





GROWTH PROMOTERS IN BANANA SEEDLINGS SUBMITTED TO SALINE IRRIGATION

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ABSTRACT

The objective of this study was to evaluate the effect of inoculation of a plant growth promoter bacteria on the growth of micropropagated banana seedlings cultivar Williams under irrigation with water at different saline levels. The experiment was carried out in a greenhouse at Embrapa Agroindústria Tropical, Fortaleza, State of Ceará. The experimental design was in randomized blocks, in a 3 x 4 factorial scheme, corresponding to the three factors for growth promotion (negative control: water; Osmocote® slow-release fertilizer and a *Bacillus* spp. bacterium) subjected to four levels of irrigation water salinity (S1 = 0.5; S2 = 1.5; S3 = 3.0 and S4 = 4.5 dS m⁻¹), and five blocks, totaling 60 experimental units. Sixty days after transplanting (DAT) and application of treatments, the following variables related to plant growth were measured: number of leaves (NL), pseudostem diameter (PD), plant height (PH), leaf area (LA), and root length (RL). The rise in saline levels in the irrigation water negatively influenced the variables number of leaves, pseudostem diameter, root length, and leaf area, showing a decreasing linear behavior. The variables number of leaves and leaf area of seedlings inoculated in association with *Bacillus* spp. did not differ from each other, regardless of the saline level. This indicated a likely increase in the response to the salinity tolerance of the seedlings. Treatment with Osmocote® fertilizer differed statistically for variables plant height, pseudostem diameter, and leaf area.

Palavras-chave:

Musa spp.

Condutividade elétrica

Bacillus spp.

Variáveis biométricas

PROMOTORES DE CRESCIMENTO EM MUDAS DE BANANEIRA SUBMETIDAS À IRRIGAÇÃO SALINA

RESUMO

Objetivou-se avaliar o efeito da inoculação de bactéria promotora de crescimento de planta no desenvolvimento de mudas micropropagadas de bananeira cultivar Williams, submetidas à irrigação com água em diferentes níveis salinos. O experimento foi conduzido em casa de vegetação na Embrapa Agroindústria Tropical, Fortaleza, Ceará. O delineamento experimental foi em blocos ao acaso, em esquema fatorial 3 x 4, referentes aos três fatores para promoção de crescimento (controle negativo: água, adubo de liberação lenta Osmocote® e a bactéria *Bacillus* spp.), submetidos à quatro níveis de salinidade da água de irrigação (S1 = 0,5; S2 = 1,5; S3 = 3,0 e S4 = 4,5 dS m⁻¹), e cinco blocos, totalizando 60 unidades experimentais. Decorridos 60 dias após o transplante (DAT) e a aplicação dos tratamentos, foram mensuradas as variáveis relacionadas ao crescimento das plantas: número de folhas (NF), diâmetro do pseudocaule (DP), altura da planta (AP), área foliar (AF) e comprimento radicular (CR). A elevação da salinidade da água de irrigação influenciou negativamente as variáveis número de folhas, diâmetro do pseudocaule, comprimento radicular e área foliar exibindo ajuste linear. As variáveis número de folhas e área foliar das mudas inoculadas com isolados de *Bacillus* spp. não diferiram entre si, independentemente do nível salino. Indicando provável incremento à resposta a tolerância à salinidade das mudas. O tratamento com o adubo Osmocote® diferiu estatisticamente dos demais tratamentos avaliados para as variáveis, altura da planta, diâmetro do pseudocaule e área foliar.

INTRODUCTION

Banana (*Musa* spp.) is one of the most consumed fresh tropical fruits in the world. It is native to Asia. Among the largest banana producers in 2018, the following stand out: India, China, Indonesia, Brazil, and Ecuador (AGRIANUAL, 2021). In the Brazilian agricultural scenario, banana production has emerged as a segment in remarkable expansion. In 2019, its production reached about 7.1 million tons (IBGE, 2020).

