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Acclimatization of banana plantlets inoculated with *Bacillus* sp. and irrigated with low-salinity water[1]

Aclimatização de mudas de bananeira inoculadas com *Bacillus* sp. e irrigadas com água de baixa salinidade

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HIGHLIGHTS:

Inoculation with Bacillus *sp. strains 186 and 109 reduced the leaf area of banana plants irrigated with high-salinity waters.*
Inoculation with Bacillus *sp. strains 186 and 109 raised phosphorus contents of banana plants irrigated with saline water.*
Banana plants inoculated with Bacillus *sp. had higher leaf contents of phosphorus and iron in the control treatment.*

ABSTRACT: Plant growth-promoting bacteria emerge as an alternative to attenuate abiotic stresses in plants such as salinity. The objective of this study was to evaluate the effects of *Bacillus* sp. strains 186 and 109 on the acclimatization of banana cv. 'Prata Catarina' irrigated with saline water. The experiment was conducted in a completely randomized design in a 4 × 2 factorial scheme, with 4 growth-promotion treatments, subjected to 2 levels of electrical conductivity of irrigation water. Irrigation with saline water decreased the number of leaves, leaf area, transpiration rate, stomatal conductance, and CO_2 assimilation rate in banana leaves, regardless of inoculation with bacterial strains. The use of *Bacillus* sp. strain 109 promoted increments in phosphorus and iron contents in banana plants irrigated with low-salinity water.

Key words: growth-promoting bacteria, nutrition, photosynthetic rate

RESUMO: Bactérias promotoras de crescimento vegetal surgem como alternativa para atenuar estresses abióticos em plantas como a salinidade. O objetivo deste estudo foi avaliar os efeitos do *Bacillus* sp. cepas 186 e 109 na aclimatização da cv. 'Prata Catarina' irrigada com água salina. O experimento foi conduzido em delineamento inteiramente casualizado em esquema fatorial 4 × 2, com 4 tratamentos promotores de crescimento, submetidos a 2 níveis de condutividade elétrica da água de irrigação. Irrigação com água salina diminuiu o número de folhas, a área foliar, a taxa de transpiração, a condutância estomática e a taxa de assimilação de CO_2 nas folhas de bananeira, independentemente da inoculação com cepas bacterianas. O uso de *Bacillus* sp. cepa 109 promoveu incrementos nos teores de fósforo e ferro em bananeiras irrigadas com água de baixa salinidade.

Palavras-chave: bactérias promotoras do crescimento, nutrição, taxa fotossintética

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Introduction

Banana (*Musa* spp.) is one of the most consumed fresh fruits worldwide. In Brazil, its cultivation is widespread throughout the territory, with the production of 7 million tons in 2021 (Ferreira et al., 2016; IBGE, 2021). In northeastern Brazil, to achieve a good crop production, it is necessary to apply irrigation almost all year round due to the scarce rainfall typical of semi-arid regions. However, the water sources available for irrigation, besides being scarce, may also contain high salt content, which most often makes them unsuitable for agricultural purposes (Lira et al., 2015).

Banana crop has low tolerance to salinity and several factors may influence its growth, such as cultivar and levels of salt stress. For example, at water salinity levels from 2 dS m^{-1} some cultivars, such as 'Princesa', are sensitive. The Prata group cultivars better tolerate higher salinity levels (Santana Júnior et al., 2020). The use of plant growth-promoting bacteria (PGPB) has been cited as a potential way to mitigate the effects of salinity on banana plants irrigated with saline water (Chen et al., 2017; Baek et al., 2020; Silva et al., 2021).

According to Banerjee et al. (2019), some PGPB protect plants from the effects of high concentrations of Na^+ in soil by producing exopolysaccharides, maintaining a high K^+/Na^+ ratio and the osmotic potential of the plants, increasing their tolerance to salinity. PGPB can also act as ACC (1-aminocyclopropane-1-carboxylate) collectors, hydrolyzing it through the ACC deaminase enzyme, reducing the level of ethylene in the plant. In this context, with the production of this enzyme by the bacteria, they supply nitrogen and energy for the plants to survive under salt stress conditions (Ramakrishna et al., 2020). The objective of this study was to evaluate the effects of *Bacillus* sp. strains on the acclimatization of banana cv. 'Prata Catarina' irrigated with saline water.

