AGRONOMY (AGRONOMIA)

Agrária —Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.21, n.1, e4441, 2026
Recife, PE, UFRPE. www.agraria.pro.br
DOI: 10.5039/agraria.v21i1e4441 - Protocol 4441
Subm. 16/10/2025 • App. 18/12/2025 • Pub. 15/02/2026

Phosphate-solubilizing bacteria as biofertilizers for upland rice

Jéssica Alves de Oliveira[1], Flávia Mendes dos Santos Lourenço[2], Juliana Trindade Martins[3], Orivaldo Arf[2], Philippe Solano Toledo Silva[4], Katia Luciene Maltoni[2], Ana Maria Rodrigues Cassiolato[2*]

1 Instituto Mato-Grossense do Algodão, Rondonópolis, MT, Brasil. E-mail: jessicaoliveira@imamt.org.br
2 Universidade Estadual Paulista "Júlio de Mesquita Filho", Ilha Solteira, SP, Brasil. E-mail: flaviamsl1@hotmail.com; o.arf@unesp.br; katia.maltoni@unesp.br; ana.cassiolato@unesp.br
3 Aarhus University, Aarhus, Denmark. E-mail: jtm@agro.au.dk
4 Universidade Estadual Paulista "Júlio de Mesquita Filho", São Paulo, SP, Brasil. E-mail: philippe.toledo@unesp.br

ABSTRACT: Rice is an important cereal in the basic diet of approximately one-third of the world's population. The inoculation of seeds with solubilizing bacteria provides a source of P, thereby reducing the need for added inputs to the crop. Soil bacteria with the potential to solubilize phosphates were evaluated in greenhouse and field trials in a 4 × 3 factorial scheme for treatments, with 4 replications. The treatments consisted of a combination of four doses of P (0, 23.3, 46.6, and 70 kg ha^{-1}), based on the use of 0, one-third, two-thirds, and full dose for upland rice, with the inoculation of three isolates of phosphate-solubilizing bacteria (AAC01 – *Pantoea* sp.; AAC02 – *Enterobacter* sp.; and AAC04 – *Klebsiella* sp.). In the greenhouse experiment, AAC02, together with a dose of 46.6 kg ha^{-1} of P, increased soil basal respiration (SBR) and shoot fresh mass. AAC01 increased soil basal respiration linearly up to 70 kg ha^{-1} of P and increased root fresh mass by 46.6 kg ha^{-1}. In the field experiment, the P doses increased the P content of rice grains, roots, shoot dry mass, and plant height. AAC04 associated with 46.6 kg ha^{-1} provided equivalent levels of P in the soil and leaves, and P levels in the grains were higher than those in non-inoculated plants fertilized with 70 kg ha^{-1} of P. The AAC04 treatment yielded significant gains in both greenhouse and field experiments, with an approximately 33% reduction in phosphate fertilization, resulting in a greater number of panicles per square meter and full grains per panicle. Based on these findings, we recommend AAC04 as a biofertilizer to ensure environmentally healthier rice production in the future.

Keywords: Bioalternative; Cerrado; *Enterobacter* sp.; *Klebsiella* sp.; *Pantoea* sp.

Bactérias solubilizadoras de fosfato em arroz de sequeiro

RESUMO: O arroz é um cereal de extrema importância na dieta básica de aproximadamente um terço da população mundial. A inoculação de sementes com bactérias solubilizadoras pode fornecer fósforo (P), diminuindo a necessidade de insumos adicionais à cultura. Bactérias do solo com potencial para solubilizar fosfatos foram avaliadas em ensaios de casa de vegetação e campo, em esquema fatorial 4 x 3 para as avaliações entre tratamentos, com 4 repetições. Os tratamentos constituíram da combinação de 4 doses de P (0; 23,3; 46,6 e 70 kg ha^{-1}), baseadas na utilização de 0, 1/3, 2/3 e 3/3 da dose recomendada para a cultura, com a inoculação dos 3 isolados de bactérias solubilizadoras (BSF) (AAC01 – *Pantoea* sp.; AAC02 – *Enterobacter* sp. e AAC04 – *Klebsiella* sp.). No experimento em casa de vegetação, AAC02, juntamente com uma dose de 46,6 kg ha^{-1} de P, aumentou a respiração basal do solo e a massa fresca da parte aérea. AAC01 aumentou a respiração basal do solo linearmente até 70 kg ha^{-1} de P e aumentou a massa fresca da raiz a 46,6 kg ha^{-1}. No experimento de campo, as doses de P utilizadas aumentaram o teor de P nos grãos de arroz, raízes, massa seca da parte aérea e altura das plantas. AAC04 associada a 46,6 kg ha^{-1} proporcionou níveis equivalentes de P no solo e nas folhas, e níveis de P nos grãos superiores aos das plantas não inoculadas e fertilizadas com 70 kg ha^{-1} de P. AAC04 apresentou ganhos significativos tanto no experimento em casa de vegetação quanto no experimento em campo, com redução de cerca de 33% na adubação fosfatada, proporcionando maior número de panículas por metro quadrado e grãos inteiros e totais por panícula, demonstrando ser um biofertilizante, recomendado para estudos futuros para garantir uma produção de arroz ambientalmente mais saudável.