In 2018, the Brazilian regions that stood out in fruit production were the Southeast (2,316,279 t) and the Northeast (2,274,779 t), led by the states of São Paulo, Bahia, Ceará, and Pernambuco, which together account for 40.34% of the domestic production (AGRIANUAL, 2021). The main cultivars produced for the domestic market belong to the subgroups Prata, Pacovan, Prata Anã, Maçã, Mysore, Terra, and Angola, while for export the most exploited are the cultivars Nanica, Nanicão and Grande Naine (MENDONÇA *et al.*, 2013).

The competitiveness of the crop in the country has been attributed to a set of measures adopted in the banana production system. Among these factors, the following can be highlighted: the use of healthy and vigorous seedlings from tissue culture (micropropagation), the availability through genetic improvement programs of different cultivars adapted to biotic stress conditions (diseases and pests), as well as, the use of management practices in pre- and post-harvest, especially those related to plant nutrition, irrigation and phytosanitary aspects (FERREIRA *et al.*, 2016).

Banana is a highly demanding crop in terms of fertilization (CREMONESI, 2016) and requires well-distributed rainfall and soil moisture availability to reach desirable levels of productivity for the market (VOSSELEN *et al.*, 2005). However, in most of the Brazilian arid and semi-arid regions, rainfall is insufficient to meet the water need by the crop, therefore impelling the use of irrigation (MEDEIROS *et al.*, 2013). The scarcity of good-quality water has resulted in the intensive use of poor-quality water, that is, saline water (GONDIM *et al.*, 2005).

Therefore, the occurrence of these problems highlights the importance of adopting management practices and technologies that allow good plant development under conditions of abiotic stress. In this sense, plant growth-promoting bacteria (PGPB) have been shown as an environmentally

viable and safe alternative. Furthermore, these microorganisms have shown to be promising in the bioremediation of saline soils and plant development under these conditions (DIMKPA *et al.*, 2009; LEITE *et al.*, 2014).

PGPB are microorganisms that colonize the rhizospheric region of plants. These microorganisms associate with plant roots and stimulate their growth using different mechanisms. Among the mechanisms, the following stand out: phosphate solubilization, biological nitrogen fixation, production of growth-regulating hormones, siderophores, and compounds related to tolerance to biotic and abiotic stresses (LUGTENBERG; KAMILOVA, 2009; GLICK, 2012).

Despite its relevance, few studies address the use of PGPB in the reduction of the effects of salinity in banana production. According to Rodrigues *et al.* (2021) besides being ecologically correct the inoculation of PGPB can attenuate the effects of salt stress, enabling, according to Soares *et al.* (2016), the use of saline water in plant production. Thus, the objective of this work was to evaluate the effect of inoculation of plant growth-promoting bacteria on the development of micropropagated seedlings of banana cultivar Williams, submitted to irrigation with water at different saline levels.

MATERIAL AND METHODS

The experiment was carried out from May to July 2019 in a greenhouse at Embrapa Agroindústria Tropical, Fortaleza, State of Ceará. The climate in the area is the Aw'-type, according to the classification of Köppen (1948) and the experimental area is located within the geographic coordinates of 3°45'S of latitude and 38°34'W longitude, located at 19.5 m above sea level. The dimensions of the greenhouse were 16m x 4m x 2.5m (length, width, and height, respectively) fully covered and closed on the side with 50% light interception shading. During the experimental test period, it was recorded an average temperature of 27.3°C, accumulated rainfall of 698.2 mm, and RH of 77.66%, according to data collected by the Weather Station of the Federal University of Ceará, Pici campus.

Micro-propagated seedlings of banana cultivar Williams, acquired from Biofactory Bioclone Produção de Mudanças LTDA (Eusébio-CE) were used. The seedlings were removed from the flasks (*in vitro* condition) and their roots were washed in

running water, in order to remove the excess of the culture medium. Before planting, the roots of the seedlings were trimmed with the aid of scissors disinfected with sodium hypochlorite (NaClO, 2%). Afterward, the seedlings were transferred to polyethylene trays composed of 162 cells.

