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Physiological, biochemical, and growth aspects in corn inoculated with endophytic bacteria under saline stress

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ABSTRACT: The Caatinga, a Brazilian biome, which has a microbiota with adaptation to both drought and salinity, within this ecosystem are bacteria such as those of the genus *Bacillus* that stimulate plant growth and increase resistance to biotic and abiotic factors. This study aimed to evaluate the development of corn irrigated with brackish water, through the inoculation of seeds with bacteria isolated from the Caatinga. The experiment was composed of 3 × 4 factorial, with bacteria: T0 (negative control); T1 - bacteria 1 (XX6.9); T2 - mix (XX6.9 + T1.1 and T11.1); and, three salinity levels: (1.5, 2.5, and 4.5 dS m^{-1}) plus control treatment and six repetitions. The following growth analyses were performed at 15 and 54 days after sowing (DAS): plant height, stem diameter, root length, fresh mass, and biochemical analyses: reducing sugars (RS), total soluble sugars (TSS), and the amino acid proline content of leaf and root. The inoculum used increased the growth of the root system, promoting a gain in the accumulation of fresh mass at the beginning of cultivation and reducing its loss at 54 DAS. There was also an increase in carbohydrate accumulation, in addition to the promotion of osmotic adjustment of the cells with the accumulation of the amino acid proline.

Key words: *Bacillus*; inoculum; salinity; tolerance

Aspectos fisiológicos, bioquímicos e crescimento em milho inoculado com bactérias endofíticas sob estresse salino

RESUMO: A Caatinga, bioma brasileiro, que possui uma microbiota com adaptação tanto à seca quanto à salinidade, dentro deste ecossistema encontram-se bactérias como as do gênero *Bacillus* que estimulam o crescimento vegetal e aumentam a resistência a fatores bióticos e abióticos. Esse trabalho teve como objetivo avaliar o desenvolvimento do milho irrigado com água salobra, através da inoculação de sementes com bactérias isoladas da caatinga. O experimento foi constituído em fatorial 3 × 4, sendo às bactérias: T0 (controle negativo), T1 - bactéria 1 (XX6.9), T2 - mix (XX6.9 + T1.1 e T11.1), e de três níveis de salinidade: (1,5, 2,5 e 4,5 dS m^{-1}) mais o tratamento controle e seis repetições. Foram realizadas as seguintes análises de crescimento aos 15 e aos 54 dias após a semeadura: altura da planta, diâmetro do caule, comprimento da raiz, massa fresca e análises bioquímicas: teor de açúcares redutores (AR), de açúcares solúveis totais (AST) e o aminoácido prolina da folha e raiz. Os inóculos utilizados incrementaram o crescimento do sistema radicular, promovendo ganho no acúmulo de massa fresca no início do cultivo e reduzindo sua perda aos 54 DAS. Houve também incremento no acúmulo de carboidratos, além de promoção do ajuste osmótico das células com o acúmulo do aminoácido prolina.

Palavras-chave: *Bacillus*; inóculo; salinidade; tolerância

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Introduction

Corn (*Zea mays* L.), belonging to the Poaceae family, is a grass grown all over the world due to its versatility of use, its great nutritional value, and its high productive potential. It can be used in the human and animal food chain, as well as, in the industry for the production of biofuels such as ethanol (Duarte & Garcia, 2021).

According to data from CONAB (2018), the North and Northeast regions are the smallest producers in numbers in Brazil, showing low percentages when compared to the other regions. In the 2020 harvest, the Northeast produced approximately 2.5 thousand kg ha^{-1}, while in the Southern region of the country, these values exceed 8.0 thousand kg ha^{-1} (CONAB, 2021).

The difficulty in technology, inadequate genotypes, problems of scarcity, and irregularity of rains, with rainfall rates below 800 millimeters per year, concentrated in just over five months of the year, directly influence the low levels of corn productivity in Northeast Brazil (Santos et al., 2020a). A possible alternative to increase production in the Northeast is the use of groundwater through artesian wells, as these increase water availability, promoting water assurance in the semiarid region of Brazil (Vieira et al., 2020).