Palavras-chave: Bioalternativas; Cerrado; *Enterobacter* sp.; *Klebsiella* sp.; *Pantoea* sp.

* Ana Maria Rodrigues Cassiolato. E-mail: ana.cassiolato@unesp.br. (Corresponding author)
Associate Editor: Mário de Andrade Lira Junior

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INTRODUCTION

The low availability of P in soils is a major challenge in modern agriculture (Withers et al., 2018). P is an essential macronutrient required in large quantities by plants; however, its fixation to soil minerals has been reported to reduce its availability for uptake considerably.

Microorganisms facilitate the acquisition of nutrients by plants (Elhaissoufi et al., 2020). Phosphate biosolubilization represents a promising strategy for releasing P immobilized in the soil, improving the sustainability of agricultural systems (Beltran-Medina et al., 2023).

The metabolic mechanisms of phosphate-solubilizing bacteria (PSB) have been observed as strategic alternatives for converting soil-fixed P into plant-available forms. These bacteria mineralize organic P by producing enzymes such as phospholipases and phytases and solubilize inorganic P primarily by secreting organic acids, e.g., gluconic, propionic, citric, and oxalic acids, as well as by releasing protons and hydroxyl or bicarbonate ions (Beltran-Medina et al., 2023). In addition, many PSBs act as plant growth promoters through several mechanisms, including the production of siderophores, antibiotics, and enzymes, as well as the induction of phytohormone synthesis (Fanai et al., 2024).

Rice (*Oryza sativa* L.) is an important cereal in the basic diet of approximately one-third of the world's population (Irfan et al., 2020). Phosphorus is essential for the growth and development of rice. However, P can also be a limiting nutrient for tillering and crop yield (Santiago et al., 2013; Lange et al., 2016). A substantial proportion (65%) of the minerals absorbed by rice plants are exported by grains, which makes them an important source of this nutrient in human nutrition (Huang et al., 2020). Thus, creating sustainable strategies aligned with the Sustainable Development Goals (SDGs), especially SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production), is essential for ensuring an adequate supply of nutrients to crops and consequently sustaining global agricultural production (ONU BR, 2015).

The inoculation of rice seeds with bacteria has the potential to provide a significant supply of P to the crop, decrease the use of added inputs, and ensure supply to the plants. Therefore, the aim of the present study was to evaluate the effects of seed inoculation with selected PSB in combination with different P doses on the upland rice cultivar 'BRS Esmeralda.'

MATERIALS AND METHODS

In this study, we conducted two experiments, the first in a greenhouse in the municipality of Ilha Solteira and the second in the field at the Teaching, Research, and Extension Farm of the School of Engineering, municipality of Selvíria. The soil used as substrate in the greenhouse experiment was collected from the 0–0.20 m layer in the area where the field experiment was installed. The soil, Typic Haplustox, with a clayey texture (Santos et al., 2018; USDA, 2022), had a pH of $CaCl_2$ = 5.0; P mg dm^{-3} = 25; MO g dm^{-3} = 18; K^+ $mmol_c$ dm^{-3} = 0.7; Ca^{2+} $mmol_c$ dm^{-3} = 19; Mg^{2+} $mmol_c$ dm^{-3} = 16; H+Al $mmol_c$ dm^{-3} = 31; Al $mmol_c$ dm^{-3} = 1; CTC $mmol_c$ dm^{-3} = 66.7; and V (%) = 54, according to Raij et al. (2001).

Randomized blocks in a 4 × 3 factorial scheme were used in both experiments. The treatments consisted of four P doses (0, 23.3, 46.6, and 70.0 kg ha^{-1}), corresponding to 0, one-third, two-thirds, and full dose of the recommended application rate for upland rice (Cantarella et al., 2022), using simple superphosphate, and the inoculation of three PSB isolates (Oliveira et al., 2024), belonging to the collection of the Molecular Phytopathology Laboratory at the Faculty of Engineering, UNESP – Ilha Solteira Campus, São Paulo State (number AAC01 – *Pantoea* sp.; AAC02 – *Enterobacter* sp.; AAC04 – *Klebsiella* sp.). The control treatments consisted of the application of two doses of P (0 and 35 mg dm^{-3} equivalent to 0 and 70 kg ha^{-1}) without inoculation. For better characterization of the isolates, calcium phosphate and iron phosphate solubilization tests, solubilization index, and germination and seedling emergence tests can be found in Oliveira et al. (2024).

Greenhouse experiment

Soil mineral fertilization at sowing consisted of 20 kg ha^{-1} ammonium sulfate [$(NH_4)_2SO_4$] and 60 kg ha^{-1} potassium chloride (KCl) applied together with P doses. In addition, N fertilization was applied as a cover at 20 and 40 days after plant emergence (DAE), with 40 kg ha^{-1} of urea in each application (Cantarella et al., 2022).

The experimental units, 15-L pots, received 16 seeds of upland rice and the cultivar 'BRS Esmeralda.' The inoculation of each BSP was carried out by seeding at a dose of 2.0 mL kg^{-1} using plastic bags, with vigorous shaking for 3.0 min, and then left to dry in the shade. The inoculants were prepared by multiplying PSB individually in the laboratory in liquid GL culture medium (glucose 10 g L^{-1} and yeast extract 2.0 g L^{-1}) to a concentration of 1.0 × 10^9 colony-forming units mL^{-1}. At 10 DAE, thinning was performed with 10 plants per pot, which were manually irrigated with public water throughout the cultivation period. Temperature and lighting time were not controlled. The experiment was conducted until the floral differentiation stage, which occurred at 77 DAE when the plants were cut close to the soil surface.