The substrate used in the trays was autoclaved and prepared in a 1:1 ratio of sieved soil and Germina Plant Horta substrate (peat and carbonized rice husk). The 60 seedlings were planted in intercalated cells, for their better development.

At this stage, the following procedures were performed: 20 seedlings were fertilized with the slow-release fertilizer – Osmocote® 14-14-14 (dose 5.0 kg m⁻³) (NOMURA *et al.*, 2008). Another 20 seedlings were inoculated through soil irrigation (4 mL per seedling, 1.2 x 10⁹ CFU mL⁻¹) with a *Bacillus* spp. strain. The other 20 plants referring to the negative control received only irrigation water. Seedling bacterization was repeated 30 days after the first inoculation. The bacterial isolate inoculum had its concentration adjusted to 1.2 x 10⁹ CFU mL⁻¹ using the MacFarland scale (LELLIOTT; STEAD, 1987).

After fertilization and bacterization, the trays were placed on benches for 45 days. Then, the seedlings were transplanted into polyethylene bags filled with 1.5 kg of the sterile substrate in a 1:1 ratio (1 autoclaved sieved soil:1 autoclaved commercial substrate) irrigated to the maximum water retention capacity by the substrate. The need for daily application of saline irrigation was established by weighing the seedling (bag + substrate + plant) on a digital scale.

Irrigations were carried out manually with the aid of a beaker on alternate days, to provide a 20% leaching fraction. The application of saline-water treatments was carried out after the seedling fertilization and bacterization procedures. Pests were controlled by monitoring the presence of insects and water spraying with liquid glycerin soap twice a week. The incidence of phytopathogens was not found in the seedlings.

The experiment was carried out in a randomized block design (DBC), in a 3x4 factorial arrangement, corresponding to three growth-promoting treatments (negative control - C; Osmocote® 14-14-14 slow-release fertilizer at a dose of 5.0 kg m⁻³ - F; and the bacteria *Bacillus* spp. - B), subjected to four levels of salinity of the irrigation water (S1 = 0.5; S2 = 1.5; S3 = 3.0 and S4 = 4.5 dS m⁻¹), defined according to the banana plant tolerance to salinity

and pre-established in the literature (BORGES *et al.*, 2004), consisting of five replications, totaling 60 experimental units. Treatment with water (C) represented the negative control (absence of application).

Except for the saline level of 0.5 dS m⁻¹, which corresponded to the salinity of the local water supply, solutions with different salt levels were prepared by adding sodium chloride, calcium chloride, and magnesium chloride, in a proportion equivalent to 7:2:1 (AQUINO *et al.*, 2007). The adjustment of electrical conductivity was performed with the aid of a portable conductivity meter (model DIST4 brand Hanna) following the relationship between the electrical conductivity of the irrigation water (EC_w) and the concentration of salts (mmol L⁻¹ = EC x 10) extracted from Rhoades *et al.* (1992).

At 30 and 60 days after transplanting (DAT), the characteristics related to growth, number of leaves (NL), pseudostem diameter (PD), and plant height (PH), were quantified. The number of leaves was characterized by manual counting, plant height (cm) was measured with the aid of a millimeter ruler and the pseudostem diameter (mm) was measured using a digital caliper.

At the end of 60 DAT, the seedlings were partitioned in the aerial part and root system, followed by drying in a forced air circulation oven at 65°C, for 72 hours. Next, the length of the root system (RL) and leaf area (LA) were quantified. Root length (cm) was measured with a millimeter ruler and leaf area (cm²) was measured with a surface meter (LI-3100, Area Meter, Li-Cor., Inc., Lincoln, 87 Nebraska, USA). The dry mass of the aerial part and root (g) was obtained by weighing in a semi-analytical scale.

Data were subjected to analysis of variance by the F test, with the application of the test of Tukey at 5% significance for comparison of treatment means. When saline levels were significant, regression analysis was performed. Statistical analyses were performed using the SAEG software (SAEG, 2000).