It is known that the soil of much of the Caatinga is calcareous and over time the infiltrated water has absorbed a large amount of dissolved salts, reaching the water table and often exceeding the limits imposed in Ordinance MS No. 2.914 of 2011 (Brasil, 2011). Irrigation with saline water can generate problems that influence crop production in all its physiological stages, this due to the high concentration of salts (Sousa et al., 2018). This management impacts the soil, reducing its water potential and causing toxic effects on plants, causing functional disturbances in crop metabolism (Rodrigues et al., 2020).

In plants, especially legumes and grasses, it is possible to use an alternative to mitigate the effects of salinity, drought, and other abiotic stresses by using the native microbiota of the semiarid region. Some of these microorganisms have the ability to perform the process of Biological Nitrogen Fixation (BNF), phosphate solubilization due to the release of organic acids, besides promoting plant growth through the availability of nutrients, minerals, and water, and the production of enzymes, proteins, and vegetal hormones (Rodrigues et al., 2020).

The use of biological inputs, such as the inoculation of plant growth promoting bacteria (PGPB) that have the ability to fix nitrogen for plants, is a good alternative, mainly because, by favoring root growth, they promote greater nutrient and water uptake (Stoll et al., 2016; Veronezi et al., 2018).

Bacteria can produce phytohormones, which induce the plant to develop a larger root system, consequently improving the nutrient uptake capacity (Rodrigues et al., 2020).

However, saline stress can lead the plant to primary stresses, consequently leading to secondary problems, for example, the overproduction of reactive oxygen species, leading to cytoplasmic membrane damage and a cascade of dysfunctions in essential metabolic processes (Wang et al., 2017). To mitigate these effects, bacteria can stimulate plant genes responsible for the production of antioxidant enzymes that act as a defense mechanism in the plant, such as superoxide dismutase, glutathione reductase, guaiacol peroxidase, glutathione peroxidase, among others (Alves et al., 2018).

Studies show that symbiotic *Bacillus* strains from nodules have valuable attributes to be exploited as biofertilizers acting on nutrient uptake such as F and P (Zhao et al., 2018).

Knowing this capacity, the use of microorganisms already adapted to saline stress conditions can bring beneficial results to the production of various crops irrigated with water taken from artesian wells. Thus, the objective of this study was to evaluate the effect of the use of bacteria isolated from the Caatinga and inoculated in irrigated corn (*Zea mays* L.) under saline conditions in the Submédio do Vale São Francisco region.

Materials and Methods

The bacterial strains used are from the culture collection of the Microbial Biotechnology Laboratory (Laboratório de Biotecnologia Microbiana - LBM), located at Universidade do Estado da Bahia, Campus III, Juazeiro, Bahia, Brazil. Three isolates previously from the Caatinga with high salinity tolerance characteristics were selected for in vitro testing. The bacteria used belong to the genus *Bacillus* and identified with codes XX6.9, T1.1 and T11.1, being isolated from the rhizosphere of Cactaceae of the genus *Pilosocereus* and *Tacinga* (Santos et al., 2020b; Dias et al., 2022). The strains used were activated in TSA (Tryptone Soy Agar) culture medium, incubated for 24 hours at 28.0 ± 2.0 °C.

A salinity tolerance test was previously performed at concentrations: 1.5, 3.0, and 4.5 dS m^{-1} L^{-1} of NaCl (w/v), along with a control with the original medium concentration (0.2% of NaCl). The bacteria were replicated in Petri dishes using a platinum loop with 3 repetitions. They were then incubated in a bacteriological oven at 28.0 ± 2.0 °C for 48 hours.

Subsequently, the bacterial isolates were activated and repotted onto TSA medium and then kept in an incubator for 48 hours at 30.0 ± 2.0 °C. Suspensions were prepared by adding saline solution (0.85%) over the colonies and scraping them with a Drigalsky loop. The suspension was adjusted to 0.5 in a spectrophotometer (600 nm) using 0.1% xanthan gum as a diluent.

The inoculum in the seeds was subdivided into 2 treatments and the control (T0 - no bacteria (negative control), T1 - bacteria 1 (XX6.9), and T2 - mix (XX6.9 + T1.1 and T11.1)). For T2, all bacteria were adjusted to 0.5 m in a spectrophotometer homogenized together before microbiolization. The negative control was prepared in xanthan gum only.

