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Biostimulant in acclimatization of micropropagated banana (*Musa* spp.) seedlings

Ana Janaína Oliveira Rodrigues[1], Christiana de Fátima Bruce da Silva[2]*, Alan Bernard Oliveira de Sousa[3], Marlos Alves Bezerra[2], Lamine Sanó[4], Genilton da Silva Faheina Júnior[2]

[1] Instituto Federal de Educação, Ciência e Tecnologia do Ceará, Crateús, CE, Brasil. E-mail: ana.janaina@ifce.edu.br
[2] Embrapa Agroindústria Tropical, Fortaleza, CE, Brasil. E-mail: christiana.bruce@embrapa.br; marlos.bezerra@embrapa.br; genilton@gmail.com
[3] Universidade Federal do Ceará, Fortaleza, CE, Brasil. E-mail: alan.sousa@ufc.br
[4] Universidade do Estado de Santa Catarina, Lages, Centro de Ciências Agroveterinárias, SC, Brasil. E-mail: lsano928@gmail.com

ABSTRACT: Microorganisms, such as growth-promoting bacteria, may favor the obtaining of better-quality banana (*Musa* spp.) seedlings. This study aimed to evaluate the effect of inoculation with growth-promoting bacteria *Bacillus* sp. on micropropagated seedlings of banana cv. 'Prata Catarina' in the acclimatization phase. The design was in randomized blocks, with 4 treatments (negative control: no inoculation and no fertilization; positive control: application of slow-release fertilizer; inoculation with *Bacillus* sp. strain 186; and inoculation with *Bacillus* sp. strain 109) and 4 replicates (14 experimental units), totaling 224 seedlings. The bacterial population in the rhizosphere was quantified and the growth of seedlings and contents of minerals were evaluated. Seedlings treated with slow-release fertilizer showed better performance for the number of leaves (6.14 ± 1.44), shoot (3.64 g ± 1.72) and root (4.76 g ± 3.23) fresh weight and root dry weight (0.72 g ± 0.43). Using strains alone did not promote the growth of seedlings, under the conditions of the present study. Nevertheless, the strains were efficient in increasing the nitrogen content in leaves (17.93 g kg^{-1} ± 0.73, strain 109) and potassium and magnesium contents in roots (6.96 g kg^{-1} ± 0.39 and 6.98 g kg^{-1} ± 0.40, strain 186).

Key words: *Bacillus* sp.; *Musa* spp.; plant growth

Bioestimulante na aclimatização de mudas micropropagadas de banana (*Musa* spp.)

RESUMO: Microrganismos, como as bactérias promotoras de crescimento podem favorecer na obtenção de mudas de bananeira (*Musa* spp.) de melhor qualidade. O estudo objetivou avaliar o efeito da inoculação com bactérias promotoras de crescimento *Bacillus* sp., em mudas micropropagadas de bananeira cv. 'Prata Catarina' na fase de aclimatização. O delineamento foi em blocos casualisados, com 4 tratamentos (controle negativo: sem inoculação e fertilização; controle positivo: aplicação de adubo de liberação lenta; inoculação com *Bacillus* sp. cepa 186; e cepa 109), 4 repetições (14 unidades experimentais), totalizando 224 mudas. A população bacteriana foi quantificada na rizosfera, avaliados o crescimento das mudas e teores de minerais. As mudas tratadas com o adubo de liberação lenta mostraram melhor desempenho para o número de folhas (6.14 ± 1.44), massa fresca de parte aérea (3.64 g ± 1.72), raiz (4.76 g ± 3.23) e seca da raiz (0.72 g ± 0.43). O uso das cepas sozinhas não promoveu o crescimento das mudas, nas condições em que o estudo foi conduzido. Entretanto, as cepas incrementaram o teor de nitrogênio nas folhas (17.93 g kg^{-1} ± 0.73, cepa 109) e nas raízes de potássio e magnésio (6.96 g kg^{-1} ± 0.39 e 6.98 g kg^{-1} ± 0.40, cepa 186).

Palavras-chave: *Bacillus* sp.; *Musa* spp.; crescimento vegetal

* Christiana de Fátima Bruce da Silva - E-mail: christiana.bruce@embrapa.br (Corresponding author)
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Introduction

Brazil is the world's fourth-largest producer of bananas (*Musa* spp.) (FAO, 2021). According to Ferreira et al. (2016), the increase in banana yield in Brazil is related to the quality of the seedlings used and to the management adopted during their cultivation. To ensure quality, the micropropagation technique has been used to produce banana seedlings. This technique consists of cultivating seedlings under aseptic conditions, where explants of stem apexes with a single bud can originate hundreds of seedlings with the same genotype of the mother plant in a few generations and free of phytosanitary problems (Ferreira et al., 2016). The seedlings produced by micropropagation are commercialized when they are 5 to 10 cm tall, requiring a pre-field step called acclimatization (Martins et al., 2011).