RESULTS AND DISCUSSION

The summary of the analysis of variance of the variables number of leaves (NL), plant height (PH), pseudostem diameter (PD), root length (RL), and leaf area (LA) are shown in Table 1. An interaction was observed between the source of variation in irrigation water salinity levels and treatments

(negative control - C, slow-release fertilizer Osmocote® - AD, and the bacteria *Bacillus* spp. - B), for the variables, NL, PD, and LA at 5 % and 1% probability by the F test (Table 2, 3 and 4), respectively.

It can be seen that the source of variation salinity containing four levels (CEW of 0.5; 1.5; 3.0; 4.5 dS.m⁻¹) showed a significant effect for all variables, except AP, showing a linear decrease with a unit increment of 10.49%, 6.35%, 11.62%, and 16.68%, for the NL, PD, RL and LA variables (Figures 1A, 1B, 1C and 1D), respectively. For the treatment (slow-release commercial fertilizer; bacteria 186 and control) we observed a significance in the variables PH, PD, and LA (Figures 2A, 2B, and 2C), respectively.

Studies report results similar to those found in this study. Bastos *et al.* (2020) found negative effects of salinity in cv. Prata Catarina banana seedlings. These effects were observed for the following variables related to seedling growth: height of the aerial part, pseudostem diameter, number of leaves, leaf area, dry mass of the aerial

part. The positive effect was found only for the water content in the leaf.

In another study, Carmo (2003) obtained similar responses when reductions in the number of leaves, height, pseudostem diameter, and leaf area of the banana cultivars Marmelo and Pacovan were observed under increasing levels of salinity of the irrigation water. Abreu *et al.* (1982) reported that the banana crop is sensitive to salinity, requiring irrigation water EC lower than 1.00 dS m⁻¹ for better vegetative development.

As for the number of leaves (NL), the interaction of mean saline concentrations within the growth promoters shows that all treatments at 0.5 and 1.5 CEW (dS m⁻¹) did not show statistical differences among themselves (Table 2). However, the salinity effect of the irrigation water was more accentuated in the negative control (C) and fertilizer (F) treatments, when submitted to the level of 4.5 dS m⁻¹. Seedlings inoculated with *Bacillus* spp. (bacterization) showed no significant difference between salinities, demonstrating greater tolerance to the effects of salinity.

Table 1. Summary of analysis of variance and regression for number of leaves (NL), plant height (PH), pseudostem diameter (PD), root length (RL), and leaf area (LA) of banana seedlings cv. Williams, irrigated with different electric conductivity

SV	DF	Mean square				
		NL	PH	PD	RL	LA
Block	4	1.373 ^{ns}	0.880 ^{ns}	15.525 ^{ns}	77.705 ^{ns}	33649.14 ^{ns}
Salinity (S)	3	22.723*	10.634 ^{ns}	42.024**	663.784*	820209.5*
Linear	1	67.135*	30.428**	110.073*	1927.058*	2165807*
Quadratic	1	1.035 ^{ns}	1.092 ^{ns}	0.025 ^{ns}	42.552 ^{ns}	292597**
Deviations	1	0.000 ^{ns}	0.380 ^{ns}	15.973 ^{ns}	21.741 ^{ns}	2224.765 ^{ns}
GP	2	4.482 ^{ns}	74.698*	204.376*	60.346 ^{ns}	1297930*
S x GP	6	3.318**	15.141 ^{ns}	34.915**	274.593 ^{ns}	303377.2*
Residue	44	1.397	7.450	12.443	127.357	68857.490
CV (%)		22.065	24.408	28.467	44.851	50.819

SV= source of variation; DF= degree of freedom; CV= coefficients of variation; GP – Growth promoters; ^{ns} – Not significant, * and ** - Significant at 5 and 1% of probability by the F rest, respectively

Table 2. Interaction between salinity (S) and growth promoter (GP) for the variable number of leaves (NL) of cv. Williams banana plants under different levels of irrigation water salinity

