

ORIGINAL RESEARCH PAPER

Morphological and nutritional performance of maize (*Zea mays* L.) inoculated with phosphate-solubilizing microorganisms

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Abstract: The use of microbial inoculants presents potential benefits for the development of crops. This study aimed to evaluate different phosphate-solubilizing inoculants on the early development of maize. The trials were conducted in pots under experimental conditions, with seeds inoculated using fungal and bacterial microbial suspensions. Morphological parameters such as root and shoot length, fresh and dry biomass of roots and shoots, and bioaccumulated phosphorus (P) content were determined up to the V8 growth stage. The solutions containing phosphate-solubilizing microorganisms showed potential to enhance plant development, fresh and dry biomass accumulation, and bioaccumulated phosphorus (P) content, especially the fungal inoculant *Scleroderma citrinum*, a microorganism of emerging agricultural interest.

Keywords: *Pseudomonas*; *Scleroderma citrinum*; Plant development; Plant nutrition.

1. Introduction

As a means of transforming insoluble phosphate into soluble phosphate available to plants, humanity already knows this process through the biochemical solubilization carried out by numerous microorganisms such as fungi and bacteria (Nahas et al., 1994). Maize (*Zea mays* L.) is a major global crop, and Brazil is one of the largest producers and exporters of this grain due to its high productive capacity (Bento et al., 2016; Lucero et al., 2021).

This crop demands a high supply of macro- and micronutrients, with NPK being the main minerals absorbed throughout the entire growth cycle. Phosphorus (P) is a limiting factor in agricultural production due to its involvement in various physiological and biochemical processes in plants, such as photosynthesis and cellular respiration (Abreu et al., 2014; Beltran-Medina et al., 2023).

Soils in tropical regions like Brazil are characterized by a high degree of weathering and low

levels of plant-available P (Rocha et al., 2005; Corrêa et al., 2008; Costa et al., 2009). The Brazilian Cerrado region, which includes extensive agricultural areas, is particularly affected by this issue (Costa et al., 2024).

Although P is present in significant amounts in soils, it is often in non-labile forms, which cannot be absorbed by plants. Therefore, various labile phosphate sources are annually added to cultivated soils, making this macronutrient costly. To address this issue, especially in the Cerrado soils, the use of microbial strains capable of converting non-labile P into labile P emerges as a more affordable and promising alternative, with benefits for both plant development and soil health (Araújo, 2011; Martins et al., 2025).

Seed inoculation with phosphate-solubilizing microorganisms (PSM) has gained popularity in Brazil and worldwide as a natural and cost-effective solution to the high expenses associated with phosphate fertilizers. Interestingly, several microbial groups possess phosphate-solubilizing capabilities (Ateş et al.,

2022).

Studies on the microbial fauna of planting regions can lead to the purification and testing of strains with potential as natural phosphate solubilizers. Thus, studying microorganisms from the local plant microbiome, particularly endophytic organisms—those that inhabit the interior of plants and are part of a highly diverse ecosystem—has proven to be an effective strategy for selecting promising strains for bioinoculant production (Silva et al., 2023).

It is well known that P naturally influences root development, improving nutrient and water uptake, and allowing maize to withstand water deficits for longer periods. PSMs in the maize rhizosphere enhance the availability of these nutrients and water absorption (Fathi, 2017).

Various fungal groups, such as *Aspergillus* and *Penicillium*, and bacterial genera such as *Pseudomonas*, *Enterobacter*, and *Erwinia*, can metabolize phosphate through natural biochemical solubilization, making P available in soils (Ali et al., 2022). Wahid & Mehana (2000) discuss that PSMs play an important role in making inorganic forms of phosphate, such as Ca-P, Al-P, and Fe-P, available, thereby increasing the P content in soil solution, improving root and shoot morphology, and enhancing crop yield, especially in maize cultivation (Rawat et al., 2021; Timofeeva et al., 2022).

In this context, several studies have focused on inoculating PSMs in both large- and small-scale crops. However, our understanding of their effects on high-yield maize hybrids and straw formation is limited, despite evidence suggesting that these practices can lead to greater agricultural productivity, improved grain yield, and increased straw for soil coverage and sustainability (Meng et al., 2024).

This study aimed to evaluate the inoculation of

different groups of phosphate-solubilizing microorganisms, including commercial fungal and bacterial strains, and an experimental isolate, *Sclerotinia citrinum*, in hybrid maize, focusing on their effects during the vegetative phase and on P content in roots and shoots.