Material and Methods

The experiment was conducted at Embrapa Tropical Agroindustry, Fortaleza, CE, Brazil (3° 45' 9.09" S, 38° 34' 31.82" W, with an average altitude of 19.5 m), using vegetatively cloned plantlets of banana cv. 'Prata Catarina'. Initially, the roots of the plantlets were washed in running water, and then they were thinned, selected based on size and vigor of the plants and transplanted into 162-cell polyethylene trays, arranged in alternated cells. The substrate used consisted of peat, which was sterilized in an autoclave set at 121 °C for one hour followed by an additional sterilization after 24 hours. Subsequently, the plantlets were pre-acclimatized in a room under controlled temperature (± 28 °C), 12-hour photoperiod and luminosity of 563 lux for seven days. Then, plants were taken to a greenhouse covered with a double layer of shade net, laterally closed with 50% shade, allowing natural ventilation. At 45 days after transplanting (DAT) to the trays, the plantlets were placed in polyethylene bags containing commercial substrate and sterilized soil in autoclave (120 °C, 1 atm for 1 hour, for two consecutive days) in a 1:1 v/v ratio. The soil was classified as Arenic Haplustults (Argissolo Vermelho-amarelo) and its chemical characteristics, before and after sterilization, are presented in Table 1. The soil was collected in the Experimental Field of Embrapa Tropical Agroindustry, located in Pacajus, CE, Brazil.

When the plantlets were transplanted into polyethylene bags, we began the application of the following growth-promotion treatments: inoculation of *Bacillus* sp. strains 186 and 109 collected from the rhizosphere of banana plants and from the collection of Embrapa Tropical Agroindustry and slow-release fertilizer. After 74 DAT, the plants were transplanted into 25 L pots containing soil classified as Arenic Haplustults (Argissolo Vermelho-amarelo). After that, the plants were subjected to two salinity levels and their growth and physiological aspects were evaluated for 155 days.

The design was completely randomized, in a 4 × 2 factorial scheme, with four growth-promotion treatments (negative control: non-inoculated and non-fertilized plants; positive control: plants fertilized with slow-release fertilizer; and plants inoculated with *Bacillus* sp. strains 186 and 109) and two levels of electrical conductivity of irrigation water (ECw of 0.5 dS m^{-1} and 2.0 dS m^{-1}, obtained by the addition of NaCl) (defined by Rodrigues et al., 2021), with 4 replicates, totaling 32 plants.

The water used was stored in 1000 L water tanks. The irrigation system used was drip irrigation, with two main lines, one for each water tank containing the two ECw levels (0.5 and 2.0 dS m^{-1}), at 74 DAT. In each row, four lateral lines were placed for positioning the drippers depending on the arrangement of treatments in the field. A pressure-compensating button type dripper with a flow rate of 2 L h^{-1} was also placed per plant. The irrigation depth was controlled by time, using an irrigation timer programmer. Irrigations were performed daily, using water balance to determine the volumes of water.

The inoculum suspensions of *Bacillus* sp. strains 186 and 109 were prepared from the collection of biomasses. The pre-inoculum was obtained from activated strains immersed in 50 mL of NYD broth (dextrose 10 g L^{-1}, meat extract 3 g L^{-1}, yeast extract 5 g L^{-1}, and distilled water), with growth for 24 hours at 30 °C and rotation of 150 rpm. A 50 µL aliquot of pre-inoculum was diluted in 100 mL of NYD broth and set to grow to obtain the inoculum. After 24 hours, the inoculum was transferred to Falcon tubes and centrifuged for 10 minutes at 3500 rpm at 25 °C. The procedure was repeated twice to remove the culture medium, using saline solution of 8.5 g L^{-1} of NaCl during washes to avoid bacterial cell plasmolysis. Finally, the content of each tube with the inoculum was diluted in 200 mL of saline solution to the concentration of 1.2 × 10^9 CFU mL^{-1}. The inoculum was applied to the soil at the dose of 75

Table 1. Chemical characteristics of non-autoclaved and autoclaved soil

Soil	OM	pH	P	K	Ca	Mg	Na	H + Al	Al^{+3}	SB	CEC	V
	(g kg^{-1})		(mg dm^{-3})	($mmol_c$ dm^{-3})								(%)
Non-autoclaved	6.2	5.5	9.4	1.2	11	5	0	21.8	0.4	18	39	45
Autoclaved	5.7	5.6	5.6	1.3	10	5	0	23.8	0.0	17	41	41

OM – Organic matter; pH – Hydrogen potential; SB – Sum of bases; CEC – Cation exchange capacity; V – Base saturation

mL per pot, with a sterile graduated cylinder. The inoculum was applied in the morning, watering the substrate, totaling 5 applications during the experiment. The inoculation process was repeated every 30 days during the experimental period.

The slow-release fertilizer was applied to the soil by placing 125 g of 14-14-14 fertilizer per pot in the positive control. All pots, regardless of the treatment, also received planting and formation fertilization (Ferreira et al., 2016). Irrigation was applied using a drip system, with one pressure-compensating dripper (2 L h^{-1}) per plant. The daily irrigation depth for both solutions was adjusted to keep the soil at field capacity, controlled with an irrigation programmer.

