

Bacteria from the Brazilian Savanna as growth promoters in upland rice¹

Aline Cristina Mendes Façanha², Carlos Augusto Cavalcante de Oliveira³,
Edmilson Júnio Medeiros Caetano², Evandro Inácio da Costa², Adriano Stephan Nascente⁴

ABSTRACT

Beneficial bacteria can significantly enhance rice development through the production of phytohormones, biological nitrogen fixation, nutrient solubilization, siderophore synthesis and biofilm formation, which improve nutrient uptake and increase tolerance to abiotic stresses. This study aimed to assess the potential of 13 rhizobacterial isolates from the Brazilian Savanna biome to promote the growth and yield of upland rice. Biochemical assays were conducted to functionally characterize the isolates, followed by controlled-condition experiments. Evaluations included shoot and root dry biomass and grain yield components. Plants inoculated with *Bacillus* sp. (IA1) exhibited greater shoot biomass, whereas those treated with *Acinetobacter* sp. (IB10) showed higher root biomass. The *Stenotrophomonas* sp. (IB8) isolate resulted in the highest values for number of panicles per pot, number of grains per pot and grain yield. The principal component analysis identified *Stenotrophomonas* sp. (IB8) and *Acinetobacter* sp. (IB6) as most closely associated with yield-related traits. *Bacillus* sp. (IA1), *B. subtilis* (IR5) and *Acinetobacter* sp. (IB10) were more strongly linked to vegetative development. The microorganisms promoted both growth and yield in upland rice, likely due to biofilm formation, indole-3-acetic acid production and phosphate solubilization.

KEYWORDS: *Oryza sativa* L., seed treatment, beneficial microorganisms.

RESUMO

Bactérias do Cerrado como promotoras de crescimento em arroz de terras altas

Bactérias benéficas podem induzir significativamente o desenvolvimento de plantas de arroz por meio da produção de fitormônios, fixação de nitrogênio, solubilização de nutrientes, produção de sideróforos e formação de biofilmes que favorecem a absorção de nutrientes e aumentam a tolerância a estresses abióticos. Objetivou-se determinar o potencial de 13 rizobactérias do Cerrado na promoção do crescimento e produção de arroz de terras altas. Foram realizados testes bioquímicos para caracterização funcional dos isolados e, posteriormente, testes em condições controladas. Avaliaram-se a biomassa seca da parte aérea e radicular e componentes de produção de grãos. As plantas tratadas com *Bacillus* sp. (IA1) apresentaram maior biomassa aérea, e as tratadas com *Acinetobacter* sp. (IB10) maior biomassa radicular. O isolado *Stenotrophomonas* sp. (IB8) proporcionou os maiores valores para número de panículas por vaso, número de grãos por vaso e produção de grãos. A análise de componentes principais indicou *Stenotrophomonas* sp. (IB8) e *Acinetobacter* sp. (IB6) como os mais relacionados às variáveis produtivas. *Bacillus* sp. (IA1), *B. subtilis* (IR5) e *Acinetobacter* sp. (IB10) apresentaram maior associação com o desenvolvimento vegetativo. Os micro-organismos promoveram o crescimento e a produção do arroz, possivelmente devido à formação de biofilme, produção de ácido indol-3-acético e solubilização de fosfato.

PALAVRAS-CHAVE: *Oryza sativa* L., tratamento de sementes, micro-organismos benéficos.

INTRODUCTION

Rice is among the most widely cultivated and consumed cereals worldwide, with an estimated production of 776 million tons in 2022

(FAO 2023). Its broad acceptance is primarily attributed to its high concentration of easily digestible carbohydrates, as well as its nutritional and organoleptic properties, which make it a staple food and an essential dietary component for nearly

¹ Received: June 18, 2025. Accepted: Aug. 18, 2025. Published: Sep. 11, 2025. DOI: 10.1590/1983-40632025v5583152.

² Universidade Federal de Goiás, Escola de Agronomia, Goiânia, GO, Brazil.

E-mail/ORCID: alinefacanha@discente.ufg.br/0000-0002-4800-4620; junio2@discente.ufg.br/0000-0002-4836-7640; evandrocosta@discente.ufg.br/0000-0003-1991-0285.

³ Universidade Federal do Pará, Instituto de Ciências Biológicas, Belém, PA, Brazil.

E-mail/ORCID: carlos.cavalcante@icen.ufpa.br/0000-0003-3058-4740.

⁴ Empresa Brasileira de Pesquisa Agropecuária (Embrapa Arroz e Feijão), Santo Antônio de Goiás, GO, Brazil.

E-mail/ORCID: adriano.nascente@embrapa.br/0000-0002-6014-3797.

Editor: Luis Carlos Cunha Junior/Data Availability Statement: Research data are only made available by authors upon request.

half of the global population (Verma & Srivastav 2020).

Globally, Brazil ranks ninth in rice production, accounting for 1.3 % of the total cultivated worldwide (Embrapa 2025). In the Americas, the country contributes with approximately 28 % of the production, consolidating its position as the leading regional producer (Embrapa 2025). The 2023 crop year yielded 10.3 million tons harvested from 1.5 million hectares, with an average yield of 6,939 kg ha⁻¹ (IBGE 2024). Nevertheless, the prevailing production model is still characterized by the intensive use of chemical inputs, such as synthetic fertilizers and pesticides, which generate significant environmental impacts and increase production costs, ultimately compromising the sustainability of the system (Cruz et al. 2023).

In this context, the use of plant growth-promoting microorganisms (PGPM) has emerged as a promising biotechnological alternative, capable of mitigating the negative effects of conventional management while simultaneously enhancing the agronomic performance of crops (Ansabayeva et al. 2025). Several studies have highlighted the benefits of PGPM inoculation in different agricultural species. For instance, Moretti et al. (2020) reported substantial improvements in both vegetative and reproductive development of soybean through coinoculation with growth-promoting bacteria. Similarly, Korir et al. (2017) observed significant increases in shoot and root dry mass, as well as in nitrogen fixation, in common bean plants subjected to inoculation.

Specifically in rice, studies such as those by Yasmin et al. (2016), Ouyabe et al. (2020) and Cruz et al. (2025) have demonstrated that PGPM application enhances vegetative growth, plant vigor and, consequently, grain yield. These findings reinforce the importance of strategies based on agricultural microbiology as sustainable tools for productivity intensification, particularly under challenging edaphoclimatic conditions.

Despite the achieved advances, there remains a need to expand the exploration of new microbial strains adapted to the specific conditions of upland rice, with the goal of diversifying the available bioinputs and optimizing crop performance. Thus, the present study aimed to evaluate the potential of 13 rhizobacterial isolates from the Brazilian Savanna in promoting the growth and yield of upland rice. To this end, biochemical tests were conducted to

assess the functional traits of the isolates, followed by controlled experiments designed to validate their agronomic effects.