After harvesting, the soil close to the roots was transported to the laboratory for analysis. Part of it was evaluated for chemical attributes (Raij et al., 2001), whereas the other part was quantified for released CO_2–C via soil basal respiration (SBR). Fresh soil (100 g) was incubated in a hermetically sealed glass bottle in a small vial containing 10 mL of 0.1 mol L^{-1} sodium hydroxide (NaOH) solution. Incubation time was determined using a calibration curve. After opening the bottles, 2.0 mL of 10% barium chloride was

added to the vial containing NaOH for CO_2 precipitation. Next, titration was performed using 0.1 mol L^{-1} hydrochloric acid, with 1% phenolphthalein as an indicator (Anderson & Domsch, 1989).

The plant roots were washed and dried using a paper towel. Subsequently, the roots and aerial parts of the plants were weighed to obtain their fresh weight. Next, the samples were dried in an oven with forced air circulation at 65 ºC until a constant weight was obtained, and then they were weighed to determine their dry weight. They were then ground (Wiley-type mill) and subjected to nitro-perchloric digestion for the analytical determination of P using metavanadate colorimetry (Malavolta et al., 1997).

Field experiment

The experimental area was established at the coordinates 20º20'47.1'' S and 51º23'53.9'' W, and temperature and precipitation data (monthly averages) were collected throughout the experimental period at the UNESP meteorological station (Figure 1).

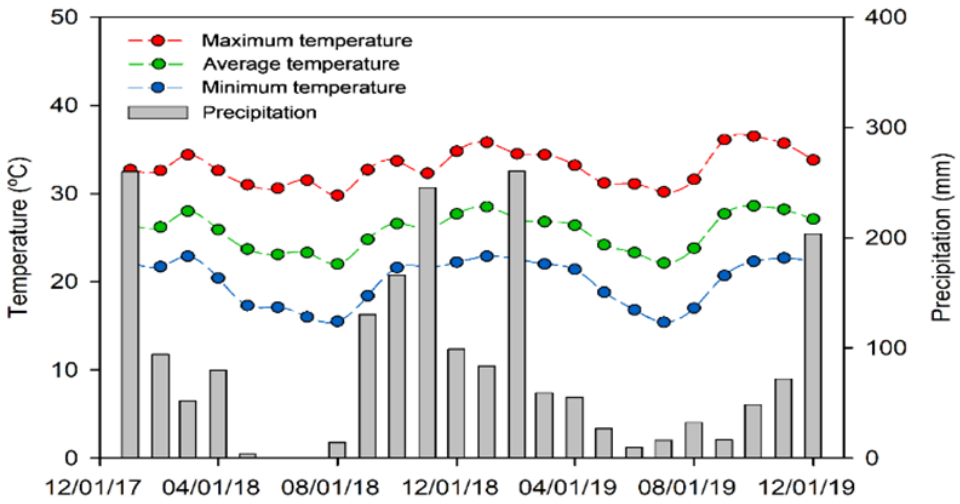

Figure 1 - Temperature and precipitation recorded in the experimental area during the experimental period. Source: Canal CLIMA UNESP.

Conventional soil preparation was used in the area, and the experiment followed a randomized block design, containing five 5.0 m long rows, spaced 0.35 m apart. The mineral fertilizer applied in the sowing and covering furrows was calculated based on the soil chemical attributes, considering the recommendations of Cantarella et al. (2022): 20 kg ha^{-1} of N in the form of ammonium sulfate and 60 kg ha^{-1} of K in the form of potassium chloride, together with doses of P. The N top dressing was applied twice, that is, at 20 and 40 DAE, with 40 kg ha^{-1} of N in the form of urea, in each application. Before sowing, rice seeds were treated with pyraclostrobin (25 g L^{-1}), methyl thiophanate (225 g L^{-1}), or fipronil (250 g L^{-1}) at a dose of 2.0 mL kg^{-1}.

At sowing, 70 kg ha^{-1} of seeds from the upland rice cultivar 'BRS Esmeralda' was manually applied. Each PSB was inoculated on the seeds in a sowing furrow in the form of a jet directed by a manual backpack sprayer at a dose of 400 mL ha^{-1}. The inoculants were prepared by individually multiplying each PSB in the laboratory in liquid GL culture medium, as explained before, reaching a concentration of 1.6 × 10^9 UFC mL^{-1}.

If necessary, a central pivot-type sprinkler irrigation system was used to supply water with an average rainfall of 13 mm. Plant emergence occurred after 4 days. Weed management was performed using the pre-emergence herbicide pendimethalin (1,400 g ha^{-1} of the active ingredient [a.i.]) and post-emergence herbicide (metsulfuron-methil, 2.0 g ha^{-1} of the a.i.). Weeds that were not affected by the herbicides were manually removed.

The harvesting of the three useful rows was manually performed at 109 DAE. The evaluation of soil chemical attributes (Raij et al., 2001), was performed on soil samples consisting of four simple samples, per experimental plot, collected at a depth of 0.0 to 0.20 m in the crop row.

