, BRM 32110 + BRM 32114 and BRM 63523 + BRM 32114, in 2020/2021. Asterisks indicate statistically significant differences according to the F-test. * and **: Significant at 5 and 1% probability levels, respectively. Vertical bars denote mean ± SE. ns: non-significant. Different letters indicate significant differences among the treatments (Duncan's multiple range test at $p<0.05$)

generally regarded as a beneficial element for plant growth, particularly for Poaceae crops such as rice (Garg et al. 2020), where it can make up to 10% of the shoot dry weight (Ma and Yamaji 2006).

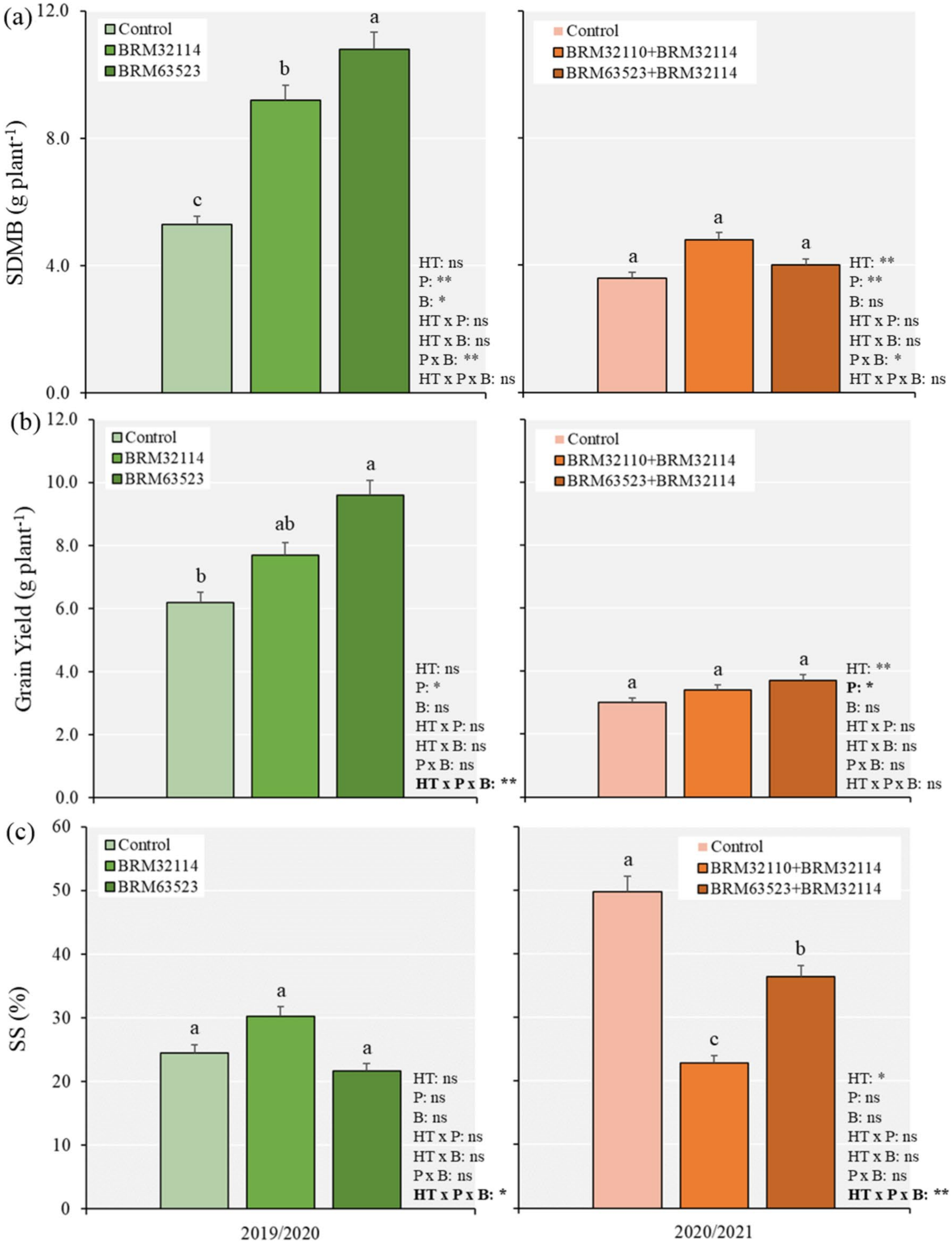
The development of the root system and its architecture are important agronomic traits that enable plants to survive under various abiotic stresses, such as drought and nutrient deficiency (Kang et al. 2022). Additionally, the root serves as a structural anchor to support the shoot and enhances resource efficiency by increasing the root absorption area in the soil (Hannan et al. 2020; Malamy 2005; Robin and Saha 2015; Robin et al. 2016). In this study, inoculated plants, especially BRM 63523 isolate and BRM 63523 + BRM 32114 consortium, induced improvement in root system (length density, surface area, volume, and the number of fine and thick roots) under stressful conditions. Early reports indicated that bacteria colonization can enhance root systems under drought (El-Mageed et al. 2022; Jochum et al. 2019; Omar et al. 2014; Zhao et al. 2023) and phosphorus deficiency (Gupta and Huang 2014; Kour et al. 2021; Richardson et al. 2009).

