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Upland rice yield and development as affected by single and combined plant growth-promoting rhizobacteria (PGPR) inoculations

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Plant growth-promoting rhizobacteria (PGPR) offer a sustainable strategy to boost upland rice productivity. This greenhouse study assessed the effects of single and combined PGPR inoculations on upland rice growth and yield, using a completely randomized design with 29 treatments and four replicates. The experiment assessed root and shoot biomass and key yield components (panicles per pot, grains per pot, 1000-grain weight, and total grain yield) at 118 days after sowing. Several single strains, including *Leclercia adecarboxylata* (M01), *Priestia megaterium* (M02), *Pseudomonas* sp. BRM69536 (M03), *Pseudomonas* species BRM69515 (M04), *Enterobacter hormaechei* (M05), and *Serratia marcescens* (M07) as well as specific combinations significantly improved growth and yield components ($p < 0.05$) when compared to the non-inoculated control. Treatments M02 (*P. megaterium*), M04 (*Pseudomonas* species BRM69515), and M05 (*E. hormaechei*) showed the best overall performance across all growth and yield parameters. These results indicate that selected individual and combined PGPR strains can improve upland rice performance under controlled conditions and should be further tested under field conditions.

Key words: *Oryza sativa*, rhizobacteria, bioinoculants, grain production, sustainable agriculture.

INTRODUCTION

Rice (*Oryza sativa*) is the staple food for half of the world's population; its overall world per capita consumption reaches 78.9 kg/year (Food and Agriculture Organization [FAO], 2020). To meet the projected population growth and ensure food security, it is essential to increase rice grain productivity (Zhang et al., 2024). The United Nations (UN, 2022) estimates that the global

population may reach 8.5 billion by 2030 and 9.7 billion by 2050. To keep up with the projected population growth, rice production must increase by at least 25% by 2030 (Li et al., 2014), which must be achieved using sustainable and environmentally responsible approaches. Within this context, rainfed upland rice has emerged as a promising alternative, as it does not require irrigation, can be

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cultivated on sloped and uneven land, and allows intercropping with other crops. However, achieving efficient productivity of upland rice grain still presents significant challenges, including a lack of scientific studies on appropriate agronomic practices for its cultivation (Hussain et al., 2022). Additionally, over the past two decades, the cultivated area of upland rice has decreased by 70%, primarily due to high levels of agroclimatic risk such as drought (Heinemann et al., 2019). In this scenario, growth-promoting bacteria, which mitigate climatic risks and enhance rice production, have gained attention as a focus of study. They represent an alternative and sustainable solution for cropping systems, potentially preventing a decline in global rice production (Ngalimat et al., 2021). Plant growth-promoting rhizobacteria (PGPR) enhance plant development mainly through interactions with the rhizosphere, particularly at root tips and root hairs, where exudation is most intense, and in some cases by colonizing cortical tissues as endophytes (Nascente et al., 2017). Therefore, the development of bioinputs based on beneficial microorganisms and growth promoters, which can improve upland rice productivity under aerobic conditions while conserving water, has emerged as a viable and sustainable approach for upland rice cultivation systems (Isawa et al., 2010).

Previous studies have demonstrated that rice production and plant growth parameters are generally enhanced in plants inoculated with PGPR compared to uninoculated controls, with strain-specific differences in efficacy (Ouyabe et al., 2020). In a controlled greenhouse experiment, single and co-inoculations of *Rhodopseudomonas palustris* and *Bacillus subtilis* led to significant yield increases ranging from 9.84 to 17.73% (Zhao et al., 2021). Similarly, Ríos-Ruiz et al. (2020) reported that inoculation with selected native bacterial consortia reduced the need for nitrogen fertilizers by up to 25% while improving rice productivity. These findings suggest the potential of microbial inoculants in sustainable rice production and highlight the importance of evaluating multiple PGPR strains under controlled conditions prior to field application.

Despite these advances, most research has focused on irrigated rice systems, where plants grow under continuous flooding, or on individual and commercial PGPR strains. In contrast, upland rice is cultivated under aerobic conditions, meaning it is grown in non-flooded soils that rely on rainfall, often on sloped or marginal lands. These environments expose plants to greater drought stress and nutrient limitations, yet have received comparatively little scientific attention regarding PGPR applications. Furthermore, the synergistic or antagonistic interactions resulting from the combination of different PGPR strains remain poorly understood. Therefore, this study aims to address these knowledge gaps by evaluating non-commercial PGPR isolates with

documented plant-beneficial traits under greenhouse conditions and their combined effects on upland rice. This approach contributes to identifying alternative bioinput sources beyond the current commercial strains. This study provides new evidence that both individual and combined PGPR strains can significantly enhance upland rice growth and yield components. By focusing on upland rice and evaluating new microbial consortia, this study provides original evidence to support sustainable strategies for improving productivity in water-limited and high-risk agroecosystems.

MATERIALS AND METHODS

Site characterization

The experiment was conducted from January 2024 to May 2024 in a greenhouse at the Fazenda Capivara headquarters of Embrapa Arroz e Feijão (CNPAP), Santo Antônio de Goiás, Goiás, Brazil, located at geographic latitude coordinates 16° 28' 00" S, longitude coordinates 49° 17' 00" W, and an altitude of 823 m. The soil type used in the trial is classified as Typic Acrustox according to the USDA Soil Taxonomy. Before the execution of the experiment, soil samples were collected at a depth of 0-20 cm for chemical analysis, following the methodology of Donagema et al. (2011). Briefly, Phosphorus (P) and Potassium (K) were extracted by Mehlich 1 extracting solution (0.05M HCl + 0.0125M H₂SO₄). From the extracted solution phosphorus was determined by colorimeter and K by flame photometer. Calcium (Ca), Magnesium (Mg), and Aluminium (Al) were extracted with 1M KCL Aluminium was determined by titration with NaOH, and Ca and Mg by titration with Ethylenediaminetetraacetic acid (EDTA) from the extracted solution. Micronutrients were determined on a portion of the extract for P by atomic absorption spectrophotometry. Soil quality was assessed by determining two enzymes: β -glucosidase and arylsulfatase. β -glucosidase activity was estimated using the method proposed by Tabatabai (1994). Arylsulfatase activity (Tabatabai and Bremner, 1970) was measured by the hydrolysis of potassium p-nitrophenyl sulfate into p-nitrophenol (PNP) after incubating soil samples for 1 h at 37°C.

Treatments and experimental design

The microorganisms used in this study were originally isolated from radish, millet cover crops and from maize. These bacterial isolates were previously tested under laboratory conditions and showed potential for plant growth promotion, including IAA production and the solubilization of potassium, phosphorus, and zinc (Duarte, 2025). The isolates are preserved in the Multifunctional Microorganisms Collection of Embrapa Rice and Beans, with their corresponding identifications listed in Table 1. The strains consisted of M01 (*Leclercia adecarboxylata*, MIT3), M02 (*Priestia megaterium* BRM69509), M03 (*Pseudomonas* species, BRM69536), M04 (*Pseudomonas* spp., BRM69515), M05 (*Enterobacter hormaechei*, BRM69541), M06 (*Bacillus toyonensis*, BRM32110), M07 (*Serratia marcescens*, BRM65920) (Table 1) and treatments M08 to M028 were strain combinations (Table 2).

Preparation of microorganisms solutions

Prior to experimentation, each bacterial isolate was cultivated on a solid medium of nutrient agar and was then suspended in a liquid

Table 1. Strains used in this study.

Isolate	Species	Crop	Location
MIT3	<i>Leclercia adecarboxylata</i>	Millet	Jataí – Go, Brazil
BRM69509	<i>Priestia megaterium</i>	Millet	Jataí – Go, Brazil
BRM69536	<i>Pseudomonas</i> spp.	Radish	Jataí – Go, Brazil
BRM69515	<i>Pseudomonas</i> spp.	Radish	Jataí – Go, Brazil
BRM69541	<i>Enterobacter hormaechei</i>	Maize	Jataí – Go, Brazil
BRM32110	<i>Bacillus toyonensis</i>	Rice	Dom Eliseu – Pa, Brazil
BRM65920	<i>Serratia marcescens</i>	Rice	Santo Antonio – Go, Brazil

Table 2. Treatments used in this study (M01–M29).