MATERIAL AND METHODS

The study was carried out at the Empresa Brasileira de Pesquisa Agropecuária (Embrapa Arroz e Feijão), in Santo Antônio de Goiás, Goiás state, Brazil, in May 2024. The local climate is classified as AW (tropical Savanna), with experimental temperatures ranging from 20 to 31 °C.

Since the microorganisms had not yet been biochemically characterized, four tests were conducted to evaluate their functions: phosphate solubilization, biofilm production, nitrogen fixation and indole-3-acetic acid (IAA) production. The microorganisms were collected from the central pivot area of the IFGoiano, in Ceres, Goiás state, Brazil, from soil adhering to the roots of *Brachiaria ruziziensis* and *Pennisetum glaucum*. The isolates evaluated were: IA1 (*Bacillus* sp.), IR1 (*Herbaspirillum seropedicae*), IR3 (*Rhizobium* sp.), IR5 (*Bacillus subtilis*), IR6 (*Lysinibacillus boronitolerans*), IR7 (*Herbaspirillum seropedicae*), IR8 (*Herbaspirillum seropedicae*), IB1 (*Bacillus velezensis*), IB5 (*Pseudomonas putida*), IB6 (*Acinetobacter* sp.), IB8 (*Stenotrophomonas* sp.), IB10 (*Acinetobacter* sp.) and IF2 (*Pantoea* sp.).

Biofilm production was assessed on Congo Red Agar (CRA), following Freeman et al. (1989), with incubation at 28 °C, for 48 h. Black, wrinkled colonies were considered positive producers. Phosphate solubilization was tested on 10 % TSA medium supplemented with CaHPO₄, with the formation of a translucent halo indicating a positive result (Cattelan 1999). Nitrogen fixation was evaluated according to Estrada de Los Santos et al. (2001), with a change in medium color after seven days indicating activity. For IAA quantification, isolates were cultivated in potato-dextrose (PD) broth, with and without L-tryptophan (100 mg L⁻¹), under agitation at 26 ± 2 °C, for eight days. Every 48 h, samples were analyzed by spectrophotometry (540 nm), based on a standard curve (Oliveira et al. 2009). All tests were performed in triplicate.

After biochemical characterization, the microorganisms were tested under partially controlled conditions (screenhouse), between September and December 2024.

The experiment followed a completely randomized design, with 13 treatments (microorganisms) and one control (no microorganisms), each replicated four times. The isolates were cultured on Nutrient Agar (NA) at 28 °C, for 24 h, in a BOD incubator, and then transferred to nutrient broth in Erlenmeyer flasks (35 mL) and shaken for 24 h, at 140 rpm. The suspension was adjusted to $A_{540} = 0.1$ (10^8 CFU) (Nascente et al. 2023).

Upland rice seeds (cultivar A502) were surface-sterilized with sodium hypochlorite (7.5 %) for 7 min, followed by 70 % ethanol for 3 min, and rinsed three times with sterilized distilled water. After drying on absorbent paper, the seeds were microbiolized following Bezerra et al. (2013), by immersion in 35 mL of the bacterial suspension for 24 h, under agitation at 140 rpm. The control was treated only with sterilized, autoclaved distilled water.

The soil chemical properties were as follows: pH (H₂O): 5.1; available P: 2.30 mg dm⁻³ (Mehlich-1); S: 0.0 mg dm⁻³; K⁺: 0.20 cmol_c dm⁻³, equivalent to 78 mg dm⁻³; Ca²⁺: 2.0 cmol_c dm⁻³; Mg²⁺: 0.9 cmol_c dm⁻³; Ca²⁺ + Mg²⁺: 2.9 cmol_c dm⁻³; Al³⁺: 0.0 cmol_c dm⁻³; H⁺ + Al³⁺: 2.7 cmol_c dm⁻³; organic C: 2.3 %; Zn²⁺: 0.5 mg dm⁻³; Cu²⁺: 1.1 mg dm⁻³; Fe³⁺: 1.4 mg dm⁻³; Mn²⁺: 0.6 mg dm⁻³; CEC (pH 7.0): 5.82 cmol_c dm⁻³; V: 53.65 %; Ca/CEC: 34.36 %; Mg/CEC: 15.46 %; K/CEC: 3.44 %; H + Al/CEC: 46.39 %; m: 0 %.

One week before sowing, 7-kg pots were filled with soil and fertilized with 2 g of N-P₂O₅-K₂O (5-30-15). Ten microbiolized seeds were sown per pot. At 10 days after emergence, thinning was performed, leaving 2 plants pot⁻¹. During tillering and panicle initiation, 2 g of urea were applied per pot. Weed control was carried out manually on a weekly basis, and irrigation was performed daily to maintain the soil at field capacity until physiological maturity.

At 104 days after sowing, at physiological maturity, the shoots were harvested at the soil level. The roots were collected and washed in fine-mesh sieves to minimize losses. Shoot and root samples were placed in paper bags and oven-dried at 65 °C, with forced air circulation, until a constant weight was reached. The dry biomass (g pot⁻¹) was determined using a precision scale.

At physiological maturity, agronomic traits were measured: number of panicles per pot, determined by counting the total number of panicles per pot; number of grains per pot, evaluated

by counting the total number of grains per pot; 1,000-grain weight (g), measured and calculated by proportional conversion; and grain yield (g pot⁻¹), determined by weighing the total grain production per pot.

The data were subjected to Anova, to assess the significance of treatment effects ($p < 0.05$). When necessary, Box-Cox transformation was applied. Means were compared using the Tukey test, and assumptions of normality and homoscedasticity were verified with the Shapiro-Wilk and O'Neill-Mathews tests. Analyses were performed in the R software (R Core Team 2023), using the packages fpp, ExpDes.pt and ggplot2.

For multivariate analysis, the principal component analysis (PCA) was applied, using the correlation matrix. The components were selected according to the Kaiser's criterion (eigenvalues > 1), and plots were generated with ggplot2.

RESULTS AND DISCUSSION

All tested microorganisms exhibited at least one functional trait related to plant growth promotion (Table 1). Biofilm production was observed in six isolates (IA1, IR5, IB1, IB8, IB10 and IF2). This trait is particularly relevant because, as biofilms mature, bacteria secrete proteins, DNA and polysaccharides that form a protective matrix, enhancing resistance to stress and favoring rhizosphere colonization (Rabin et al. 2015, Rodrigues et al. 2019). An example is *Bacillus subtilis* (IR5), whose biofilm formation plays a central role in biocontrol processes and plant growth promotion, contributing to microbial adaptation and rhizosphere community stability (Arnaouteli et al. 2021).