Despite the significant influence of microbial treatments on the root system of BRS Esmeralda plants, major effects were observed in the shoot. Limited moisture and phosphorus availability results in a complex response characterized by a decrease in soil water potential, which affects tissue nutrition and leads to changes in various plant processes (Rosales et al. 2012; Suriyagoda et al. 2014). The reduction in leaf water potential (*LWP*) was on average 74.7% lower for plants associated with beneficial bacteria compared to uninoculated plants, with BRM 32114 (2019/2020) and BRM 63523 + BRM 32114 (2020/2021) showing the most significant differences. The more pronounced gradient in *LWP* likely improved water absorption, potentially mitigating severe internal damage in reproductive organs during drought periods. According to Rêgo et al. (2018), beneficial bacteria can help plants detect changes in moisture availability and fine-tune their water status in response to environmental conditions.

The gradient in *LWP* may result from osmotic adjustment (*OA*) in the rice cells. OA, typically achieved through the accumulation of compatible solutes and maintenance of relative water content, can significantly impacts grain yield, although this component was not evaluated in our study. According to Nounjan et al. (2018), high levels of compatible solutes or osmoprotectants (e.g., proline and total sugars) in rice help to correct osmotic imbalance under drought and salinity conditions. During the reproductive phase, this mechanism enables upland rice plants to maintain an adequate physiological state, keeping the leaves green and cool for a longer period, supporting spikelet establishment and retention, and thereby sustaining grain yield (Fischer et al. 2003).

Regarding *iWUE*, Sheshshayee et al. (2012) highlighted the importance of water use efficiency (*WUE*) in improving drought tolerance. In this study, plants associated with beneficial bacteria, under combined stresses, demonstrated greater water use efficiency compared to uninoculated plants, despite the negative impact of water deficit on gas exchange. Notably, plants associated with BRM 63523 (2019/2020) and BRM 32110 + BRM 32114 (2020/2021) improved their *iWUE* by 53.0% and 59.6%, respectively, compared to well-watered (*WW*) plants under low phosphorus (*LP*) conditions. This was likely due to higher stomatal control efficiency, maintaining approximately 73.1% of the photosynthetic process while reducing stomatal conductance by 55.4% through stomatal closure. In comparison, the average reduction in stomatal conductance for uninoculated plants was only 17.5% compared to the *WW* plants under *LP* conditions.

The carboxylation efficiency (*CE*) can be considered an estimate of Rubisco activity, highlighting its limitations under stress conditions (Niinemets et al. 2009). In this study, we observed that inoculated plants demonstrated a greater ability to overcome limitations in CO_2 diffusion through stomata and mesophyll and to fix CO_2 more effectively (0.035 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$) compared to uninoculated plants (0.025 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$).


