







Effects of beneficial microorganisms on upland rice performance¹

Efeito de microrganismos na performance do arroz de terras altas

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HIGHLIGHTS:

Use of biological resources as a biostimulant in rice cultivation.

Inoculations in rice as a way to enhance performance in gas exchange.

Increase in biomass and nutrient content, after inoculations with beneficial microorganisms.

ABSTRACT: Inoculating plants with a mix of beneficial microorganisms can significantly improve plant growth and contribute to sustainable agriculture. However, it is unclear if mixed inoculations of microorganisms provide better results than inoculations of a single beneficial microorganism. Thus, the objective of this study was to evaluate the effects of beneficial microorganisms on the performance of upland rice in a greenhouse experiment using a completely randomized design with three repetitions and 26 treatments. The treatments consisted of inoculations of *Bacillus* sp. (BRM32110), *Serratia* sp. (BRM32114), *Azospirillum* sp. (1381), *Bacillus* sp. (1301), *A. brasilense* (Ab-V5) and a pool of *Trichoderma asperellum* in isolation or in a mixture in addition to a control treatment (without application of microorganisms). Microorganisms significantly affected gas exchange, biomass production and nutrient accumulation in upland rice plants. The mixed treatments of BRM32114 + pool of *T. asperellum* and Ab-V5 + pool of *T. asperellum* were more effective than the other treatments, with improved gas exchange variables and nutrient concentration. The 1301 + Ab-V5 mixed treatment showed higher averages for biomass production than the other treatments, and these values were significantly different from those of the control treatment.

Key words: *Oryza sativa*, inoculation, bioagents, growth promoters

RESUMO: As inoculações em plantas com mix de microrganismos benéficos podem melhorar significativamente o crescimento dessas e contribuir para uma agricultura sustentável. Entretanto, ainda existem questionamentos se inoculações em mix poderiam proporcionar resultados ainda melhores do que na forma isolada, sendo necessários estudos adicionais. Desta forma, objetivou-se avaliar os efeitos de microrganismos benéficos no desempenho da cultura do arroz de terras altas, em experimento conduzido em casa de vegetação, usando delineamento inteiramente casualizado, com três repetições e 26 tratamentos, que consistiram da inoculação de microrganismos isolados ou em mix de *Bacillus* sp., *Serratia* sp., *Azospirillum* sp., *Bacillus* spp., *Azospirillum brasilense* (Ab-V5) e um pool de *Trichoderma asperellum*, além do tratamento controle (sem aplicação de microrganismos). Os microrganismos afetaram significativamente as trocas gasosas, produção de biomassa e teor de nutrientes em plantas de arroz de terras altas. Os tratamentos mix BRM32114 + pool de *T. asperellum*, e Ab-V5 + pool de *T. asperellum*, foram mais efetivos, com destaque para as variáveis de trocas gasosas e teor de nutrientes. O tratamento mix 1301 + Ab-V5, apresentou maiores médias para produção de biomassa, com diferença significativa em relação ao tratamento controle.

Palavras-chave: *Oryza sativa*, inoculação, bioagentes, promotores de crescimento

INTRODUCTION

Inoculating plants with beneficial microorganisms directly influences the promotion of plant growth, especially due to the phytohormonal effect and the greater absorption of nutrients by plants, resulting in greater biomass production and significant increases in the grain yield of agricultural crops (Nascente et al., 2017a,b; Pereira et al., 2020; Rosa et al., 2020). Studies carried out at Embrapa Rice and Beans (CNPAB) found promising results with the use of different rhizobacterial isolates for two flood-irrigated rice cultivars (Sousa et al., 2019). In addition, in research carried out at the Federal Rural University of the Amazon (UFRA), in a greenhouse environment and under field conditions, four beneficial fungal isolates were identified that constitute a pool of *Trichoderma asperellum* (UFRA. T06, UFRA. T09, UFRA. T12 and UFRA. T52) (França et al., 2014).