Phosphate solubilization was detected in the isolates IR3, IB5, IB6 and IB10 (Table 1). This capacity increases soil phosphorus availability, an essential nutrient often found in insoluble forms, and therefore poorly assimilated by plants (Timofeeva et al. 2022). *Pseudomonas putida* (IB5) is notable for its ability to solubilize insoluble phosphates such as tricalcium and rock phosphate, mainly through the production of organic acids (gluconic, oxalic and malic) that acidify the medium and release phosphorus (Singh et al. 2024). A strain has been reported to solubilize up to 367.54 µg mL⁻¹ of phosphorus, highlighting its potential as a biofertilizer in P-deficient soils (Khan et al. 2022).

Table 1. Biochemical characterization of the isolated microorganisms.

Code	Isolate	Biofilm	Phosphate	Nitrogen	IAA ($\mu\text{g mL}^{-1}$)
IA1	<i>Bacillus</i> sp.	+	-	-	4.14
IR1	<i>Herbaspirillum seropedicae</i>	-	-	+	25.51
IR3	<i>Rhizobium</i> sp.	-	+	-	14.82
IR5	<i>Bacillus subtilis</i>	+	-	+	10.38
IR6	<i>LysiniBacillus boronitolerans</i>	-	-	-	8.39
IR7	<i>Herbaspirillum seropedicae</i>	-	-	+	32.45
IR8	<i>Herbaspirillum seropedicae</i>	-	-	+	28.94
IB1	<i>Bacillus velezensis</i>	+	-	-	4.66
IB5	<i>Pseudomonas putida</i>	-	+	-	1.63
IB6	<i>Acinetobacter</i> sp.	-	+	-	6.02
IB8	<i>Stenotrophomonas</i> sp.	+	-	-	14.42
IB10	<i>Acinetobacter</i> sp.	+	+	+	2.04
IF2	<i>Pantoea</i> sp.	+	-	+	29.93

+ and - : presence and absence of the attribute, respectively; IAA: indole-3-acetic acid.

Nitrogen fixation was confirmed in the isolates IR1, IR3, IR5, IR7, IR8, IB10 and IF2 (Table 1). *Herbaspirillum seropedicae* (IR1, IR7 and IR8) is an endophytic diazotrophic bacterium capable of fixing atmospheric nitrogen and assimilating nitrate and ammonium via systems such as NtrY-NtrX and assimilatory nitrate reductase (Bonato et al. 2016). Its inoculation improves nitrogen uptake and increases plant biomass (Breda et al. 2019). *Rhizobium* sp. (IR3) is well known for symbiotic nitrogen fixation in legumes (Radwan et al. 2002). *Bacillus subtilis* (IR5), *Acinetobacter* sp. (IB10) and *Pantoea* sp. (IF2) also participate in the nitrogen cycle, although their mechanisms remain less well understood (Loaces et al. 2011).

Indole-3-acetic acid (IAA) production, a key auxin in plant growth promotion, was observed in all isolates, with the highest levels recorded for IR7 ($32.45 \mu\text{g mL}^{-1}$), IF2 ($29.93 \mu\text{g mL}^{-1}$), IR8 ($28.94 \mu\text{g mL}^{-1}$) and IR1 ($25.51 \mu\text{g mL}^{-1}$), respectively (Table 1). IAA directly promotes cell elongation, lateral root formation and tissue differentiation, making it one of the main mechanisms of microbial plant growth promotion (Etesami & Glick 2024). Strains of *Herbaspirillum seropedicae* (IR1, IR7, IR8) have been reported to produce more than $100 \mu\text{M}$ of IAA in media supplemented with tryptophan (Radwan et al. 2002), and production can be increased by up to 370 % through cultivation optimization (Scheidt et al. 2020). The IF2 (*Pantoea* sp.) isolate also showed a high potential, surpassing even bacteria traditionally recognized for this function, such as *Azospirillum brasilense* (Loaces et al. 2011).

The analysis of Figure 1A shows that the *Bacillus* sp. (IA1) isolate promoted the highest accumulation for shoot dry matter, with $41.83 \text{ g plant}^{-1}$, representing an increase of approximately 109 %, when compared to the control, thereby demonstrating its strong potential to stimulate shoot development in rice. The treatments IR1, IR3, IR5, IR6, IR7, IR8, IB1, IB5, IB10 and IF2 showed intermediate values, with no significant differences, if compared with the control.

These findings corroborate those of Fernandes et al. (2020), who reported significant increases in rice shoot biomass following inoculation with microbial consortia containing *Bacillus* species. Similarly, Nascente et al. (2016) observed an increase in shoot biomass from 18.54 g in the control to 21.53 g with the BRM32114 (*Bacillus* spp.) isolate, reinforcing the potential of microorganisms in plant growth promotion. More recent studies, such as that of Kulkova et al. (2023), also highlight the beneficial effects of *Bacillus* inoculation in rice cultivation.

In this context, the *Bacillus* sp. (IA1) isolate, despite presenting low levels of IAA and lacking phosphate solubilization or nitrogen fixation, still promoted shoot growth. This effect may be attributed to the production of siderophores such as bacillibactin, widely synthesized by species like *B. velezensis* and *B. subtilis* (Liu et al. 2024), which increase iron availability and activate transporters such as YSL15, thereby favoring shoot development (Singh et al. 2023).

Regarding root dry mass, a distinct response was observed. *Bacillus subtilis* (IR5) and