(a)
SDMB (g plant-1)
12.0
8.0
4.0
0.0
Control
BRM32114
BRM63523
c
b
a
HT: ns
P: **
B: *
HT x P: ns
HT x B: ns
P x B: **
HT x P x B: ns
Control
BRM32110+BRM32114
BRM63523+BRM32114
a
a
a
HT: **
P: **
B: ns
HT x P: ns
HT x B: ns
P x B: *
HT x P x B: ns
(b)
Grain Yield (g plant-1)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Control
BRM32114
BRM63523
b
ab
a
HT: ns
P: *
B: ns
HT x P: ns
HT x B: ns
P x B: ns
HT x P x B: **
Control
BRM32110+BRM32114
BRM63523+BRM32114
a
a
a
HT: **
P: *
B: ns
HT x P: ns
HT x B: ns
P x B: ns
HT x P x B: ns
(c)
SS (%)
60
50
40
30
20
10
0
Control
BRM32114
BRM63523
a
a
a
HT: ns
P: ns
B: ns
HT x P: ns
HT x B: ns
P x B: ns
HT x P x B: *
Control
BRM32110+BRM32114
BRM63523+BRM32114
a
c
b
HT: *
P: ns
B: ns
HT x P: ns
HT x B: ns
P x B: ns
HT x P x B: **
2019/2020
2020/2021

◂**Fig. 4** Shoot dry matter biomass (*SDMB*) (**a**), grain yield (*GY*) (**b**), and spikelet sterility (*SS*) (**c**) of the BRS Esmeralda plants cultivated under low phosphorus (20 mg dm.$^{-3}$) and drought deficit-affected soil ($n=4$). Moreover, microbial treatments: no microorganism (control) and single isolates, BRM 32114 and BRM 63523, in 2019/2020, and combined isolates, BRM 32110 + BRM 32114 and BRM 63523 + BRM 32114, in 2020/2021. Asterisks indicate statistically significant differences according to the F-test. * and **: Significant at 5 and 1% probability levels, respectively. Vertical bars denote mean ± SE. ns: non-significant. Different letters indicate significant differences among the treatments (Duncan's multiple range test at $p<0.05$)

Notably, the combined effect of water and phosphorus deficit led to a reduction in BRS Esmeralda grain yield (*GY*). However, plants associated with beneficial bacteria showed an average increase in yield of 39.5% in 2019/2020 and 18.8% in 2020/2021 compared to uninoculated plants under combined stresses. This effect was particularly pronounced in plants associated with BRM 63523 isolate (2019/2020) and the BRM 63523 + BRM 32114 consortium (2020/2021). The improved photosynthetic activity in inoculated plants compared to uninoculated plants likely contributed to the higher grain yield. Additionally, *GY* stability was higher in inoculated plants than in uninoculated plants under combined stresses suggesting that beneficial bacteria enhanced the tolerance of upland rice plants to these stresses.

Bacteria inoculation significantly influences stomatal behavior in host plants. This behavior regulates water vapor efflux and CO_2 influx, which in turn affects photosynthetic activity and biomass production (Augé et al. 2015). In this study, plants associated with beneficial bacteria under combined stresses exhibited greater dry biomass accumulation compared to uninoculated plants, with BRM 63523 (2019/2020) and the BRM 32110 + BRM 32114 consortium (2020/2021) showing the most significant differences. Additionally, changes in stomatal conductance due to beneficial bacteria inoculation are closely linked to leaf water potential and water use efficiency in host plants.

It is important to note that the environmental conditions of the SITIS phenotyping platform differed significantly between the two years of experimentation. In 2020/2021, in addition to the artificially imposed combined stresses of drought and phosphorus deficiency, the maximum light intensity was 598 lum/ft^2, which was 38.3% lower than the 969 lum/ft^2 recorded in 2019/2020. According to Heinemann et al. (2021), reduced solar radiation decreases the photosynthetic rate of leaves, leading to decreased dry matter accumulation, with the most significant impact occurring during the grain-filling phase and resulting in reduced yield. This suggests that the lower grain yield (*GY*) of BRS Esmeralda observed in 2020 can be partly attributed to the reduced solar radiation during the period of combined stresses.