In another study, upland rice plants inoculated with rhizobacterial isolates and the pool of *T. asperellum* showed a higher photosynthetic rate, higher dry biomass and accumulation of nutrients in the aerial parts (Nascente et al., 2017a,b). Research has shown that beneficial microorganisms inoculated as mixtures can provide greater plant growth than microorganisms inoculated in isolation (Dalolio et al., 2018); however, there are still questions that have not been fully answered on the topic of inoculations of mixtures of beneficial microorganisms (Fernandes et al., 2020; Silva et al., 2020), and additional studies are needed. Therefore, the objective of this study was to determine the effects of beneficial microorganisms inoculated alone or in combination on gas exchange, dry biomass production and macronutrient content (nitrogen, phosphorus and potassium) in the aerial parts and roots of upland rice plants.

MATERIAL AND METHODS

The experiment was conducted from May to July 2018 in a greenhouse at Fazenda Capivara, headquarters of the Embrapa Rice and Beans (CNPAB), Santo Antônio de Goiás, Goiás, Brazil, located at geographic latitude coordinates 16° 28' 00" S, longitude coordinates 49° 17' 00" W and altitude of 823 m. The soil used came from the arable layer (0-0.20 m) of a medium-textured Oxisol. Before the experiment was initiated, a soil fertility analysis was carried out and revealed a pH (H₂O) = 6.1; Ca²⁺ = 78.4 mmol_c dm⁻³; Mg²⁺ = 20.9 mmol_c dm⁻³; H + Al³⁺ = 12 mmol_c dm⁻³; Al³⁺ = 0 mmol_c dm⁻³; P = 35.9 mg dm⁻³; K⁺ = 203 mg dm⁻³; Cu²⁺ = 2.4 mg dm⁻³; Zn²⁺ = 2.9 mg dm⁻³; Fe³⁺ = 39 mg dm⁻³; Mn²⁺ = 28 mg dm⁻³ and organic matter = 24.7 g kg⁻¹. These values were determined following the methods proposed by Teixeira et al. (2017).

Three weeks before upland rice sowing, the BRS A501 CL cultivar was cultivated, and 7 kg pots were filled with soil and fertilized with 70 mg dm⁻³ of N (urea), 400 mg dm⁻³ of P₂O₅ (simple superphosphate) and 200 mg dm⁻³ of K₂O (potassium chloride).

The experiment was performed according to a completely randomized design with 26 treatments and three replications that consisted of the inoculation of the following beneficial microorganisms in isolation or in a mixture: *Bacillus* sp.

(BRM32110), *Serratia* sp., (BRM32114), *Azospirillum* sp. (1381), *Bacillus* sp. (1301), *A. brasilense* (Ab-V5) and *T. asperellum* (pool of UFRA. T06 + UFRA. T09 + UFRA. T12 + UFRA. T52). All microorganisms, isolated or mixed, were inoculated at three times during the experiment: 1. microbiolization of seeds before sowing; 2. application of a microbial suspension to the soil during watering at 7 days after sowing (DAS); and 3. application of a microbial suspension to the aerial parts of the plant by spraying at 21 DAS. The bacterial isolates and the pool of *T. asperellum* are integral parts of the Agricultural Microbiology laboratories of CNPAB and UFRA. The biochemical characteristics and taxonomic classification of rhizobacteria are available in Nascente et al. (2017a), and those of the pool of *T. asperellum* are available in Silva et al. (2011).

The bacterial suspensions were prepared in 523 liquid medium (Kado & Heskett, 1970) for 24 hours at 28 °C in a shaking incubator. The concentration of the suspension was adjusted with a spectrophotometer to an absorbance of 0.7 at a wavelength of 540 nm, corresponding to 1 x 10⁸ colony-forming units (CFUs) per mL. The rice seeds were immersed in the suspensions and, for the control treatment, in water for a period of 24 hours and a temperature of 28 °C under constant agitation in an incubator chamber with orbital agitation (Shaker), following the methodology proposed by Filippi et al. (2011).

Microbiolization with a pool of *T. asperellum* was performed with viable conidia multiplied and preserved at the Agricultural Microbiology Laboratory of CNPAB. In all, 0.5 g of conidia were used for each isolate, and 6.7 mL of white glue solution (1%) was prepared for each 200 g of rice seeds, which was placed in a plastic bag and stirred with the isolates according to the methodology of França et al. (2014).

