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Research Article

Beneficial Bacteria Associated With Silica Nanoparticles for Growth Promotion of *Paspalum notatum*

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Plant growth–promoting bacteria (PGPB) can play an essential role as biofertilizers to increase pasture efficiency and reduce the application of agrochemicals. Plant growth can be potentialized when these bacteria are combined with silica nanoparticles (SiNPs). The present study aimed to evaluate the effect of PGPB associated with SiNPs on the growth of bahiagrass (*Paspalum notatum*) seedlings. The PGPB were isolated from rhizospheric soils and leaves of *Paspalum* spp. grown in the tropical high-altitude region of Brazil and selected by their ability to fix nitrogen, solubilize phosphate, and synthesize indoleacetic acid (IAA). They were identified as *Alcaligenes faecalis*, *Enterobacter asburiae*, and *Serratia marcescens* by 16S rDNA sequencing. Spherical SiNPs (85 nm in diameter) were synthesized by the hydrolysis of the silicon precursor tetraethyl orthosilicate (TEOS), characterized by infrared spectroscopy and scanning electron microscopy (SEM) and applied at 5% (0.05 mg·mL^{-1}) and 10% (0.1 mg·mL^{-1}) concentrations. Disinfected *P. notatum* seeds were treated with PGPB, SiNPs, and PGPB + SiNPs and cultivated in magenta boxes containing peat, sand, and perlite. The seedlings were evaluated for their germination percentage, root length, shoot length, root dry weight, and shoot dry weight. Disinfected seeds subjected to the same treatments were also grown in Petri dishes containing 0.7% agarose. The roots of the seedlings in Petri dishes were stained with diaminobenzidine tetrahydrochloride (DAB) and visualized using a light microscope to confirm bacterial colonization. The three strains without SiNPs promoted the growth of *P. notatum* seedlings. *S. marcescens* treatment presented the greatest shoot length, and both concentrations of nanosilica with PGPB improved or maintained root lengths. Treatments of *S. marcescens* and *E. asburiae* with 10% SiNPs showed 100% seed germination. Seedlings inoculated with 10% SiNPs with *S. marcescens* and *E. asburiae* alone showed the highest shoot dry weight, and all treatments increased root dry weight compared to the control. The 10% SiNPs' concentration inoculated with *S. marcescens* and *A. faecalis* positively affected *P. notatum* seedlings' growth. This study suggests that nanosilica can be applied with PGPB to improve the development of bahiagrass and reduce the need for applications of agrochemicals.

Keywords: bahiagrass; biofertilizer; endophytes; nanosilica; plant growth

1. Introduction

Conventional agriculture requires the use of high amounts of chemical fertilizers and pesticides to meet the growing world's food demand. The consequences of this excessive use of agrochemicals include environmental degradation, climate change, soil damage, and biodiversity loss. Thus, we must find sustainable approaches to produce more and healthier food while reducing the adverse impacts on natural resources [1].

Beneficial microorganisms such as plant growth-promoting bacteria (PGPB) have been valuable alternatives to agrochemicals because they naturally dwell in the rhizosphere or tissues of plants and promote plant growth by fixing nitrogen, solubilizing phosphate, synthesizing phytohormones, and protecting against phytopathogens, among other mechanisms [2–5]. The list of bacteria with plant growth-promoting activity is extensive, including the Gram-positive genera *Bacillus* and *Clostridium* and the Gram-negative *Azospirillum, Azotobacter, Acinetobacter, Burkholderia, Citrobacter, Enterobacter, Klebsiella, Pantoea, Pseudomonas, Rhizobium,* and *Serratia* [6, 7].

Biofertilizers are formulations containing live or latent PGPB, such as nitrogen fixers, phosphate solubilizers, and biocontrollers, that can be applied to seeds, leaves, or the soil to improve plant health [8–10]. Although biofertilizers are becoming more popular globally each year, there are some obstacles regarding bacterial stability, field applications, and reproducibility due to several biotic and abiotic factors influencing bacterial activity in the field [11, 12].

Given this context, nanotechnology can be an efficient tool for supporting and enhancing bacterial performance in plants. Studies have shown that different nanomaterials, such as silica, silver, zinc, titanium, and gold, can increase the PGPB populations and improve their beneficial traits in terms of their effects on various plant species [13]. Furthermore, given that they provide resistance to heat and desiccation, nanoformulations can enhance the stability and reproducibility of biofertilizers in natural environments [14, 15]. Therefore, the association of silica nanoparticles (SiNPs) and PGPB can bring economic and environmental benefits to agricultural systems [13].

Nanoscale materials have specific properties that differ from those of their bulk forms, such as a higher surface-area-to-volume ratio and being more soluble, reactive, and adherent [16, 17]. Silicon dioxide (SiO_2) or SiNPs have been used in agriculture to enhance plant health and protection because of the advantageous features of these particles, such as their low-cost production, biocompatibility, nontoxicity, chemical inertness, and large surface area [18–22]. Recent investigations have indicated that the coinoculation of SiNPs and PGPB can increase bacterial populations and viability [23, 24]; improve soil nutrient contents, biomass, and productivity of maize plants [24, 25]; and promote the growth of land cress [26].