Treatment	Combination of species
M01	<i>Leclercia adecarboxylata</i>
M02	<i>Priestia megaterium</i>
M03	<i>Pseudomonas</i> spp.
M04	<i>Pseudomonas</i> spp.
M05	<i>Enterobacter hormaechei</i>
M06	<i>Bacillus toyonensis</i>
M07	<i>Serratia marcescens</i>
M08	<i>L. adecarboxylata</i> + <i>Priestia megaterium</i>
M09	<i>L. adecarboxylata</i> + <i>Pseudomonas</i> spp. BRM69536
M10	<i>L. adecarboxylata</i> + <i>Pseudomonas</i> spp. BRM69515
M11	<i>L. adecarboxylata</i> + <i>E. hormaechei</i>
M12	<i>L. adecarboxylata</i> + <i>B. toyonensis</i>
M13	<i>L. adecarboxylata</i> + <i>S. marcescens</i>
M14	<i>P. megaterium</i> + <i>Pseudomonas</i> spp. BRM69536
M15	<i>P. megaterium</i> + <i>Pseudomonas</i> spp. BRM69515
M16	<i>P. megaterium</i> + <i>E. hormaechei</i>
M17	<i>P. megaterium</i> + <i>B. toyonensis</i>
M18	<i>P. megaterium</i> + <i>S. marcescens</i>
M19	<i>Pseudomonas</i> spp. BRM69536 + <i>Pseudomonas</i> spp. BRM69515
M20	<i>Pseudomonas</i> spp. BRM69536 + <i>E. hormaechei</i>
M21	<i>Pseudomonas</i> spp. BRM69536 + <i>B. toyonensis</i>
M22	<i>Pseudomonas</i> spp. BRM69536 + <i>S. marcescens</i>
M23	<i>Pseudomonas</i> spp.2 + <i>E. hormaechei</i>
M24	<i>Pseudomonas</i> spp. BRM69515 + <i>B. toyonensis</i>
M25	<i>Pseudomonas</i> spp. BRM69515 + <i>S. marcescens</i>
M26	<i>E. hormaechei</i> + <i>B. toyonensis</i>
M27	<i>E. hormaechei</i> + <i>S. marcescens</i>
M28	<i>B. toyonensis</i> + <i>S. marcescens</i>
M29	Sterile control group

medium of nutrient broth consisting of casein hydrolysate, magnesium sulphate heptahydrate, potassium phosphate monobasic, yeast extract, sucrose, and agar (Kado and Heskett, 1970) and finally incubated for 24 h at 28°C in a shaking incubator. Bacterial concentrations were then standardized to 1×10^8 colony-forming units (CFU) per mL by adjusting the spectrophotometric reading of $A_{540} = 0.05$, and combinations between bacteria were

proportionally adjusted to maintain the same final concentration. The bacterial suspensions were not tested for viability (CFU counts) prior to each application.

Inoculation

Inoculation was performed at three stages during the experiment:

Table 3. Soil analyses.

Parameter	Result
pH (H ₂ O)	5.8
Organic matter (O.M.)	33.8 g kg ⁻¹
P (Mehlich)	12.9 mg dm ⁻³
K	2 mmolc dm ⁻³
Ca ²⁺	14.2 mmolc dm ⁻³
Mg ²⁺	6.1 mmolc dm ⁻³
Al ³⁺	1 mmolc dm ⁻³
H + Al	27 mmolc dm ⁻³
SB (Sum of bases)	22.3 mmolc dm ⁻³
Cu ²⁺	1.4 mg dm ⁻³
Zn ²⁺	4.3 mg dm ⁻³
Fe ³⁺	21 mg dm ⁻³
Mn ²⁺	13.7 mg dm ⁻³
β-glucosidase	19.6 mg g ⁻¹ h ⁻¹
Arylsulfatase	13.9 mg g ⁻¹ h ⁻¹

(1) seed inoculation of the upland rice cultivar A504 CL, developed by Embrapa, where "CL" denotes Clearfield®, a herbicide-tolerant technology that allows the selective control of weedy rice and grasses; (2) application of the bacterial suspension to the soil together with irrigation at 7 days after sowing (DAS); and (3) foliar application at 21 DAS. For the control treatment, sterile distilled water was used instead of bacterial suspensions. Prior to seed inoculation, upland rice seeds were disinfected by immersion in 7.5% sodium hypochlorite for 7 min. The seeds were then soaked in 70% alcohol for 3 min, drained, and rinsed three times with distilled water. Finally, the seeds were dried on filter paper in trays in a drying room maintained at 29°C. Seed inoculation was carried out for 24 h at 25°C under constant agitation. Distilled water was used for seed immersion in the control treatment, following the methodology described by Filippi et al. (2011).

Rice management

Before sowing, 116 pots (each with a capacity of 7 kg) were filled with soil and fertilized with 70 mg dm⁻³ of nitrogen (urea), 400 mg dm⁻³ of P₂O₅ (single superphosphate), and 200 mg dm⁻³ of K₂O (potassium chloride). A completely randomized design was employed, consisting of 29 treatments and four replicates per treatment; each pot served as one biological replicate. Each experimental unit consisted of ten inoculated upland rice seeds sown per pot. Thinning was carried out seven days after emergence, leaving two plants per pot. Nitrogen (N) fertilization (2.0 g urea per pot) was applied twice during the experiment, at 48 and 80 DAS. Weed control was performed manually on weekly basis, and no interventions were necessary for pest or disease management because no incidence of pests or diseases was observed during the experiment, and plants remained healthy throughout the growth cycle. Irrigation was conducted daily to maintain soil moisture near field capacity until physiological maturity, when plants were harvested for evaluation. Soil moisture was monitored daily by weighing the pots, and water lost through evapotranspiration was replenished whenever levels dropped to 85% of field capacity.

Dry shoot, root biomass and accumulation of nutrients in rice plants

At 118 DAS, plant shoots were harvested by cutting them near the soil surface, and the roots were carefully collected and washed under running water to remove all adhering soil. To minimise root loss during washing, a fine mesh sieve was employed. Samples of both shoots and roots were placed in paper bags and dried in a forced-air oven at 65 °C until constant weight was achieved. The dry biomass of shoots and roots (expressed in g pot⁻¹) was determined using a precision balance. Subsequently, dried material was ground, and the nutrient content (P, K, Ca, Mg, and S) was analysed in accordance with the methodology described by Malavolta et al. (1997). P was determined by colorimetry, Ca and Mg by atomic absorption spectrophotometry, potassium by flame emission photometry, and sulfur by turbidimetry. Nutrient accumulation in shoots and roots was calculated by multiplying the concentration of each nutrient by their respective dry biomass.

Yield components and grain yield

At the physiological maturity stage of upland rice (118 DAS), the agronomic traits evaluated were grain yield determined by weighing the grain from each pot after standardizing the moisture content to 13% on a wet basis expressed as g pot⁻¹; number of panicles per pot, obtained by counting the total number of panicles in each pot; number of grains per pot, calculated by counting the total number of grains in each pot; and 1000-grain weight, measured by counting 1000 grains, recording their weight; at 13% moisture level. The 1000-grain weight was determined by manually counting and weighing 1000 grains from each sample, after adjusting grain moisture to 13%.

Statistical analysis

The data were subjected to analysis of variance (ANOVA), and when significant differences were detected, mean comparisons were conducted using the Scott-Knott test at the 5% significance level ($p < 0.05$). Statistical analyses were performed using Sisvar software, version 5.6 (Ferreira, 2019). Principal Components (PCs) were employed as response variables when the correlation coefficient was $r \geq 0.50$. The first two PCs accounted for 54.3% of the total variation in the dataset. A biplot (two-dimensional graph) was constructed to illustrate the correlation between the isolated microorganisms and the response variables, using Scilab statistical software for this analysis.

RESULTS

The soil is slightly acidic pH (5.8). Organic matter was high, calcium, magnesium, and micronutrients were adequate, while potassium was low and phosphorus moderate. Exchangeable aluminium was low, and enzyme activity was present (Table 3).

The inoculation of upland rice with PGPR clearly affected both root (Figure 1) and shoot (Figure 2) development. Inoculation with treatments M01, M02, M03, M04, M05, M06, M07, M09, M10, M11, M12, M14, M19, M20, and M26 resulted in the greatest increases in the total dry biomass of upland rice, expressed as the sum of root and shoot dry weights. The control treatment