2. Material and Methods

2.1. Experimental Site

The experiment was conducted in the experimental area of the UniBRAS University Center in Rio Verde, Goiás, Brazil, at coordinates (17°48'16.7'' S and 50°56'04.6'' W) during the first semester of 2025.

2.2. Climate and Experimental Design

The climate of the experimental region, according to the Köppen-Geiger classification (Cardoso; Marcuzzo, 2014), is type Aw, with a rainy season in summer from October to March and a dry season in winter from April to September. The average temperature ranges between 22 and 25 °C, and annual precipitation varies from 1,400 to 1,800 mm.

The experimental design was conducted using a completely randomized design (CRD) with seven suspended microorganism solutions (fungi and bacteria) as inoculants, six replicates per treatment, plus a control (without inoculum addition).

2.3. Soil type and analysis of the planting area

The soil used in the experiment was classified as a Dystroferic Red Latosol (LVdf), with a clayey texture, and the following chemical characteristics (Table 1).

Table 1. Chemical and physicochemical parameters of the planting soil.

Cmol _c dm ⁻³					mg dm ⁻³			
Ca	Mg	Ca+Mg	Al	K	K	S	P	pH
2.11	0.64	4.4	0.0	0.1	38.59	11.4	2.0	5.8
Micronutrients mg dm ⁻³					g dm ⁻³		Cmol _c dm ⁻³	
Na	Fe	Mn	Cu	Zn	B	O.M	CTC	SB
8.0	40.8	8.4	9.95	0.59	0.11	8.6	88.34	11.25
Texture			Relationships base			% bases CEC		
Clay	Silt	Sand	Ca/Mg	Ca/K	Mg/K	Ca/CEC	Mg/CEC	K/CEC

42.5	15.1	42.4	3.3	21.1	1.97	21.1	18.2	2.9
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Note: CEC = Cation Exchange Capacity. Extractants P (Mel), K, Na, Cu, Fe, Mn, and Zn = Mehlich¹; Ca, Mg, and Al = 1N KCl; S = Ca(H₂PO₄)₂ in HOAc (acetic acid); Organic Matter (O.M.) = Colorimetric method; Total P = Sulfuric acid digestion, and B = BaCl₂. Source: Authors, 2025.

2.4. Soil amendment, sowing, and maize hybrid description

After soil analysis, correction was performed considering the area as having high fertility, with over 30 years of continuous cultivation (soybean, maize, and sorghum). Liming was carried out to raise base saturation to 70%. A total of 600 kg of soil was collected at two depths (0–20 cm and 20–40 cm) and transported to the experimental area. Based on the soil analysis results and the interpretations of Ribeiro et al. (2019), the basal fertilization was carried out with 120 kg ha⁻¹ of P₂O₅ and 120 kg ha⁻¹ of K₂O. After soil analysis, the soil was transferred to 20 L experimental units (pots), and five maize seeds were sown per unit. After germination, thinning was performed at the V2 growth stage, leaving three plants per experimental unit.

The maize hybrid used was the transgenic Pioneer P3601 PWU. This hybrid has an early growth cycle and is intended for grain production. It has a plant height of 2.70 m, an ear height of 1.46 m, a flowering GDU of 1593, and a semi-hard yellow-orange kernel type. It presents high yield potential and stability, good foliar disease resistance, and nematode tolerance: high resistance factor (RF) reduction for *Pratylenchus brachyurus* and *Meloidogyne incognita*, and moderate RF reduction for *Meloidogyne javanica*. It also exhibits high responsiveness to crop management practices.

2.5. Preparation and inoculation

Microorganisms *Ralstonia* sp., *Sclerotinia citrinum*, *Rhizobium miluonense*, *Brevibacillus nitrificans*, *Pseudomonas fluorescens*, *Bacillus subtilis* + *Bacillus megaterium*, in the form of stabilized suspended solutions—either individually or as a microbial mixture—were applied directly to maize seeds for each treatment.

For each kilogram of seeds, 2 mL of the commercial suspension and the experimental fungal solution of *S. citrinum* were used. After inoculation, the seeds were manually homogenized and, after 15 min, sown.

2.6. Cultural practices

At the V4 stage, the commercial product FICAM® (Bendiocarb, Brazil) was used for pest control, as

Diabrotica speciosa was observed. Irrigation was performed every six days or when low soil moisture was detected with an electronic moisture probe (BOM, model 6810, China).