The corn seeds (*Zea mays* L.) used were hybrid variety AG 1051 from SEMINIS®. Seeds prior to microbiolization were surface disinfected using a 70% alcohol solution for 1 minute, followed by 1% hypochlorite (NaCl) for 1 minute and, three consecutive washes of sterile distilled water, leaving to dry naturally on absorbent paper. After drying, the seeds were transferred to 50 mL Falcon tubes containing the bacterial inoculum and held for 60 minutes. Then the seeds were dried at room temperature for approximately 30 minutes.

Saline water was prepared by adding salts (NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$)) to the water supply in a ratio equivalent to 7:2:1 between Na, Ca, and Mg ions, respectively. The electrical conductivities were monitored using a Kasvi DDS-307[a] conductivity meter and checked by the Waterproof Pen Ms/cm tester.

Before sowing, the pots were weighed at field capacity. The irrigation was performed on alternate days manually based on the weighing method, where the pots were weighed before irrigation to obtain the average value of the blade to be applied, always raising the soil moisture to its field capacity.

The microbiologized seeds were sown in 4 L pots filled with soil from the experimental area of the Universidade do Estado da Bahia (UNEB), class Neossolo Flúvico, characterized by Laboratory of Soil, Water, Plant, Fertilizers and Correctives (Laboratório de Solo, Água, Planta, Fertilizantes e Corretivos - LASAC) from UNEB. At 15 days, the plant was thinned, leaving only one plant per pot.

Growth evaluations occurred at 15 and 54 days after sowing (DAS). The analyses were: stem diameter (pachymeter), aerial plant length, and root length (millimeter ruler). The weighing of fresh matter of root and leaf tissue was also performed at 15 and 54 DAS.

Three biochemical analyses were performed in leaf tissues of the crop at 54 DAS for quantification of total soluble sugars (TSS) according to Yemm & Willis (1954), reducing sugars (RS) according to the methodology described by Miller (1959), and the quantification of the amino acid proline was performed in the leaf and root tissue of the plant according to Bates et al. (1973).

The experiment was conducted in the greenhouse of the Universidade do Estado da Bahia - UNEB, Campus III, in 2021, located in the municipality of Juazeiro, BA, Brazil (9° 25' 44" S, 40° 32' 14" O, altitude of 384 m) the local climate is of type Bswh. Fertilization was done according to the crop recommendation. The application of fungicides, insecticides, and other cultural and management practices were carried out according to the needs of the crop.

The design was completely randomized in a 3 × 4 factorial, consisting of three treatments referring to bacteria (T0, T1, and T2) and three salinity levels (1.5, 3.0, and 4.5 dS m^{-1}) along with a negative control (river water) and six repetitions, totaling seventy-two pots. The data collected in the present study were submitted to analysis of variance (ANOVA), using Tukey test and regression at the 5% probability level for comparison of the means of the treatments employed, using the SISVAR statistical program (Ferreira, 2019).

Results and Discussion

In the analysis of variance of the factors studied (Table 1), it was observed that there was no significant effect of the interaction between the levels of salinity and the inoculants applied in the variables: plant height, stem diameter, and RS content. In this case, they were analyzed separately. The interaction between bacteria and salinity levels occurred in the variables: root length, root fresh weight, and content of total soluble sugars and proline.

The inoculation of seeds with bacteria of the genus *Bacillus* did not increase the plant height, which was corroborated with the result presented by Alcantara et al. (2021). However, we can see that salinity reduced plant size significantly at 15 DAS (Figure 1A) and at 54 DAS (Figure 1B). This reduction was accentuated and linear as salinity increased at 54 DAS, leading to a 15% reduction in plant height when compared to the control.

According to Rodrigues et al. (2020), salt can affect plants by influencing their growth and development, causing modifications throughout their structure morphological, metabolic, and structural. The high salt index affects the plant osmotically, mainly reducing the growth of the aerial part through the accumulation of salts in the root system and through an ion-specific response, leads to damage to the permeability of cell membranes and cytoplasmic organelles (Gomes et al., 2019). This damage brings about imbalance in the photosynthetic apparatus, stomatal closure, cellular alterations in amino acids and consequently in the production of proteins, enzymes, and phytohormones (Willadino & Camara, 2005).

Salinity is one of the abiotic stresses that most compromises crop growth and productivity worldwide (Silva et al., 2020). Water scarcity and soil salinization are the two main limiting factors for sustainable agriculture in arid and semiarid regions worldwide (Pedrotti et al., 2015).