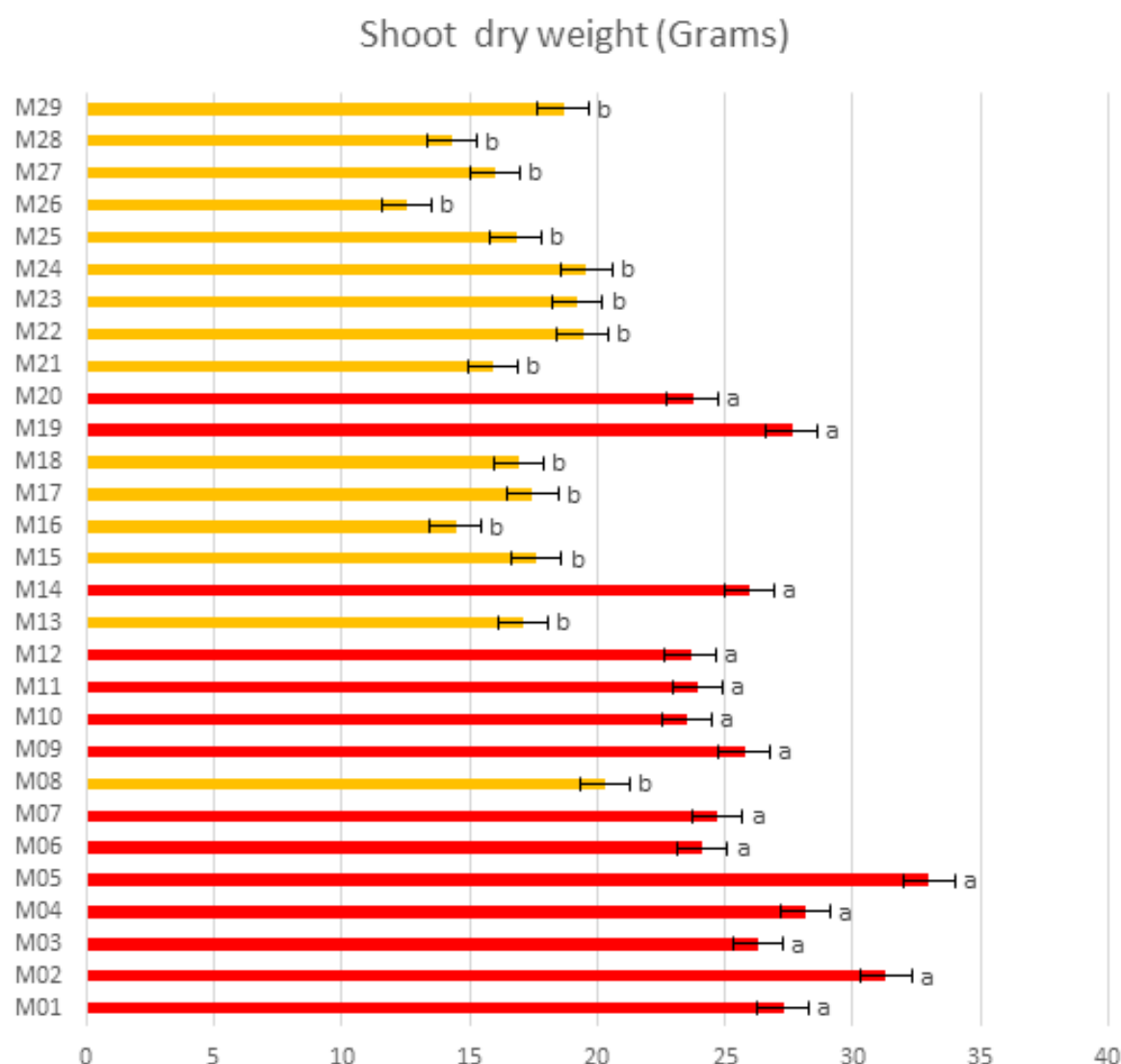


Figure 1. Effect of sole and combined microorganisms on the dry weight of shoots of upland rice cultivar A504 CL. *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$.

recorded a significantly lower total dry biomass of 23.02 g, representing only 57.62% of the highest value observed in treatment M05.

The highest number of panicles per pot in upland rice was recorded in treatments M01 (11.75), M02 (11.75), M05 (11.25), and M04 (10.74) (Figure 3), all involving single microorganism inoculations. These treatments exceeded the control, which produced 4.25 panicles per pot. Increases were 176.5% for M01 and M02, 164.7% for M05, and 152.7% for M04. In addition, treatments M03, M06, M07, M12, M13, M14, and M19 also resulted in high panicle numbers and did not differ statistically from the top-performing treatments, suggesting a

consistent positive effect of inoculation. All these treatments produced significantly higher panicle numbers than the control. The most effective treatments for the number of grains per pot were M04, M03, M01, M02, M19, M14, M12, M05, M13, M09, M07, M11, M20, M23, and M06, although they did not differ significantly from each other (Figure 4). Among them, *Pseudomonas* spp. BRM69515 (M04) and *Pseudomonas* spp. BRM69536 (M03) significantly increased the number of grains when applied as single inoculants.

Specifically, treatment M04 produced 1054 grains per pot, which corresponds to 410.9% of the control (257 grains). Likewise, M03 yielded 1008 grains, equal to a

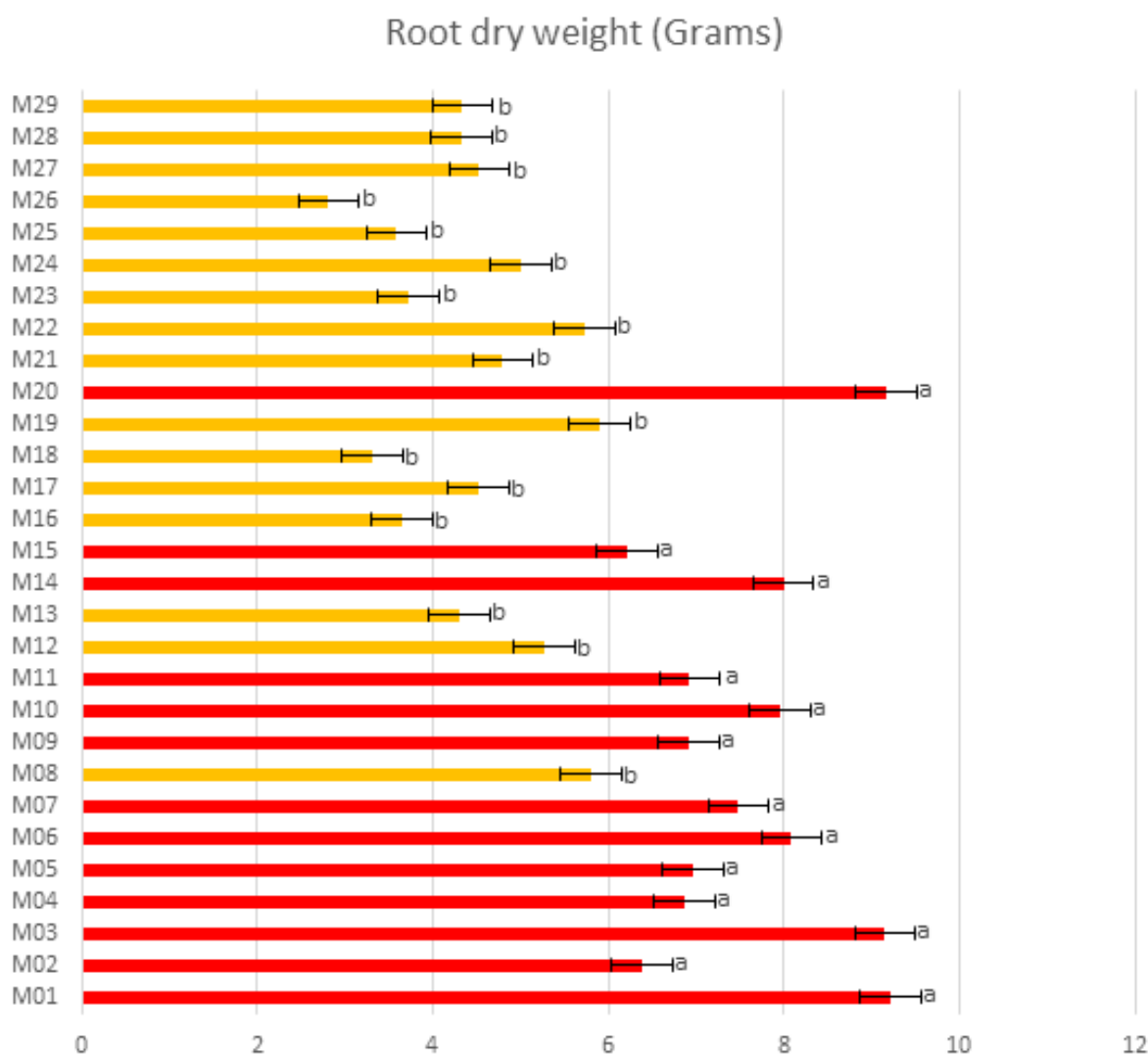


Figure 2. Effect of sole and combined microorganisms on the dry weight of roots of upland rice cultivar A504 CL. *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$.

292.2% increase. In contrast, all other treatments, although numerically higher, did not differ statistically from the control. Furthermore, the 1000-grain weight of upland rice was not affected by any inoculation treatments. Both single and combined applications of rhizobacteria showed no significant differences compared to the control (Figure 5).

The total grain yield per pot was enhanced respectively by treatments M04 (26.66 g), M03, M01, M02, M19, M14, M09, M05, M07, M20, M12, M13, and M11, although the differences were not statistically significant (Figure 6). Treatment M04 (*Pseudomonas* sp. BRM69515) showed the highest increase, with a yield of 26.66 g compared to 6.47 g in the control, representing a 312% increase.

These results were primarily influenced by the presence of beneficial bacteria from the genera *Bacillus*, *Priestia*, *Pseudomonas*, *Enterobacter*, and *Serratia*.