Paspalum L. is a relevant genus of the Poaceae family and comprises 330 species [27]. *Paspalum notatum*, commonly known as bahiagrass, is a perennial, rustic, and warm-season grass distributed across tropical and subtropical regions, predominantly in South American pastures, and used as a forage and turf worldwide [28, 29]. In the Southeast of the United States, due to its high nutritional quality and adaptability, *P. notatum* is the most widely used forage for cow–calf production [30, 31]. Since nitrogen is the most limiting nutrient to bahiagrass growth, it is crucial to apply proper fertilization for its establishment, high forage production/crude protein content, and pasture renovation [32, 33]. Thus, an efficient microbial biofertilizer could reduce or even replace chemical inputs, providing the same benefits without causing environmental damage.

Given the potential advantages of beneficial bacteria and nanomaterials for sustainable agriculture, the investigation of PGPB-nanoparticle–plant interactions is highly relevant. From this perspective, the present work aimed to investigate the impacts of the PGPB *Alcaligenes faecalis*, *Enterobacter asburiae*, and *Serratia marcescens* isolated from *Paspalum* spp. associated with SiNPs on *P. notatum* growth. This is the first study on the association of PGPB and SiNPs in *Paspalum* spp.

2. Materials and Methods

2.1. Bacterial Isolation and Purification. Bacteria were isolated from the rhizospheric soils and leaves of *Paspalum rojasii* Hack. (BGP 272-VRcMmSv 14,536), *Paspalum lenticulare* Kunth (BGP 281-VRcMmSv 14,559), and *Paspalum compressifolium* Swallen (BGP 380) collected from the Germplasm Bank of *Paspalum* at the Embrapa Southeast Livestock, São Carlos, São Paulo, Brazil (21°57′ S and 47°56′ W), in October 2017.

The isolation of the rhizospheric bacteria was performed according to Mohite [34]. Samples of 10 g of rhizospheric soil of each plant genotype were collected and placed into sterilized Erlenmeyer flasks filled with 90 mL of phosphate-buffered saline (PBS) for 30 min under constant agitation. After being subjected to serial dilution from 10^0 to 10^{-3} in PBS, the soil suspensions were placed on Petri dishes containing trypticase soy agar (TSA) and 50 μg·mL^{-1} benomyl ($C_{14}H_{18}N_4O_3$), to avoid fungal contamination, and incubated at 28°C for 48 h. The bacterial colonies were selected, transferred to new Petri dishes filled with TSA, and incubated for 48 h for purification. The purified bacterial strains were then inoculated in tryptic soy broth (TSB) for 48 h and subsequently stored in cryotubes containing 500 μL of the bacterial suspension and 500 μL glycerol at −80°C.

For the isolation of endophytic bacteria, samples of 10 g of leaf and root tissue from each plant genotype were superficially disinfected with 70% ethanol for 2 min, followed by 3% sodium hypochlorite for 3 min and then 70% ethanol for 1 min, after which they were washed twice in sterile distilled water [35]. The disinfected leaves were macerated in 10 mL of PBS, and the roots were cut into small portions. The leaf material and root fragments were incubated in PBS at 28°C for 1 h under constant agitation. Serial dilution, incubation, and purification were performed according to the same methods used for the rhizospheric bacteria.

The bacterial isolates used in this study were selected according to their capacity to fix nitrogen, synthesize IAA, and solubilize phosphate, and therefore, classified as PGPBs.

2.2. Bacterial Identification. The bacteria were identified by 16S ribosomal DNA (rDNA) sequencing. The total genomic DNA was extracted with a DNA extraction kit (Sigma-Aldrich, St. Louis, Missouri), and the sequences were amplified using the universal primers 16SF (5′-AGAGTTTGATCCTGGCTCAG-3′), 16SR (5′-CTACGGCTACCTTGTTACGA-3′), 1492R (5′-GGTTACCTTGTTACGACTT-3′) [36], V3F (5′-ACTCCTACGGGAGGCAGCAG-3′), and

V6R (5′ ACAGCCATGCANCACCT 3′) [37]. The PCR products were purified and sequenced by staff at Genewiz, Inc. (South Plainfield, New Jersey, USA).

The forward and reverse sequences for each isolate were aligned by DNA Baser sequence assembly software (Heracle BioSoft S.R.L.), and the consensus sequences among the different amplified regions were obtained by MEGA software (Version 10.1.5) [38]. For bacterial isolate identification, the sequences were compared to those of GenBank accessions using BLASTn [39, 40] and to those housed in the EzBioCloud 16S database using 16-base IDs [41].

2.3. Synthesis and Characterization of SiO_2 Nanoparticles. Spherical nanoparticles of SiO_2 were obtained based on the procedure reported by Stöber, Fink, and Bohn [42]. The Stöber method is widely used to obtain spherical nanoparticles with a thoroughly controlled size. The nanoparticles are formed through the hydrolysis of the silicon (Si) precursor tetraethyl orthosilicate (TEOS). This reaction is catalyzed by ammonium hydroxide and results in $Si(OH)_4$ tetrahedra, which subsequently condense to form the three-dimensional structure of silica.