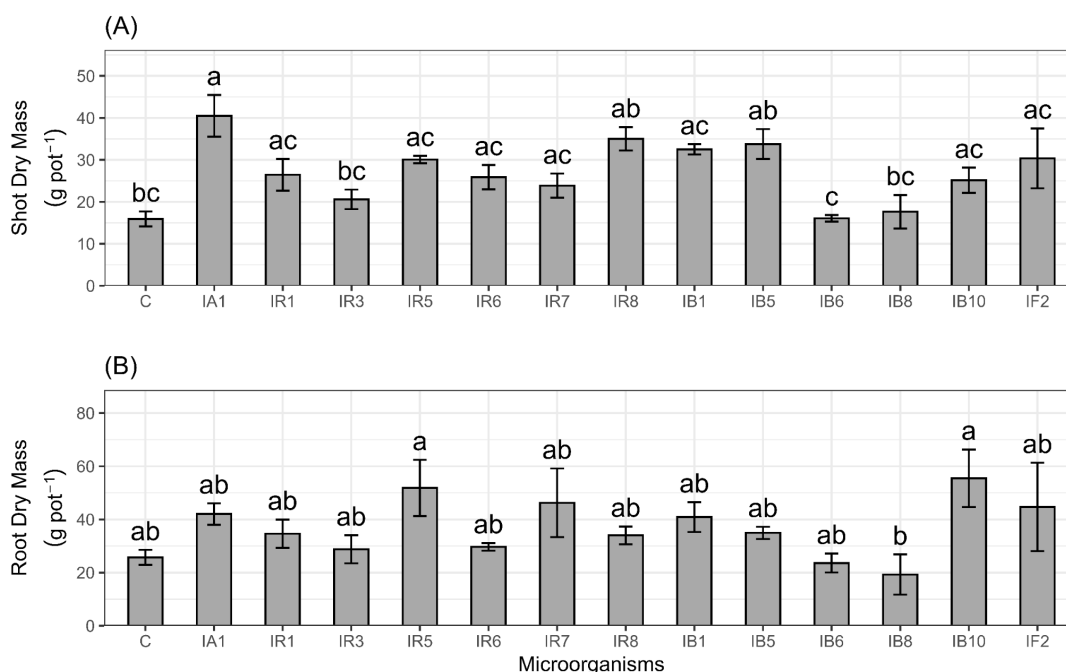


Figure 1. Shoot (A) and root dry mass (B) in upland rice plants, cultivar BRS A502. Treatments: C: control; IA1: *Bacillus* sp.; IR1: *Herbaspirillum seropedicae*; IR3: *Rhizobium* sp.; IR5: *Bacillus subtilis*; IR6: *Lysinibacillus boronitolerans*; IR7: *Herbaspirillum seropedicae*; IR8: *Herbaspirillum seropedicae*; IB1: *Bacillus velezensis*; IB5: *Pseudomonas putida*; IB6: *Acinetobacter* sp.; IB8: *Stenotrophomonas* sp.; IB10: *Acinetobacter* sp.; IF2: *Pantoea* sp.

Acinetobacter sp. (IB10) stood out, with values of 51.86 and 55.49 g, respectively (Figure 1B). These correspond to increases of 101.39 and 115.49 %, when compared with the control (25.75 g), clearly demonstrating the positive effect of inoculation on root development. This response can be explained by the biochemical traits of these microorganisms. Both demonstrated biofilm formation and nitrogen fixation, and, in the case of *Acinetobacter* sp. (IB10), also phosphate solubilization (Table 1), providing multiple mechanisms for root growth promotion.

The performance of *Bacillus subtilis* (IR5) is consistent with reports for rice (*Oryza sativa*) (Zhu et al. 2022), *Brassica juncea* (brown mustard) (Zaidi et al. 2006) and various other crops reviewed by Blake et al. (2021), all emphasizing IAA production and biofilm formation as key mechanisms of root growth promotion. Although less studied in rice, *Acinetobacter* sp. (IB10) seems promising. Its ability to form biofilm, fix nitrogen, solubilize phosphate and produce high levels of IAA enhances its potential effects on the root system (Bhattacharjee et al. 2023).

The *Stenotrophomonas* sp. (IB8) isolate was the most effective in promoting the productive

traits of rice, showing the highest number of panicles per pot (Figure 2A) and grains per pot (Figure 2B). It resulted in an average increase of 133.33 % in the number of panicles and 305.39 % in the number of grains per pot, when compared to the control, indicating its strong potential as a plant growth-promoting agent. Biofilm formation by *Stenotrophomonas* sp. supported stable rhizosphere colonization and protection against environmental stresses (Zhao et al. 2024), while also enabling the release of beneficial compounds such as organic acids, siderophores, auxins, hydrolytic enzymes and volatile organics (García-Fraile et al. 2015). Additionally, the production of IAA stimulated root growth and enhanced the uptake of essential nutrients (Singh et al. 2023). The integrated action of these mechanisms significantly contributed to the increase in rice panicles, even in the absence of other functions. Previous studies also report similar effects of *Stenotrophomonas*, such as an increase in the number of panicles in rice (Saechow et al. 2018, Suárez-Moreno et al. 2019) and yield improvements in crops such as sugarcane and tomato (Singh et al. 2020, Tuong et al. 2022). Furthermore, auxin production by *Stenotrophomonas* may be associated

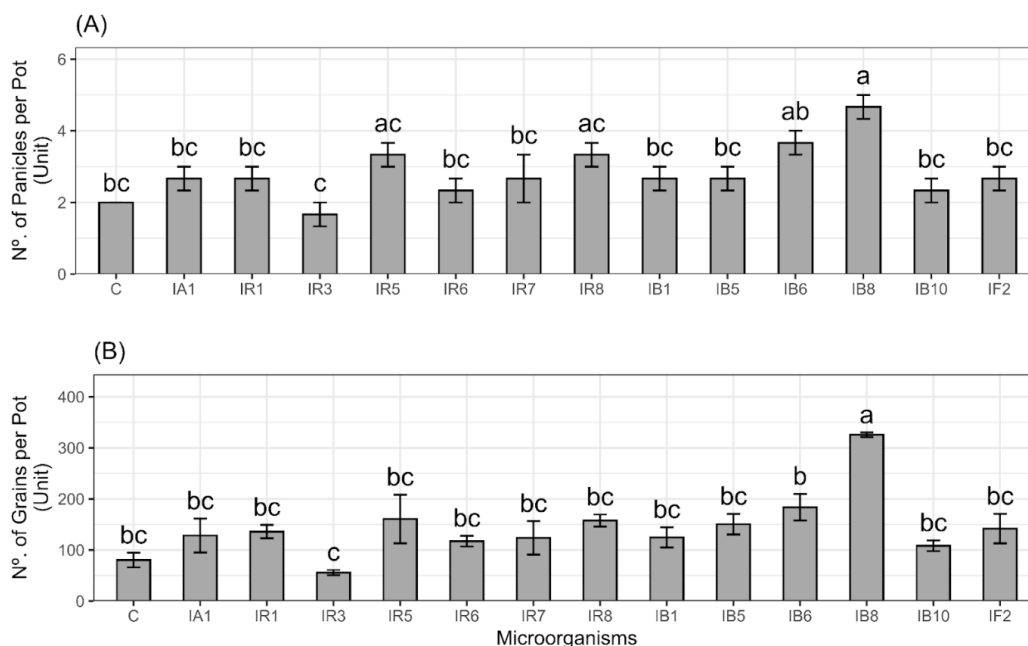


Figure 2. Number of panicles per pot (A) and number of grains per pot (B) in upland rice plants, cultivar BRS A502. Treatments: C: control; IA1: *Bacillus* sp.; IR1: *Herbaspirillum seropedicae*; IR3: *Rhizobium* sp.; IR5: *Bacillus subtilis*; IR6: *Lysinibacillus boronitolerans*; IR7: *Herbaspirillum seropedicae*; IR8: *Herbaspirillum seropedicae*; IB1: *Bacillus velezensis*; IB5: *Pseudomonas putida*; IB6: *Acinetobacter* sp.; IB8: *Stenotrophomonas* sp.; IB10: *Acinetobacter* sp.; IF2: *Pantoea* sp.

with the stimulation of reproductive growth (Ulrich et al. 2021) (Table 1).