Nutrient accumulation in roots was led by M10 and M20 with the highest accumulation of P, K and Ca, while M06 stood out with the highest accumulation of Ca and Mg and M14 with K and Ca (Table 4). Concerning the shoots, treatment M05 was the only one with significant accumulation in all four nutrients (K, Ca, Mg and P) though phosphorus did not differ statistically from the control (Table 4). Treatment M02 provided highest value of K, Ca, and Mg; M09 P and K; M10 and M11 P and K; M14 P, K and Ca. M19 K and Ca. These treatments provided more nutrients in upland rice plants than the

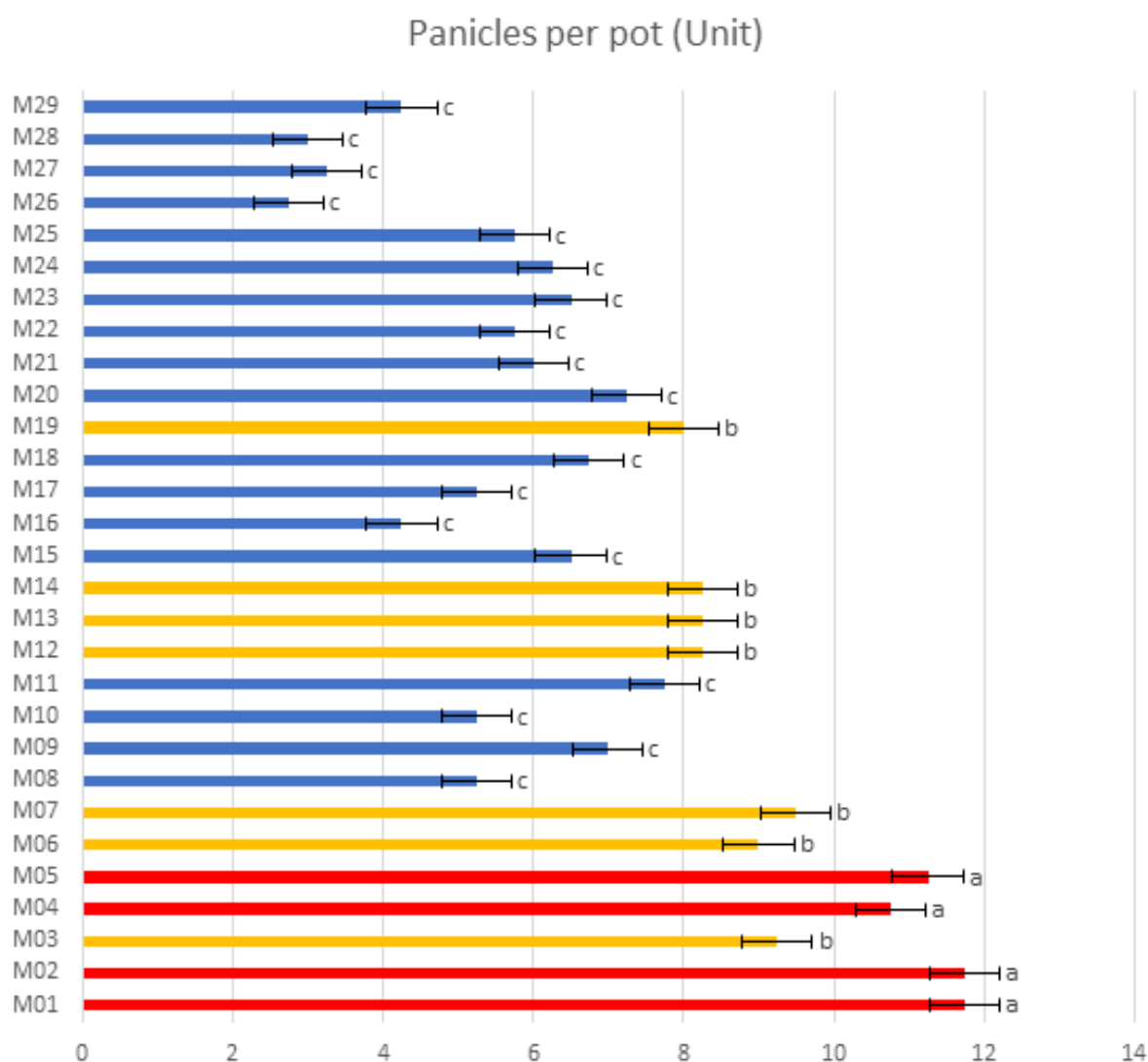


Figure 3. Effect of sole and combined microorganisms on the panicle per pot (PPP) of upland rice cultivar A504 CL.
 *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$.

control. Interestingly, several of the treatments that promoted greater nutrient accumulation, such as M02, M05, M11, M12, M14 and M20 also contributed to higher grain yield, suggesting a direct relationship between nutrient availability and productivity.

In the principal component analysis (PCA), it was observed that the variability of treatments with microorganisms, both isolated and combined, in relation to biomass production, root dry mass (DRM) and shoot dry mass (DSM), nutrient accumulation in the shoot (P_Shoot, K_Shoot, Ca_Shoot, and Mg_Shoot) and root (P_Root, K_Root, Ca_Root, and Mg_Root), as well as production components (number of panicles per pot – Pan/Pot, number of grains per panicle – G/P, 1000-grain

weight – M1000G, and grain mass per pot – GM/Pot) in upland rice plants, was best represented by two principal components (PCs), which explained 72.1% of the data variation, with 54.3% attributed to PC1 and 17.8% to PC2. The PCA biplot (factor map) illustrates the relationships among the evaluated variables and their contributions to the principal components, as well as the association of these variables with the inoculation treatments. The direction of the vectors indicates positive or negative correlations with each principal component, while vector length reflects the relative contribution of each variable to the overall variability. Principal Component 1 (PC1) was positively associated with the number of grains per panicle, whereas it showed

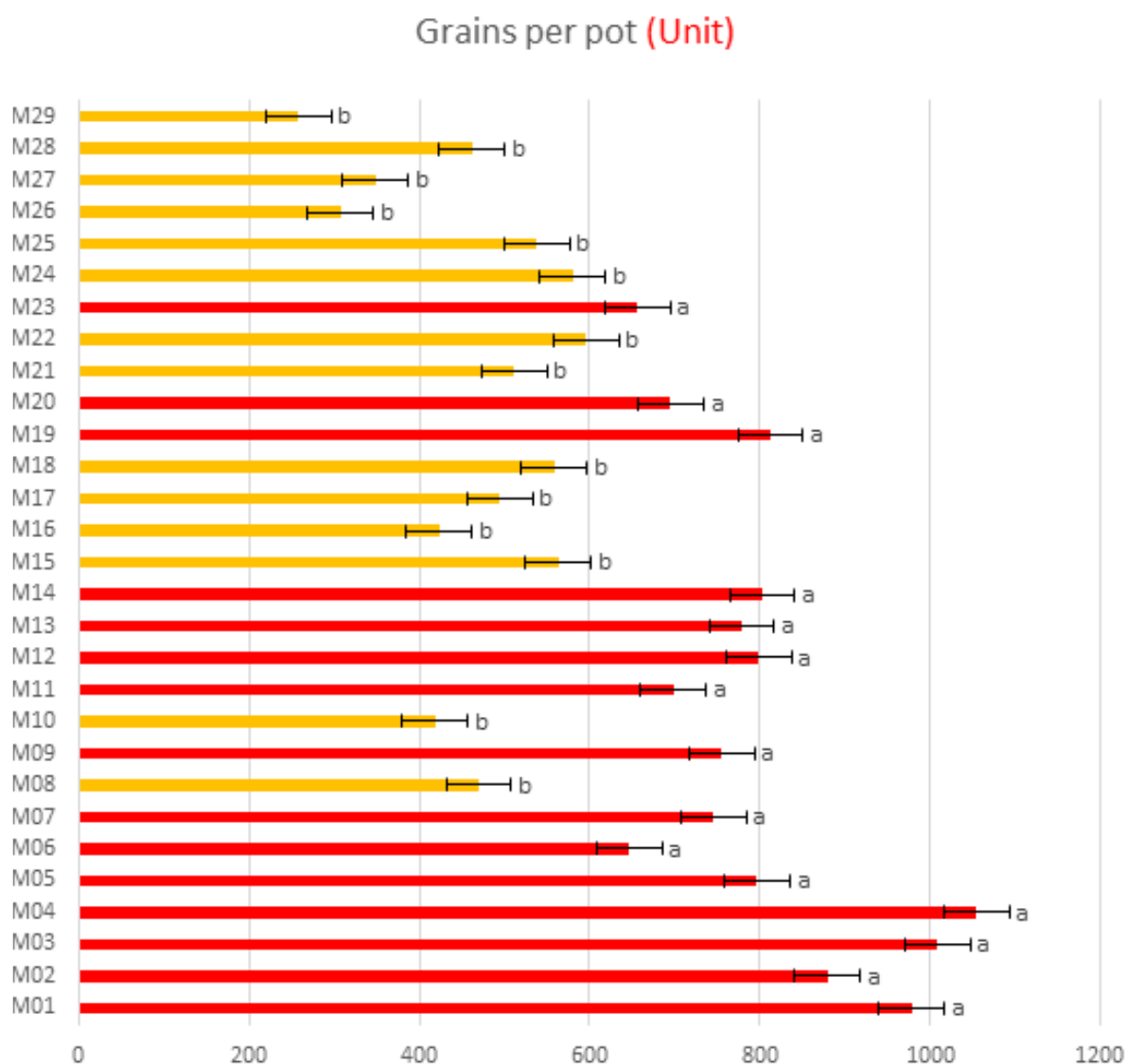


Figure 4. Effect of sole and combined microorganisms on the grains per pot (GPP) of upland rice cultivar A504 CL. *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$.

negative correlations with grain mass per pot, number of panicles per pot, number of grains per pot, potassium (K), phosphorus (P), calcium (Ca), and magnesium (Mg) contents in the shoot, as well as K, Ca, and Mg contents in the root, shoot dry mass, root mass, and 1000-grain weight. This component therefore represents a trade-off between grain number per panicle and overall biomass accumulation and nutrient uptake. In contrast, Principal Component 2 (PC2) was positively correlated with grain mass per pot, number of panicles per pot, number of grains per pot, K, Ca, and Mg contents in the shoot, and shoot dry mass. Negative correlations along PC2 were

observed with root mass, number of grains per panicle, 1000-grain weight, K, Ca, and Mg contents in the root, and P content in the shoot. These patterns suggest that PC2 captures variation related to yield performance and nutrient allocation in response to inoculation treatments. Overall, the PCA indicates that the inoculations influenced plant growth, yield components, and nutrient distribution, allowing the discrimination of treatments based on their effects on biomass production and grain yield attributes.