2.7. Sampling and variable analysis

Sampling was carried out on maize plants at the V8 phenological stage. The roots were washed with running water to remove soil particles. The plants were then transferred to the Soil and Foliar Laboratory at UniBRAS Rio Verde, where vegetative parameters were analyzed, including Root Length (RL) and Shoot Length (SL) both expressed in centimeters (cm), Shoot Fresh Mass (SFM), Root Fresh Mass (RFM), Shoot Dry Mass (SDM), and Root Dry Mass (RDM), all expressed in grams (g). Phosphorus analysis was performed on root (RPC) and shoot (SPC) samples from each treatment.

2.8. Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA), according to the adopted experimental design. When statistical significance was observed, means were compared using the *Scott-Knott* test at a 5% probability level. All statistical analyses were performed using the SISVAR software (Ferreira, 2019).

3. Results

3.1. Morphological parameters

Significant differences were observed in RL, forming two statistical groups according to the applied test. Roots shorter than 53 cm formed group (b), which included the microorganisms *Rhizobium miluonense*, *S. citrinum*, and the *A. brasilense* + *P. fluorescens* solution. The microorganism *Ralstonia* sp. showed the greatest SL, reaching 105 cm, followed by *S. citrinum* with 80 cm. Similar values were also observed for the commercial products *B. nitrificans*, *R. miluonense*, and the *A. brasilense* + *P. fluorescens* solution (Table 2).

Our experimental microorganism, *S. citrinum*, proved to be superior in terms of RFM, forming a data group with *B. nitrificans* and *Ralstonia* sp., with values exceeding 11 g. Regarding SFM, two statistical groups were identified. The best results for this parameter were observed for *B. nitrificans* and *Ralstonia* sp., with fresh shoot mass exceeding 0.04 g (Table 2).

Table 2. Morphological parameters, including root and shoot length, as well as root and shoot fresh biomass, in maize

(*Zea mays* L.) at the V8 phenological stage, inoculated with different phosphate-solubilizing microorganisms.

Microorganisms	RL	SL	RFM	SFM
Control	55.87 a	72.52 d	6.37 b	0.02 b
<i>B. nitrificans</i>	62.38 a	96.53 b	11.98 a	0.04 a
<i>Ralstonia</i> sp.	57.03 a	105.87 a	11.98 a	0.04 a
<i>R. miluonense</i>	44.30 b	93.71 b	9.12 b	0.03 b
<i>S. citrinum</i>	46.36 b	80.70 c	15.36 a	0.02 b
<i>A. brasilense</i> + <i>P. fluorescens</i>	48.53 b	84.89 c	6.95 b	0.02 b
<i>B. subtilis</i> + <i>B. megaterium</i>	53.36 a	90.53 b	8.17 b	0.03 b
<i>P. fluorescens</i>	56.20 a	96.21 b	8.06 b	0.03 b
CV (%)	16.93	9.01	31.44	30.56

Note: RL = root length, SL = Shoot length. RFM = root fresh mass. SFM = Shoot fresh mass. Different letters in the same column indicate significant differences according to the *Scott-Knott* test at the 5% probability level. Source: Authors, 2025.

For the parameter RDM, the solutions containing *Ralstonia* sp. and *S. citrinum* showed the greatest root residue accumulation, with results exceeding 2 g (1.95 and 2.38 g, respectively). No significant differences were observed among treatments and control for SDM.

Regarding bioaccumulated phosphorus (P) content in roots (RPC), the highest value was recorded for the *B. subtilis* + *B. megaterium* solution, with 2.30 mg kg⁻¹. For bioaccumulated P in the shoot (SPC), the solution containing *R. miluonense* showed the highest value, exceeding 2 mg kg⁻¹ (Table 3).

Table 3. Morphological parameters, root dry mass, shoot dry mass, root phosphorus content, and shoot phosphorus content in maize at the V8 growth stage inoculated with different phosphate-solubilizing microorganisms.