The amounts of TEOS and ammonium hydroxide employed were based on the work published by Arantes et al. [43], which showed that it is possible to vary the size of the spheres by changing the TEOS/NH_3 ratio. In this synthesis, 70 mL of anhydrous ethanol (Synth 98.9%), 25 mL of distilled water, 1 mL of ammonium hydroxide (Synth 27%), and 6 mL of TEOS (98%, Sigma-Aldrich) were added sequentially in a beaker. The mixture was then stirred at room temperature for 24 h. The nanoparticles were subsequently centrifuged at 23,000 rpm for 15 min three times together with anhydrous ethanol to remove the ammonium hydroxide. Their infrared spectral information was collected via diffuse reflectance infrared Fourier transform (DRIFT) in the range of 4000–400 cm^{-1} in a Bruker Equinox 55 instrument to confirm silica formation. The size and morphology were determined via SEM with an SEM-FEG Zeiss model SUPRA 35.

2.4. Bacterial Growth in the Presence of SiO_2 Nanoparticles. Each bacterial isolate was grown in Luria–Bertani (LB) broth without SiNPs and in the presence of 5% and 10% SiNPs (85 nm) at 28°C for 24 h with constant agitation (Table 1). The bacterial suspensions were centrifuged, rinsed with sterile water to remove all the media from the bacterial pellets, and standardized to a concentration of 10^6–10^8 cells mL^{-1}.

2.5. Plant Material. The present study was performed with seeds of bahiagrass (*P. notatum* Flüggé cultivar Argentine) obtained from the company Wonderlawn (lot no. 8340).

The bahiagrass seeds were treated with 98% H_2SO_4 for 10 min to break dormancy, rinsed with sterile water several times, and surface disinfected with 4% NaOCl (Clorox) for 30 min with constant agitation. After the seeds were disinfected, they were washed several times with sterile double-distilled water to remove the NaOCl completely [44].

2.6. Seedling Growth Promotion Experiments in Agarose Plates and Magenta Boxes. Surface-disinfected seeds were soaked in all the treatment suspensions (Table 1) for 2 h in Petri plates (1 mL per 50 seeds) and plated on 0.7% agarose plates (10 seeds per plate) [45]. For the magenta box experiment, the seeds subjected to the different treatments were placed in boxes filled with 15 g of potting media comprising peat, sand, and perlite at a 2:1:1 ratio and 40 mL of sterile water [44]. Each box contained 10 seeds, and the control was set up with surface-disinfected seeds without bacteria. The agarose plate and magenta box experiments were replicated three times, after which the boxes were incubated in a controlled environment with a temperature of 30°C during the day, 20°C at night, and a 12-h photoperiod. Seed germination (G) was evaluated, and root length (RL) and shoot length (SL) were measured with a ruler and recorded after 25 days of the magenta box experiment, according to de Paula et al. [46] with modifications. The seedlings were then transferred to an oven at 70°C for 48 h and weighed on an electronic scale to obtain their root dry weight (RDW) and shoot dry weight (SDW).

2.7. Reactive Oxygen Species (ROS) Staining and Visualization of Bacteria and SiO_2 NPs in Roots. After 7 days of incubation on 0.7% agarose, the roots of the seedlings were stained by flooding the plates with 2.5 mM diaminobenzidine tetrachloride (DAB; Sigma-Aldrich, Saint Louis, Missouri, USA) for 15 h. DAB is used to visualize the reactive oxygen (H_2O_2) produced around inter- and intracellular bacteria [3]. The roots were stained with aniline blue, a counterstain to visualize bacterial rods.

2.8. Statistical Analysis. One-way ANOVA followed by Tukey's post hoc test at the 0.05 level of probability was executed via IBM SPSS Statistics software Version 21.0 [47] to compare significant differences among the RL and SL of *P. notatum* seedlings in the magenta box experiments. Principal component analysis (PCA) was performed for all the traits analyzed using PAST software Version 2.17c [48]. Statistical analysis was not performed for the RDW and SDW traits because the seedlings could not be weighed on the scale separately due to their tiny size.

3. Results

3.1. Characterization of SiNPs. The spectrum of the synthesized nanoparticles is shown in Figure 1(a). The main bands related to silica are the -OH group stretch in the 3700 – 3200 cm^{-1} region, the stretch of the Si-O-Si antisymmetric group in the 1320 – 1000 cm^{-1} region, the Si-OH group stretch in the region of 980 – 880 cm^{-1}, and the deformation of the -OH group in the 845 – 765 cm^{-1} region. The average diameter of the synthesized nanoparticles was

Table 1: Treatments applied to *Paspalum notatum* seeds and control.

Treatment	NPs' concentration (%)	Bacterium	Accession no.
Control	—	—	
1	5	—	
2	10	—	
3	—	*Serratia marcescens*	OK396664
4	5	*Serratia marcescens*	OK396664
5	10	*Serratia marcescens*	OK396664
6	—	*Alcaligenes faecalis*	OK396670
7	5	*Alcaligenes faecalis*	OK396670
8	10	*Alcaligenes faecalis*	OK396670
9	—	*Enterobacter asburiae*	OK396667
10	5	*Enterobacter asburiae*	OK396667
11	10	*Enterobacter asburiae*	OK396667

Note: The identification number of each treatment, SiO_2 NP concentration, and bacterial species are indicated. "—," absence.
Abbreviation: NPs, nanoparticles.