Fifteen rice seeds were sown per pot. Seedling emergence started six days after sowing, and thinning was carried out seven days after emergence (DAE), with four plants per pot. During tillering (28 DAE) and floral primordium (48 DAE), nitrogen (2.0 g of ammonium sulfate) and potassium (1.0 g of potassium chloride) fertilizers were applied per pot and released as described by Fageria (2017). Weed control was performed weekly, and manually, there was no need for intervention to control pests and diseases. During the experiment, irrigation was performed daily while keeping the soil saturated until the full flowering phase, when the plants were cut and samples were evaluated. Gas exchanges were obtained with a portable gas meter in the IRGA infrared wavelength (LCpro +, ADC BioScientific), with the following variables being determined: photosynthetic rate (A, μmol CO₂ m⁻² s⁻¹); transpiration rate (E, mmol H₂O m⁻² s⁻¹); stomatal conductance (gs, mol H₂O m⁻² s⁻¹); internal concentration of CO₂ (Ci, μmol mol⁻¹) and instant carboxylation efficiency (EiC). The instant carboxylation efficiency (EiC) was calculated by the ratio between A and Ci [(μmol m⁻² s⁻¹) (μmol mol⁻¹)⁻¹] (Silva et al., 2013). The evaluation period was 95 and 96 DAE (stage R3), between 08 h 30 min and 10 h 30 min, with an average leaf temperature of 33 °C.

The readings were taken in the central third of the first fully expanded leaf (from top to bottom) in the rice plants during the reproductive period, with 50% of the total plants showing full bloom. The IRGA photosynthesis chamber was

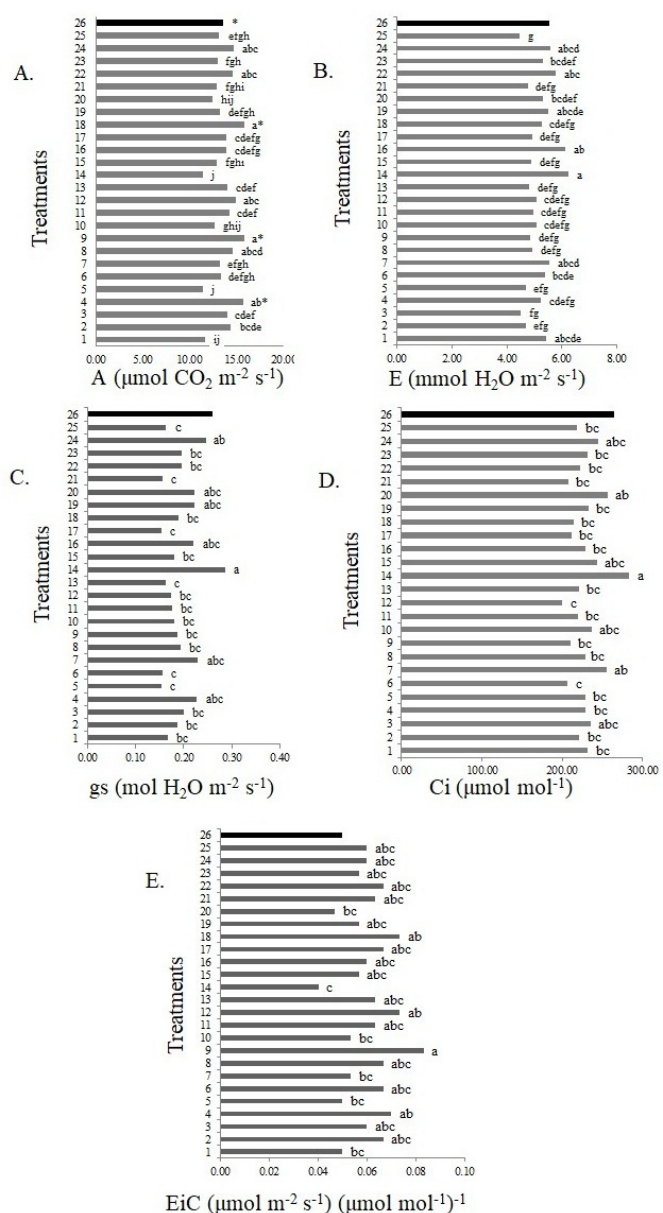
maintained with concentrations of 370 - 400 mol mol⁻¹ CO₂ in the reference air and with a density of photosynthetically active photon flux (DFFFA) of 1200 μmol [quanta] m⁻² s⁻¹ according to Nascente et al. (2017a).