Leaf P content was assessed at flowering by collecting 20 "flag leaves" per plot; however, grain P was analyzed from samples of 100 g of polished grains from each plot. The leaves and grains were dried in an oven with forced air circulation at 65 °C until constant weight was obtained, ground (Wiley-type mill), and subjected to nitric-perchloric digestion followed by the analytical determination of P using metavanadate colorimetry (Malavolta et al., 1997).

All results were subjected to the analysis of variance using the *F*-test ($p \leq 0.05$). The effects of the inoculations were compared using the Tukey test ($p \leq 0.05$), and the effects of P doses were evaluated using regression analysis, adopting the equation with the highest R^2.

RESULTS AND DISCUSSION

Greenhouse experiment

Under greenhouse conditions, no changes in response to the treatments were observed in soil pH, organic matter content, exchangeable aluminum content, sum of bases, cation exchange capacity, or base saturation (Table 1). Changes in soil fertility were expected following the application of a single superphosphate because of its P, Ca, and sulfur content. Such effects have been reported under high application rates, as demonstrated by Schenfert et al. (2015), who applied 444 kg P_2O_5 ha^{-1}, and Homenchuk & Fonseca (2018), who used 500 kg ha^{-1}, both to a Typic Haplustox. These doses far exceed those used in this study, justifying the absence of changes in soil fertility.

Table 1 - Analysis of variance for soil pH, P, organic matter (OM), and aluminum (Al) contents, sum of bases (SB), cation exchange capacity (CEC), and base saturation (V); leaf P content (foliar P content); root P content (grain P content); shoot fresh mass (SFM) and shoot dry mass (SDM); root fresh mass (RFM) and root dry mass (RDM); and soil basal respiration (SBR; mg CO_2 kg^{-1}), for rice ('BRS Esmeralda' cultivar), as a function of P doses (PR) and the inoculations of P-solubilizing bacteria, under greenhouse conditions.

Sources of variation	pH	P resin	OM mg dm^{-3}	Al	SB	CEC	V %	P leaf g kg^{-1}	P root kg^{-1}	SFM g	SDM g	RFM g	RDM g	SBR
				----- $mmol_c$ dm^{-3} ------										
P doses (kg ha^{-1})														
0.0	5.9	15.3	16.1	16.0	29.9	45.9	64.7	1.49	0.18	10.4	2,4 b	3.2	1.4	1.1
23.3	5.9	16.1	16.0	16.3	28.9	45.2	63.7	1.51	0.25	11.7	2.7ab	4.2	1.7	1.2
46,.6	5.9	15.3	16.5	16.2	26.6	42.7	62.1	1.57	0.33	12.3	2.8 a	5.3	2.4	1.3
70.0	5.9	17.0	16.7	17.1	28.8	45.9	62.0	1.64	0.39	11.9	2.8 a	4.6	2.5	1.2
Inoculations (Inoc)														
AAC01[1]	5.9	15.1	15.9	16.5	28.3	44.8	62.9	1.51	0.2 b	11.4	2.6	3.9	1.9	1.2
AAC02	5.9	15.1	16.4	16.2	28.6	44.8	63.5	1.57	0.3 ab	11.2	2.6	4.0	1.8	1.2
AAC04	5.8	18.3	16.7	16.4	28.4	44.8	62.9	1.59	0.3 a	12.2	2.8	5.1	2.5	1.3
F Value														
Pr	0.06^{ns}	2.09^{ns}	0.60^{ns}	0.86^{ns}	1.22^{ns}	2.20^{ns}	0.58^{ns}	0.26^{ns}	6.05**	9.62**	5.61**	6.66**	5.48**	4.78**
Inoc	1.64^{ns}	098^{ns}	1.30^{ns}	0.08^{ns}	0.02^{ns}	0.00^{ns}	0.05^{ns}	0.16^{ns}	3.44*	6.52**	2.94^{ns}	7.98**	4.03*	6.90**
Pr xInoc	1.68^{ns}	3.08*	0.40^{ns}	1.68^{ns}	1.78^{ns}	1.52^{ns}	1.68^{ns}	0.62^{ns}	0.46^{ns}	3.01*	1.32^{ns}	3.96*	3.71*	3.38*
Means	5.9	16.3	16.3	16.4	28.5	44.8	63.1	1.56	0.29	11.60	2.68	4.37	2.06	1.26
C.V.(%)	3	21	9	11	16	7	9	29	44	8	13	23	37	5

Means followed by the same letter within a column from each source of variation do not differ from each other according to Tukey's test ($p \leq 0.05$). ns = not significant. The single "*" and double "**" asterisk symbols indicate significance at 5.0 and 1.0% probability levels, respectively, according to the *F*-test. [1]AAC01: *Pantoea* sp.; AAC02: *Enterobacter* sp.; and AAC04: *Klebsiella* sp.

Root P content varied as a function of PSB inoculation, with higher values observed in the presence of the AAC04 isolate (*Klebsiella* sp.) (Table 1). Additionally, root P content increased linearly with the applied P dose, with the lowest values recorded in the absence of P fertilization (Figure 2).