85 ± 11 nm (Figure 1(b)) and a spherical morphology was observed (Figure 1(c)).

3.2. Effects of PGPB and SiNPs on Paspalum notatum Seedlings. Molecular characterization based on 16S rDNA partial sequence showed that the bacterial strains are shared between 99% and 100% gene similarity with *Serratia marcescens*, *Enterobacter asburiae*, and *Alcaligenes faecalis* (Table 2).

Inoculation with 5% SiNPs + *S. marcescens* (Figure 2(f)) promoted the greatest RL for *P. notatum* seedlings, statistically significant, according to Table 3. Each of the three PGPB species associated with both the concentrations of SiNPs increased or maintained the RL. Except for Treatment 5 (10% SiNPs + *S. marcescens*), these same treatments also increased RDWs.

Concerning shoot traits, the inoculation with *S. marcescens* without SiNPs (Figure 2(d)) emerged as presenting the greatest SL, followed by Treatment 9 (*E. asburiae*). The concentrations of 5% and 10% SiNPs maintained and slightly increased the SL, respectively, when associated with *A. faecalis*. On the other hand, both SiNP concentrations reduced the SL when inoculation with *S. marcescens* and *E. asburiae* occurred.

Treatments 5 (10% SiNPs + *S. marcescens*) and 9 (*E. asburiae*) resulted in the highest SDW. The G percentage was relatively high for Treatment 3 (*S. marcescens*), Treatment 5 (10% SiNPs + *S. marcescens*), Treatment 7 (5% SiNPs + *A. faecalis*), Treatment 9 (*E. asburiae*), and Treatment 11 (10% SiNPs + *E. asburiae*). Treatments 5 and 11 resulted in 100% germinated seeds.

Considering treatments with only bacteria, the bacterium *E. asburiae* (Treatment 9) showed good performance for the evaluated traits, confirming its growth-promoting potential for *P. notatum* seedlings.

The seedlings inoculated with *S. marcescens* presented a high G percentage and increased SL and RL and dry weight compared to the controls. Either the 5% or 10% SiNP concentrations in association with *S. marcescens* improved the seedling RL and RDW, while the 10% SiNP concentration enhanced both the G and the RDW of *S. marcescens*.

The bacterium *A. faecalis* improved the G, RL, RDW, and SDW. Only Treatment 8 (10% SiNPs + *A. faecalis*) slightly increased the SL compared to those without nanosilica.

Regarding the treatments with SiNPs without bacteria, the 5% SiNP concentration (Figure 2(b)) reduced seed G, although compared with the control, it slightly increased the RDW and SDW. Treatment with 10% SiNPs (Figure 2(c)) improved the seed G, RL, RDW, SL, and SDW. The 10% SiNP concentration promoted 100% seed G in the treatments with *E. asburiae* and *S. marcescens*.

According to the PCA, Principal component 1 (PC1) had a strong positive loading for RL, followed by RDW, SDW, G, and SL. PC2 presented a strong positive loading for SL. PC2 contributed the most to the variance among the treatments, was positively associated with SDW, and was negatively associated with RL, RDW, and G.

Regarding the distribution of all the treatments applied to *P. notatum* seeds shown in the biplot graph of the principal components (Figure 3), the control and treatments composed of SiNPs without bacteria were distributed on the graph's upper left side and opposite the vectors of G, RL, and RDW, since they presented the lowest values for those traits (Table 3). Therefore, treatments characterized by higher G, RL, and RDW rates were scattered on the lower-right side of the biplot graph. The treatments with higher levels for SL and SDW were distributed on the upper-right side of the graph. On the other hand, those characterized by low SL and SDW were scattered on the lower-left side.

3.3. Microscopy Analysis of Bacteria and SiNPs Within Paspalum notatum Roots. The bacteria colonized the seedling roots and induced the release of reactive oxygen, indicated by the brown color of the root hairs (Figures 4 and 5). Transparent spots were observed within the root hairs, both of seedlings treated with SiNPs only and within those treated with PGPB plus SiNPs (Figure 4), which may be an aggregation of nanosilica absorbed by the seedlings.

Figure 5 shows the bacteria (blue-stained) around and inside the root hairs of *P. notatum* seedlings, indicating that bacterial colonization in the plant occurred. Unlike in the microscopy images of the seedlings inoculated with