Growth promotor	Number of leaves			
	0.5 (dS m ⁻¹)	1.5(dS m ⁻¹)	3.0(dS m ⁻¹)	4.5(dS m ⁻¹)
C	6.00 A ab	5.60 A a	6.00 A a	3.25 B a
F	7.80 A a	7.00 AB a	5.00 BC a	3.75 C a
B	5.80 A c	5.60 A a	4.25 A a	4.25 A a

C= negative control (water); F= Osmocote® fertilizer; B= *Bacillus* spp. Means followed by the same upper-case letter in the lines and lower-case letter in the columns are not different from each other by the test of Tukey at 5% of probability

In salt stress conditions, one of the possibilities of the plants to maintain water absorption is also related to the reduction in the number of leaves, as a consequence of morphological and anatomical changes, reflecting in the reduction of transpiration (OLIVEIRA *et al.*, 2011).

There was no significant difference between the seedlings treated with the negative control and those inoculated with *Bacillus* spp. when observing the effect of salinity under the pseudostem diameter variable (Table 3).

For the variable LA, there was no significant difference between the salinity levels for the negative control and *Bacillus* spp. (Table 4). The treatment with Osmocote® fertilizer irrigated with a saline concentration of 0.5 dS m⁻¹ showed a higher mean for LA, differing statistically from the other levels of salinity.

Munns *et al.* (2008) in studying salinity tolerance mechanisms, explain that the reduction in the leaf area is also caused by the deviation of the energy necessary for the activation and maintenance of metabolic activities associated with salinity tolerance. Accordingly, Silveira *et al.* (2016) claim that salinity causes metabolic disturbances in plants, resulting in restrictions on growth and water transport, the latter being, according to

Carmo *et al.* (2003) and Teixeira, (2014) one of the main factors of cell wall expansion and, under limitations, would cause a reduction in the number of leaves, leaf area, and height of the banana tree.

The analysis in the salinity levels in the irrigation water (Figure 1A, 1B, 1C, 1D) showed in both variables number of leaves (NL), pseudostem diameter (PD), root length (RL), and leaf area (LA) a decreasing linear behavior, with coefficients of determination of 0.98; 0.87; 0.97; 0.88 respectively, revealing that, while the salinity levels in the irrigation water increased, there were decreases of 10.49%, 6.35%, 11.62%, and 16.68%, respectively, per unit increment of electrical conductivity.

The same result was also observed by Teixeira *et al.* (2014), who observed in the experiment that the effect of salinity on the biometric parameters of plants follows a decreasing linear behavior, in which as the water salinity increases, biometric measurements are reduced. Munns (2002) highlighted that NaCl especially alters the activity of certain enzymes which results in the reduction in the synthesis and translocation of hormones synthesized in the root, required for leaf metabolism, resulting in less leaf growth, and thus, smaller photosynthetic area of the plant.

Based on the graphs (Figure 2A, 2B, and 2C),

Table 3. Interaction between salinity (S) and growth promoter (GP) for the variable pseudostem diameter (PD) of cv. Williams banana plants under different levels of irrigation water salinity

Growth promotor	Pseudostem diameter			
	0.5 (dS m ⁻¹)	1.5(dS m ⁻¹)	3.0(dS m ⁻¹)	4.5(dS m ⁻¹)
C	10.80 A b	10.42 A b	13.54 A a	9.10 A a
AD	18.84 A a	19.99 A a	11.26 B a	14.13 AB a
B	11.50 A b	11.40 A b	8.98 A a	8.75 A a

C= negative control (water); F= Osmocote® fertilizer; B= *Bacillus* spp. Means followed by the same upper-case letter in the lines and lower-case letter in the columns are not different from each other by the test of Tukey at 5% of probability

Table 4. Interaction between salinity (S) and growth promoter (GP) for the variable leaf area (LA) of cv. Williams banana plants under different levels of irrigation water salinity