Based on the representational quality of the treatments with isolated and combined microorganisms for the

Table 4. Effect of sole and combined microorganisms on nutrient content in the roots (R) and shoots (S) of upland rice cultivar A504 CL.

Treatment	R - P	R - K	R - Ca	R - Mg	S - P	S - K	S - Ca	S - Mg
M01	0.005 ^c	0.041 ^b	0.008 ^a	0.003 ^b	0.025 ^b	0.602 ^b	0.131 ^b	0.076 ^b
M02	0.003 ^c	0.032 ^c	0.006 ^b	0.003 ^b	0.031 ^b	0.885 ^a	0.152 ^a	0.084 ^a
M03	0.006 ^c	0.042 ^c	0.007 ^a	0.003 ^b	0.027 ^b	0.631 ^b	0.121 ^b	0.064 ^b
M04	0.004 ^c	0.025 ^c	0.005 ^c	0.002 ^c	0.028 ^b	0.676 ^b	0.149 ^a	0.061 ^b
M05	0.004 ^c	0.027 ^c	0.004 ^c	0.002 ^c	0.050 ^a	0.816 ^a	0.184 ^a	0.095 ^a
M06	0.005 ^c	0.031 ^c	0.007 ^a	0.005 ^a	0.029 ^b	0.630 ^c	0.127 ^b	0.066 ^b
M07	0.005 ^c	0.030 ^c	0.006 ^b	0.003 ^b	0.037 ^a	0.592 ^c	0.133 ^b	0.070 ^b
M08	0.003 ^c	0.024 ^c	0.002 ^c	0.002 ^c	0.025 ^b	0.411 ^c	0.113 ^b	0.040 ^c
M09	0.008 ^b	0.035 ^c	0.006 ^b	0.002 ^c	0.041 ^a	0.459 ^c	0.150 ^a	0.068 ^b
M10	0.012 ^a	0.058 ^a	0.007 ^a	0.003 ^b	0.046 ^a	0.519 ^a	0.118 ^b	0.063 ^b
M11	0.007 ^b	0.046 ^b	0.007 ^a	0.003 ^b	0.034 ^a	0.596 ^a	0.134 ^b	0.066 ^b
M12	0.005 ^c	0.043 ^b	0.005 ^c	0.003 ^b	0.023 ^b	0.554 ^a	0.122 ^b	0.064 ^b
M13	0.003 ^c	0.029 ^c	0.004 ^c	0.002 ^c	0.018 ^b	0.408 ^c	0.081 ^c	0.039 ^c
M14	0.008 ^c	0.062 ^a	0.007 ^a	0.003 ^b	0.036 ^a	0.614 ^a	0.142 ^a	0.060 ^b
M15	0.006 ^c	0.053 ^a	0.004 ^c	0.003 ^b	0.023 ^b	0.423 ^c	0.080 ^c	0.038 ^c
M16	0.003 ^c	0.024 ^c	0.003 ^c	0.001 ^c	0.020 ^b	0.288 ^d	0.072 ^c	0.036 ^c
M17	0.005 ^c	0.036 ^c	0.004 ^c	0.002 ^c	0.023 ^b	0.390 ^c	0.077 ^c	0.040 ^c
M18	0.003 ^c	0.021 ^c	0.003 ^c	0.002 ^c	0.018 ^b	0.332 ^d	0.076 ^c	0.032 ^c
M19	0.006 ^c	0.029 ^c	0.006 ^b	0.002 ^c	0.029 ^b	0.586 ^a	0.153 ^a	0.066 ^b
M20	0.011 ^a	0.061 ^a	0.009 ^a	0.003 ^b	0.032 ^a	0.468 ^c	0.116 ^b	0.062 ^b
M21	0.006 ^c	0.036 ^c	0.004 ^c	0.002 ^c	0.018 ^b	0.391 ^c	0.071 ^c	0.033 ^c
M22	0.007 ^b	0.042 ^b	0.005 ^c	0.002 ^c	0.028 ^b	0.459 ^c	0.075 ^c	0.040 ^c
M23	0.003 ^c	0.023 ^c	0.003 ^c	0.001 ^c	0.022 ^b	0.453 ^c	0.102 ^c	0.049 ^c
M24	0.004 ^c	0.029 ^c	0.004 ^c	0.002 ^c	0.026 ^b	0.455 ^c	0.113 ^b	0.046 ^c
M25	0.005 ^c	0.030 ^c	0.004 ^c	0.002 ^c	0.019 ^b	0.378 ^c	0.089 ^c	0.045 ^c
M26	0.004 ^c	0.023 ^c	0.003 ^c	0.001 ^c	0.018 ^b	0.244 ^d	0.063 ^c	0.020 ^c
M27	0.005 ^c	0.036 ^c	0.003 ^c	0.002 ^c	0.026 ^b	0.344 ^d	0.086 ^c	0.039 ^c
M28	0.005 ^c	0.047 ^b	0.003 ^c	0.001 ^c	0.027 ^b	0.278 ^d	0.068 ^c	0.037 ^c
M29	0.006 ^c	0.043 ^b	0.004 ^c	0.002 ^c	0.040 ^a	0.368 ^c	0.108 ^b	0.040 ^c

Data are presented as means only.

analyzed variables, treatments M02, M04, and M05 showed the highest positive correlation with the PPP, GPP, and GY (Figure 7). The variables shoot dry mass and K, Ca, and Mg content in the aerial part were positively correlated with treatments M01, M03, M06, M07, M10, and M19. The 1000-grain weight was positively correlated with treatment M09, while the number of grains per panicle was significantly correlated with treatments M15 and M22. Root dry mass, Ca and Mg content in the root, and P content in the aerial part were equally correlated with treatments M11 and M14. Finally, P and K contents showed a positive correlation with combinations M10 and M20. The remaining treatments did not show a positive correlation within the representational quality of the PC1 x PC2 interaction. A Pearson's correlation analysis revealed significant relationships among productive traits, biomass variables,

and nutrient contents in shoots and roots (Table 5). Grain yield (GY) was strongly and positively correlated with pods per plant (PPP; $r = 0.90$, $p \leq 0.05$) and thousand-grain weight (M1000G; $r = 0.98$, $p \leq 0.05$). Dry shoot mass (DSM) also showed positive correlations with PPP ($r = 0.64$), GY ($r = 0.62$), and M1000G ($r = 0.63$), indicating an association between aboveground biomass and yield performance. Root phosphorus (R-P) exhibited strong positive correlations with PPP ($r = 0.86$), GY ($r = 0.78$), and M1000G ($r = 0.81$), as well as with shoot calcium (S-Ca; $r = 0.94$) and shoot magnesium (S-Mg; $r = 0.95$). Root magnesium (R-Mg) was positively correlated with DSM ($r = 0.87$) and with yield-related traits ($r \approx 0.56$ – 0.57). In contrast, root potassium (R-K) and calcium (R-Ca) showed weak associations with yield traits but were strongly intercorrelated ($r = 0.82$). Shoot nutrient contents were moderately associated with