Regarding P accumulation in BRS Esmeralda plants, significant main effects were observed for water conditions and soil P availability, as well as their interaction, on P concentration and uptake in grains. An interaction between P availability and bacteria inoculation was noted only for P concentration in grains. As expected, P concentration and uptake in shoot dry matter (*SDMB*) and grains were much lower under *LP* compared to *NP* conditions, while *IPE* was higher in *LP* conditions, indicating that phosphorus deficit stress was effectively induced. Since *DR* was applied during the reproductive phase, P concentration and uptake in *SDMB* were not significantly affected by water conditions or inoculation with the bacteria evaluated. Consequently, the treatments had a more pronounced impact on P concentration and uptake in grains. Furthermore, the P concentration and uptake in shoots were more significantly affected by soil P availability than by water stress. This is likely because plants experienced low P stress throughout the crop cycle, whereas water stress was only applied during a specific period at the reproductive stage. In *NP* conditions, the negative effect of drought and the beneficial effect of bacteria inoculation on P nutrition were observed. Specifically, inoculation with BRM 32110 + BRM 32114 resulted in an 18.7% increase in P content in grains compared to uninoculated plants.

P concentration in grains was about five times higher than in SDMB, consistent with the grain-to-shoot P ratios reported in rice by Fageria et al. (2013). While P concentration and uptake in SDMB showed no significant correlation with grain yield, P uptake in grains was positively correlated with grain yield ($R=0.76$, $n=45$, $p<0.001$), root length ($R=0.86$), root surface area ($R=0.84$), and root volume ($R=0.83$). This highlights the importance of improving grain P uptake to boost upland rice yield,

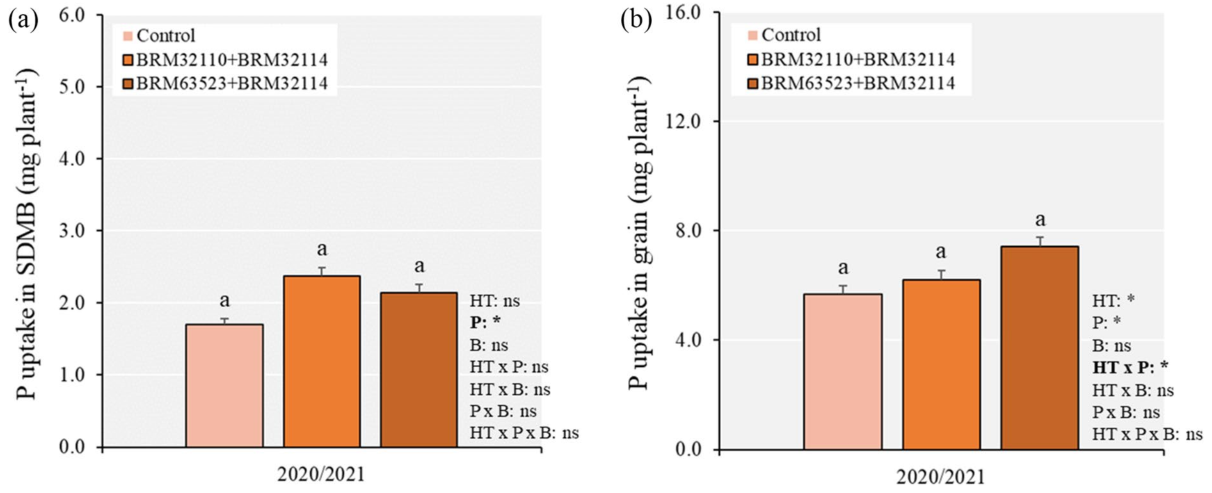


Fig. 5 Phosphorus in the shoot (**a**) and grain (**b**) of BRS Esmeralda plants cultivate under low phosphorus (20 mg dm.$^{-3}$) and drought deficit-affected soil ($n=4$). Moreover, microbial treatments: no microorganism (control) and combined isolates, BRM 32110 + BRM 32114 and BRM 63523 + BRM 32114, in 2020/2021. Asterisks indicate statistically significant differences according to the F-test. * and **: Significant at 5 and 1% probability levels, respectively. Vertical bars denote mean ± SE. ns: non-significant. Different letters indicate significant differences among the treatments (Duncan's multiple range test at $p < 0.05$)

achievable through root growth-promoting practices, such as inoculation with beneficial bacteria.