In the acclimatization phase, to accelerate the development and provide good vegetative growth of seedlings, commercial substrates, as well as slow- or controlled-nutrient release fertilizers should be used (Rodrigues et al., 2021). Inadequate use of fertilizers may lead to increase in substrate salinity (Medeiros et al., 2010). Growth-promoting microorganisms are an alternative to replace or reduce the use of fertilizers in seedling production (Maheshwari et al., 2013).

These microorganisms play a fundamental role in soil, participating in organic matter decomposition, making nutrients available and favoring their absorption by plants (Balota, 2018). Among the microorganisms, plant growth-promoting bacteria (PGPB) colonizing the roots of plants stand out. In this environment, PGPB multiply and promote numerous benefits, such as increased plant yield and quality. In addition, they can provide nutrients and protect plants from biotic and abiotic stresses (Glick, 2012; Patel et al., 2018). Among the PGPB, *Bacillus* stands out with excellent results for promoting the growth of crops (Pérez-García et al., 2011), such as bananas (Silva et al., 2018).

Therefore, the hypothesis tested in the present study was that the use of bacterial strains of the genus *Bacillus* promotes increments in the growth of micropropagated banana seedlings during the acclimatization phase. Thus, the objective of this study was to evaluate the effect of inoculation with growth-promoting bacteria, *Bacillus* sp. strains, on micropropagated seedlings of banana cv. 'Prata Catarina', during their acclimatization phase.

Materials and Methods

Description of the experimental site, treatments, and experimental design

The experiment was conducted in a screened greenhouse belonging to Embrapa Tropical Agroindustry, Fortaleza, Ceará, Brazil. According to Köppen's classification, the climate of the region is Aw' type, characterized as rainy tropical, of tropical savannah, with the driest season in winter and maximum rainfall in autumn. The greenhouse was 16 meters long by 4 meters wide, 2.5 meters high, being covered and closed on the sides with a 50% black shade net, allowing natural ventilation. During the experimental period, an average temperature of 27.8 °C and an average relative humidity of 72% were recorded. The experiment lasted 74 days. The banana (*Musa* spp.) seedlings, cv. 'Prata Catarina', were produced by micropropagation and acquired from a private seedling production company. The substrate used was Germina Plant Horta®, with a composition based on peat and charred rice husk, which underwent steam sterilization in a vertical autoclave, at a temperature of 121 °C and pressure of 1 atm, for one hour, a process that was repeated after 24 hours. The substrate was analyzed for its chemical characteristics, before and after sterilization (Table 1).

The seedlings were transplanted to the substrate 7 days after sterilization. For transplanting, the seedlings were removed from the glass containers containing culture medium, and their roots were washed in running water and cut to a maximum length of 1.0 cm. The seedlings were transplanted into 162-cell polyethylene trays, filled with sterile commercial substrate. After transplanting, the seedlings underwent a period of pre-acclimatization in a room under controlled temperature (28 °C) and artificial light. After seven days, they were taken to the greenhouse and arranged under reduced lighting, which was achieved with a black shade net placed above them. The shade net was removed after seven days, totaling 14 days after transplantation (DAT), and the seedlings were subjected to treatments. Irrigation in the greenhouse was performed by micro-sprinklers installed at the top of the structure. The micro-irrigation system was automatically actuated three times a day, for 15 minutes each.

The experimental design was in randomized blocks, with four treatments (1- negative control: no inoculation and no fertilization; 2- positive control: application of slow-release fertilizer 14-14-14; 3- inoculation with *Bacillus* sp., strain 186; 4- inoculation with *Bacillus* sp. strain 109) and four replicates of 14 experimental units, totaling 224 seedlings.

The *Bacillus* sp. strains (186 and 109) were collected from the rhizosphere of healthy banana trees from plantations in the Northeast region and are preserved in the working collection of Bacteria and Fungi of the Laboratory of Soil Microbiology, Embrapa Tropical Agroindustry. For the assay, the inoculum suspensions of the strains were prepared through the dilution of the colonies with 24 hours of growth in the exponential phase, until reaching the concentration of 1.2 x 10^9 CFU mL^{-1}, with the aid of the McFarland scale. Bacterial suspensions

Table 1. Chemical characteristics of the Germina Plant Horta® substrate before and after the autoclaving process.

Commercial substrate	pH	EC (dS m^{-1})	N-total (g kg^{-1})	Ca	Mg	K	Na	P	S	$N\text{-}NH_4$	$N\text{-}NO_3$
				(mg L^{-1})							
Not autoclaved	6.0	0.241	15.2	2197	375	27	63	1	1333	11	122
Autoclaved	5.7	0.249	15.1	2106	406	48	106	2	1466	47	63

were applied to the substrate in the collar region of the seedlings, by placing 2 mL per cell of the tray containing each seedling, using a sterile syringe. The slow-release fertilizer was applied to the planting substrate, by placing approximately 0.25 g of slow-release fertilizer 14-14-14 (5.0 kg m^{-3}) per plant cell (Rodrigues et al., 2021).