At 98 and 99 DAE (stage R3), the aerial parts of the plants were cut close to the soil, and the roots were collected and cleaned with water using fine mesh sieves to avoid loss of roots in the cleaning process. The aerial and root samples were stored in a paper bag and dehydrated in an oven with forced air at 65 °C until constant biomass. The mass of the samples was determined using a precision scale, obtaining the dry biomass values of the aerial parts roots and the total (aerial parts + roots) (g pot⁻¹). Subsequently, these samples were ground in a knife mill and sent for analysis to determine the contents of nitrogen, phosphorus and potassium (g kg⁻¹) in the aerial parts and the roots according to the recommendations of Malavolta et al. (1997).

The data were analyzed for normality of errors (Shapiro-Wilk test) and homogeneity of variance (Bartlett test). Then, the data were subjected to analysis of variance, and the means were compared using the LSD test ($p \leq 0.05$). In addition, each treatment was compared with the control using the Dunnett test at $p \leq 0.05$. These analyses were performed using the SAS statistical package (2002). In addition, principal component analysis (PCA) was performed to describe the correlation between response variables (gas exchange, biomass and nutrients). The variables were considered constituents of a given main component when $r \geq |0.50|$. The biplots were created with the “FactoExtra” package (Kassambara, 2015) in R software.

RESULTS AND DISCUSSION

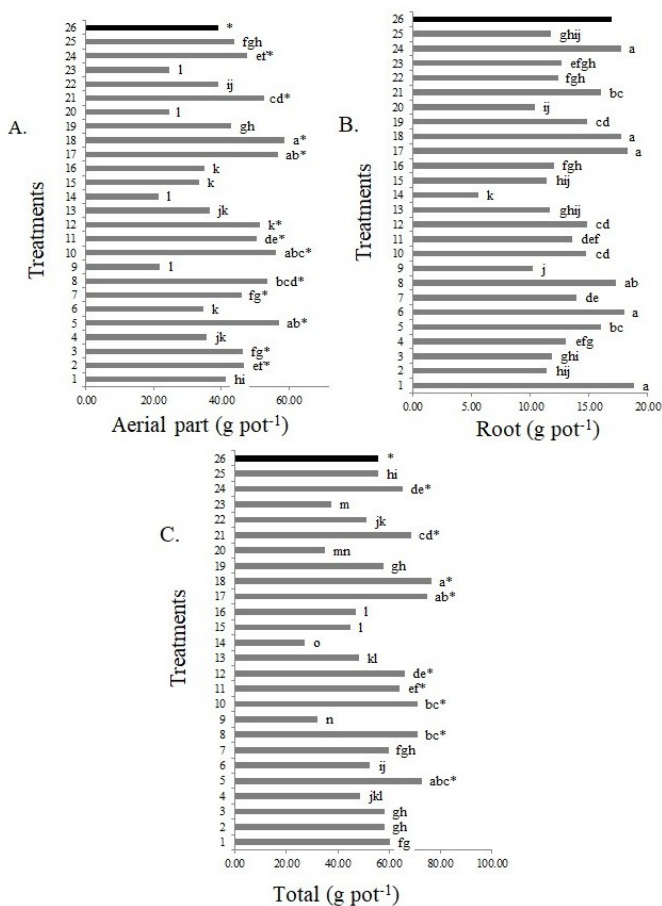
In plants treated with BRM32114 + pool of *T. asperellum* or 1301 + Ab-V5, the photosynthetic rate, A (15.83 and 15.80 μmol CO₂ m⁻² s⁻¹, respectively) values were higher than those for the other treatments and differed significantly from those of the control treatment (Figure 1). The highest transpiration rate, E (6.26 mmol H₂O m⁻² s⁻¹), stomatal conductance, gs (0.29 mol H₂O m⁻² s⁻¹), and internal CO₂ concentration, Ci (282 μmol mol⁻¹), values were found for plants treated with the Ab-V5 + pool of *T. asperellum* mixture, but these values did not differ significantly from those of the control treatment. Additionally, for instant carboxylation efficiency, EiC, the highest value was obtained with the BRM32114 + pool of *T. asperellum* mixture (0.08 (μmol m⁻² s⁻¹) (μmol mol⁻¹)⁻¹); however, there was no significant difference from that of the control treatment. The increase in enzymatic activity associated with photosynthesis, enhanced by biotic factors such as the introduction of beneficial microorganisms to the culture medium (Galmés et al., 2011; Nascente et al., 2017a,b; Santini et al., 2018; Buzo et al., 2019; Santos Júnior et al., 2019), can result in greater growth, which can increase productivity. Therefore, the mixed treatments of BRM32114 + pool of *T. asperellum*, 1301 + Ab-V5 and Ab-V5 + pool of *T. asperellum* stood out and provided the upland rice plants with higher values both in the photosynthetic rate and in the carboxylation efficiency.