After 155 days of irrigation with saline water, the following growth variables were evaluated: plant height (cm); pseudostem diameter (mm); number of leaves, obtained by counting fully expanded and photosynthesizing leaves and excluding senescent leaves; length of largest root (cm), leaf area (cm^2), shoot and root fresh mass (g per plant) and shoot and root dry mass (g per plant).

Physiological monitoring of the plants was also performed at 155 days after plants started to be irrigated with saline water, for five times. Measurements of CO_2 assimilation rate (A), stomatal conductance (gs), transpiration rate (E), and internal CO_2 concentration (Ci) were carried out. The evaluations were always performed on the youngest fully expanded leaf, of all plantlets of all treatments, between 8:00 and 12:00 a.m., using a portable infrared gas analyzer (IRGA) (LCi, ADC, BioScientific), with artificial radiation source of 1500 µmol m^{-2} s^{-1} at ambient temperature and relative humidity of air.

For the leaf mineral concentrations, leaves were dried in an oven with forced air circulation at 65 °C for at least 72 hours, and then the leaves were crushed in an analytical mill until obtaining small particles (*mesh*). For nitrogen (N), extraction was performed using 0.2 g of the sample with digesting solution: 175 mL of milli-Q water, 21.39 g of sodium sulfate (Na_2SO_4), 4.0 g of copper sulfate ($CuSO_4.5H_2O$), plus 200 mL of concentrated sulfuric acid (H_2SO_4). After digestion, N was determined by steam drag distillation and titration with dilute acid (0.01 N H_2SO_4). For the macronutrients K, P, Ca, and Mg and Na, and the micronutrients Mn, Zn, Fe, and Cu, 0.5 g of the sample was weighed, and 8 mL of acid mixture (HNO_3:$HClO_4$, 3:1) was added. The mixture was kept cold for 3-4 hours and taken to the digestion block at 60 ºC, with temperature increased every 30 minutes up to 250 ºC. After being removed from the block and cooled, the samples (4 replicates per treatment) were shaken in vortex and transferred to a 50 mL volumetric flask, whose volume was checked using distilled water filtered through low-speed filter paper. Minerals were determined with an inductively coupled plasma-optical emission spectrometer (Agilent, ICP-OES 5100) (Miyazawa et al., 2009).

The results were subjected to analysis of variance and the means were compared by the F test ($p \leq 0.05$); when necessary, the data were transformed to $\sqrt{x}$ to meet normality assumptions. When the variables showed significant differences, their means were compared by the Scott-Knott test ($p \leq 0.05$). The statistical programs SAEG version 5.0 and Sisvar 5.6 were used.

Results and Discussion

For the variables related to the growth of banana plants, both growth-promotion and salinity treatments caused no changes, except for number of leaves (Table 2). The exceptions were also leaf area and number of leaves, which were affected by the interaction between factors ($p = 0.0385$, and $p = 0.0414$, respectively) (Table 2).

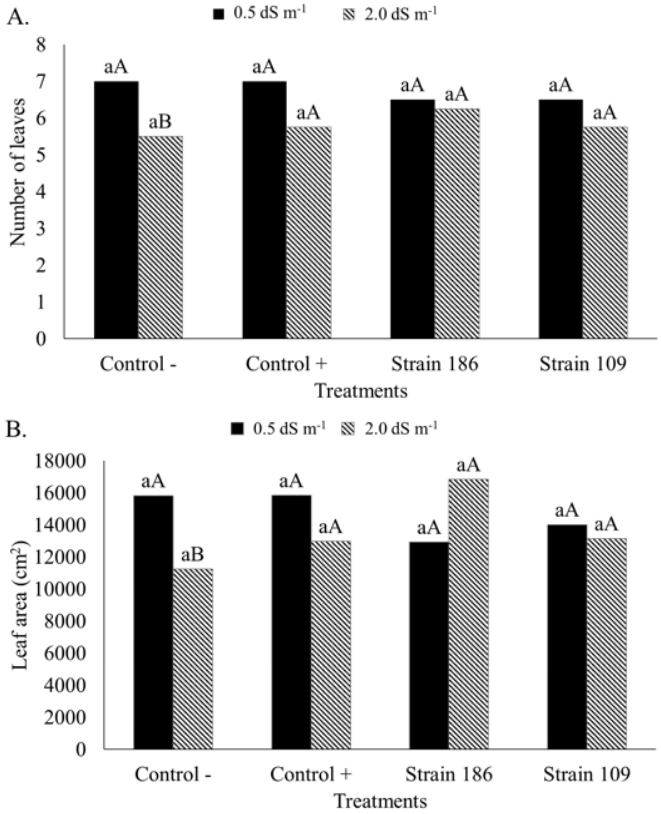
For the electrical conductivity of irrigation water (ECw), only the number of leaves ($p = 0.0127$) showed significant differences, with negative influence of salinity. Plants irrigated with low salinity water of 0.5 dS m^{-1} had an average of 6.7 leaves, while plants irrigated with 2.0 dS m^{-1} water had an average of 5.8 leaves (Figure 1A). Results similar to ours were reported by Silva et al. (2021), who found that the increases in salinity levels (0.5, 1.5, 3.0, and 4.5 dS m^{-1}) in the irrigation of banana plants had significant negative effects on the number of leaves. However, plants inoculated with *Bacillus* sp. strains at the highest salinity level (4.5 dS m^{-1}) were able to produce the highest average numbers of leaves. Inoculated plants even under conditions of high salinity levels were able to produce more leaves, evidencing the beneficial effect of bacteria on plant development under conditions of abiotic stress. In the present study, this effect of bacterial strains on the increase in the number of leaves was not observed. According to Silveira et al. (2016), salinity causes disorders in plant metabolism, triggering growth restrictions. As water is one of the essential factors for cell wall expansion, its limitation implies lower growth of banana cells and tissues (Ravi et al. 2013).