Quantification and isolation of bacterial strains from the rhizosphere of seedlings

Strains 186 and 109 associated with the roots of micropropagated banana seedlings were quantified 60 days after inoculation. For this, 10g of roots + rhizospheric substrate of the seedlings were homogenized, under shaking, with 90 mL of saline solution at 0.85% (8.5 g L^{-1}). Serial dilutions were performed up to 10^{-5} and heated in a water bath at 80 °C. Aliquots of the dilutions were inoculated in Petri dishes containing Kado and Heskett medium in triplicate. The Petri dishes were incubated in a BOD chamber at 35 °C for 48 h. After this period, the plates with the number of colonies were counted using a colony counter and the results were expressed in colony-forming units per gram (CFU g^{-1}) of the rhizosphere (roots + rhizospheric substrate). The colonies were confirmed as being of the *Bacillus* strains, through visualization of bacterial cells under optical microscope (100x).

Variables evaluated and statistical analyses

Evaluations related to growth promotion were performed at 74 days after transplantation (DAT), by measuring the following variables: a) plant height (cm): measured with a ruler from the substrate level to the last apical bud; b) pseudostem diameter (mm): measured with a digital caliper at about 1 centimeter above the plant collar; c) number of leaves: nominal count of fully expanded and photosynthesizing leaves, disregarding senescent leaves; d) root system length (cm): a ruler was used to measure the distance from the beginning of the rhizome to the extreme end of the root; e) leaf area (cm^2); determined using a leaf area meter (LI - 3100, Area Meter, Li-Cor., Inc., Lincoln, 87 Nebraska, USA); f) shoot and root fresh weight (g): determined by weighing fresh plant material on a precision scale; g) shoot and root dry weight (g): determined by weighing the plant material dried by the oven drying method at 65 °C on a precision scale until reaching constant mass.

For the analysis of minerals in leaves and roots, after drying in an oven with forced air circulation at a temperature of 65 °C, for a minimum period of 72 hours, the plant samples were crushed with an analytical mill (IKA A11 Analytical Mill) until obtaining small particles. For nitrogen analysis, the extraction was carried out using 0.2 g of plant material with digesting solution (175 mL of milli-Q water, 21.39 g of Na_2SO_4, 4.0 g of $CuSO_4.5H_2O$, plus 200 mL of concentrated H_2SO_4). After digestion, nitrogen was determined by steam drag distillation, followed by titration with diluted acid (0.01 N H_2SO_4). For analysis of the other minerals (macronutrients: K, P, Ca, Mg, Na; micronutrients: Mn, Zn, Fe, Cu), approximately 0.5 g of plant material was weighed, and 8 ml of an acid mixture (HNO_3 - $HCLO_4$, 3:1 ratio) were added. The mixture was kept cold for 3 to 4 hours and then taken to the digester block, where it was subjected to the initial temperature of 60 °C, which was increased every 30 minutes, until reaching the maximum temperature of 250 °C. After being removed from the block and back at room temperature, the digested samples were stirred in a vortex and transferred to a 50-mL volumetric flask, and the volume was completed with distilled water for subsequent filtering through slow-speed filter paper, thus being ready for determination. Mineral determination was performed using an inductively coupled plasma optical emission spectrometer (Agilent, ICP-OES 5100). This device made the readings simultaneously for all analytes (Miyazawa et al., 2009).

The data of all tests were subjected to analysis of variance (ANOVA) and the means were compared by F test ($p < 0.05$). When the variables showed significant differences, their means were compared by Tukey test. The statistical programs SAEG version 5.0 and Sisvar 5.6 were used to perform the analyses.

Results and Discussion

Quantification and isolation of bacterial strains of the rhizosphere of seedlings

There was a significant effect ($F = 9.40$, $p = 0.0064$) on the colony count between the two strains used, with strain 109 having a higher population mean (4.93 x 10^4 CFU g^{-1}) (Figure 1A) compared to strain 186 (3.8 x 10^4 CFU g^{-1}) (Figure 1B). Both inoculum populations decreased in comparison to the initial