Growth promotor	Leaf area			
	0.5 (dS m ⁻¹)	1.5(dS m ⁻¹)	3.0(dS m ⁻¹)	4.5(dS m ⁻¹)
C	490.61 A b	310.20 A b	391.72 A a	174.55 A a
F	1501.20 A a	813.33 B a	480.67 B a	439.50 B a
B	488.40 A b	570.40 A ab	207.40 A a	328.27 A a

C= negative control (water); F= Osmocote® fertilizer; B= *Bacillus* spp. Means followed by the same upper-case letter in the lines and lower-case letter in the columns are not different from each other by the test of Tukey at 5% of probability

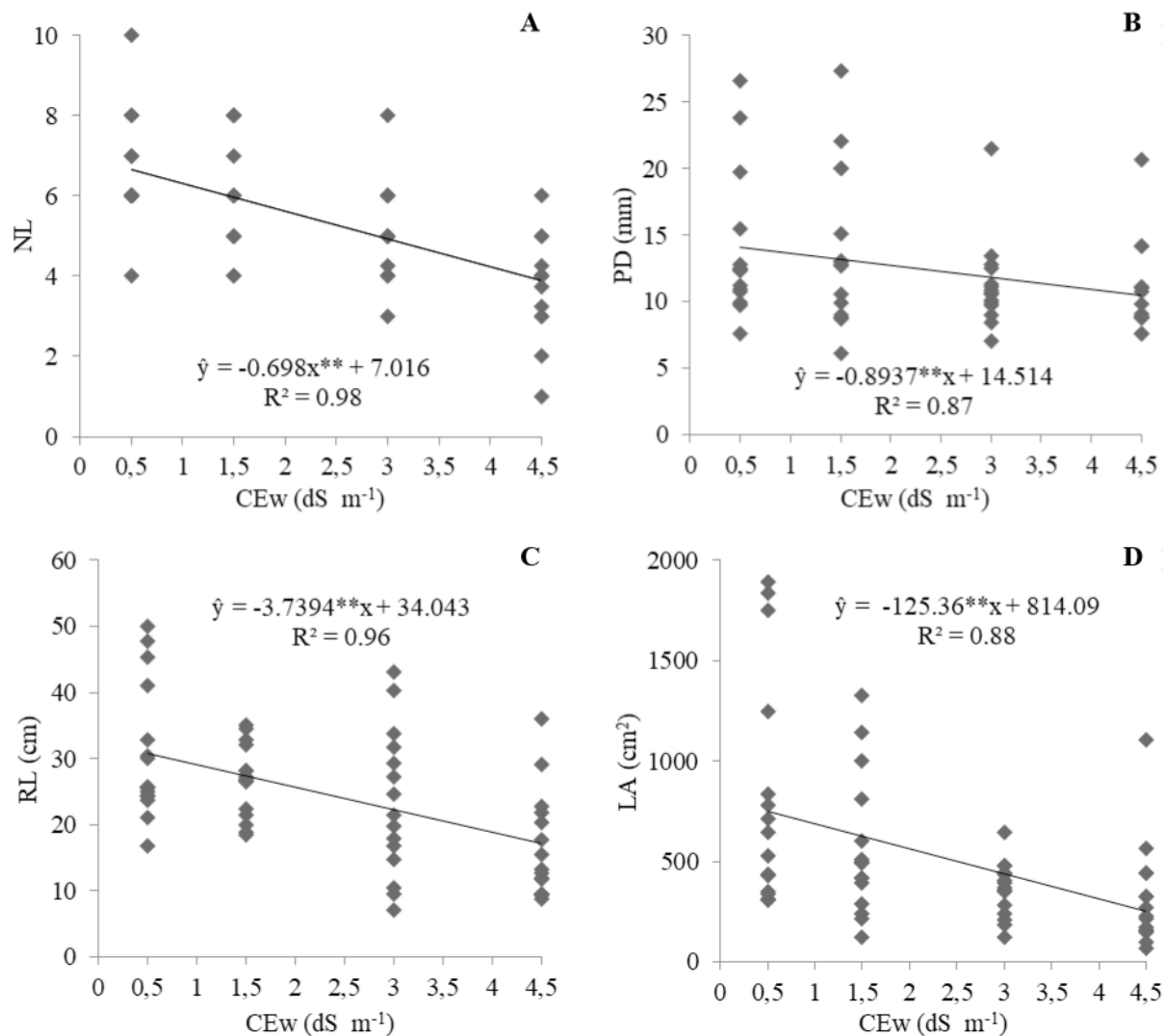


Figure 1. (A) Plant height; (B) Pseudostem diameter; (C) Leaf area of cv. Williams banana plants, irrigated with water of the different salt concentrations (CEw of 0.5; 1.5, 3.0; 4.5 dS m⁻¹). * and **: significant at 5 and 1% probability, respectively

inoculation with *Bacillus* spp. did not show a significant effect for the parameters PH, PD, and LA in seedlings of cv. Williams bananas. In both analyzed growth variables, the seedlings treated with the commercial fertilizer Osmocote® showed the highest mean values. The other treatments did not differ from each other.

The results differ from those presented by Ramadoss *et al.* (2013) who worked with halotolerant bacteria from saline environments to mitigate salt stress in wheat seedlings, and concluded that these bacteria had the potential to increase plant growth under saline stress. The authors explained that this is possible through 68 direct and indirect mechanisms, thus being a suitable bio-inoculant for saline conditions. The

collection region of the strains used in this work does not have saline soil characteristics, which is a likely limitation in the performance of bacteria under saline stress conditions.

The bacterization effect on the enhancement of plant growth in this work differs from Gamez *et al.* (2019) who obtained increases in the number of leaves, height, leaf area, root length, and pseudostem diameter in the evaluation of the implication of *Bacillus* and *Pseudomonas* bacteria on the growth of banana seedlings.

Mayak *et al.* (2004), in a study on tomato crops and on the inoculation of bacteria that promote the growth of plants that confer resistance to salt stress, also obtained similar results to this work. The authors observed greater suppression of