Inoculation with microorganisms did not significantly influence the mass of 1,000 grains, which ranged from 24.70 (IB8) to 27.80 g (IR7), values statistically similar to the control (Figure 3A). This result confirms the observations of Fernandes et al. (2020), indicating that grain mass is a highly heritable trait, predominantly determined by cultivar genotype. Environmental or microbiological factors tend to affect the number of grains or total biomass rather than the individual grain mass, which remains stable and reflects the plant's genetic potential (Morais Júnior et al. 2017, Streck et al. 2017).

In contrast, grain yield was significantly affected by the treatments (Figure 3B), with *Stenotrophomonas* sp. (IB8) producing values statistically higher than the control. This performance may be related to its ability to form biofilms, which favor rhizosphere colonization and improve nutrient uptake (Ajjah et al. 2023). Moreover, this feature contributes to moisture retention around the roots and protection against abiotic stresses such as drought (Nascimento 2025). Biofilms are structures composed of exopolysaccharides, proteins and volatile compounds produced by bacteria

that modulate plant physiological responses such as stomatal closure and accumulation of osmoprotective solutes, thereby increasing resilience and directly enhancing yield (Gomes et al. 2022). Tuong et al. (2022) observed an increase in the number of fruits in tomato with *Stenotrophomonas* sp., whereas Singh et al. (2020) reported agronomic gains in sugarcane. Together with the results obtained in this study, these findings confirm the potential of the *Stenotrophomonas* genus as a yield-promoting agent in different crops, including upland rice.

The first two principal components (PC1 and PC2) together explained 69.74 % of the variability in the data (Figure 4), meeting the Kaiser's criterion (eigenvalues > 1). PC1 showed a direct association with production-related variables - number of grains per pot, number of panicles per pot and yield - whereas shoot dry matter, root dry matter and 1,000-grain weight exhibited inverse correlations. PC2, on the other hand, was mainly associated with vegetative variables, particularly root dry matter (40.48 %) and shoot dry matter (37.43 %), followed by 1,000-grain weight, yield, number of panicles per pot and number of grains per pot, characterizing it as an index of vegetative development.

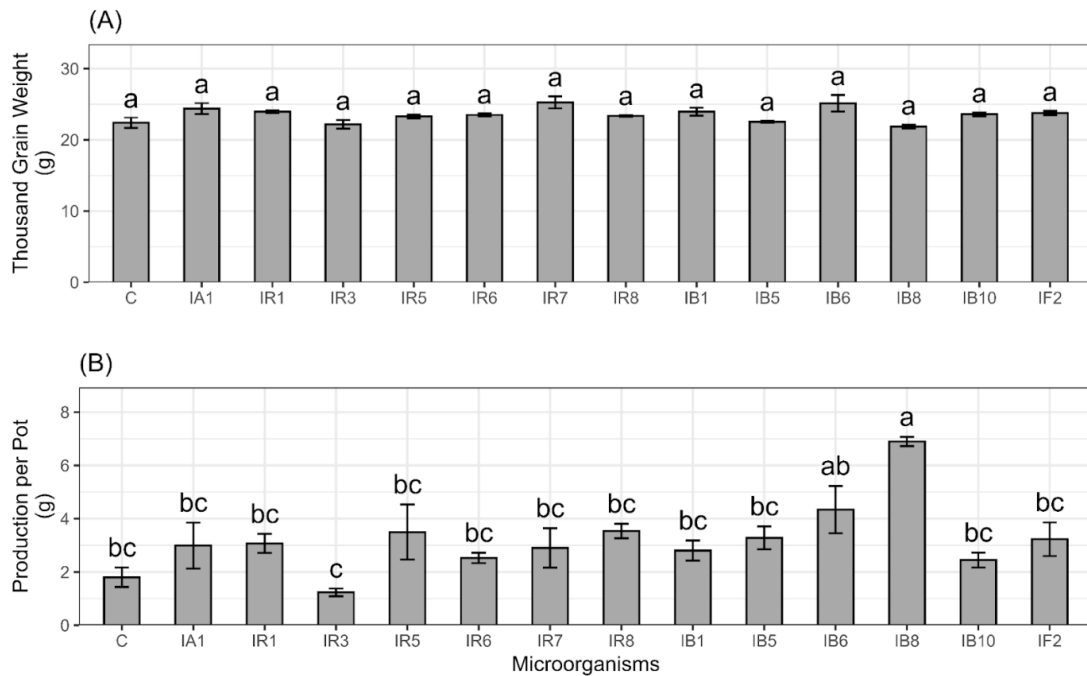


Figure 3. One thousand grain weight (A) and grain yield (B) in upland rice plants, cultivar BRS A502. Treatments: C: control; IA1: *Bacillus* sp.; IR1: *Herbaspirillum seropedicae*; IR3: *Rhizobium* sp.; IR5: *Bacillus subtilis*; IR6: *Lysinibacillus boronitolerans*; IR7: *Herbaspirillum seropedicae*; IR8: *Herbaspirillum seropedicae*; IB1: *Bacillus velezensis*; IB5: *Pseudomonas putida*; IB6: *Acinetobacter* sp.; IB8: *Stenotrophomonas* sp.; IB10: *Acinetobacter* sp.; IF2: *Pantoea* sp.