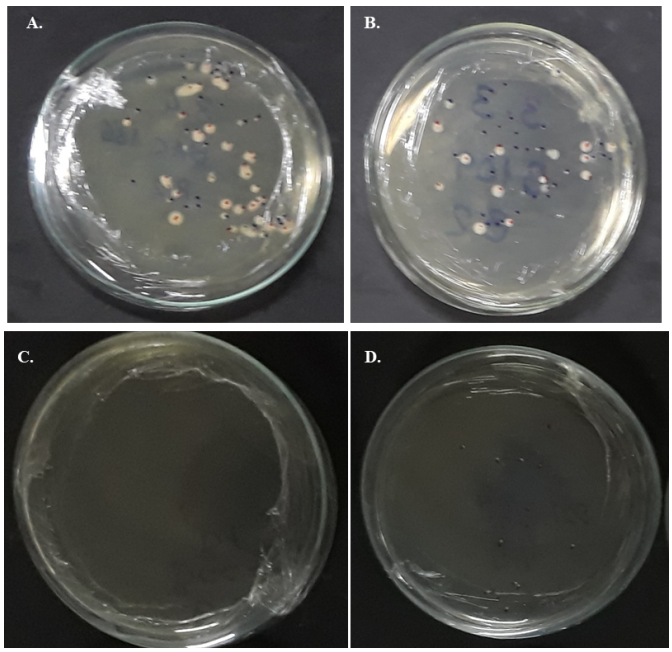


Figure 1. Count of colonies per plate, of the rhizosphere region of banana seedlings cv. 'Prata Catarina' in the acclimatization phase after 60 days, subjected to the treatments: A (inoculation with *Bacillus* sp. strain 109), B (inoculation with *Bacillus* sp. strain 186), C (water) and D (slow-release fertilizer). Count performed in triplicate (3 plates) per treatment, at dilution of 10^{-5}.

concentration of the suspension used (1.2 x 10^9 CFU mL^{-1}). In the negative control (seedlings inoculated with water) (Figure 1C) and positive control (fertilized seedlings) (Figure 1D), the presence of colonies of *Bacillus* strains was not detected.

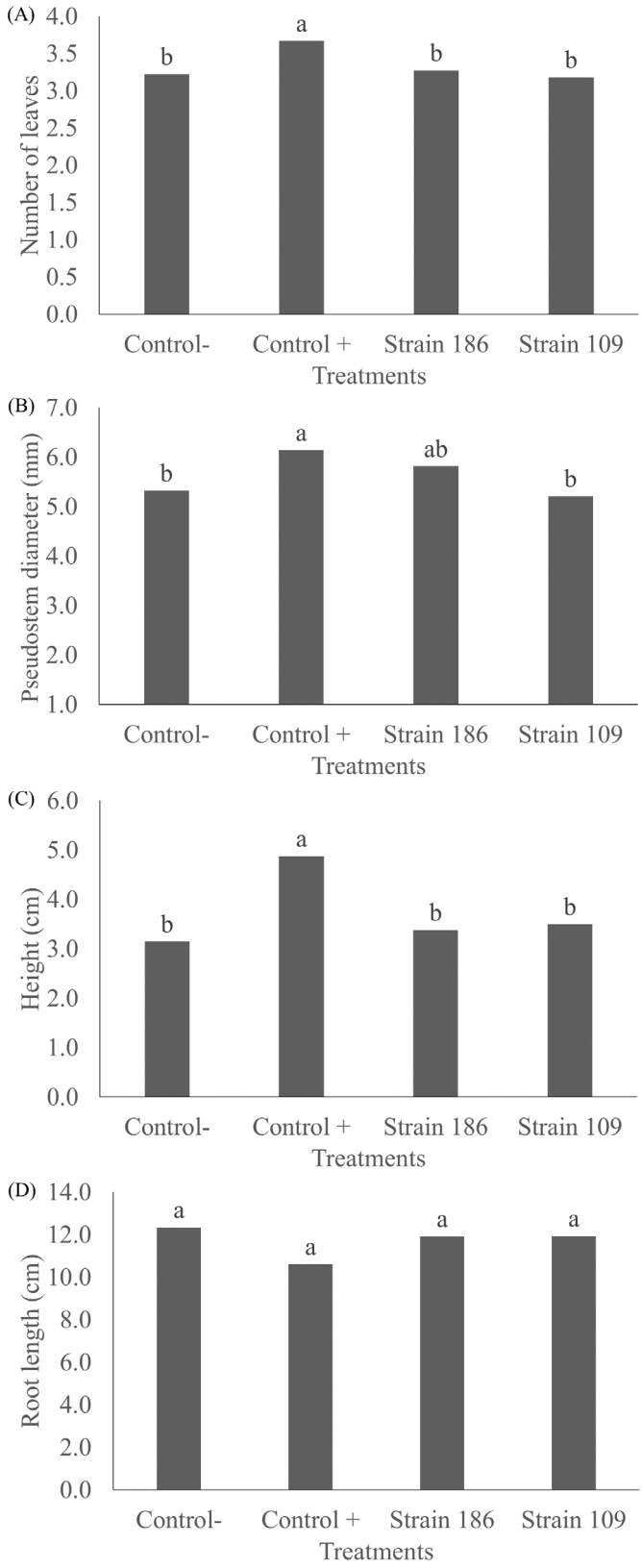
In the present study, the bacterial population in the rhizosphere of banana plants decreased over time. The initial inoculum of *Bacillus* sp. strains 186 and 109 supplemented to the micropropagated seedlings of banana cv. 'Prata Catarina' was 1.2 x 10^9 CFU mL^{-1}. This reduction is common because bacteria begin to enter the stationary phase. However, still in this phase these bacteria can express their potential, producing hormones such as indoleacetic acid (IAA), which promotes increments in some variables related to the growth of micropropagated seedlings (Maheshwari et al., 2013).