Table 1. Summary analysis of variance for plant height (cm), stem diameter (mm), root length (cm), root fresh weight (g), total soluble sugars (TSS, mg g^{-1} of fresh mass), reducing sugars (RS, mg g^{-1} of fresh mass), and proline (u g^{-1}).

SV	Plant height		Stem diameter		Root			TSS		RS		Proline	
					Length	Fresh mass							
	Days after sowing							Plant component					
	15	54	15	54	54	15	54	Root	Leaf	Root	Leaf	Root	Leaf
	F												
I	0.57^{ns}	0.39^{ns}	0.05*	0.38^{ns}	0.000**	0.458^{ns}	0.1311^{ns}	0.0003**	0.0000**	0.049*	0.379^{ns}	0.0000**	0.647^{ns}
S	0.005**	0**	0.04*	0.02**	0.001**	0.016*	0.0000**	0.0000**	0.0000**	0.0023**	0.384^{ns}	0.0000**	0.005*
I × S	0.56^{ns}	0.22^{ns}	0.324^{ns}	0.285^{ns}	0.000**	0.016*	0.021*	0.0001**	0.0000**	0.095^{ns}	0.117^{ns}	0.0000**	0.027*
CV (%)	8.27	6.64	13.79	10.91	10.61	22.27	14.39	20.49	7.23	80.82	91.05	10.7	56.5

I - Inoculum; S - Salinity; I × S - I and S interaction; ns - Not significant ($p \geq 0.05$); * - Significant ($p < 0.05$) and *** - Significant ($p < 0.01$) by the F test.

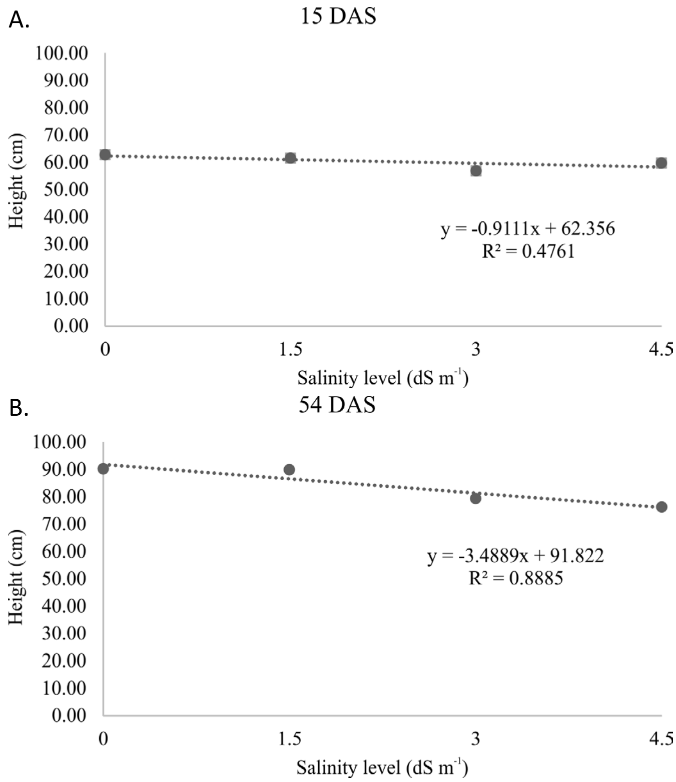


Figure 1. Interaction of corn (*Zea mays*) plant height, hybrid variety AG 1051 from SEMINIS® company in relation to salinity at 15 (A) and 54 (B) days after sowing (DAS) - Juazeiro, BA, Brazil, 2022.