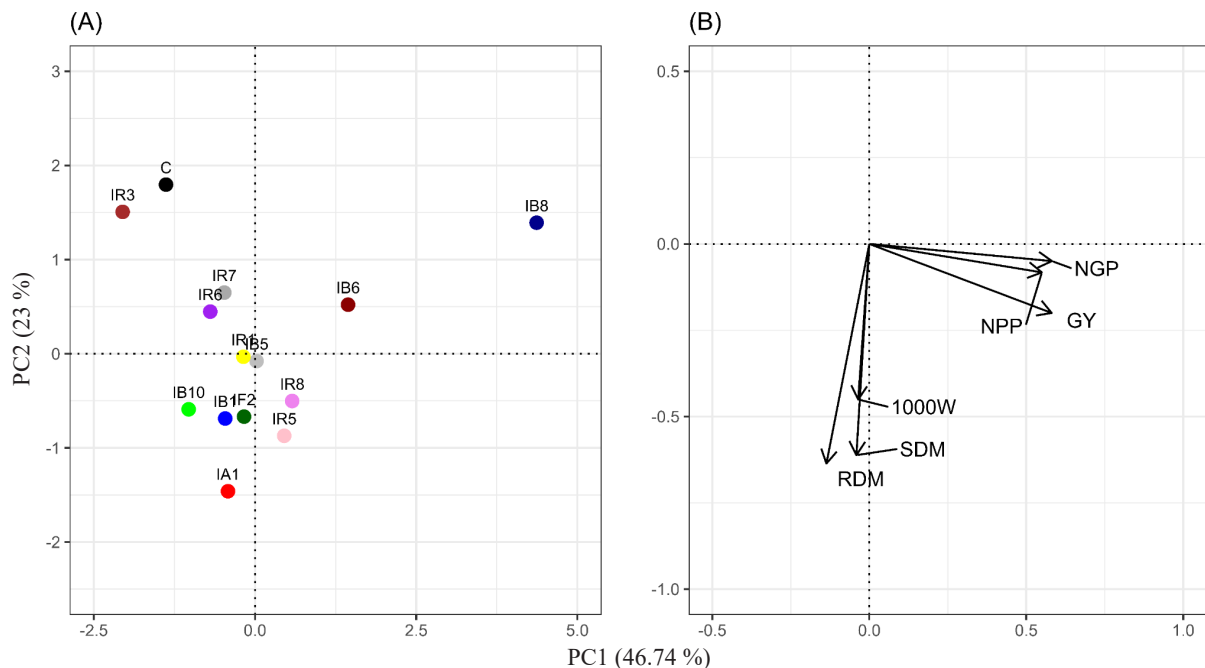


Figure 4. Score plot (A) and loading plot (B) showing the correlations between variables and treatments. Variables: GY: grain yield; NPP: number of panicles per pot; 1000W: 1,000-grain weight; NGP: number of grains per pot; SDM: shoot dry mass; RDM: root dry mass. Treatments: C: control; IA1: *Bacillus* sp.; IR1: *Herbaspirillum seropedicae*; IR3: *Rhizobium* sp.; IR5: *Bacillus subtilis*; IR6: *Lysinibacillus boronitolerans*; IR7: *Herbaspirillum seropedicae*; IR8: *Herbaspirillum seropedicae*; IB1: *Bacillus velezensis*; IB5: *Pseudomonas putida*; IB6: *Acinetobacter* sp.; IB8: *Stenotrophomonas* sp.; IB10: *Acinetobacter* sp.; IF2: *Pantoea* sp.

The score plot (Figure 4A) revealed that treatments with *Stenotrophomonas* sp. (IB8) and *Acinetobacter* sp. (IB6) had the highest values for PC1, indicating a strong association with productive variables. This suggests that these isolates have a greater potential to promote grain yield and reproductive structures in rice. In contrast, the control treatment presented negative scores for this component, reinforcing the positive effect of microbial inoculation.

The results are consistent with those shown in Figures 3A and 3B, which already indicated the strong performance of IB8 and IB6 in production-related variables. Multivariate analysis, therefore, confirms and complements the univariate findings, highlighting the biotechnological potential of these isolates in promoting the growth and yield of upland rice.

The IA1 (*Bacillus* sp.), IB10 (*Acinetobacter* sp.) and IR5 (*Bacillus subtilis*) isolates presented low mean scores for PC2, indicating that these treatments are promising for enhancing vegetative development in upland rice (Figure 3). These results are consistent with previous findings (Figures 1A and 1B), reaffirming the potential of these bacteria. Moreover, the control lies in the diametrically opposite direction from the treatments IA1, IB10 and IR5, indicating that microbial application is an effective strategy to improve these variables, when compared to the control.

Therefore, inoculation with multifunctional microorganisms positively influenced rice plant development, promoting gains in yield components such as number of panicles per pot and grain mass, as well as grain yield, while also favoring vegetative shoot growth. This indicates a greater efficiency in resource use and biomass accumulation, resulting in more vigorous and productive plants.

CONCLUSIONS

1. All tested microorganisms exhibited at least one functional trait associated with plant growth promotion, such as indole-3-acetic acid production, biofilm formation, phosphate solubilization or nitrogen assimilation;
2. *Stenotrophomonas* sp. (IB8) and *Acinetobacter* sp. (IB6) were associated with increases in number of grains per pot, number of panicles per pot and grain yield;

3. *Bacillus* sp. (IA1), *Bacillus subtilis* (IR5) and *Acinetobacter* sp. (IB10) showed potential to promote vegetative development.

REFERENCES

- AJIJAH, N.; FIODOR, A.; PANDEY, A. K.; RANA, A.; PRANAW, K. Plant growth-promoting bacteria (PGPB) with biofilm-forming ability: a multifaceted agent for sustainable agriculture. *Diversity*, v. 15, e112, 2023.
- ANSABAYEVA, A.; MAKHAMBETOV, M.; REBOUH, N. Y.; ABDELKADER, M.; SAUDY, H. S.; HASSAN, K. M.; NASSER, M. A.; ALI, M. A. A.; EBRAHIM, M. Plant growth-promoting microbes for resilient farming systems: mitigating environmental stressors and boosting crops productivity: a review. *Horticulturae*, v. 11, n. 3, e260, 2025.
- ARNAOUTELI, S.; BAMFORD, N.; STANLEY-WALL, N.; KOVÁCS, Á. *Bacillus subtilis* biofilm formation and social interactions. *Nature Reviews Microbiology*, v. 19, n. 9, p. 600-614, 2021.
- BEZERRA, G. A.; MACEDO, D. A.; NASCIMENTO, I. O.; SOUSA, T. P. de; COSTA, N. B.; SOUSA, L. F. R. A. Uso de *Bacillus* spp. no controle de fitopatógenos em sementes de soja variedade BRS Valiosa RR. *Revista Agroecossistemas*, v. 5, n. 1, p. 68-73, 2013.
- BHATTACHARJEE, K.; BARUA, S.; CHRUNGGOO, N.; JOSHI, S. Characterization of biomineralizing and plant growth-promoting attributes of lithobiontic bacteria. *Current Microbiology*, v. 80, n. 2, e80, 2023.
- BLAKE, C.; CHRISTENSEN, M. N.; KOVÁCS, Á. T. Molecular aspects of plant growth promotion and protection by *Bacillus subtilis*. *Molecular Plant-Microbe Interactions*, v. 34, n. 1, p. 15-25, 2021.
- BONATO, P.; ALVES, L.; OSAKI, J.; RIGO, L.; PEDROSA, F.; SOUZA, E.; ZHANG, N.; SCHUMACHER, J.; BUCK, M.; WASSEM, R.; CHUBATSU, L. The NtrY-NtrX two-component system is involved in controlling nitrate assimilation in *Herbaspirillum seropedicae* strain SmR1. *The FEBS Journal*, v. 283, n. 21, p. 3919-3930, 2016.
- BREDA, F. da F.; SILVA, T. da; SANTOS, S.; ALVES, G.; REIS, V. Modulation of nitrogen metabolism of maize plants inoculated with *Azospirillum brasilense* and *Herbaspirillum seropedicae*. *Archives of Microbiology*, v. 201, n. 4, p. 547-558, 2019.
- CATTELAN, A. J. *Modos qualitativos para determinação de características bioquímicas e fisiológicas associadas com bactérias promotoras de crescimento vegetal*. Londrina: Embrapa Soja, 1999.