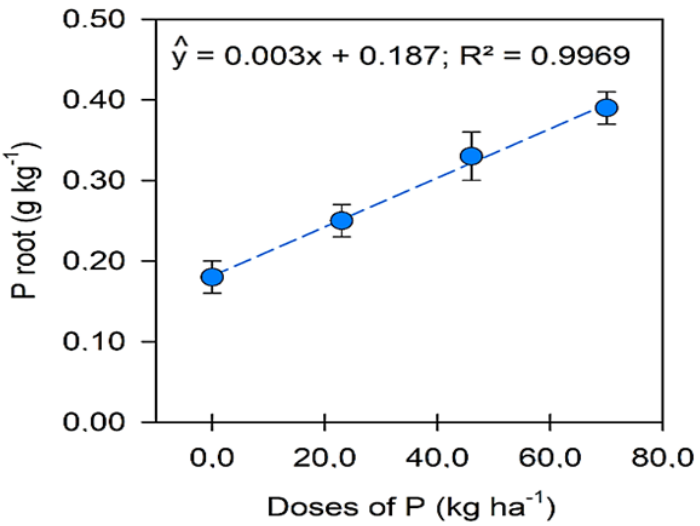

Figure 2 - Phosphorus content in the roots of rice plants ('BRS Esmeralda' cultivar) as a function of phosphorus doses, under greenhouse conditions.

However, these levels did not translate into significant increases in P-leaf concentration, possibly due to P redistribution within the plant, as reported by Irfan et al. (2020), particularly in metabolically active tissues. In cereal roots, P concentration is directly associated with soil P availability because their root systems are highly responsive to P nutrition (Liu, 2021). The significant interaction between P doses and PSB inoculation indicates that the application of the AAC04 isolate (*Klebsiella* sp.), combined with P doses of 46.6 and 70 kg ha^{-1}, increases P availability in the soil (Table 2). The P concentrations observed at both doses did not differ significantly, suggesting that the isolate enhanced P solubilization, even at a 33% lower phosphate fertilization rate.

Table 2 - Analysis of the interactions between soil P content and soil basal respiration (SBR) in rice ('BRS Esmeralda' cultivar) as a function of P doses and the inoculations of P-solubilizing bacteria, under greenhouse conditions

Inoculation	P doses (kg ha^{-1})				Regression analysis	F values
	0	23.3	46.6	70.0		
	---------- P resin (mg dm^{-3}) ----------					
AAC01	15.7 a	15.5 ab	13.5 b	15.5 b	^{ns}	0.385^{ns}
AAC02	15.7 a	14.7 b	14.7 b	15.0 b	^{ns}	0.533^{ns}
AAC04	14.2 a	18.0 a	20.5 a	20.5 a	ŷ= 0.091x + 15.128 (R^2= 0.8620)	4.222*
F values	0.26^{ns}	3.46*	4.36*	3.24*		
	---------- SBR (mg CO_2 kg^{-1}) ---------					
AAC01[1]	1.12 a	1.22 a	1.21 c	1.26 ab	ŷ= 0.0018x + 1.14 (R^2= 08019)	4.173*
AAC02	1.19 a	1.31 a	1.34 b	1.19 b	ŷ= -0.0001x^2 + 0.0087x + 1.19 (R^2= 0.9843)	6.831**
AAC04	1.21 a	1.31 a	1.45 a	1.34 a	ŷ= -0.0001x^2 + 0.009x + 1.20 (R2= 0.8554)	9.533**
F values	2.81^{ns}	2.98^{ns}	9.24**	5.99**		

Means followed by the same letter within a column from each source of variation do not differ from each other according to Tukey's test ($p \leq 0.05$). ns = not significant. The single "*" and double "**" asterisk symbols indicate significance at 5.0 and 1.0% probability levels, respectively, according to the *F*-test. [1]AAC01: *Pantoea* sp.; AAC02: *Enterobacter* sp.; and AAC04: *Klebsiella* sp.

SBR increased in response to P application when inoculated with the AAC01 isolate, whereas isolates AAC02 and AAC04 exhibited higher respiration values at approximately 46.6 kg ha^{-1} of P (Table 2), with peak responses at 43.5 kg ha^{-1} and 45.0 kg ha^{-1} for AAC02 and AAC04, respectively. The increase in SBR following PSB inoculation was likely due to the indirect stimulation of native soil microbial populations through changes in plant root exudates. Additionally, the application of insoluble phosphate may contribute to the growth of soil microorganisms by providing nutrients for their survival and development (Li et al., 2023).

The highest mean values of shoot fresh mass were observed with the AAC02 and AAC04 isolates, with the maximum responses of 52.8 kg ha^{-1} and 51.45 kg ha^{-1} of P, respectively (Table 1). In contrast, shoot dry mass varied according to the P application rate, increasing with higher P doses (Figure 3).