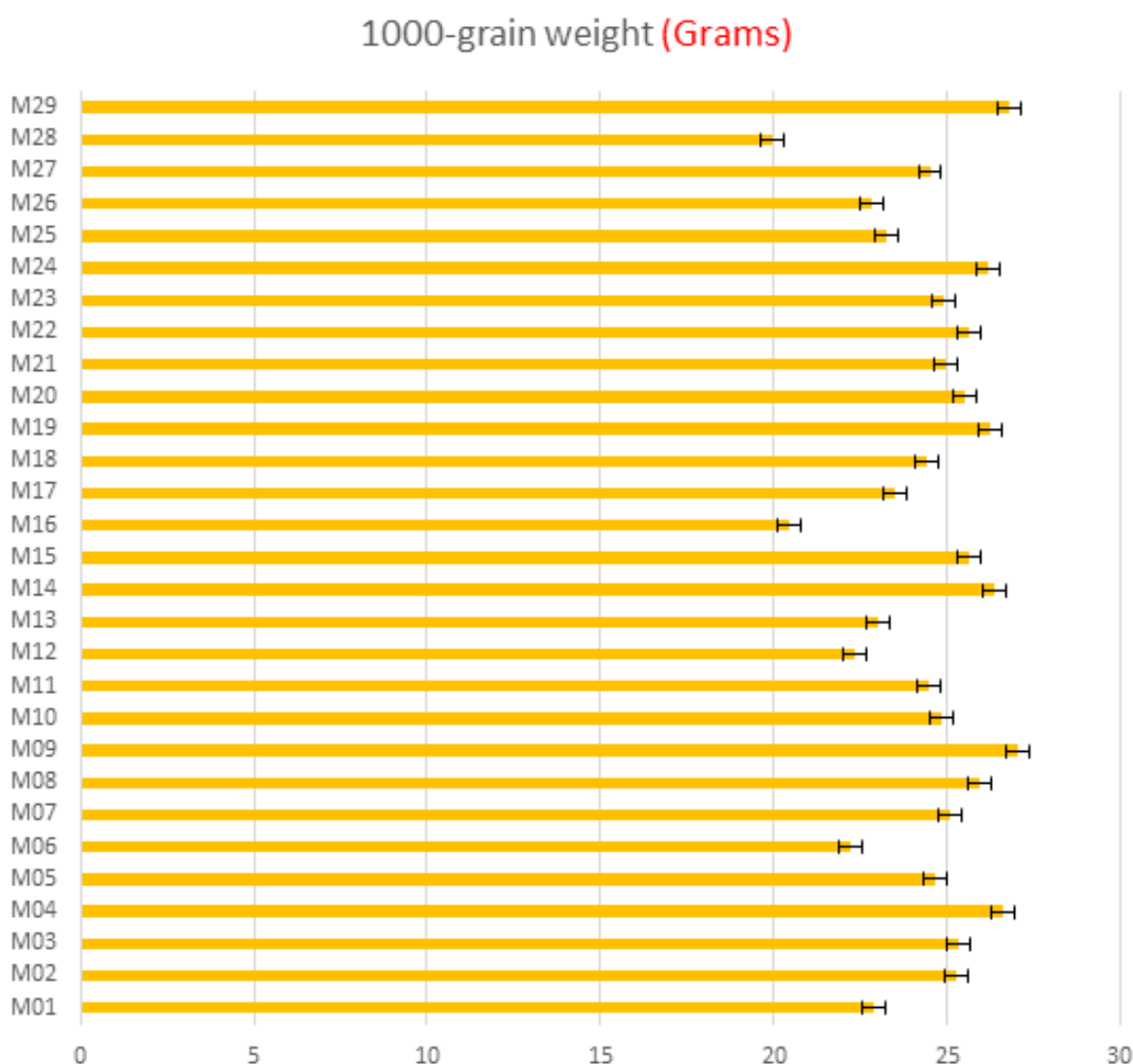


Figure 5. Effect of sole and combined microorganisms on 1000-grain weight (1000GW) of upland rice cultivar A504 CL. *Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$.

productive traits. Shoot phosphorus (S-P) correlated positively with PPP ($r = 0.54$) and GY ($r = 0.39$), while shoot calcium (S-Ca) showed strong correlations with PPP ($r = 0.89$) and GY ($r = 0.76$).

The strong correlations observed between grain yield and its primary components, particularly pods per plant and thousand-grain weight, reinforce the central role of reproductive efficiency in determining crop productivity. The positive association between dry shoot mass and yield-related traits indicates that greater aboveground biomass accumulation likely enhances assimilate availability for grain filling, supporting higher yield formation. Phosphorus emerged as a key nutrient

associated with both biomass production and yield. The strong correlations between root phosphorus content and productive traits suggest that enhanced P acquisition at the root level plays a decisive role in supporting reproductive development. Moreover, the close association between root P and shoot Ca and Mg indicates a coordinated nutrient uptake and translocation process, which may reflect improved root activity and nutrient-use efficiency. Magnesium also showed consistent associations with biomass and yield traits, particularly through its correlation with dry shoot mass. Given the role of Mg in chlorophyll structure and photosynthetic metabolism, these relationships suggest

Table 5. Pearson's correlation coefficients among productive traits, biomass variables, and nutrient contents in shoots and roots.

PPP	GPP	GY	M1000G	DRM	DSM	R - P	R - K	R - Ca	R - Mg	S - P	S - K	S - Ca	S - Mg
—													
-0.37*	—												
0.90*	-0.03	—											
0.87*	-0.05	0.98*	—										
0.24	-0.30	0.23	0.39*	—									
0.64*	-0.14	0.62*	0.63*	0.32	—								
0.86*	-0.25	0.78*	0.81*	0.42*	0.75*	—							
-0.09	0.14	-0.04	0.02	0.30	0.55*	0.19	—						
-0.04	0.10	0.02	0.03	0.13	0.53*	0.13	0.82*	—					
0.57*	-0.10	0.57*	0.56*	0.22	0.87*	0.65*	0.66*	0.58*	—				
0.54*	-0.40*	0.39*	0.36	0.14	0.73*	0.52*	0.36	0.42*	0.72*	—			
0.25	-0.13	0.13	0.20	0.44*	0.58*	0.62*	0.59*	0.43*	0.50*	0.334	—		
0.89*	-0.33	0.76*	0.77*	0.33	0.67*	0.94*	0.06	0.08	0.57*	0.57*	0.49*	—	
0.75*	-0.236	0.67*	0.71*	0.46*	0.65*	0.95*	0.16	0.06	0.55*	0.43*	0.68*	0.86*	—

*They indicate strong correlations ($p \leq 0.05$).

that adequate Mg availability contributes to increased assimilate production and, consequently, improved yield performance. In contrast, potassium and calcium physiological functions than directly to yield formation under the studied conditions. Overall, the correlation patterns highlight phosphorus and magnesium as central elements linking nutrient uptake, biomass accumulation, and yield, reinforcing their importance in strategies aimed at improving crop productivity and nutrient-use efficiency.

DISCUSSION

The soil presents moderate to high fertility, with a slightly acidic pH (5.8) suitable for most crops, though liming may enhance nutrient use. Organic matter is high, and calcium, magnesium and micronutrients are adequate. Potassium is low and phosphorus moderate, indicating possible need for supplementation. Low aluminium reduces toxicity risk, while enzyme activity reflects active microbial processes that support carbon and sulphur cycling (Sousa and Lobato, 2004). Several PGPR treatments, including both single and combined inoculations, significantly improved key yield components of upland rice, such as the number of panicles per pot, number of grains per pot, and overall grain yield. These enhancements were likely associated with improved root and shoot development, as well as increased nutrient accumulation, as observed across multiple treatments. Such improvements in plant architecture and physiological performance are consistent with the known mechanisms of PGPR action, including phytohormone production, nutrient solubilization, and biological nitrogen fixation. The number of panicles per pot, a critical

contents in roots were weakly associated with yield traits, despite their strong intercorrelation, indicating that their contribution may be more related to structural or determinant of grain yield, was significantly increased by microbial inoculation. This result aligns with findings from previous studies, where the application of *Pseudomonas* strains led to improvement in yield components such as panicle number, independent of application method (Sultana et al., 2024). Similarly, the increased number of grains per pot observed in this study can be attributed to the plant growth-promoting attributes of *Pseudomonas*, including siderophore production, phosphate solubilization, and nitrogen fixation (Gopalakrishnan et al., 2017). *Pseudomonas aeruginosa* BRRh-5 has previously been shown to enhance both growth and grain yield in rice (Khan et al., 2017).

While grain filling and final grain mass are largely governed by genetic factors, the proportion of filled grains is strongly influenced by environmental and physiological conditions (Sakamoto and Matsuoka, 2008)]. In this context, the observed improvements in grain yield under PGPR inoculation can be interpreted as a result of improved plant growth conditions, such as greater root development, better nutrient availability, and increased nutrient uptake. This is further supported by the accumulation of nutrients in inoculated plants in our study, highlighting the role of plant-growth-promoting microorganisms in enhancing nutrient uptake efficiency and, consequently, crop productivity (Fageria and Baligar, 2005).

Several studies support the yield-enhancing potential of various PGPR genera on plant biomass and yield. For instance, *E. hormaechei* has been reported to significantly increase shoot and root weight, in part due to