- CRUZ, D. R. C.; NASCENTE, A. S.; FERREIRA, I. V. L.; MONTEIRO, N. O. da C.; SILVA, M. A.; DUARTE, G. A.; ROCHA, I. de S.; BARROSO NETO, J. Rizobactérias multifuncionais no crescimento inicial da raiz de mudas de soja, feijão comum, milho e arroz de terras altas. *Semina: Ciências Agrárias*, v. 46, n. 2, p. 383-400, 2025.
- CRUZ, D. R.; SILVA, M. A.; NASCENTE, A. S.; FILIPPI, M. C. de; FERREIRA, E. P. D. B. Use of multifunctional microorganisms in corn crop. *Revista Caatinga*, v. 36, n. 2, p. 349-361, 2023.
- EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA (Embrapa). *Arroz: estatística de produção*. 2025. Available at: <https://www.embrapa.br/agencia-de-informacao-tecnologica/cultivos/arroz/pre-producao/socioeconomia/estatistica-de-producao>. Access on: Aug. 19, 2025.
- ESTRADA DE LOS SANTOS, P.; BUSTILLOS CRISTALES, R.; CABALLERO MELLADO, J. *Burkholderia*, a genus rich in plant-associated nitrogen fixers with wide environmental geographic distribution. *Applied Environmental Microbiology*, v. 67, n. 6, p. 2790-2798, 2001.
- ETESAMI, H.; GLICK, B. R. Bacterial indole-3-acetic acid: a key regulator for plant growth, plant-microbe interactions, and agricultural adaptive resilience. *Microbiological Research*, v. 281, e127602, 2024.
- FERNANDES, J. P. T.; NASCENTE, A. S.; FILIPPI, M. C. C. de; LANNA, A. C.; SOUSA, V. S.; SILVA, M. A. Physio-agronomic characterization of upland rice inoculated with mix of multifunctional microorganisms. *Revista Caatinga*, v. 33, n. 3, p. 679-689, 2020.
- FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS (FAO). *Agricultural production statistics 2000-2022*. Rome: FAO, 2023.
- FREEMAN, D. J.; FALKINER, F. R.; KEANE, C. T. New method for detecting slime production by coagulase negative staphylococci. *Journal of Clinical Pathology*, v. 42, n. 8, p. 872-874, 1989.
- GARCÍA-FRAILE, P.; MENÉNDEZ, E.; RIVAS, R. Role of bacterial biofertilizers in agriculture and forestry. *AIMS Bioengineering*, v. 2, n. 3, p. 183-205, 2015.
- GOMES, E.; LANA, U. D. P.; ALVES, V.; OLIVEIRA-PAIVA, C. A.; GODINHO, B. de; SOUSA, S. M.; TINOCO, S. M. D. S. *Mecanismos das bactérias promotoras do crescimento de plantas na mitigação dos efeitos do déficit hídrico*. Sete Lagoas: Embrapa Milho e Sorgo, 2022. (Documentos, 267).
- INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE). *Produção agrícola municipal - PAM 2023: safra bate recorde, mas valor da produção cai*. Rio de Janeiro: IBGE, 2024.
- KHAN, I.; ZADA, S.; RAFIQ, M.; SAJJAD, W.; ZAMAN, S.; HASAN, F. Phosphate solubilizing epilithic and endolithic bacteria isolated from clastic sedimentary rocks, Murree lower Himalaya, Pakistan. *Archives of Microbiology*, v. 204, n. 6, e332, 2022.
- KORIR, H.; MUNGAI, N. W.; THUITA, M.; HAMBA, Y.; MASSO, C. Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. *Frontiers in Plant Science*, v. 8, e141, 2017.
- KULKOVA, I.; DOBRZYŃSKI, J.; KOWALCZYK, P.; BEIŠECKI, G.; KRAMKOWSKI, K. Plant growth promotion using *Bacillus cereus*. *International Journal of Molecular Sciences*, v. 24, n. 11, e9759, 2023.
- LIU, Y.; DAI, C.; ZUO, Y.; QIAO, J.; SHEN, J.; YIN, X.; LIU, Y. Characterization of siderophores produced by *Bacillus velezensis* YL2021 and its application in controlling rice sheath blight and rice blast. *Phytopathology*, v. 114, n. 12, p. 2491-250, 2024.
- LOACES, I.; FERRANDO, L.; SCAVINO, A. Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. *Microbial Ecology*, v. 61, n. 3, p. 606-618, 2011.
- MORAIS JÚNIOR, O. P. de; MELO, P. G. S.; MORAIS, O. P. de; COLOMBARI FILHO, J. M. Variabilidade genética durante quatro ciclos de seleção recorrente em arroz. *Pesquisa Agropecuária Brasileira*, v. 52, n. 11, p. 1033-1041, 2017.
- MORETTI, L. G.; CRUSCIOL, C. A. C.; KURAMAE, E. E.; BOSSOLANI, J. W.; MOREIRA, A.; COSTA, N. R.; ALVES, C. J.; PASCOALOTO, I. M.; RONDINA, A. B. L.; HUNGRIA, M. Effects of growth-promoting bacteria on soybean root activity, plant development, and yield. *Agronomy Journal*, v. 112, n. 1, p. 418-428, 2020.
- NASCENTE, A. S.; ISHOLA, Z. T.; FILIPPI, M. C. C. de; SILVA, M. A.; CRUZ, D. R. C.; BEZERRA, G. de A. Biochemical characterization of individual and combined plant growth-promoting microorganisms. *Pesquisa Agropecuária Tropical*, v. 53, e75376, 2023.
- NASCENTE, A.; FILIPPI, M. C. C. de; LANNA, A.; SOUZA, A. de; LOBO, V. da S.; SILVA, G. da. Biomass, gas exchange, and nutrient contents in upland rice plants affected by application forms of microorganism growth promoters. *Environmental Science and Pollution Research*, v. 24, n. 3, p. 2956-2965, 2016.
- OLIVEIRA, C. A.; ALVES, V. M. C.; MARRIEL, I. E.; GOMES, E. A.; SCOTTI, M. R.; CARNEIRO, N. P.; GUIMARÃES, C. T.; SCHAFFERT, R. E.; SÁ, N. M. H. Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an Oxisol of the