* - Significant difference from the control treatment by the Dunnett test at $p \leq 0.05$; Means followed by the same letter do not differ by the LSD test ($p \leq 0.05$); 1 to 26 - Number of treatments; The black bar refers to the control treatment; Treatments: isolated BRM32114 (1); isolate BRM32110 (2); pool of *T. asperellum* (3); isolate 1381 (4); Ab-V5 isolate (5); isolate 1301 (6) and mix BRM32114 + BRM32110 (7); mix BRM32114 + 1381 (8); mix BRM32114 + pool of *T. asperellum* (9); mix BRM32114 + 32110 + Ab-V5 (10); mix 32110 + Ab-V5 (11); mix 32110 + *T. asperellum* pool (12); mix 32110 + Ab-V5 + pool of *T. asperellum* (13); mix Ab-V5 + pool of *T. asperellum* (14); mix BRM32114 + BRM32110 + Ab-V5 + pool of *T. asperellum* (15); mix 1381 + 1301 (16); mix 1381 + Ab-V5 (17); mix 1301 + Ab-V5 (18); mix 1381 + BRM32114 (19); mix 1301 + BRM32114 (20); mix 1381 + 32110 (21); mix 1301 + 32110 (22); mix 1381 + *T. asperellum* pool (23); mix 1301 + pool of *T. asperellum* (24); mix BRM32114 + BRM32110 + 1381 + pool of *T. asperellum* + 1301 + Ab-V5 (25) and control (without microorganism) (26)

Figure 1. Gas exchange of upland rice plants, cultivar BRS A501 CL: photosynthesis (A), transpiration (E), stomatal conductance (gs), internal CO₂ concentration (Ci) and instant carboxylation efficiency (EiC) submitted inoculation of microorganisms alone or in mixture

Plants treated with the 1301 + Ab-V5 mixture showed higher average values of dry shoot biomass (58.64 g pot⁻¹), differing significantly from those of the control treatment (Figure 2). For dry root biomass, the highest value was obtained with plants treated with the BRM32114 isolate; however, this value did not differ significantly from that of the control treatment. In the case of total dry biomass production (aerial