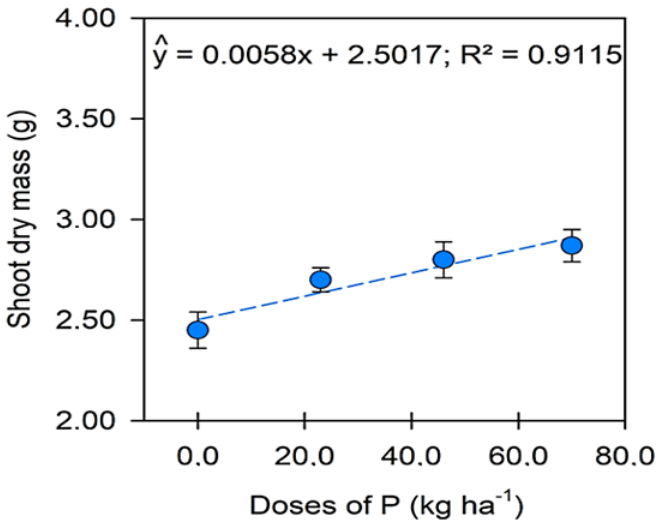

Figure 3 - Regression analysis for the shoot dry mass of rice ('BRS Esmeralda' cultivar) as a function of phosphorus doses, under greenhouse conditions.

In the presence of the AAC01 and AAC04 isolates, the highest root fresh mass values were recorded at 4.8 g and 6.7 g, respectively, at the 46.6 kg ha^{-1} P dose (Table 3), with peak values at 40.5 kg ha^{-1} and 45.8 kg ha^{-1} of P, respectively. The AAC04 isolate also positively impacted root dry mass, reaching 3.37 g at a P dose of 46.6 mg dm^{-3}, with a maximum response at 55.5 kg ha^{-1} of P.

Table 3 - Analysis of the significant interactions from the analysis of variance for shoot fresh mass (SFM), root fresh mass (RFM), and root dry mass (RDM) of rice ('BRS Esmeralda' cultivar) as a function of P doses and the inoculations of P-solubilizing bacteria, under greenhouse conditions.

Inoculation	P doses (kg ha^{-1})				Regression analysis	F values
	0	23.3	46.6	70		
---------- SFM (g) ----------						
AAC01	10.96 a	11.41 ab	11.54 a	11.55 a	^{ns}	0.408^{ns}
AAC02	9.83 a	10.85 b	12.56 a	11.59 a	ŷ = -0.0009x² + 0.0950x + 9.66 (R²= 0.8617)	7.029**
AAC04	10.49 a	12.69 a	12.86 a	12.81 a	ŷ= -0.001x² + 0.1029x + 10.58 (R²= 0.9586)	7.199**
F values	1.69^{ns}	5.89**	2.25^{ns}	2.70^{ns}		
---------- RFM (g) ----------						
AAC01	2.60 a	4.49 a	4.88 b	3.76 b	ŷ= -0.0014x² + 0.1133x + 2.60 (R²= 0.9990)	3.911*
AAC02	3.26 a	3.51 a	4.49 b	4.97 a	^{ns}	2.511^{ns}
AAC04	3.90 a	4.68 a	6.72 a	5.19 a	RQ(4) ŷ= -0.0011x² + 0.1007x + 3.66 (R²= 0.7288)	5.487**
F values	1.65^{ns}	1.53^{ns}	9.25**	4.09*		
---------- RDM (g) ----------						
AAC01[1]	1.41 a	1.79 a	1.94 b	2.26 a	^{ns}	0.837^{ns}
AAC02	1.27 a	1.49 a	1.94 b	2.60 a	^{ns}	2.328^{ns}
AAC04	1.78 a	2.01 a	3.37 a	2.86 a	ŷ= -0.0004x² + 0.0444x + 1.63 (R2= 0.7265)	3.735*
F values	0.46^{ns}	0.45^{ns}	4.60*	0.62^{ns}		

Means followed by the same letter within a column from each source of variation do not differ from each other according to Tukey's test ($p \leq 0.05$). ns = not significant. The single "*" and double "**" asterisk symbols indicate significance at 5.0 and 1.0% probability levels, respectively, according to the *F*-test. [1]AAC01: *Pantoea* sp.; AAC02: *Enterobacter* sp.; and AAC04: *Klebsiella* sp.

The highest dry mass observed in the presence of the highest doses of P in association with inoculation reinforces the pivotal role of P in biomass accumulation in rice plants, given its essential contribution to vegetative growth, for instance, by expanding the photosynthetically active leaf area and thereby enhancing CO_2 assimilation (Soares et al., 2023). On average, the shoot fresh mass increased by 20% in the inoculated treatments without additional P input. Notably, these treatments received only 66% of the recommended P rate for rice, underscoring the contribution of AAC02 and AAC04 isolates to P availability for plant uptake.

Field Experiment

As observed in the greenhouse experiment, P application doses and inoculation with the three PSB isolates did not significantly affect soil pH, organic matter content, exchangeable aluminum content, sum of bases, cation exchange capacity, or base saturation (Table 4). A change in pH was expected, considering that the release of organic acids is an important, albeit not exclusive, mechanism involved in nutrient mobilization.

Table 4 - Analysis of variance for soil pH, P, organic matter (OM), aluminium (Al) contents, sum of bases (SB), cation exchange capacity (CEC), base saturation (V); foliar P content (leaf P content); grain P content (grain P content); and plant height (H), for rice ('BRS Esmeralda' cultivar), as a function of P doses (PR) and P-solubilizing bacteria inoculation, under field conditions.