In the present study, plants that did not receive inoculation or fertilization (control -) had reduced leaf area in response to the higher irrigation water salinity. Although the growth-promotion treatments of fertilization (control +) or inoculation (strains 186 and 109) had no single effect on leaf area, it tended to be larger with the use of strain 186 when compared to the other treatments, when plants were subjected to salinity of 2.0 dS m^{-1} (Figure 1B). The results differ from those found in

Table 2. Analysis of variance of the growth and development variables of banana cultivar 'Prata Catarina', subjected to growth promoter with inoculation with *Bacillus* sp. strains 186 and 109, when compared to controls (non-fertilized/non-inoculated plants and fertilized plants), at 155 days of irrigation with saline water

Sources of variation		Mean squares							
		Number of leaves	Plant height	Pseudostem diameter	Length of largest root	Leaf area	Shoot fresh mass	Root fresh mass	Shoot dry mass
Growth promoter	3	0.11^{ns}	34.21^{ns}	65.57^{ns}	5.61^{ns}	0.35^{ns}	0.51^{ns}	2.21^{ns}	0.0049^{ns}
ECw	1	7.03*	36.12^{ns}	8.39^{ns}	5.69^{ns}	0.097^{ns}	0.028^{ns}	5.15^{ns}	0.000052^{ns}
Growth promoter × ECw	3	0.61*	22.71^{ns}	12.70^{ns}	0.15^{ns}	0.27*	0.15^{ns}	0.52^{ns}	0.00077^{ns}
Residual	24	0.97	26.50	32.18	23.42	0.082	0.30	1.48	0.0028
CV (%)	-	15.67	6.07	5.40	12.39	20.34	17.87	24.14	16.71

ns - Not significant; *Significant at $p \leq 0.05$ by F test



Same lowercase letters do not differ between inoculation treatments within salinity levels. Same uppercase letters do not differ between salinity levels within the same inoculation treatment ($p \leq 0.05$)

Figure 1. Number of leaves - A, and leaf area - B, of banana plants cv. 'Prata Catarina', subjected to growth promoters with inoculation of *Bacillus* sp. strains 186 and 109, and subjected to salinity levels of irrigation water, compared to non-inoculated controls (non-fertilized/non-inoculated plants – control - and fertilized plants– control +)

others studies in the promotion of plant growth, which may be due to several factors, such as environmental conditions, plant age, banana cultivar, inoculation method and origin of strains. According to Gao et al. (2016), despite stimulating plant growth, PGPB may have some of their mechanisms limited due to low rhizosphere colonization. In a study carried out by Gamez et al. (2019) with banana plants cv. Williams in a greenhouse, the use of PGPB promoted an increase in leaf area (69 to 80%), when compared to non-inoculated plants, values much more significant than those found in the present study.

With regard to gas exchange variables, only salinity levels caused significant effects (Table 3).

The CO_2 assimilation rate (A) of plants under irrigation with 0.5 dS m^{-1} (lowest level of salinity) water was 19.3 µmol CO_2 m^{-2} s^{-1}, a value much higher than that of plants subjected to 2.0 dS m^{-1} (highest level of salinity), which showed a reduction of 93.0% in their photosynthetic rate (Figure 2A), similar to that found by Almeida et al. (2016), who observed a decrease in the photosynthetic rate with electrical conductivity above 1.5 dS m^{-1} in 'Prata' banana colonized with arbuscular mycorrhizal fungi.