Phosphorus use efficiency is assessed by internal phosphorus efficiency (*IPE*) and phosphorus harvest index (*PHI*) (SPRPN 2023). *IPE* reflects a plant's capacity to convert soil phosphorus into grain yield, while *PHI* indicates phosphorus allocation to grains. Despite drought stress, *IPE* remained unaffected due to BRS Esmeralda's drought tolerance (Lanna et al. 2021), with values ranging from 197 to 446 g g^{-1}, higher than other rice cultivars (Deng et al. 2020, 2021). *LP* increased *IPE* by 56%, demonstrating BRS Esmeralda's adaptability to low-phosphorus soils. Conversely, *PHI* was reduced by water stress, declining from 82% in *WW* plants to 74% in DR plants. Comparisons to other cultivars show *PHI* values between 45 and 73% (Deng et al. 2020, 2021; Fageria et al. 2013, 2015), further evidencing BRS Esmeralda's resilience under low phosphorus and water-stress conditions.

Phosphorus (P) is a crucial nutrient for plants, essential for metabolic processes such as photosynthesis, respiration, adenosine triphosphate (ATP) production, cell division, signal transduction, and stress mitigation (Rawat et al. 2022). Although this study did not reveal significant differences ($p > 0.05$) in P uptake in *SDMB* and grains between inoculated plants (BRM 32110 + BRM 32114 and BRM 63523 + BRM 32114) and uninoculated plants (control treatment) under combined stresses, the mean values indicated a potential increase in inoculated plants. More promising results regarding P uptake might be achieved by implementing re-inoculation strategies in upland rice plants over the long term. In this study, we used virgin soil, which represented the initial interaction between the soil and the bacteria populations (BRM 32114, BRM 63523, BRM 32110 + BRM 63523, BRM 32114 + BRM 63252). These populations might have faced competition from native microorganisms in tropical soils. In Brazil, re-inoculation is estimated to increase soybean grain yield by an average of 8% (Hungria and Mendes 2015) and by 6.8% (Leggett et al. 2017) to 14% (Hungria et al. 2016) in Argentina. Interactions within the plant growth-promoting bacteria (PGPB)-root system can alter root exudate composition and influence the release of bioactive molecules, such as phenolic compounds and organic acids, especially under stress conditions. These changes in the microbiome structure can develop gradually over time, even if the survival rate of PGPB in the soil and their ability to re-colonize plant tissues are limited (Yaghoubi Khanghahi et al. 2024). According to Santos et al. (2019), re-inoculation facilitates the qualitative renewal of beneficial microorganisms in production systems, complemented by the quantitative action of applied inoculants.

Finally, effective research strategies should include the development of new upland rice varieties with high productive potential and the establishment of appropriate management practices, such as using bacteria and silicon (Si). These approaches could form a technological package to support upland rice cultivation under conditions of limited water and low phosphorus availability. Consequently, beneficial bacteria are considered environmentally friendly technologies for managing upland rice crops.

Conclusions

Upland rice inoculation with *Serratia* sp. increased shoot and root traits under combined stresses (drought and low phosphorus). This increase in plant growth may be attributed to the expression of one or more plant-growth-promoting characteristics. These effects are likely correlated with the production of indoleacetic acid (IAA), exopolysaccharides (EPS), phosphorus solubilizers, and the presence of ACC deaminase detected in these isolates. Therefore, these isolates have significant potential for promoting upland rice growth and mitigating the adverse effects of water and phosphorus deficits. For this reason, the use of *Serratia marcenses* in rainfed agriculture, particularly as a component of new biofertilizers for upland rice cultivation, shows great promise.

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Authors' contributions ACL, MGM, APC, MCCF, MCSC, RS conceived the idea and designed the experiment; NBC, GAB, ACL conducted the analyzes and collections at SITIS. ACL and NBC analyzed the data and led the writing of the manuscript. All authors contributed to the drafts and gave final approval.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that influenced the work reported in this paper.

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