Source of variation	pH $CaCl_2$	Presin mg dm^{-3}	OM g dm^{-3}	Al	SB	CEC	V %	P leaf g kg^{-1}	P grain g kg^{-1}	H cm
				---------- $mmol_c$ dm^{-3} ----------						
P doses (kg ha^{-1})										
0.0	5.0	13.9	16.2	1.6	27.0	50.3	53.6	2.27	3.00	97.50
23.3	5.1	17.7	16.5	1.0	30.4	52.2	58.2	2.33	3.37	97.83
46.6	5.0	27.7	16.4	1.7	27.8	51.8	53.5	2.33	3.47	97.06
70.0	5.0	26.8	16.2	1.8	27.6	51.9	53.9	2.29	3.72	96.78
Inoculation (Inoc)										
AAC01[1]	5.0	20.4	16.2	1.9	26.1	49.9	52.1	2.21	3.16 b	96.52
AAC02	5.1	20.1	16.2	1.0	29.8	52.0	57.1	2.30	3.23 ab	96.98
AAC04	5.0	24.1	16.6	1.6	28.8	52.0	55.2	2.40	3.78 a	98.37
Valores de F										
PD	1.00^{ns}	8.31^{**}	0.29^{ns}	0.97^{ns}	1.15^{ns}	0.98^{ns}	1.00^{ns}	0.72^{ns}	3.83^{*}	0.13^{ns}
Inoc	1.46^{ns}	3.22^{*}	1.39^{ns}	2.25^{ns}	2.45^{ns}	2.95^{ns}	1.70^{ns}	11.84^{**}	3.23^{*}	0.72^{ns}
PD x Inoc	0.56^{ns}	3.14^{*}	0.87^{ns}	0.79^{ns}	0.70^{ns}	1.22^{ns}	0.41^{ns}	3.56^{*}	1.35^{ns}	0.38^{ns}
Means	5.00	21.50	16.35	1.52	28.21	51.32	54.79	2.30	3.39	97.3
C.V. (%)	5	18	5	36	17	6	14	5	22	5

Means followed by the same letter within a column from each source of variation do not differ from each other according to Tukey's test ($p \leq 0.05$). ns = not significant. The single "*" and double "**" asterisk symbols indicate significance at 5.0 and 1.0% probability levels, respectively, according to the *F*-test. [1]AAC01: *Pantoea* sp.; AAC02: *Enterobacter* sp.; and AAC04: *Klebsiella* sp.

These isolates were previously selected for their phosphatase activity, particularly in relation to the solubilization of inorganic P compounds (Ibarra-Galeana et al., 2017).

Neither the P application doses nor PSB inoculations impacted plant height (Table 4). Other studies have also reported no response in plant height to P doses and inoculation (Oliveira et al., 2015; Bento et al., 2016), as well as reported no significant differences in maize plant height following PSB inoculation and mineral P fertilization. The plant heights observed in the present study were within the expected range for the 'BRS Esmeralda' cultivar, between 95 and 108 cm (Castro et al., 2014).

The soil P content matched the recommendation proposed by Sousa et al. (2016), which suggested that soil P values above 35 mg dm^{-3} are necessary to achieve maximum

yields in annual crops under irrigated systems.

The mean soil P content of 36.25 mg dm^{-3}, recorded at the 46.6 kg ha^{-1} P dose with the inoculation of the AAC04 isolate, with a calculated maximum at 55.28 kg ha^{-1}, met the recommendation proposed by Sousa et al. (2016). These authors suggested that soil P content above 35 mg dm^{-3} is necessary to achieve maximum yields in annual crops under irrigated systems. The highest foliar P concentration was also found under this treatment, reaching 2.46 g kg^{-1} of dry matter with AAC04 inoculation, with a peak at 44.17 kg ha^{-1}.

The quadratic regression model underscored the remarkable efficacy of the AAC04 isolate in solubilizing soil P and facilitating its uptake by plants in sufficient quantities, thereby reducing phosphate fertilization requirements (Table 5).

Table 5 - Analysis of the significant interactions from the analysis of variance for soil P and leaf P contents in rice ('BRS Esmeralda' cultivar) as a function of P doses and P-solubilizing bacteria inoculation, under field conditions.

Inoculation	P doses (kg ha^{-1})				Regression analysis	F values
	0	23.3	46.6	70.0		
	--------- P resin (mg dm^{-3}) ----------					
AAC01[1]	12.2a	16.5a	24.5ab	28.2a	$\hat{y} = 0.24 + 11.98$ (R^2= 0.9795)	3.041*
AAC02	18.7a	20.0a	22.2b	19.2b	ns	0.136ns
AAC04	10.7a	20.5a	36.2a	29.5a	$\hat{y} = -0.0032x^2 + 0.6233x + 8.99$ (R^2=0.8777)	8.793**
F values	1.03ns	0.23ns	3.22*	3.78*		
	---------- P leaf (g kg^{-1}) ----------					
AAC01	2.2a	2.2a	2.2b	2.2b	ns	0.237ns
AAC02	2.3a	2.3a	2.3ab	2.3ab	ns	0.361ns
AAC04	2.3a	2.4a	2.5a	2.4a	$\hat{y} = -0.00006x^2 + 0.0053x + 2314$ (R^2= 092)	3.294*
F values	1.26ns	2.32ns	5.36**	5.04*		

Means followed by the same letter within a column from each source of variation do not differ from each other according to Tukey's test ($p \leq 0.05$). ns = not significant. The single "*" and double "**" asterisk symbols indicate significance at 5.0 and 1.0% probability levels, respectively, according to the *F*-test. [1]AAC01: *Pantoea* sp.; AAC02: *Enterobacter* sp.; and AAC04: *Klebsiella* sp.