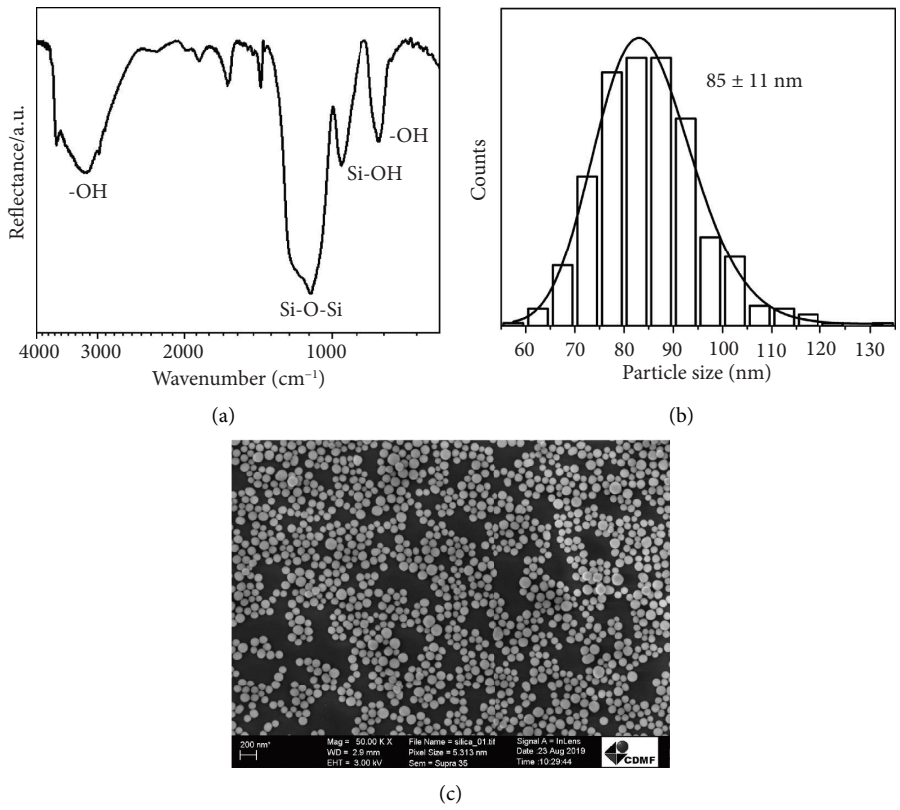


Figure 1: Characterization of synthesized silica nanoparticles (NPs). (a) Infrared spectrum of the synthesized NPs. (b) Diameters of the NPs. (c) Spherical morphology of the NPs obtained via scanning electron microscopy (SEM).

Table 2: Identification and characterization of three selected bacterial species isolated from *Paspalum* spp.

Plant species/genotype	Origin	Selected bacterium	Accession no.	% Similarity
Paspalum lenticulare/BGP 281	Rhizospheric soil	*Serratia marcescens*	OK396664	100
Paspalum rojasii/BGP 272	Leaves	*Enterobacter asburiae*	OK396667	99.6
Paspalum compressifolium/BGP 380	Rhizospheric soil	*Alcaligenes faecalis*	OK396670	99.2

nanosilica, transparent spots were not observed in the roots without nanosilica (Figure 5).

4. Discussion

The genera *Alcaligenes* and *Enterobacter* have been previously described as endophytic and rhizospheric bacteria of *Paspalum* spp. Nitrogen-fixing bacteria *Alcaligenes faecalis* and *Enterobacter* were found associated with *P. vaginatum* Sw. [49, 50], and *Enterobacter* isolated from different *Paspalum* spp. also showed the ability to fix nitrogen and produce IAA [46, 51]. The genus *Serratia* has been reported as a biocontrol agent and plant growth promoter in several plant species, including grasses, such as rice, grass pea, and pangolão grass [52–55]. Likewise, all three strains used in the present study can fix nitrogen, solubilize phosphate, and synthesize IAA *in vitro*. This work shows that inoculations of each bacterium without SiNPs improved the growth of *P. notatum* seedlings (Table 3), which supported the *in vitro* findings.

In the present study, the solutions comprising 10% SiNPs added to *E. asburiae* improved G and promoted the greatest RDW (0.31 mg). These results are supported by those of Boroumand, Behbahani, and Dini [26], who also reported the highest RDW in land cress (*Barbarea verna*) plants inoculated with phosphate-solubilizing bacteria and nanosilica.

The bacterial species *E. asburiae*, *S. marcescens*, and *A. faecalis*, without SiNPs, promoted the growth of *P. notatum* seedlings. Both concentrations of nanosilica improved or maintained the RL, although the SL was reduced when inoculation with PGPB occurred. The increased RL and root surface area are significant advantages to plants because they allow greater water and nutrient uptake. Through its ability to induce root branching and increase root surface area, the phytohormone IAA produced by PGPB is involved in root architecture development [56, 57]. Studies suggest that Si promotes phytohormone homeostasis in plants, thus