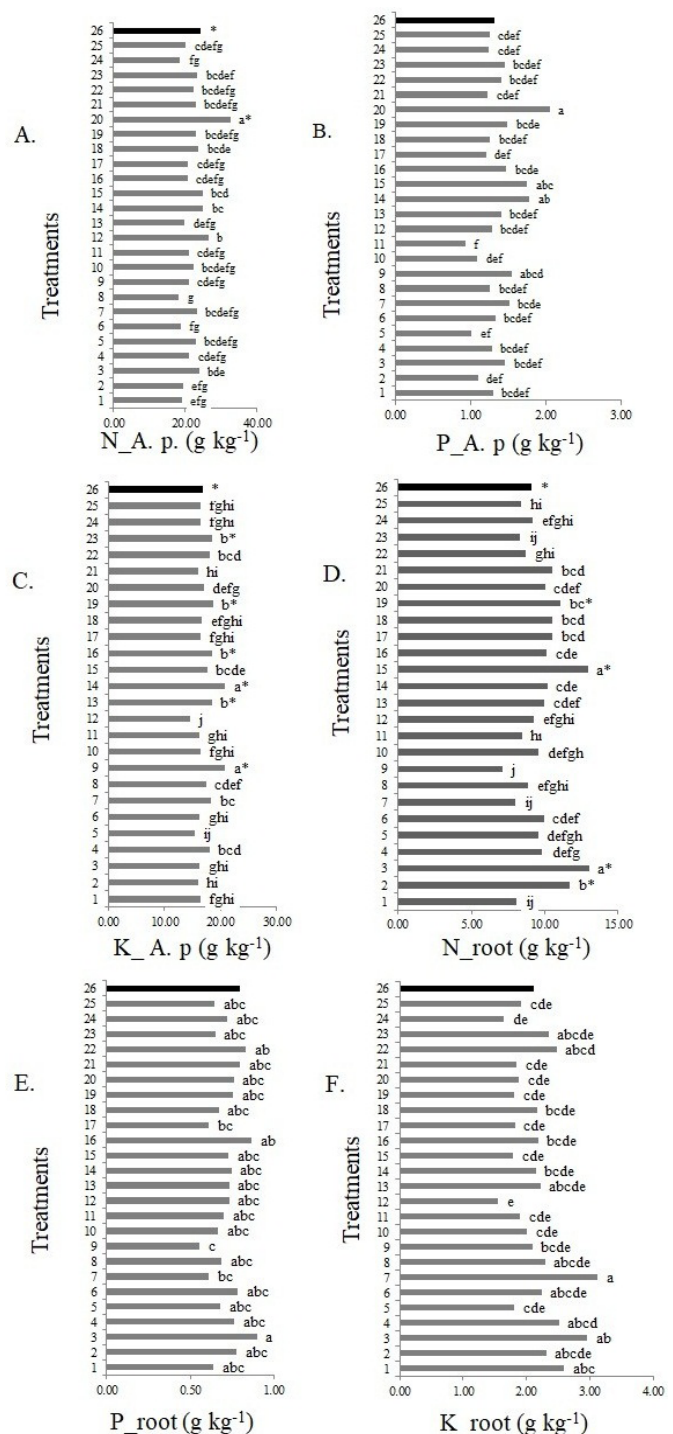
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Figure 2. Production of dry biomass from upland rice plants, cultivar BRS A501 CL, submitted to the inoculation of beneficial microorganisms, isolated or in mixture

parts + roots), the highest value (76.38 g pot⁻¹) occurred with plants treated with the 1301 + Ab-V5 mixture, and this value differed significantly from that of the control treatment. A similar result was reported by Fernandes et al. (2020), who observed superior performance in morphophysiological and productivity characteristics in rice plants inoculated with different beneficial microorganisms. Other authors have also recorded significant increases in biomass production and grain productivity in grasses and legumes with the inoculation of beneficial microorganisms. These authors observed that plants treated with these microorganisms had a higher content of nutrients and phytohormones (Louzada et al., 2009; Kapri & Tewari, 2010; Chagas et al., 2016; Araújo et al., 2020).

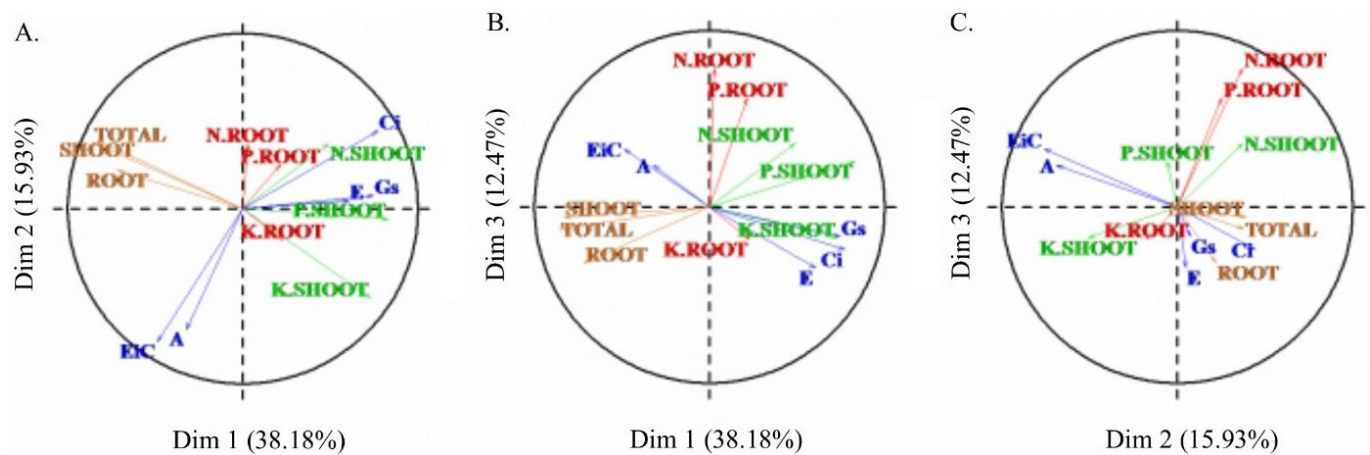
The highest levels of macronutrients in the shoot of the rice were obtained in plants treated with the 1301 + BRM32114 mixture (32.71 g kg⁻¹). The highest N content was obtained with the 1301 + BRM32114 mixture (2.05 g kg⁻¹); the highest P content with the Ab-V5 + pool of *T. asperellum* mixture (20.77 g kg⁻¹), and the highest K content with the BRM32114