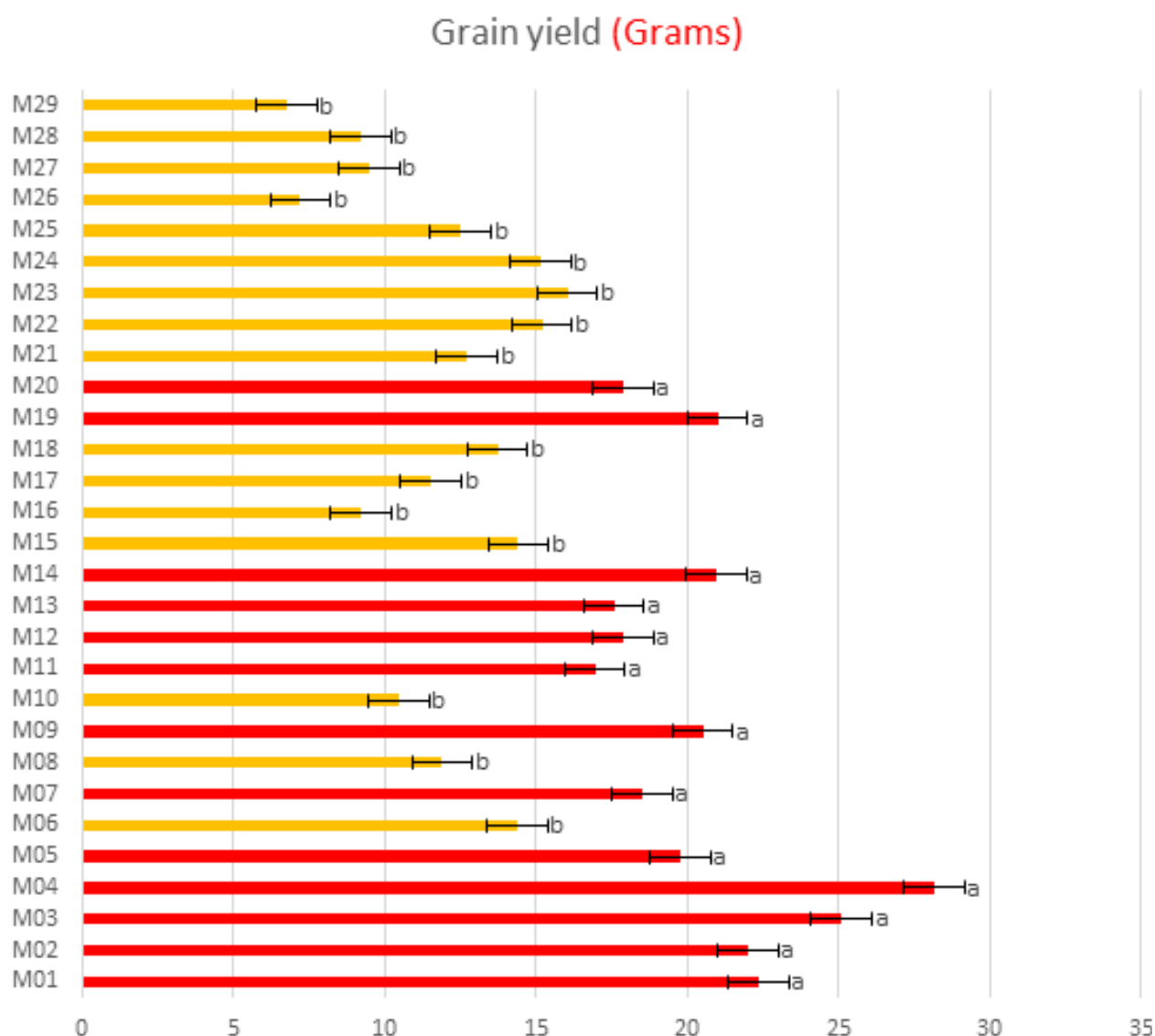


Figure 6. Effect of sole and combined microorganisms on the grains yield (GY) of upland rice cultivar A504 CL.

*Treatments with the same alphabet and colour do not differ from each other from the Scott-Knott test at $p < 0.05$.

its high IAA production (Ranawat et al., 2021; Abadi et al., 2020). Likewise, inoculation with *P. megaterium* KAH109 resulted in a 26.96% increase in plant dry weight (Srithaworn et al., 2023), while *Bacillus* strains enhanced shoot and total dry weight in maize crops (De Sousa et al., 2020). Several genes in *L. adecarboxylata* QDSM01 related to plant growth promotion has been identified, including those for biofilm formation, chemotaxis, nutrient solubilization, and IAA production, indicating its promise as a biofertilizer for enhancing plant development (Chen et al., 2023). Similarly, the beneficial effects of growth-promoting bacteria have also been reported in tomato, where *Bacillus licheniformis* increased plant dry weight by

39% and yield per plant by 51.94%, while *P. megaterium* enhanced photosynthetic rate by 9.9%. Moreover, the application of *B. subtilis*, *Bacillus amyloliquefaciens*, *P. megaterium*, and *B. licheniformis* increased mean fruit weight per plant by 26.78 to 30.70% compared with the control, nearly all bacterial strains results in improving fruit quality (Marchão et al., 2025).

In line with our findings, recent studies also report that post-emergence applications of *Pseudomonas*, *Priestia*, and *Bacillus* to soybean resulted in a 4–7% increase in grain yield (Katsenios et al., 2021). The yield-enhancing potential of *Bacillus* spp. has also been associated with their biocontrol properties, particularly against rice blast

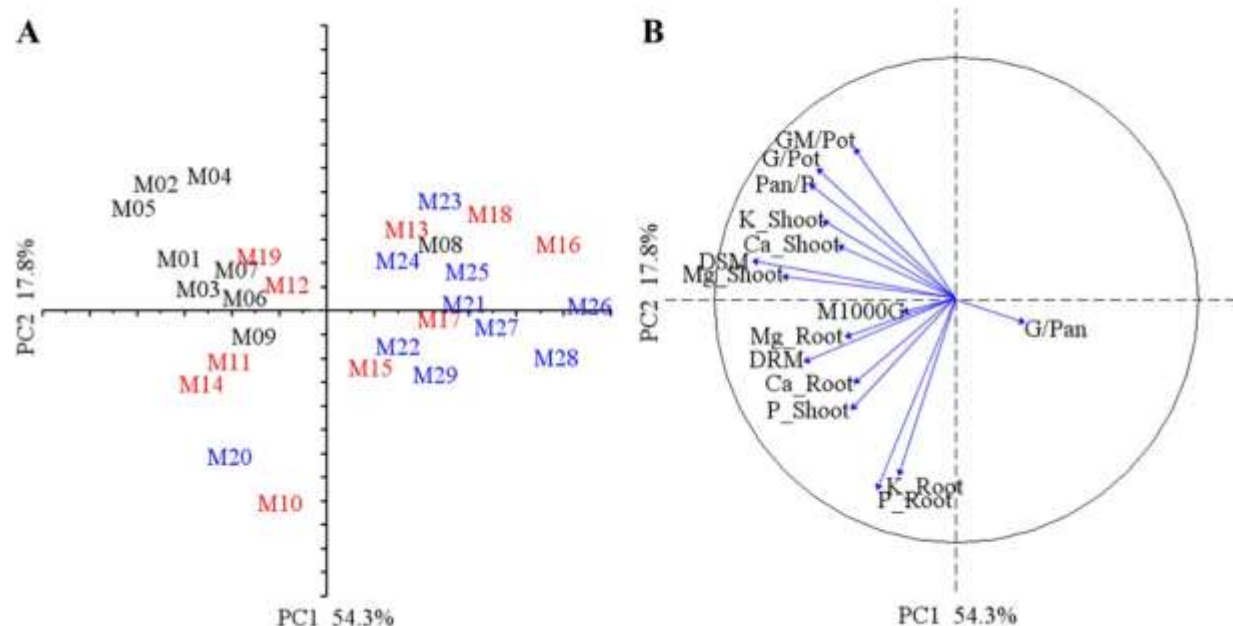


Figure 7. Principal Component Analysis (PCA) PCA1 x PCA2, demonstrating the correlations between the evaluated variables and the twenty-nine treatments with isolated multifunctional microorganisms, as well as the control (without microorganisms).** Two principal components (PCs) accounted for 98.9% of the data variation. (A) Biplot graph for treatments.

disease (Rais et al., 2018). Moreover, *Enterobacter* strains increased rice yield by 22.53% to 26.13% compared with control treatments (Suprpta et al., 2014). *Pseudomonas* spp. has similarly been recognised for their positive effects on yield across various crops, including soybean, wheat, mustard, *Rubus*, and tomato (Mehmood et al., 2023). Additionally, the *Serratia* strain BRM32114 was found to increase rice grain yield by 19% relative to untreated plants (Nascente et al., 2020). The positive impact on nutrient accumulation observed in this study suggests the role of PGPMs in enhancing nutrient use efficiency. Beneficial microorganisms contribute to plant development not only through direct growth promotion but also by increasing nutrient availability in the rhizosphere and facilitating nutrient uptake and translocation within the plant (Das et al., 2022; Ruiz and Salas, 2022).

Findings suggest that strategies based on the use of microorganisms can be an effective tool to sustainably increase productivity, aligned with principles of integrated management and resilient agricultural practices. In summary, the results highlight that the tested microorganisms have the potential to meet different cultivation demands, either by improving productivity or efficiently managing the nutrients. This diversity of responses reinforces the importance of selecting appropriate microorganisms according to its mechanisms to the specific needs of production systems. This study

has some limitations that should be acknowledged. First, the experiment was conducted under greenhouse conditions, which may not fully represent the complexity of field environments and their associated stresses. Second, the viability of bacterial suspensions was not confirmed by CFU counts prior to each inoculation, which could have influenced the consistency of microbial activity. Third, although several treatments showed numerical increases in grain yield, these differences were not statistically significant, highlighting the need for further validation under field conditions. Nevertheless, the nutrient-accumulation findings provide important insights: treatments such as M02, M05, M11, M14, and M20 promoted higher uptake of K, Ca, Mg, and P, and these improvements were generally associated with enhanced growth and yield components. This suggests that PGPR inoculation can improve nutrient dynamics in upland rice, thereby contributing to more sustainable production systems