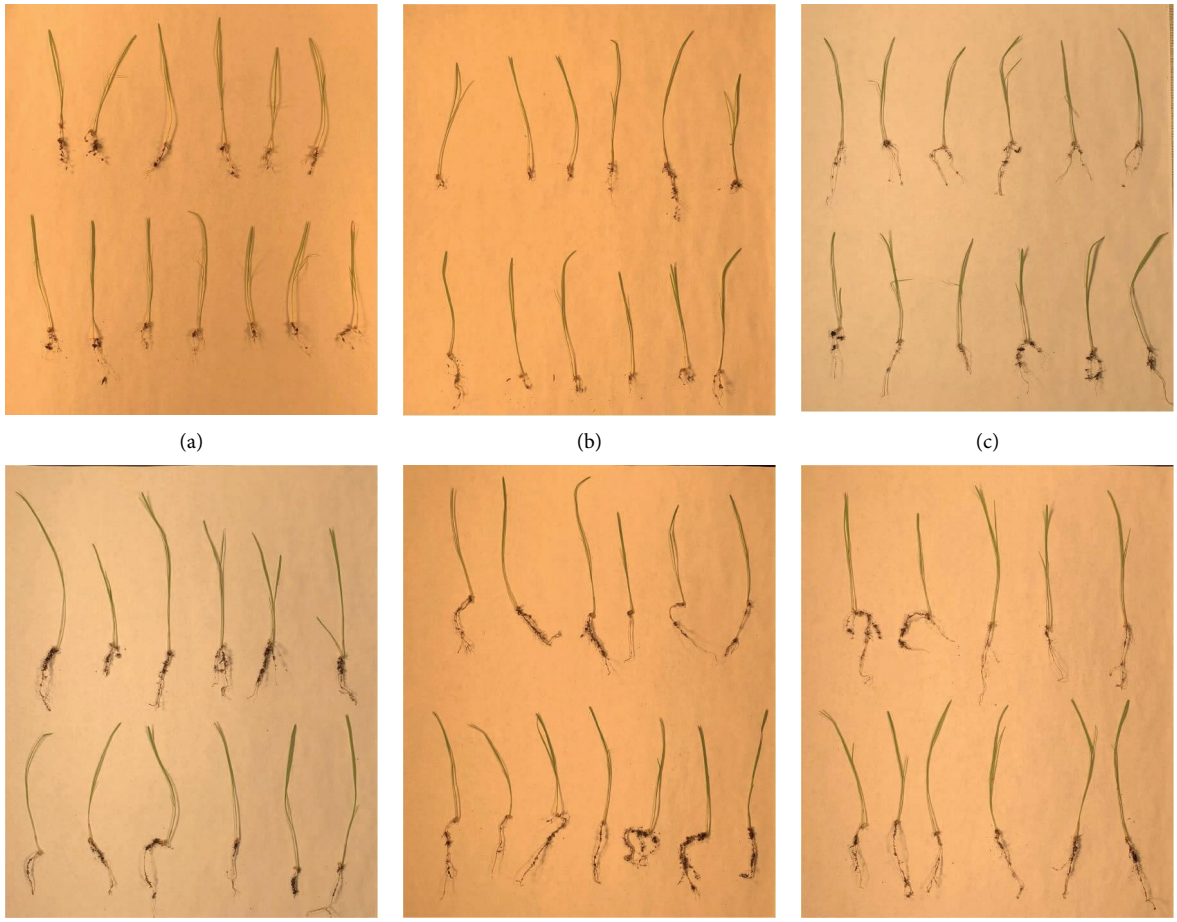


FIGURE 2: *Paspalum notatum* seedlings inoculated with different treatments and the control. (a) Control. (b) 5% SiNPs without bacteria. (c) 10% SiNPs without bacteria. (d) *S. marcescens* without SiNPs. (e) *S. marcescens* + 5% SiNPs. (f) *S. marcescens* + 10% SiNPs.

TABLE 3: Effects of different treatments on the germination (G), root length (RL), root dry weight (RDW), shoot length (SL), and shoot dry weight (SDW) of *P. notatum* seedlings.

Treatment	G (%)	RL (cm)	RDW (mg)*	SL (cm)	SDW (mg)*
C	80.0	1.70 ± 0.71^{d}	0.092	5.96 ± 1.14^{d}	0.77
1	76.66	1.63 ± 1.02^{d}	0.16	6.36 ± 1.03^{cd}	0.83
2	86.66	2.24 ± 1.05^{cd}	0.19	6.41 ± 0.83^{cd}	0.88
3	96.66	2.92 ± 0.79^{bc}	0.21	7.68 ± 1.22^{a}	0.91
4	93.33	3.72 ± 0.75^{a}	0.28	6.32 ± 0.59^{cd}	0.88
5	100.0	3.47 ± 0.68^{ab}	0.24	7.04 ± 1.03^{abc}	1.01
6	90.0	2.80 ± 0.87^{bc}	0.23	6.18 ± 1.05^{cd}	0.87
7	96.66	3.15 ± 1.25^{ab}	0.21	6.21 ± 1.10^{cd}	0.86
8	83.33	3.38 ± 0.76^{ab}	0.28	6.75 ± 0.95^{bcd}	0.87
9	96.66	3.44 ± 0.56^{ab}	0.29	7.40 ± 0.89^{ab}	1.01
10	86.66	3.31 ± 0.59^{ab}	0.28	6.97 ± 0.70^{abc}	0.92
11	100.0	3.44 ± 0.71^{ab}	0.31	6.64 ± 0.78^{abc}	0.83

Note: The different letters indicate statistically significant differences among the means according to Tukey's test ($p \leq 0.05$). The standard deviations (SDs) are presented next to the means of RL and SL.
*Average of the total weight divided by the number of seedlings.

inducing plant resistance under stress conditions [58]. Akhtar et al. [59] found that the combined application of PGPB and nanosilica improved relative water content and biomass, photosynthetic potential, nutrient uptake, and phytohormones level in wheat under drought conditions. These authors suggested that SiNPs and PGPB together improved plant defense responses and induced plant systemic resistance when facing drought stress [59].