+ pool of *T. asperellum* mixture (20.74 g kg⁻¹) (Figure 3). Beneficial microorganisms can provide greater production of dry biomass in plants due to greater absorption and use of



* - Significant difference from the control treatment by the Dunnett test at $p \leq 0.05$; averages followed by the same letter do not differ by the LSD test ($p \leq 0.05$); 1 to 26 - Number of treatments; The black bar refers to the control treatment. Treatments: isolated BRM32114 (1); isolate BRM32110 (2); pool of *T. asperellum* (3); isolate 1381 (4); Ab-V5 isolate (5); isolate 1301 (6) and mix BRM32114 + BRM32110 (7); mix BRM32114 + 1381 (8); mix BRM32114 + pool of *T. asperellum* (9); mix BRM32114 + 32110 + Ab-V5 (10); mix 32110 + Ab-V5 (11); mix 32110 + *T. asperellum* pool (12); mix 32110 + Ab-V5 + pool of *T. asperellum* (13); mix Ab-V5 + pool of *T. asperellum* (14); mix BRM32114 + pool of *T. asperellum* (15); mix 1381 + 1301 (16); mix 1381 + Ab-V5 (17); mix 1301 + Ab-V5 (18); mix 1381 + BRM32114 (19); mix 1301 + BRM32114 (20); mix 1381 + 32110 (21); mix 1301 + 32110 (22); mix 1381 + *T. asperellum* pool (23); mix 1301 + pool of *T. asperellum* (24); mix BRM32114 + BRM32110 + 1381 + pool of *T. asperellum* + 1301 + Ab-V5 (25) and control (without microorganism) (26)

Figure 3. Content of macronutrients in biomass of upland rice plants, cultivar BRS A501 CL, submitted to the inoculation of beneficial microorganisms, isolated or in mixture



Treatments (1 to 26): Isolated BRM32114 (1); isolate BRM32110 (2); pool of *T. asperellum* (3); isolate 1381 (4); Ab-V5 isolate (5); isolate 1301 (6) and mix BRM32114 + BRM32110 (7); mix BRM32114 + 1381 (8); mix BRM32114 + pool of *T. asperellum* (9); mix BRM32114 + 32110 + Ab-V5 (10); mix 32110 + Ab-V5 (11); mix 32110 + *T. asperellum* (12); mix 32110 + Ab-V5 + pool of *T. asperellum* (13); Ab-V5 + pool of *T. asperellum* (14); mix BRM32114 + BRM32110 + Ab-V5 + pool of *T. asperellum* (15); mix 1381 + 1301 (16); mix 1381 + Ab-V5 (17); mix 1301 + Ab-V5 (18); mix 1381 + BRM32114 (19); mix 1301 + BRM32114 (20); mix 1381 + 32110 (21); mix 1301 + 32110 (22); mix 1381 + *T. asperellum* pool (23); mix 1301 + pool of *T. asperellum* (24); mix BRM32114 + BRM32110 + 1381 + pool of *T. asperellum* + 1301 + Ab-V5 (25) and control (without microorganism) (26)

Figure 4. Dispersion of variables and treatments based on the scores of the first three principal components (Dim 1, Dim 2 and Dim 3)

water and nutrients, which can provide greater resistance to environmental stresses, such as increased temperature, reduced relative humidity, reduced porous soil space, and increased salt concentration (Chagas et al., 2016; Rosa et al., 2020; Pereira et al., 2020; Santini et al., 2018; Buzo et al., 2019; Santos Júnior et al., 2019).