The CO_2 assimilation rate of banana plants can range from 17 to 25 µmol CO_2 m^{-2} s^{-1} (Ramos et al., 2018). Under salinity, the use of water by the plant is limited due to the lower transpiration. There is an increase in stomatal resistance and, indirectly, the photosynthetic rate is reduced, influencing plant growth (Silveira et al., 2016).

The stomatal conductance of banana plants was also reduced by salinity; plants under irrigation water of 2.0 dS m^{-1} showed gs of 0.04 mol H_2O m^{-2} s^{-1}, while those under 0.5 dS m^{-1} showed gs of 0.47 mol H_2O m^{-2} s^{-1} (Figure 2B). Stomatal conductance is linked to stomatal control and is inverse to resistance (Vieira et al. 2010). For banana, Arantes et al. (2016) reported stomatal conductance (gs) values from 1.01 (at 08:00 hours) to 0.12 mol H_2O m^{-2} s^{-1} (at 14:00 hours), under non-saline conditions, results that are similar to those found in the present study. Santana Junior et al. (2020) found decreases in stomatal conductance and transpiration of 'Prata Anã', 'BRS Platina', 'Princesa' and 'Pacovan' banana plants with increasing salinity of irrigation water.

Transpiration rate was also negatively affected by the increase in the salinity level of irrigation water, being reduced by more than 83% with the application of saline water (5.73 mmol H_2O m^{-2} s^{-1} for banana plants irrigated with water of 0.5 dS m^{-1} and 0.97 mmol H_2O m^{-2} s^{-1} in banana plants irrigated with water of 2.0 dS m^{-1}) (Figure 2C). This result is related to the stomatal response, as confirmed by the conductance data.

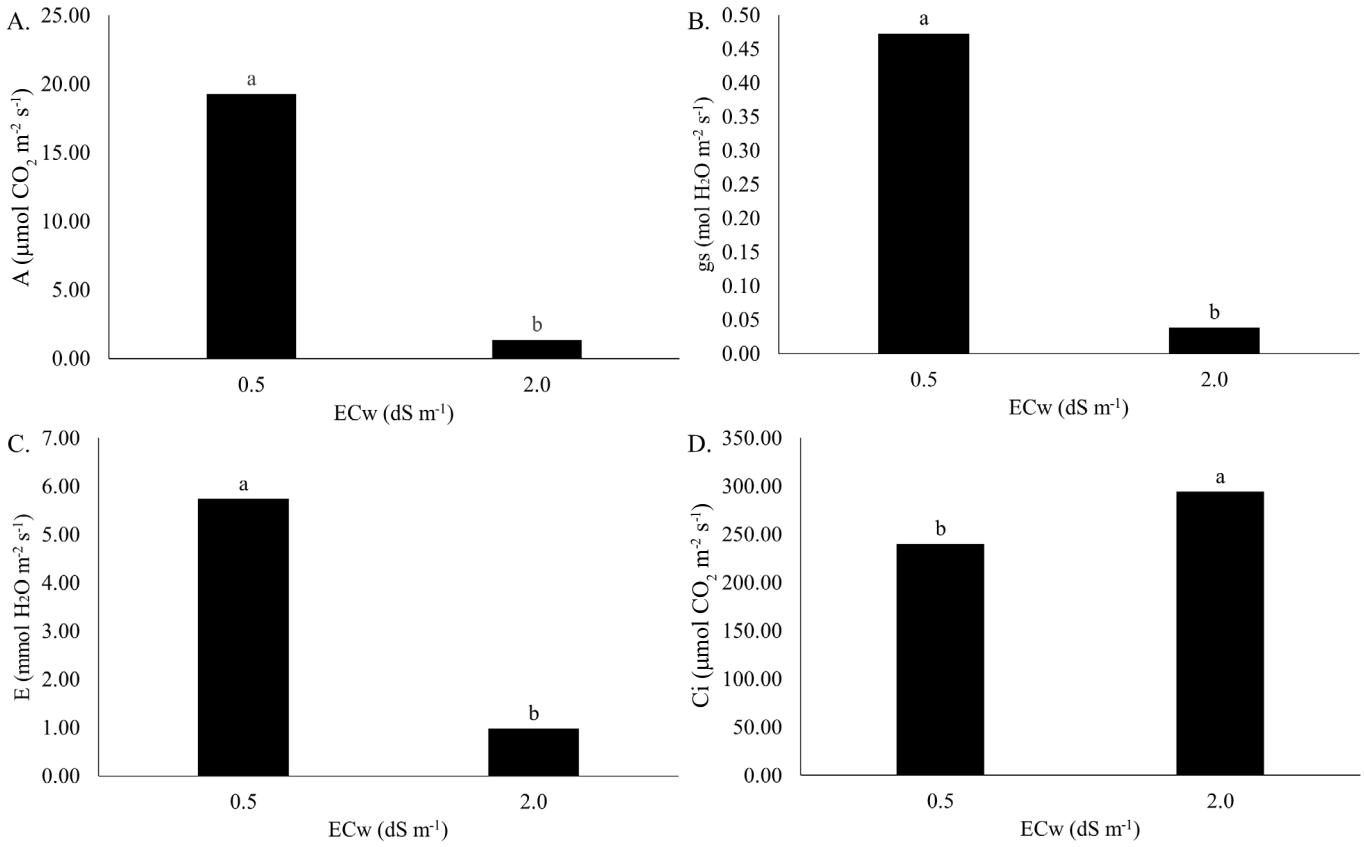
For banana plants in the semi-arid region, transpiration rates of 3.34 and 11.96 mmol H_2O m^{-2} s^{-1} were observed by Arantes et al. (2016). The results of the present study, at the lowest salinity levels, are within this ideal range of transpiration rate.