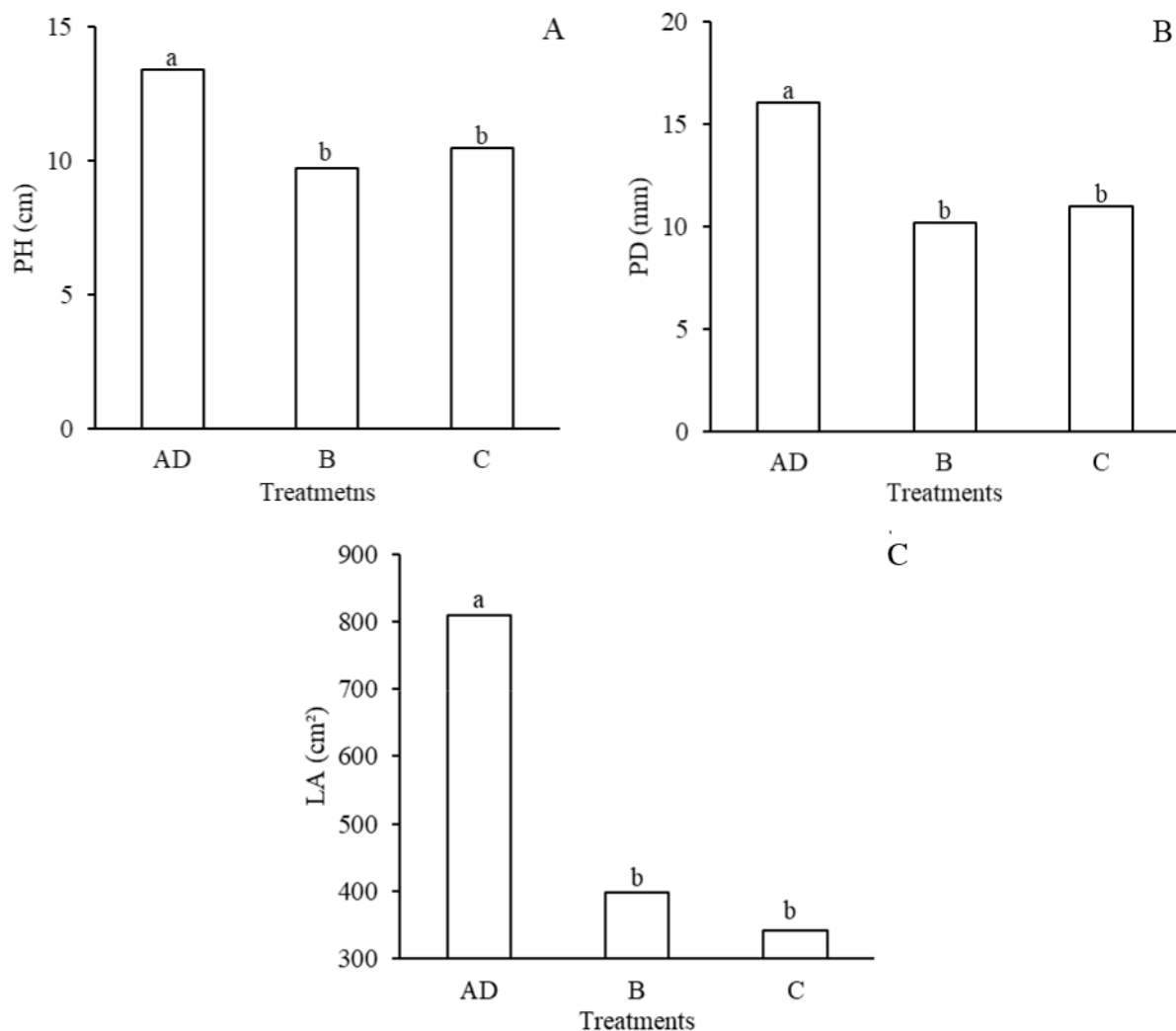


Figure 2. (A) Plant height; (B) pseudostem diameter; (C) leaf area of banana plants cv. Williams according to the different treatments ((AD) slow-release fertilizer – Osmocote® 14-14-14 (5.0 kg m⁻³); (B) Growth promoting bacteria – *Bacillus* spp; (C) negative control). Columns correspond to the mean and bars to the mean standard error. Bars with different letters are significant at 5% probability by the test of Tukey

root length when compared to aerial part growth in increasing levels of saline solution during irrigation. In addition, they explained that the observed effects are caused by the close contact of the roots with the saline solution, while the aerial part experienced only the concentration absorbed by the roots. However, an increase in the efficiency of water use due to reduced transpiration or increased photosynthesis was observed after the bacterization of tomato seedlings.

An obstacle discussed in the literature in studies on PGPB according to Silveira and Freitas (2007) concerns the variability in the results, often

being related to variable root colonization, due to unfavorable conditions for the microorganism or inoculum survival issues. Selection pressure in stressed environments can influence the tolerance of these bacteria to several abiotic factors (FERNANDES JUNIOR *et al.*, 2012).

CONCLUSION

- The salinity of irrigation water negatively affected the variables, number of leaves, pseudostem diameter, root length, leaf area. On the other hand, it positively affected plant

height.

- Seedlings inoculated with *Bacillus* spp. did not differ from each other, regardless of the saline level, for the variables leaf number and leaf area. Such a fact indicated a likely increase in the response to the salinity tolerance of the seedlings.

AUTHORSHIP CONTRIBUTION STATEMENT

SILVA, D.A.: Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing; **BASTOS, R.L.G.:** Investigation, Methodology; **SILVA, C.F.B.:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision; **SOUZA, A.B.O.:** Data curation, Formal Analysis, Investigation, Supervision, Writing – review & editing.

DECLARATION OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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