The low population level of bacteria in this experiment (in the treatments with inoculation of *Bacillus* sp. strains 186 and 109) may have been due to factors such as substrate moisture conditions and lack of nutrients for bacterial growth. Several factors can affect the growth and colonization of the bacterial population. According to Santoyo et al. (2017), it is difficult to specify all the factors that interfere in the soil microbiota, but they include abiotic factors, such as soil structure and texture concerning moisture and nutrient contents, aeration, and pH values, as well as biotic factors, such as the natural microbiota itself. The small number of bacteria in the soil may have affected the ability of these microorganisms to interfere with plant growth, since the ideal scenario is to have at least a concentration of 10^7 to 10^8 CFU mL^{-1} (Posada et al., 2018). In this study, the authors obtained positive results in promoting the growth of banana seedlings cv. Williams, inoculated with *B. subtilis*. The results of the present study also suggest the need for reapplication of bacteria at shorter time intervals, as well as better control of the conditions necessary for growth for more efficient colonization of the rhizosphere, and consequent improvement in the promotion of growth of banana plants in this stage of development.

Variables evaluated: promotion of growth of micropropagated seedlings of banana cv. 'Prata Catarina'

There were effects of the treatments on all variables related to the growth of micropropagated seedlings of banana cv. 'Prata Catarina'. Fertilization promoted the highest number of leaves, compared to the negative control, strains 186 and 109 (Figure 2A). This result may be related to the constant release of fertilizer along the experiment, which favored the maintenance of leaves on the seedlings. In addition, it reaffirms the need for reapplications of bacterial suspensions throughout plant development for growth improvements.

Pseudostem diameter as a function of the treatments showed a behavior similar to that of the number of leaves, with the largest diameter (6.14 mm ± 1.44) in the treatment that received fertilization, followed by the use of strain 186 (5.81 mm ± 2.58), which were superior to the negative control and use of strain 109 (Figure 2B). Seedling height was higher in the treatment with fertilizer, while the other treatments did not differ (Figure 2C). Several authors working with growth-



Control - (non-inoculated and non-fertilized plants); Control + (fertilized plants); Strain 186 and Strain 109 (*Bacillus* sp.), in which different lowercase letters indicate difference at 5% probability level by Tukey test.

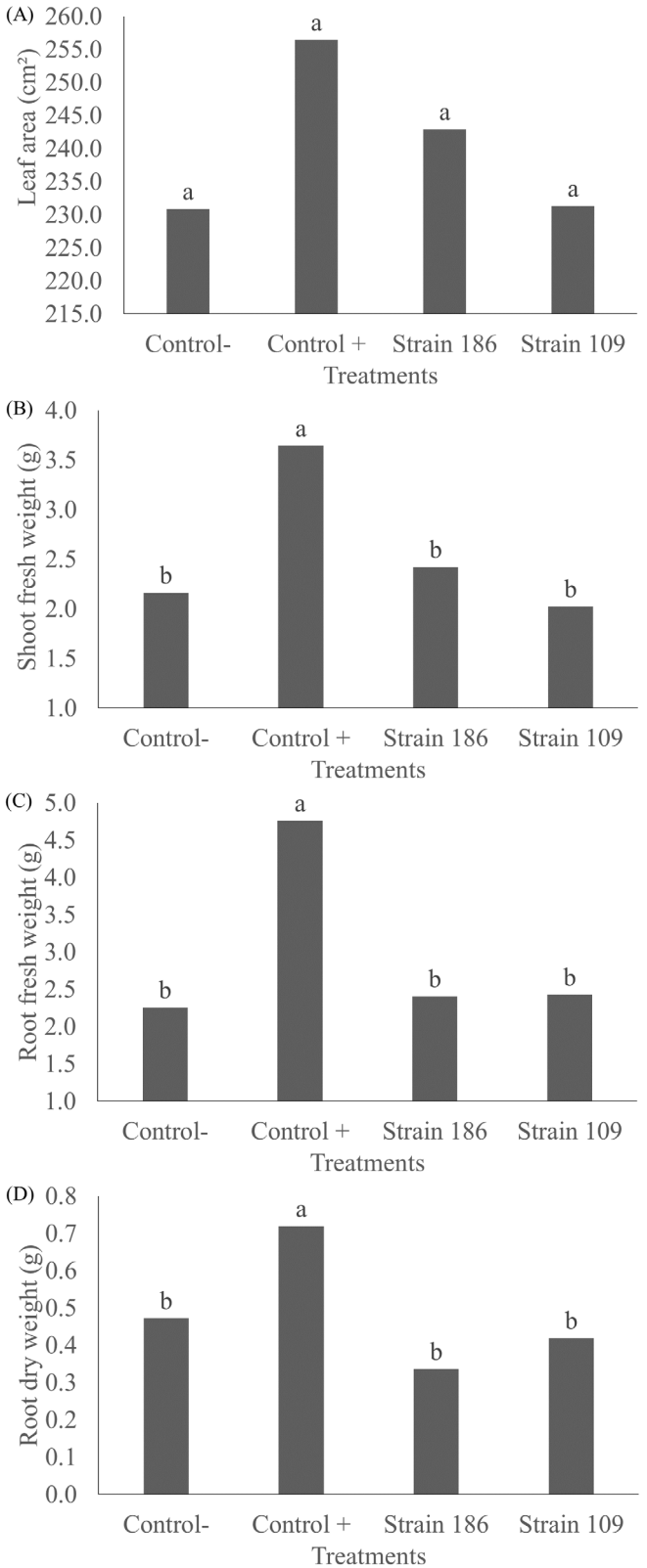
Figure 2. (A) Number of leaves, (B) Pseudostem diameter, (C) Height, (D) Root length of micropropagated seedlings of banana cv. 'Prata Catarina', subjected to treatment with inoculation of *Bacillus* sp. strains 186 and 109, evaluated at 74 days after transplanting (DAT), in the acclimatization phase.