Unlike the other variables related to gas exchange, the increase in salinity levels of irrigation water to 2.0 dS m^{-1} led to increments in the internal CO_2 concentration (Ci) of banana plants, with mean value of 294.2 µmol CO_2 m^{-2} s^{-1} for 2.0 dS m^{-1}, compared to 240.3 µmol CO_2 m^{-2} s^{-1} at the ECw of 0.5 dS m^{-1} (Figure 2D). Ramos et al. (2018) evaluated the gas exchange of banana plants grown in the field in the semi-arid region and found that the cv. 'Maçã' had the highest Ci value in March during the morning period (232.53 µmol CO_2 m^{-2} s^{-1}). For this

Table 3. Analysis of variance of the gas exchange variables of banana cultivar 'Prata Catarina', subjected to growth promoter with inoculation with *Bacillus* sp. strains 186 and 109, when compared to controls (non-fertilized/non-inoculated plants and fertilized plants), at 155 days of irrigation with saline water

Sources of variation		Mean squares			
		CO_2 assimilation rate (A)	Stomatal conductance (gs)	Transpiration rate (E)	Internal CO_2 concentration (Ci)
Growth promoter	3	9.54 ^{ns}	0.010 ^{ns}	0.18 ^{ns}	1920.78 ^{ns}
ECw	1	25.32 **	2.12**	181.07 **	23274.03 **
Growth promoter × ECw	3	1.75 ^{ns}	0.011 ^{ns}	0.34 ^{ns}	403.78 ^{ns}
Residual	24	4.64	0.009	0.63	1058.36
CV (%)	-	20.90	22.44	23.63	12.17

CO_2 assimilation rate (A), stomatal conductance (gs), transpiration rate (E), and internal CO_2 concentration (Ci); ns - Not significant; **Significant at $p \leq 0.01$ by F test



Columns identified with different letters indicate difference between salinity levels of irrigation water at $p \leq 0.05$ by Tukey test

Figure 2. CO_2 assimilation rate (A) - A, stomatal conductance (gs) - B, transpiration rate (E) - C and internal CO_2 concentration (Ci) - D of banana cv. 'Prata Catarina', when subjected to different salinity levels of irrigation water

experiment the plants were evaluated between flowering of the first and second cycle in different months.

In a study conducted by Lúcio et al. (2013) with melons associated with mycorrhizal fungi, stomatal conductance, transpiration rate, and photosynthesis were also reduced when plants were subjected to increasing salinity levels, which was attributed to stomatal and non-stomatal causes associated with osmotic and toxic effects of excess salts. In another study, micropropagated banana plants under greenhouse conditions, when supplemented with *Bacillus* strains and subjected to saline water irrigation, showed no changes in gas exchange (Rodrigues et al., 2021). These results are similar to that observed in the present study, when *Bacillus* sp. strains were inoculated, under conditions of cultivation in unsterilized soil.

The decrease in gas exchange, especially the photosynthesis of plants subjected to the highest level of salinity, without the concomitant reduction of plant growth, indicates that the salinity level employed will hamper the growth and development of plants, being more associated with the osmotic effect of salt stress (Vieira et al., 2010).

For leaf minerals, the growth-promotion treatments influenced the contents of Na, Mg, P, and Fe. The electrical conductivity of the water influenced Na, Ca, and P and there was significant interaction between the growth promotion and ECw factors for Na, P, Ca, Mg, and Fe. The other minerals in the plants were statistically equal, regardless of the growth promoters and electrical conductivity of the irrigation water used (Table 4).

According to Carvalho Júnior et al. (2019), in descending order, banana absorbs macronutrients in the following sequence: K > N > Ca > Mg > P = S. In the present study, the predominant sequence of extraction found for the leaves of banana plants was: K > N > Ca > Mg > P > S > Na, with change only in the predominance of phosphorus over sulfur.