- Brazilian Cerrado biome. *Soil Biology and Biochemistry*, v. 41, n. 9, p. 1782-1787, 2009.
- OUYABE, M.; IRIE, K.; TANAKA, N.; KIKUNO, H.; PACHAKKIL, B.; SHIWACHI, H. Response of upland rice (*Oryza sativa* L.) inoculated with non-native plant growth-promoting bacteria. *Agronomy*, v. 10, n. 6, e903, 2020.
- R CORE TEAM. *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing, 2023.
- RABIN, N.; ZHENG, Y.; OPOKU-TEMENG, C.; DU, Y.; BONSU, E.; SINTIM, H. Biofilm formation mechanisms and targets for developing antibiofilm agents. *Future Medicinal Chemistry*, v. 7, n. 4, p. 493-512, 2015.
- RADWAN, T. E. E.; ZEINAT, K. M.; REIS, V. M. Production of indole-3-acetic acid by different strains of *Azospirillum* and *Herbaspirillum* spp. *Symbiosis*, v. 32, n. 1, p. 39-53, 2002.
- RODRIGUES, M. E.; GOMES, F.; RODRIGUES, C. F. *Candida* spp./bacteria mixed biofilms. *Journal of Fungi*, v. 6, n. 1, e5, 2019.
- SAECHOW, S.; THAMMASITTIRONG, A.; KITTAKOOP, P.; PRACHYA, S.; THAMMASITTIRONG, S. Antagonistic activity against dirty panicle rice fungal pathogens and plant growth-promoting activity of *Bacillus amyloliquefaciens* BAS23. *Journal of Microbiology and Biotechnology*, v. 28, n. 9, p. 1527-1535, 2018.
- SCHEIDT, W.; PEDROZA, I.; FONTANA, J.; MELEIRO, L. da C.; SOARES, L. de B.; REIS, V. Optimization of culture medium and growth conditions of the plant growth-promoting bacterium *Herbaspirillum seropedicae* BR11417 for its use as an agricultural inoculant using response surface methodology (RSM). *Plant and Soil*, v. 451, n. 1, p. 75-87, 2020.
- SINGH, P.; KUMAR, R.; KHAN, A.; SINGH, A.; SRIVASTAVA, A. Bacillibactin siderophore induces iron mobilisation responses inside aerobic rice variety through YSL15 transporter. *Rhizosphere*, v. 27, e100724, 2023.
- SINGH, R. K.; SINGH, P.; LI, H.; GUO, D.; SONG, Q.-Q.; YANG, L.-T.; MALVIYA, M. K.; SONG, X.-P.; LI, Y.-R. Plant-PGPR interaction study of plant growth-promoting diazotrophs *Kosakonia radicincitans* BA1 and *Stenotrophomonas maltophilia* COA2 to enhance growth and stress-related gene expression in *Saccharum* spp. *Journal of Plant Interactions*, v. 15, n. 1, p. 427-445, 2020.
- SINGH, T.; BISHT, N.; ANSARI, M.; CHAUHAN, P. *Pseudomonas putida* triggers phosphorus bioavailability and P-transporters under different phosphate regimes to enhance maize growth. *Plant Physiology and Biochemistry*, v. 217, e109279, 2024.
- STRECK, E. A.; AGUIAR, G. A.; MAGALHÃES, A. M. D.; FACCHINELLO, P. H. K.; OLIVEIRA, A. C. D. Variabilidade fenotípica de genótipos de arroz irrigado via análise multivariada. *Revista Ciência Agronômica*, v. 48, n. 1, p. 101-109, 2017.
- SUÁREZ-MORENO, Z.; VINCHIRA-VILLARRAGA, D.; VERGARA-MORALES, D.; CASTELLANOS, L.; RAMOS, F.; GUARNACCIA, C.; DEGRASSI, G.; VENTURI, V.; MORENO-SARMIENTO, N. Plant-growth promotion and biocontrol properties of three *Streptomyces* spp. isolates to control bacterial rice pathogens. *Frontiers in Microbiology*, v. 10, e290, 2019.
- TIMOFEEVA, A.; GALYAMOVA, M.; SEDYKH, S. Prospects for using phosphate-solubilizing microorganisms as natural fertilizers in agriculture. *Plants*, v. 11, n. 16, e2119, 2022.
- TUONG, H. M.; MENDEZ, S. G.; VANDECASTEELE, M.; WILLEMS, A.; LUO, D.; BEIRINCKX, S.; GOORMACHTIG, S. *Stenotrophomonas* sp. SRS1 promotes growth of *Arabidopsis* and tomato plants under salt stress conditions. *Plant and Soil*, v. 473, n. 1-2, p. 547-571, 2022.
- ULRICH, K.; KUBE, M.; BECKER, R.; SCHNECK, V.; ULRICH, A. Genomic analysis of the endophytic *Stenotrophomonas* strain 169 reveals features related to plant-growth promotion and stress tolerance. *Frontiers in Microbiology*, v. 12, e687463, 2021.
- VERMA, D. K.; SRIVASTAV, P. P. Bioactive compounds of rice (*Oryza sativa* L.): review on paradigm and its potential benefit in human health. *Trends in Food Science & Technology*, v. 97, n. 1, p. 355-365, 2020.
- YASMIN, S.; ZAKA, A.; IMRAN, A.; ZAHID, M. A.; YOUSAF, S.; RASUL, G.; ARIF, M.; MIRZA, M. S. Plant growth promotion and suppression of bacterial leaf blight in rice by inoculated bacteria. *Plos One*, v. 11, n. 8, e0160688, 2016.
- ZAIDI, S.; USMANI, S.; SINGH, B. R.; MUSARRAT, J. Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. *Chemosphere*, v. 64, n. 6, p. 991-997, 2006.
- ZHAO, Y.; DING, W. J.; XU, L.; SUN, J. Q. A comprehensive comparative genomic analysis revealed that plant growth promoting traits are ubiquitous in strains of *Stenotrophomonas*. *Frontiers in Microbiology*, v. 15, e1395477, 2024.
- ZHU, H.; ZHOU, H.; REN, Z.; LIU, E. Control of magnaporthe oryzae and rice growth promotion by *Bacillus subtilis* JN005. *Journal of Plant Growth Regulation*, v. 41, n. 6, p. 2319-2327, 2022.