The increase in P in the grains was consistent with the results of other studies (Song et al., 2019) and increased with increasing P application doses (Figure 4). They reported a linear enhancement in grain P concentration in rice with soil P applications ranging from 0 to 90 kg ha^{-1}. Reis et al. (2018) observed significant improvements in both P accumulation and grain yield in rice plants following the direct application of high doses of triple superphosphate (TSP) to the planting furrow.

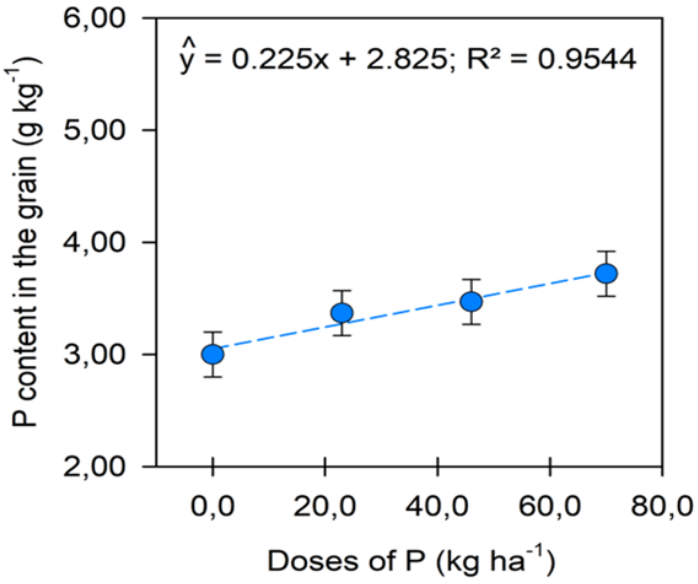

Figure 4 - Regression analysis for foliar phosphorus content in rice cultivar 'BRS Esmeralda' as a function of phosphorus doses, under field conditions.

In addition, inoculation with the AAC04 isolate resulted in the highest grain P concentrations (Table 4). Inoculation alone increased the P content in rice grains, similar to the increases in grain P content following PSB inoculation, as documented by Estrada et al. (2013) and Sousa et al. (2020) in studies involving rice and maize, respectively. The AAC04 isolate showed increased P concentrations in the soil, leaves, and grains, demonstrating good field adaptation and ensuring greater efficiency of P utilization by plants. The AAC04 isolate belongs to the genus *Klebsiella*, which has received considerable attention in recent years in agricultural research. Strains of *Klebsiella* spp. have shown high solubilization of iron and aluminum phosphates and significant improvements in the growth of inoculated plants, including increased root and shoot length, dry matter, and synthesis of oxalic acid and auxin.

Strains of *Klebsiella* spp. stimulate the production of auxin and ACC deaminase, which enhance plant resistance to environmental stressors (Rajput et al., 2015; Leite et al., 2018; Khalifa & Aldayel, 2022). The performance of AAC04 was better with alternative P sources than with TSP. Inoculation treatments highlighted the potential of using PSB as an alternative P source to ensure agricultural productivity while also enhancing sustainability.

CONCLUSIONS

In a greenhouse experiment, inoculation with the AAC04 isolate (*Klebsiella* sp.) increased the soil P concentration, SBR, and fresh and dry plant mass, achieving an approximately 33% reduction in phosphate fertilizer requirements. The AAC02 and AAC01 isolates, combined with a P dose of 46.6 kg ha^{-1}, increased shoot and root fresh mass, respectively.

In the field experiment, inoculation with AAC04 combined with a 46.6 kg ha^{-1} P dose resulted in soil and foliar P concentrations comparable to, and grain P concentrations exceeding, those observed in uninoculated plants fertilized with 70 kg ha^{-1} of P. These results suggest that AAC04 is a promising biofertilizer candidate for enhancing the sustainability of rice production systems.

This approach is strategically crucial because, in addition to contributing to soil and water preservation through a reduction in the application of phosphate mineral fertilizers, our results provide a basis for reducing the external dependence of Brazil on these inputs, while ensuring national food security.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contributions: Conceptualization: JAO, OA, KLM, and AMRC; Data curation: JAO, FMSL, and JTM; Formal analysis: JAO and PSTS; Investigation: JAO; Project administration: OA, KLM, and AMRC; Resources: OA, KLM, and AMRC; Supervision: OA, KLM, and AMRC; Validation: JAO, OA, and AMRC; Manuscript – original draft: JAO, FMSL, JTM, OA, PSTS, KLM, and AMRC; Manuscript – review & editing: JAO, OA, KLM, and AMRC.

Conflict of interest: There are no conflicts of interest between the authors and a public or private entity that could compromise the credibility of the publication, authors, or science itself.

Funding: The Coordination of Improvement of Higher Education Personnel (CAPES), Finance Code 001, and the National Council for Scientific and Technological Development - CNPq (fourth author - PQ-2 – Funding award no. 309777/2013-5).

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