As for the standard contents of nutrients in the leaf defined for banana, Carvalho Júnior et al. (2019) under conditions of cultivation in northern Minas Gerais (MG), found the following values: 23.8 g of N kg^{-1}; 1.7 g of P kg^{-1}; 35.6 g of K kg^{-1}; 6.6 g of Ca kg^{-1}; 2.9 g of Mg kg^{-1} and 1.7 g of S kg^{-1}. These values are close to those found in the present study, but not within the ideal range, even though the plants were fertilized, indicating that other factors may have affected the absorption or availability of nutrients, such as growing conditions in the greenhouse: light, pot size, type of substrate, etc. (Martins et al., 2011; Oliveira et al., 2014; Rodrigues et al., 2022).

The average P content of banana plants inoculated with strain 109 and irrigated with water of 0.5 dS m^{-1} differed from that of fertilized plants, being 16.55% higher. When plants were irrigated with water of 2 dS m^{-1}, there were no differences between growth-promotion treatments (Table 5). According to Abhilash et al. (2016), plant growth-promoting bacteria can act on the solubilization of nutrients, such as phosphorus, in soil or substrate, making them more readily available to plants, and/or inoculated plants may outperform the others in the acquisition of nutrients in their tissues. In another study, Rodrigues et al. (2022) concluded that *Bacillus* strains

Table 4. Analysis of variance of the mineral contents in shoots of banana cultivar 'Prata Catarina', subjected to growth promoter with inoculation with *Bacillus* sp. strains 186 and 109, and levels of electrical conductivity of irrigation water (ECw) at 155 days of irrigation with saline water

Sources of variation	DF	Mean squares						
		Mineral contents in shoots						
		N	P	K	Ca	Mg	S	Na [1]
Growth promoter	3	1.77^{ns}	0.03^{ns}	1.93^{ns}	1.17^{ns}	0.23^{ns}	0.010^{ns}	3.46*
ECw	1	20.70^{ns}	0.06^{ns}	8.47^{ns}	0.38^{ns}	0.11^{ns}	0.046^{ns}	56.34*
Growth promoter × ECw	3	0.37^{ns}	0.10*	10.04^{ns}	10.42*	0.33*	0.016^{ns}	3.09*
Residual	24	4.91	0.02	13.58	3.07	0.12	0.011	0.58
CV (%)	-	11.85	10.07	19.03	24.40	18.87	9.47	44.38
		Micronutrients in shoots						
		Cu[1]		Fe		Zn		Mn
Growth promoter	3	3.64^{ns}		75.09^{ns}		19.24^{ns}		732.22^{ns}
ECw	1	0.69^{ns}		22.21^{ns}		22.22^{ns}		7.34^{ns}
Growth promoter x ECw	3	1.22^{ns}		581.29*		18.62^{ns}		2554.38^{ns}
Residual	24	0.37		152.03		10.97		1096.71
CV (%)	-	22.36		17.14		28.10		20.19

N - Nitrogen; P - Phosphorus; K - Potassium; Ca - Calcium; Mg - Magnesium; S - Sulfur; Na - Sodium; Cu - Copper; Zn - Zinc; Fe - Iron; Mn - Manganese. [1]Variable transformed into √x. ns - Not significant; *Significant at p ≤ 0.05 by F test

were effective in increasing nitrogen content in leaves (17.93 g kg^{-1}) and potassium and magnesium contents in roots (6.96 and 6.98 g kg^{-1}).

Regarding saline treatments, the only change was that, for plants inoculated with strain 109, the P content was higher in those that did not receive saline water (Table 5). When analyzing the contents in each treatment, it can be observed that the change was the increase in P content in plants inoculated with strain 109 without saline water irrigation.

In relation to Na, plants irrigated with 0.5 dS m^{-1} water showed no significant differences between growth-promotion treatments. With the increase in the electrical conductivity of the irrigation water to 2.0 dS m^{-1}, there was a significant difference between the growth-promotion treatments, and plants inoculated with strain 186 showed lower content of this ion (1.43 g of Na kg^{-1}). However, even in this treatment, as in the other treatments, plants that received higher salinity levels showed higher Na content in the leaves. Santos et al. (2017) report that the increase of NaCl in the soil solution hampers root absorption of nutrients, mainly K and N, which was not observed for these two nutrients in this study.