Microorganisms	RDM	SDM	RPC	SPC
Control	0.44 d	0.47 a	1.20 h	2.34 f
<i>B. nitrificans</i>	1.64 b	0.34 a	1.70 f	2.45 e
<i>Ralstonia</i> sp.	1.95 a	0.26 a	2.10 b	2.48 d
<i>R. miluonense</i>	1.49 b	0.30 a	1.74 e	2.70 a
<i>S. citrinum</i>	2.38 a	0.32 a	1.88 d	2.58 c
<i>A. brasilense</i> + <i>P. fluorescens</i>	1.04 c	0.24 a	1.66 g	2.64 b
<i>B. subtilis</i> + <i>B. megaterium</i>	0.41 d	0.23 a	2.30 a	2.28 g
<i>P. fluorescens</i>	0.95 c	0.25 a	2.06 c	2.62 b
CV (%)	27.87	62.78	0.39	0.52

Note: RDM = root dry mass. SDM = shoot dry mass. RPC = root phosphorus content. SPC = Shoot phosphorus content. Different letters in the same column indicate significant differences according to the *Scott-Knott* test at the 5% probability level. Source: Authors, 2025.

4. Discussion

Our results demonstrated potential across most agronomic parameters evaluated in the tested maize hybrid Pioneer P3601 PWU. In particular, the commercial

products showed superior performance in several physiological parameters during the vegetative stage up to V8. The inoculation with *Sclerotinia citrinum* demonstrated promising potential for further research as a novel phosphate-solubilizing microorganism, especially regarding fresh root biomass, as well as root and shoot

residue (straw) accumulation.

Several studies have reported promising results regarding the use of microbial inoculants in maize cultivation and other crops. In the study by Bento et al. (2016), the researchers did not observe significant differences in plant height or stem diameter between inoculated and non-inoculated treatments across different doses of P_2O_5 ha^{-1} . However, results similar to ours were described for plant dry mass when biological inoculation included *Bacillus*, *Pseudomonas*, *Nitrosomonas*, and *Nitrobacter*.

Zamariolli et al. (2019) also reported positive results regarding different phosphorus sources and microbial inoculation; however, no positive interaction was observed between these factors.

Maize plants inoculated with *P. fluorescens* showed higher productivity compared to those inoculated with another *Pseudomonas* strain (SB), although no significant difference was observed among non-inoculated plants.

Chaves et al. (2013) found similar results, reporting no significant response when maize seeds were inoculated with *P. fluorescens* using either reactive or non-reactive natural phosphate as the phosphorus source. These comparative findings strongly support our results regarding the use of *P. fluorescens* inoculation. Further studies should be conducted to better understand the microorganism–plant interactions and their antagonistic or synergistic effects in maize crops.

Regarding other parameters evaluated in our study, encouraging results were also reported by Napp et al. (2024), who inoculated maize seeds with *A. brasilense* and a solution containing *B. megaterium* and *B. subtilis*, resulting in increased plant height, stem diameter, leaf area index, and higher productivity.

According to Chagas et al. (2018) and Napp et al. (2024), microorganisms from the *Bacillus* genus provide multiple benefits, especially when their populations are enhanced through combination with proper management practices, such as NPK fertilization. This integration of different strategies helps ensure a biologically active and nutrient-rich soil, ultimately improving the quality and yield of agriculturally important crops.

An important finding in our study was that *S. citrinum* led to greater fresh and dry root biomass in maize. Larger root systems contribute to improved soil aeration and decompaction. Our hypothesis is supported by Martins et al. (2022), who reported that phosphate-solubilizing bacteria assist in the decomposition of organic matter and the production of commercially valuable secondary metabolites. These microorganisms also promote plant growth through the synthesis of siderophores and/or indole-3-acetic acid (IAA), and have

been widely used for the biocontrol of soil-borne fungal pathogens.

5. Conclusion

The use of microbial inoculants showed positive effects on the development of high-efficiency hybrid maize plants, particularly during the V8 phenological stage. The microorganism *Sclerotinia citrinum* emerged as a promising candidate for future studies due to its potential to solubilize inorganic phosphates. Additionally, its possible ability to enhance nitrogen accumulation in plants should be further investigated.

The other commercial microbial inoculants also demonstrated satisfactory performance, reinforcing their agronomic potential. However, treatments containing *Pseudomonas* showed signs of antagonism compared to the other tested strains, highlighting the need for more in-depth studies on microorganism–plant interactions and their synergistic or antagonistic effects on maize development.

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Author's Contributions

Monique Hellen Ananias Vital: soil collection, project design, experimental procedures, and manuscript writing. *Thacylla Alves Fernandes*: soil collection, project design, experimental procedures, and manuscript writing. *Matheus Vinícius Abadia Ventura*: data analysis, manuscript revision, and co-supervision. *Antonio Carlos Pereira de Menezes Filho*: supervision, project and manuscript writing, revision, and publication.

Ethics

There are no conflicts of interest between the authors.