promoting bacteria have found promising results, such as Cruz-Martín et al. (2015) when testing the use of growth-promoting bacteria *B. pumilus* in the acclimatization of 'Grande Naine' banana. In this work, the authors observed that there was a promotion of growth through the increase in height and pseudostem diameter. However, the inoculated plants did not show differences in the number of leaves per plant or root length in comparison to the non-inoculated control.

The length of the roots of the micropropagated seedlings of banana cv. 'Prata Catarina' averaged 11.70 cm, with similar performance among all treatments (F = 0.935, p = 0.463) (Figure 2D). Similar behavior occurred with leaf area, root fresh weight and shoot dry weight. Although the treatments did not differ, there were increments of 12.35% and 12.44% in the root length of plants inoculated with strains 109 and 186, respectively, compared to non-inoculated and non-fertilized plants. Leaf area showed no difference between treatments (F = 0.602, p = 0.629), and seedlings belonging to the positive control (fertilizer) had the highest mean values (256.41 cm² ± 52.06) (Figure 3A). As for root length, the use of strains 186 and 109 led to increases of 5.21% and 0.19%, respectively, in leaf area measurements compared to non-inoculated plants.

Regarding the fresh weight, there were statistically significant differences for shoots (Figure 3B) (F = 9.11, p = 0.004) and roots (Figure 3C) (F = 3.91, p = 0.048), with the positive control (fertilized plants) having the highest means for both variables (3.64 g ± 1.72 and 4.76 g ± 3.23, respectively). The other treatments did not differ from each other (Figure 3B and 3C). When the results obtained for these variables in inoculated plants were compared to those of non-inoculated and non-fertilized plants (control -), it was possible to note increments of 7.71% and 6.44% for shoot fresh weight and 7.55% and 6.66% for root fresh weight in plants inoculated with strains 109 and 186, respectively. On the other hand, root dry weight differed significantly (F = 5.53, p = 0.019), and the positive control treatment (fertilized plants) had a higher mean weight (0.72 g ± 0.43), while the other treatments did not differ from one another (Figure 3D).

For the variables root length, leaf area, shoot fresh weight, root fresh weight and shoot dry weight, increments were obtained in plants inoculated with strains 186 and 109, when compared to non-inoculated and non-fertilized plants. Gamez et al. (2019), evaluating the effect of nine PGPB of *Bacillus* and *Pseudomonas* on the growth of banana seedlings, obtained increments in plant height, number of leaves, leaf area, pseudostem diameter, root length and development, as well as shoot and root fresh and dry weight, compared to non-inoculated plants. In the same work, plants inoculated with rhizobacteria showed growth similar to that of plants under the application of 100% fertilization. According to Souza et al. (2015), a problem reported very frequently in studies with PGPB is the variability of the results. This may be related to variable root colonization, inoculum survival problems, or unfavorable conditions for the bacteria.



(A) Leaf area, (B): Shoot fresh weight, (C): Root fresh weight, (D): Root dry weight. Control - (non-inoculated and non-fertilized plants); Control + (fertilized plants); Strain 186 and Strain 109 (*Bacillus* sp.), in which different letters indicate difference at 5% probability level by Tukey test.