The highest results for N, P and K concentration in the roots were obtained in plants treated with the pool of *T. asperellum* (13.01 g kg⁻¹). The highest N content was obtained with the BRM32114 + pool of *T. asperellum* mixture (0.75 g kg⁻¹). The highest P content was found with the *T. asperellum* pool (0.90 g kg⁻¹). Plants treated with the BRM32114 + BRM32110 mixture or the pool of *T. asperellum* presented the highest K contents, with values of 3.12 and 2.96 g kg⁻¹, respectively. Beneficial microorganisms inoculated onto plants can provide significant increases in nutrient uptake by plants. The types of microorganisms and inoculation methods have positive yet different effects and result in greater absorption of nutrients by plants (Lima et al., 2011; Nascente et al., 2017a,b; Rosa et al., 2020; Pereira et al., 2020).

Regarding the analysis of the principal components, it was found that the variability of treatments with isolated and mixed microorganisms in gas exchange, production of aerial parts, root and total biomass and N, P and K levels can be described by three principal components (PCs), that is, PC1 (38.18%), PC2 (15.93%) and PC3 (12.47%), representing 66.58% of the data variation (Figure 4).

The biplot shows groups of variables (arrows) denoting positive and negative correlations with each main component (PC), with the length of the arrow indicating the magnitude of each response for each PC (Figure 4). For example, the largest contributions in % of the variables in the component projection were obtained with Dim. 1, gs (0.74) and P. Shoot (0.82); Dim. 2, Ci (0.44); Dim. 3, N. Root (0.78); and Dim. 4, P. Root (0.56). In addition, the largest contributions in % of the variables in the component projection are represented by Dim. 1, Shoot and P. Shoot (12.86 and 12.87%); Dim. 2, A and EiC (21.27 and 25.83%); Dim. 3, N. Root and P. Root (35.26 and 21.76%); and Dim. 4, P. Root (22.00%). Regarding the contribution in %

of the treatments in the projection of the components, it was observed that for Dim. 1, the highest value was found with the Ab-V5 + pool of *T. asperellum* treatment (14) (36.46%); for Dim. 2, the BRM32114 + pool of *T. asperellum* (9) obtained the highest value (45.14%); and for Dim. 3, the pool of *T. asperellum* (3) and the BRM32114 (1) treatments produced the highest values of 18.80 and 16.23%, respectively.

Based on the results, it appears that inoculations with beneficial microorganisms contributed significantly to the growth of upland rice plants, particularly inoculations with the BRM32114 + pool of *T. asperellum* (9) and Ab-V5 + pool of *T. asperellum* (14) treatments, which contributed 81.60% in relation to the contribution in % of the treatments in the projection of the components. In addition, for the BRM32114 + pool of *T. asperellum* (9), the transpiration rate (A) and K_P.area presented average increments of 16.22 and 23.89%, respectively, superior to that of the control treatment. Additionally, the Ab-V5 + pool of *T. asperellum* (14) mixture obtained 23.85% higher K_P.area than the control treatment in upland rice plants. Studies must be carried out under field conditions to validate the results obtained under the controlled conditions of this study.

CONCLUSIONS

1. Inoculations with beneficial microorganisms, specifically mixtures of BRM32114 + pool of *T. asperellum* and 1301 + Ab-V5, significantly affected gas exchange due to the higher photosynthetic rate.

2. The highest concentrations of N, P and K in the aerial parts of upland rice were obtained in plants treated with the 1301 + BRM32114, 1301 + BRM32114 and Ab-V5 + pool of *T. asperellum* mixtures, respectively, due to the interspecific interaction of these microorganisms with the plants.

3. Upland rice plants inoculated with the 1301 + Ab-V5 mixture showed the highest mean values of aerial and total dry biomass, influenced by the higher photosynthetic rate, and these values differed significantly from those of the control treatment.

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