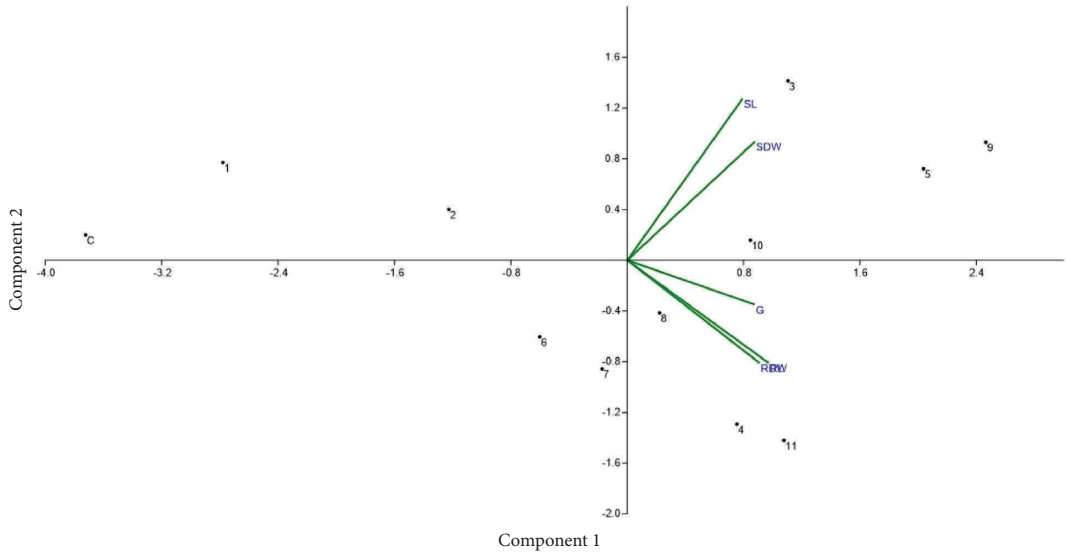
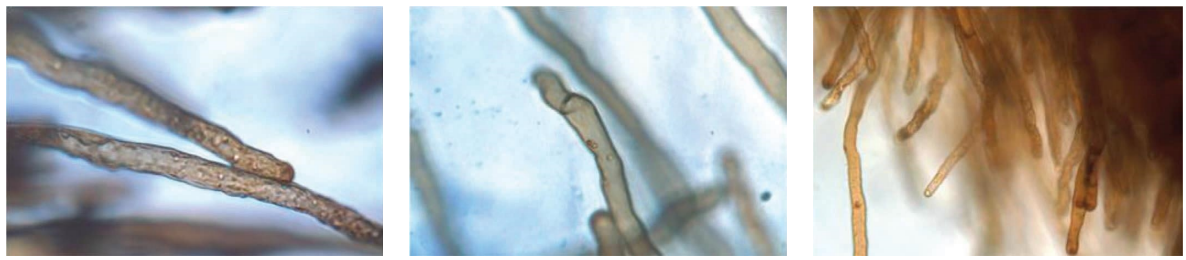


FIGURE 3: Biplot of Principal components 1 (*y* axis) and 2 (*x* axis) considering the following traits: shoot length (SL), shoot dry weight (SDW), root length (RL), root dry weight (RDW), and germination (G). The seedlings subjected to the 11 treatments and the control are distributed across the graph. The image was generated by PAST software version 2.17c.



(a) (b) (c)

FIGURE 4: Root hairs of *P. notatum* seedlings treated with SiNPs and observed under a light microscope. The images show transparent spots in the root hairs (arrows). (a) Seedling inoculated with 5% SiNPs. (b) Seedling inoculated with *S. marcescens* + 5% SiNPs. (c) Seedling inoculated with *E. asburiae* 5% SiNPs.

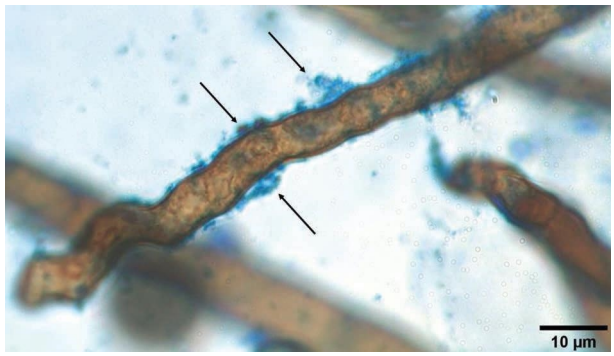


FIGURE 5: Bacteria around and in the root hairs of *P. notatum* seedlings (arrows) inoculated with *S. marcescens* and observed under a light microscope.

Karunakaran et al. [23] reported that the higher the concentration of nanosilica is the greater the viability and growth of PGPB. Furthermore, SiNPs were shown to promote 100% seed G and increase nitrogen, phosphorous, and calcium (NPK) contents in maize (*Zea mays*) plants. Similarly, Moradipour et al. [60] found improved RL, plant biomass, and seedling length of pistachio UCB-1 sprouts inoculated with PGPB encapsulated in SiNPs. The UCB-1 is an F1 hybrid of specific *Pistacia atlantica* (female) × *P. integerrima* (male) trees that were established in the 1980s for vigor, resistance to the fungal pathogen *Verticillium*, and cold tolerance [61]. In the present study, the improved seed G and root traits resulting from some treatments combining PGPB and SiNPs could be attributed to the presence of SiNPs that boosted bacterial activity since they can act as substrates or stimulants for microorganisms [26] and also to the synergistic effect of PGPB and SiNPs as both can induce the development of roots and increase nutrient uptake [59, 62].