Conclusion

The inoculation of upland rice with single and combined microorganisms significantly improved yield components and grain yield. Among the treatments, M02 (*P. megaterium*), M04 (*Pseudomonas* spp. BRM69515), and M05 (*E. hormaechei*) showed the most consistent and

superior performance across all growth and yield parameters. Taken together, these findings reinforce the potential of microbial inoculants as sustainable tools for improving crop performance and yield, particularly under field conditions where nutrient limitations and environmental stresses may constrain productivity. Thus, to validate the effectiveness under real agricultural conditions, further field studies should be conducted to assess their performance in an uncontrolled environment.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Abadi VAJM, Mozafaripour M, Alikhani HA, Ramezani A, Akbari A (2020). Role of dominant phyllosphere bacteria with plant growth-promoting characteristics on growth and nutrition of maize (*Zea mays* L.). *Journal of Soil Science and Plant Nutrition* 20(4):2348-2363.
- Chen W, Li H, Liu S, Shi J, Lu F, Li C (2023). Genome sequence of *Leclercia adecarboxylata* QDSM01 with multiple plant growth promoting properties. *Plant Growth Regulation* 102(2):445-459.
- Das PP, Saikia K, Bora SS, Dutta D (2022). Plant-soil-microbes: A tripartite interaction for nutrient acquisition and better plant growth for sustainable agricultural practices. *Environmental Research* 214:113821.
- De Sousa SM, De Campos ARR, Silva GCF, Dos Santos RN, De Paula LM, Costa JCB, Soares MAA (2020). Tropical *Bacillus* strains inoculation enhances maize root surface area, dry weight, nutrient uptake and grain yield. *Journal of Plant Growth Regulation* 40(2):867-877.
- Donagema KD, Campos DVB, Calderano SB, Teixeira WG, Viana JHM (2011). Manual de métodos de análise de solo. 2nd Edition, Embrapa Solos, Rio de Janeiro, RJ.
- Duarte GA (2025). Rizobactérias benéficas na promoção de crescimento em arroz de terras altas (*Oryza* sp.). (Dissertação de Mestrado). Universidade Federal de Goiás. Available at: <http://repositorio.bc.ufg.br/tede/handle/tede/14168>
- Fageria NK, Baligar VC (2005). Nutrient availability. In: Hillel D (ed.), *Encyclopedia of soils in the environment*. San Diego: Elsevier. pp. 63-71.
- Food and Agricultural Organization (FAO) (2020). Food balance sheets. Available at: <https://www.fao.org/faostat/en/#data/FBS>
- Ferreira DF (2019). SISVAR: A computer analysis system to fixed effects split plot type designs. *Revista Brasileira De Biometria* 37(4):529.
- Filippi MCC, da Silva GB, de Melo LC, Maciel JLN, de Moraes PV (2011). Leaf blast (*Magnaporthe oryzae*) suppression and growth promotion by rhizobacteria on aerobic rice in Brazil. *Biological Control* 58(2):160-166.
- Gopalakrishnan S, Srinivas V, Samineni S (2017). Nitrogen fixation, plant growth and yield enhancements by diazotrophic growth-promoting bacteria in two cultivars of chickpea (*Cicer arietinum* L.). *Biocatalysis and Agricultural Biotechnology* 11:116-123.
- Heinemann AB, De Araújo L, Stone LF, De Oliveira FN (2019). Upland rice breeding led to increased drought sensitivity in Brazil. *Field Crops Research* 231:57-67.
- Hussain T, Khan IA, Ahmad T, Khan MJ (2022). Impact of Nitrogen Application Rates on Upland Rice Performance, Planted under Varying Sowing Times. *Sustainability* 14(4):1997.
- Isawa T, Hoshino Z, Akao Y, Komatsu S, Hasebe A (2010). *Azospirillum* sp. Strain B510 Enhances Rice Growth and Yield. *Microbes and Environments* 25(1):58-61.
- Kado CI, Heskett MG (1970). Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas*, and *Xanthomonas*. *Phytopathology* 60(6):969.
- Katsenios N, Andreou V, Sparangis P, Djordjevic N, Giannoglou M, Chanioti S, Stergiou P, Xanthou MZ, Kakabouki I, Vlachakis D, Djordjevic S (2021). Evaluation of plant growth promoting bacteria strains on growth, yield and quality of industrial tomato. *Microorganisms* 9(10):2099.
- Khan MMA, Islam F, Islam MT, Chowdhury MAZ, Chowdhury SH, Islam MR, Nandi SP (2017). Enhancement of growth and grain yield of rice in nutrient deficient soils by rice probiotic bacteria. *Rice Science* 24(5):264-273.
- Li J, Wang J, Zeigler RS (2014). The 3,000 rice genomes project: New opportunities and challenges for future rice research. *GigaScience* 3(1):8.
- Malavolta E, Vitti GC, de Oliveira SA (1997). Avaliação do estado nutricional das plantas: Princípios e aplicações.
- Marchão RL, Bezerra WF, Diel D, Lira RFPF, da Costa JPP, Silva SLR, Sampaio EVSB (2025). Improving soybean development and grain yield by complementary inoculation with growth-promoting bacteria *Azospirillum*, *Pseudomonas*, *Priestia*, and *Bacillus*. *Plants* 14(3):402.
- Mehmood N, Ahmad R, Shah M, Shah GM, Farooq S, Ullah R, Mehmood S (2023). Multifaceted impacts of plant-beneficial *Pseudomonas* spp. in managing various plant diseases and crop yield improvement. *ACS Omega* 8(25):22296-22315.
- Nascente AS, Li Y, Kluthcouski J, Huergo M, Viana DC (2017). Effects of beneficial microorganisms on lowland rice development. *Environmental Science and Pollution Research* 24(32):25233-25242.
- Nascente AS, Li Y, Kluthcouski J, Huergo M (2020). Rhizobacterium *Serratia* sp. and phosphorus fertilization rates affect aerobic rice development. *Australian Journal of Crop Science* 14(10):1654-1661.
- Ngalimat MS, Roslan NF, Azman NI (2021). Plant growth-promoting bacteria as an emerging tool to manage bacterial rice pathogens. *Microorganisms* 9(4):682.
- Ouyabe M, Nascente AS, Cordeiro MAA, Santos AG, Li Y (2020). Response of upland rice (*Oryza sativa* L.) inoculated with non-native plant growth-promoting bacteria. *Agronomy* 10(6):903.
- Rais A, Jabeen Z, Hassan N, Zaidi S, Shakoor S (2018). Antagonistic *Bacillus* spp. reduces blast incidence on rice and increases grain yield under field conditions. *Microbiological Research* 208:54-62.
- Ranawat B, Mishra S, Singh A (2021). *Enterobacter hormaechei* (MF957335) enhanced yield, disease and salinity tolerance in tomato. *Archives of Microbiology* 203(5):2659-2667.
- Ríos-Ruiz WF, Chanamé-Aguirre JF, Panta-de la Cruz SC, De La Cruz EM, Loli-Ponciano DF, Villanueva-Talledo D, Alarcón SO, Fernández-Silva HC (2020). Inoculation of bacterial consortium increases rice yield (*Oryza sativa* L.) reducing applications of nitrogen fertilizer in San Martín region, Peru. *Rhizosphere* 14:100200.
- Ruiz JL, Salas SMDC (2022). The use of plant growth promoting bacteria for biofertilization; effects on concentrations of nutrients in inoculated aqueous vermicompost extract and on the yield and quality of tomatoes. *Biological Agriculture and Horticulture* 38:1-17.
- Sakamoto T, Matsuoka M (2008). Identifying and exploiting grain yield genes in rice. *Current Opinion in Plant Biology* 11(2):209-214.
- Sousa DMG, Lobato E (2004). Cerrado: soil correction and fertilization. Brasília, DF: Embrapa Technological Information. Planaltina, DF: Embrapa Cerrados.
- Strithaworn M, Wang R, Panichayupakaranant P, Prom-u-thai S (2023). Zinc solubilizing bacteria and their potential as bioinoculant for growth

- promotion of green soybean (*Glycine max* L. Merr.). Peer Journal 11:e15128.
- Sultana R, Sultana A, Jamil A, Jamil M, Shakoor S, Khan AR, Rais A (2024). Bacterial endophyte *Pseudomonas mosselii* PR5 improves growth, nutrient accumulation, and yield of rice (*Oryza sativa* L.) through various application methods. BMC Plant Biology 24(1):1030.
- Suprpta DN, Maulina NMI, Khalimi K (2014). Effectiveness of *Enterobacter cloacae* to promote the growth and increase the yield of rice. Journal of Biology, Agriculture and Healthcare 4(1):44-50.
- Tabatabai MA (1994). Soil enzymes. In: Weaver RW, Angle JS, Bottomley PS (eds.), Methods of soil analysis: Microbiological and biochemical properties. Soil Science Society of America pp. 775-833.
- Tabatabai MA, Bremner J. (1970). Factors affecting soil arylsulfatase activity. Soil Science Society of America Journal 34(3):427-429.
- United Nations (UN) (2022). World population prospects 2022: Summary of results. United Nations, Department of Economic and Social Affairs, Population Division. Available at: https://www.un.org/development/desa/pd/sites/www.un.org.development.desa.pd/files/wpp2022_summary_of_results.pdf
- Zhao Y, Feng B, Wang X, Feng K, Xu Y, Sun B, Zhu B, Zhang Y (2021). Enhanced rice yields are related to pronounced shifts in soil resident bacterial community structures in response to *Rhodopseudomonas palustris* and *Bacillus subtilis* inoculation. Journal of Soils and Sediments 21(6):2369-2380.
- Zhang H, Liu W, Xu J, Luo J, Fan T, Liu H, Li G, Wang Y, Dong Y (2024). Response of rice production to rising CO₂ and its adaptive cultivation strategies. Fundamental Research. <https://doi.org/10.1016/j.fmre.2024.11.025>