Figure 3. Variables related to the growth of micropropagated seedlings of banana cv. 'Prata Catarina', subjected to treatment with inoculation of *Bacillus* sp. strains 186 and 109, when compared to the control treatments (water and fertilizer) in the acclimatization phase at 74 DAT.

Variables evaluated: analysis of minerals in the leaves and roots of micropropagated seedlings of banana cv. 'Prata Catarina'

Regarding the mineral contents in the shoots, differences between treatments were observed only for nitrogen (F = 4.12, p = 0.043) and phosphorus (F = 15.53, p = 0.007) (Table 2). Regarding the roots, there were differences between the treatments for potassium (F = 6.01, p = 0.015), calcium (F = 10.8, p = 0.002) and magnesium (F = 5.9, p = 0.016), while the other minerals showed no differences (Table 2).

For the nitrogen content in the shoots, seedlings inoculated with strain 109 (17.93 g kg^{-1} ± 0.73) showed an increase of 16.7% in comparison to the positive control, without differing from the other treatments. For phosphorus in the shoots, a higher mean was observed in seedlings treated with slow-release fertilizer (0.88 g kg^{-1} ± 0.26) (Table 2). The N and P contents present in the leaves of the seedlings in the treatments with both application of slow-release fertilizer and *Bacillus* sp. strains (Table 2) were below the critical range for 'Prata-Anã' banana leaves recorded by Carvalho Júnior et al. (2019) (23.8 and 1.7 g kg^{-1}, respectively). This indicates that the seedlings were not within the adequate nutrition levels, regarding the nutrients of greatest requirement, which are recommended by Borges et al. (2016) in the following descending order: K > N > S > Mg > Ca > P. In the present study, the extraction pattern found for the shoot was: N > Ca > Mg > K > S > P > Na. For the roots, the extraction pattern observed, in descending order, was K > Mg > Ca > S > P (Table 2).

Banana is a very demanding plant in terms of nutrition. The amount of nitrogen required by bananas is continuous during most of their cycle, especially during the period of vegetative development. N favors the production and development of tillers, in addition to increasing the amount of dry matter. The lack of nutrient reduces the number of leaves and increases the number of days for the emergence of a leaf. P favors the vegetative development and the root system, and the lack of this nutrient causes stunted plant growth and poor root development (Borges et al., 2016). Paula et al. (2015) mention that the process of potassium absorption can be affected in situations of high concentrations of Mg^{2+}, and there may be inhibition of its absorption. The analysis of the chemical composition of the substrate used after autoclaving shows that the Ca and Mg contents were 43.8 and 8.46 times higher, respectively, than the K content, which justifies the pattern of nutrient extraction by the seedlings, as well as their contents in the plants being lower than that proposed by Carvalho Júnior et al. (2019), which is 35.6 g kg^{-1}. The same occurs for the phosphorus content present in the chemical composition of the substrate, where there was a small increase of 2 times the initial value (1 mg L^{-1}) after autoclaving. However, this value remained much lower than those of the other exchangeable cations (K, Ca and Mg), which justifies its position in the pattern of nutrient extraction by the seedlings and its low contents in the plants, lower than that proposed by Carvalho Júnior et al. (2019), which is 1.7 g kg^{-1}. The sulfur contents are also lower than the ideal (Table 2), which is below 1.8 g kg^{-1}, possibly attributed to its low content in the substrate (1466 mg L^{-1}).

When analyzing the minerals in the roots, it was observed that seedlings inoculated with strain 186 showed increments of 53.3% and 52.2% in potassium and magnesium contents, respectively, compared to the positive control (6.96 g kg^{-1} ± 0.39 and 6.98 g kg^{-1} ± 0.40), not differing from the other treatments. The highest calcium content was found in seedlings that received mineral fertilization (6.03 g kg^{-1} ± 0.72), with no difference between the other treatments (Table 2). Growth-promoting bacteria can act on the solubilization of nutrients, such as phosphorus and potassium, in the soil or substrate, making them more readily available to plants, and can promote improvements in the uptake of water and nutrients (Abhilash et al., 2016). Paungfoo-Lonhienne et al. (2019), investigating the use of growth-promoting bacteria, concluded that these in combination with inorganic and organic fertilizers have the potential to improve nitrogen absorption efficiency and reduce the environmental risks associated with its leaching. In another study, the use of rhizobacteria (*Bacillus* sp.) as

Table 2. Means of macronutrient contents in leaves and roots of micropropagated seedlings of banana cv. 'Prata Catarina', subjected to treatment with inoculation of *Bacillus* sp. strains 186 and 109, when compared to the control treatments (non-inoculated plants and fertilized plants).