The positive effect of nanosilica on PGPB's performance is supported by Ferrusquía-Jiménez et al. [63], who found that SiNPs at 100 ppm concentration increased the bacterial population, phosphate solubilization, and gibberellin production of *Bacillus cereus*. The specific mechanisms by which nanoparticles influence bacterial physiology are still not fully understood. Nevertheless, interactions between nanosilica

and bacteria may include gene expression alterations, ion exchanges, and cell membrane interactions [64]. The hydration property of SiNPs may facilitate bacterial attraction and increase their resistance to acidic conditions [65, 66]. Therefore, we hypothesize that SiNPs in biofertilizer formulations would increase bacterial populations and consequently enhance/extend the PGPB's beneficial properties to host plants in natural environments. Here, even when the nanosilica associated with bacteria did not significantly improve a plant growth trait, it could still be advantageous for crops in the long term, considering that seedlings were analyzed at 25 days old. However, further studies must be conducted to confirm this hypothesis. Furthermore, although the bacteria in the present study were isolated from other *Paspalum* species, the results show they can be suitable growth promoters for *P. notatum* plants.

In agreement with the data found in the present work, Siddiqui and Al-Whaibi [67] reported that SiNPs benefited tomato (*Lycopersicum esculentum*) plants through their ability to increase G and SDW. When absorbed by plants, Si is naturally plentiful in soils [68] and supports root growth through the promotion of cell wall extension [62, 69, 70]. In the present study, compared with both the control and the 5% SiNP treatment, the 10% SiNP treatment promoted root growth. However, the greatest RLs were observed when nanosilica was inoculated with PGPB.

Plants generally need silica to withstand biotic and abiotic stresses Ma and Yamaji [71]. Si improves water uptake efficiency and photosynthetic potential and promotes mechanical strength and stiffness of leaves, preventing plants from lodging and pathogen attacks [72–74]. Grasses (Poaceae family) such as *Paspalum* spp. have 10–20 times more silica within their structures than legumes (*Lathyrus sativus*) and other dicots [52, 75]. In the present work, the seedlings inoculated with SiNPs were more vigorous and upright, and their roots were thicker and more robust, whereas the roots of the controls were extremely thin and fragile. Plants absorb soluble Si from the soil through their roots and acropetally translocate it to the leaves, where this element is deposited in the form of silica bodies (SiO_2) [76, 77]. Therefore, the increased RDWs of the seedlings treated with SiNPs without bacteria can be partially explained by the primary accumulation of nanoparticles in the roots and by the improved root development triggered by the enhanced water uptake capacity due to the Si role in plant osmotic regulation [78, 79].

Furthermore, it is well known that silica promotes plant defense against biotic stress. Rangaraj et al. [24] found that a treatment composed of SiNPs and PGPB enhanced leaf maize stiffness and phenolic compound production, providing a physical barrier and inducing resistance against diseases. In addition, those same authors and Karunakaran et al. [23] reported that SiNPs associated with PGPB were more efficient than other Si sources in maize plants. The results found in the present work show that SiNPs can improve the efficiency of some species of PGPB in a dose-dependent manner.

Plants directly absorb some nutrients from endophytic microorganisms through a process called the rhizophagy cycle [80]. In the rhizophagy cycle, free-living microorganisms take nutrients from the soil and penetrate the plant root tips, becoming endophytic/intracellular. After the host plant extracts these nutrients through an oxidative process, the microbes' exhausted of nutrients leave the plant through the root hairs and recharge in the rhizosphere, starting the cycle again [80]. Considering the rhizophagy cycle dynamics, we hypothesize that the bacteria in the present study carried the SiNPs into the host plant, which produced reactive oxygen when colonized by the bacteria. As a result, the nanoparticles remained inside the root tissues.

In conclusion, the present investigation shows that SiNPs can be added to microbial biofertilizer formulations to improve the establishment and initial growth of bahiagrass pastures and broaden the knowledge concerning the interaction of nanosilica and PGPB for agricultural purposes. In summary, nanosilica could be used in nanobiofertilizer formulations with *A. faecalis* and *S. marcescens* for *P. notatum*. Nonetheless, investigations regarding the potential harm of these bacteria to living beings must be conducted to ensure their safety.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available in the UFSCAR repository at https://repositorio.ufscar.br/handle/ufscar/15187.

Ethics Statement

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

A.C.P.M. and P.T.L. conceived and designed the study. A.C.P.M., K.L.K., and L.S.R. conducted the experiments. L.S.R., E.R.C., J.F.W., and B.B.Z.V. contributed new reagents and analytical tools. A.C.P.M., K.L.K., J.F.W., B.B.Z.V., and A.P.F. analyzed the data. A.C.P.M. wrote the manuscript. All the authors read, revised, and approved the study.

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