Table 5. Mineral contents in leaves of banana cv. 'Prata Catarina' subjected to the interaction between factors growth promoter with inoculation with *Bacillus* sp. strains 186 and 109, and levels of electrical conductivity of irrigation water

Salinity	Means			
	0.5	2.0	0.5	2.0
Treatment	P (g kg^{-1})		Na (g kg^{-1})	
Control -	1.37 bA	1.48 aA	0.08 aB	3.63 aA
Control +	1.45 abA	1.48 aA	1.16 aB	2.99 aA
Strain 186	1.38 bA	1.30 aA	0.06 aB	1.43 bA
Strain 109	1.69 aA	1.28 aB	0,23 aB	4.10 aA
	Ca (g kg^{-1})		Fe (mg kg^{-1})	
Control -	7.79 aA	7.10 aA	61.25 aB	82.25 aA
Control +	5.72 aB	9.16 aA	74.00 aA	69.33 aA
Strain 186	6.64 aA	6.63 aA	62.50 aA	71.33 aA
Strain 109	8.17 aA	6.30 aA	83.50 aA	65.00 aB
	Mg (g kg^{-1})		Cu (mg kg^{-1})	
Control -	1.64 bA	1.98 aA	14.00 aA	12.00 aA
Control +	2.30 aA	1.74 aB	10.30 aA	3.66 bB
Strain 186	1.65 bA	1.74 aA	2.75 bA	6.00 abA
Strain 109	1.67 bA	1.70 aA	9.25 abA	7.66 abA

Control - (non-inoculated and non-fertilized plants); Control + (fertilized plants); Strain 186 and Strain 109 (*Bacillus* sp.). P - Phosphorus; Ca - Calcium; Mg – Magnesium; Na - Sodium; Cu - Copper; Fe - Iron. Means followed by different lowercase letters differ from each other in the column at p ≤ 0.05. Means followed by different uppercase letters differ from each other in the same row at p ≤ 0.05

For Ca, there was no change with the growth-promotion treatments, regardless of salinity level. On the other hand, when comparing saline treatments, fertilized plants showed a 60.14% increase in the nutrient content, when irrigated with water of 2 dS m^{-1}, compared to those irrigated with water of 0.5 dS m^{-1} (Table 5).

For Mg, the change was observed in fertilized plants that did not receive water of higher salinity, with value higher than those found in the other growth-promotion treatments and when compared with plants subjected to salinity within the same growth-promotion treatment (Table 5).

For copper accumulation in plant tissues, there were differences between growth promoters, evaluated at each irrigation water electrical conductivity. As the electrical conductivity of irrigation water increases, it can be seen strain 186 showed lower concentrations of copper in the tissues, not differing from the negative control (non-inoculated and non-fertilized plants) underhigh salinity water (Table 5). According to Rodrigues Filho et al. (2021) the ideal levels of copper for 'Prata Anã' banana are around 2.7-5.3 mg kg^{-1}. Thus, even with the increase in the conductivity of the irrigation water, none of the treatments showed less than the ideal content in the present study.

For Fe contents, at the water salinity of 0.5 dS m^{-1}, plants inoculated with strain 186 and the negative control (without fertilization) showed lower mean values, compared to the other two treatments, which did not differ. In plants subjected to the higher salinity, the growth-promotion treatments did not cause significant changes (Table 5). However, with the increase in salinity from 0.5 to 2.0 dS m^{-1}, there were increments in Fe content in plants of the negative control (non-inoculated/non-fertilized plants) and reductions in plants inoculated with strain 109, with no difference between the other growth promoters (Table 5).

According to Carvalho Júnior et al. (2019), the ideal Fe contents for banana cv. 'Prata-Anã' is close to 62.3 mg kg^{-1}. Thus, at the low electrical conductivity of the irrigation water, plants of the negative control (non-fertilized and non-inoculated)

were the only ones with values below the ideal. Regarding Zn and Mn contents, there were no significant differences between the factors evaluated, although plants showed levels below those considered ideal (zinc - 17.9 mg kg^{-1} and manganese - 280 mg kg^{-1}) in all treatments.

According to Santoyo et al. (2016), plant growth-promoting bacteria can facilitate the acquisition of nutrients by plants, by making them available for absorption, which is a direct mechanism for growth promotion, besides being able to increase the contents of nutrients in plant tissues. However, these same microorganisms can also compete for some minerals, such as iron, making it unavailable to plants (Santos et al., 2021). In our study, *Bacillus* strains were collected and selected from the rhizosphere of banana plants. These microorganisms are well adapted to this microbiota, but some cultivation conditions, such as type of substrate, cultivars, and number of applications of these microorganisms influence their performance in banana plants. In this study, in addition to these factors, the influence of abiotic stress caused by salinity was another factor to be tested to prove the benefits of these bacterial strains in banana cultivation. Despite this, the gains were evident with a slight reduction in the leaf area of the plants subjected to this stress condition.

Conclusions

1. Irrigation with saline water decreased the number of leaves, leaf area, transpiration rate, stomatal conductance, and CO_2 assimilation rate in banana leaves, regardless of inoculation with bacterial strains.

2. The use of *Bacillus* sp. strain 109 promoted increments in phosphorus and iron contents in banana plants irrigated with low-salinity water.

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