	Means						
	Macronutrients in leaves (g kg^{-1})						
	N	P	K	Ca	Mg	S	Na
Control -	16.88 AB	0.34 B	7.21 A	7.29 A	7.22 A	0.62 A	0.27 A
Control +	15.37 B	0.88 A	7.72 A	9.16 A	7.72 A	0.67 A	0.18 A
Strain 186	17.70 AB	0.32 B	7.40 A	8.18 A	7.41 A	0.59 A	0.26 A
Strain 109	17.93 A	0.32 B	7.72 A	6.69 A	7.72 A	0.56 A	0.28 A
	Macronutrients in roots (g kg^{-1})						
		P	K	Ca	Mg	S	
Control -		0.38 A	6.37 AB	4.19 B	6.38 AB	1.24 A	
Control +		0.46 A	4.55 B	6.03 A	4.58 B	1.30 A	
Strain 186		0.34 A	6.96 A	3.87 B	6.98 A	1.32 A	
Strain 109		0.34 A	6.42 AB	4.15 B	6.43 AB	1.15 A	

Control - (non-inoculated and non-fertilized plants); Control + (fertilized plants); Strain 186 and Strain 109 (*Bacillus* sp.). In the columns, means followed by different letters indicate difference between treatments at 5% probability level by Tukey test.

Table 3. Means of micronutrient contents in leaves and roots of micropropagated seedlings of banana cv. 'Prata Catarina', subjected to treatment with inoculation of *Bacillus* sp. strains 186 and 109, when compared to the control treatments (non-inoculated plants and fertilized plants).

	Means						
	Micronutrients in leaves			Micronutrients in roots			
	(mg kg^{-1})						
	Cu	Fe	Mn	Cu	Fe	Zn	Mn
Control -	16.88ns	0.34 ns	7.21 ns	4.75 ns	1289.00 ns	68.13 ns	93.38 ns
Control +	15.37 ns	0.88 ns	7.72 ns	7.25 ns	2271.88 ns	61.00 ns	65.13 ns
Strain 186	17.70 ns	0.32 ns	7.40 ns	7.13 ns	1087.13 ns	73.75 ns	86.63 ns
Strain 109	17.93 ns	0.32 ns	7.72 ns	5.75 ns	1276.63 ns	55.75 ns	91.25 ns

Control - (non-inoculated and non-fertilized plants); Control + (fertilized plants); Strain 186 and Strain 109 (*Bacillus* sp.). In the columns, means followed by different letters indicate a difference between treatments at 5% probability level by Tukey test. ns: not significant.

biostimulant and biofertilizer for growth of banana seedlings, associated with a minimum supply of fertilizer, was effective to increase plant growth and nutrient absorption (N and P in roots) (Moreira et al., 2021). The use of strains 109 and 186 may have contributed to the release of nutrients to the seedlings. The concentrations of minerals in the leaves (N, K, Ca, Mg, S and Na) and in the roots (P, K, Mg and S) were either higher or equal in these treatments, compared to that with the use of slow-release fertilizer. Thus, it demonstrates the possible potential of using these microorganisms to improve the utilization of nutrients by plants.

For all treatments (seedlings not treated, seedlings fertilized, and seedlings inoculated with *Bacillus* sp. strains) applied, there were no significant differences between the contents of micronutrients (Table 3). Only Fe and Mn contents in leaf tissues were below the ideal ones according to Carvalho Júnior et al. (2019) (Table 3). According to these authors, the critical levels of micronutrients for banana crop are Fe - 62.3; Cu - 7.1; Mn - 280.3; Zn - 17.9 mg kg^{-1}. For the banana crop, the micronutrient extraction pattern in descending order is Mn > Fe > B > Zn > Cu (Borges et al., 2016). In the present study, different contents can be observed, with the following nutrients being absorbed, in descending order, for leaves and roots, respectively: Cu > Mn > Fe, and Fe > Mn > Zn > Cu. According to Bindraban et al. (2015), for plants to have normal growth, it is necessary to provide both macro and micronutrients, as well as water and solar energy so that they can synthesize all the necessary compounds. In another study, Santos et al. (2021) also found non-significant effects on some minerals (Cu and Mn) in treatments inoculated with *B. subtilis* and *B. megaterium* strains. In turn, Fe showed a reduction, probably due to the competition with bacteria for the nutrient, and manganese showed the opposite behavior. In the present study, inoculation with the bacterial strains had no influence (positive or negative) on the contents of micronutrients in the micropropagated seedlings of banana cv. 'Prata Catarina'.

Conclusions

Micropropagated seedlings of banana cv. 'Prata Catarina' fertilized with slow-release fertilizer showed better performance for height, number of leaves, shoot and root fresh weight and root dry weight. The use of *Bacillus* sp. strains 186 and 109 did not promote the growth of micropropagated seedlings of banana cv. 'Prata Catarina' at the end of the acclimatization phase. Bacterial strain 109 promoted an increase in leaf nitrogen content, while strain 186 increased potassium and magnesium contents in the roots.

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Compliance with Ethical Standards

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