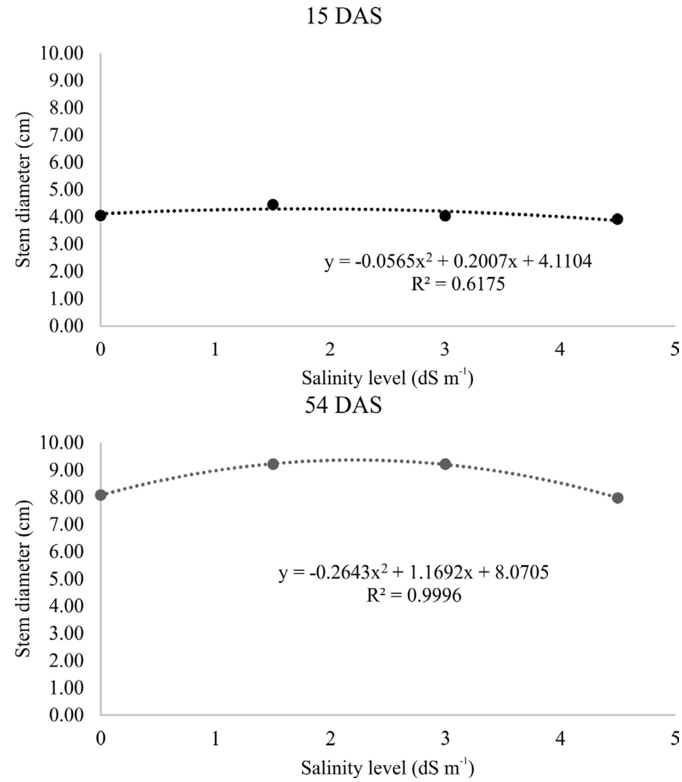


Figure 2. Stem diameter of corn (*Zea mays*) plants, hybrid variety AG 1051 from SEMINIS® company in relation to salinity at 15 (A) and 54 (B) days after sowing (DAS) - Juazeiro, BA, Brazil, 2022.

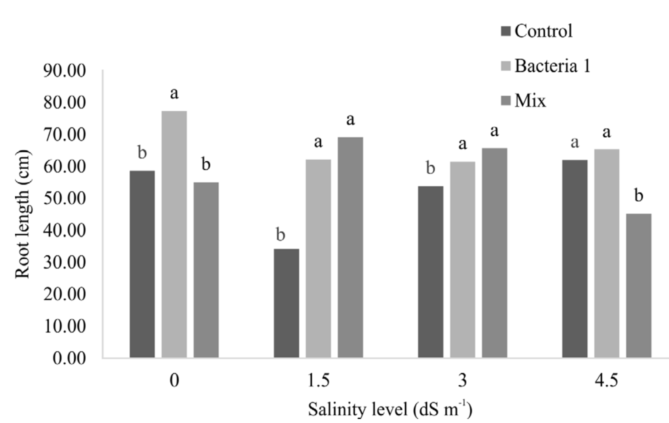
Osmotic stress affects plant growth immediately upon increasing salt concentration around the roots (Munns et al., 2016). The excessive accumulation of salts in the soil causes a reduction in the osmotic potential of the soil solution, restricting water uptake by the roots and promoting dehydration of the plant (Bartels & Sunkar, 2005).

The use of bacteria also did not interfere with respect to stem diameter, similarly, as reported earlier for plant height. Corn seed inoculated with *Azzospirillum* strains in association with nitrogen promoted an increase in plant stem diameter (Alcantara et al., 2021), attributing this superiority over the negative control to the growth hormones excreted by PGPB. However, increasing salinity interfered significantly between treatments at 15 DAS (Figure 2A) and 54 DAS (Figure 2B) with levels between 2.0 and 3.0 dS m^{-1}, providing an increase of 12.5 and 16.0%, respectively. In this sense, Rodrigues et al. (2020) report that soils with excess salts can lead to growth inhibition by reducing the osmotic potential of the soil solution.

The stem, also called the thatch, is the part of the plant responsible for providing support to the entire aerial part, usually composed of four internodes called meritals. It is considered a source of sugar reserves and photoassimilates that are used by the plant in the senescence period (Barros & Calado, 2014).

Plant growth-promoting rhizobacteria that show the ability to fix atmospheric nitrogen, can supply it to plants by two mechanisms: symbiotic and nonsymbiotic (Gupta et al., 2015). In addition, they produce phytohormones, such as auxins, cytokinins, gibberellins, which can affect cell proliferation in the root architecture by overproducing lateral roots and roots.

Inoculation of corn seeds with bacteria in combination with brackish water increased the length of the root system. It can be seen in Figure 3, that bacterium 1 in the absence of salinity provided a significant gain in root system length compared to the other treatments. Therefore, when evaluating the root length when irrigated with brackish water with 1.5 and 3.0 dS m^{-1}, there was a gain of the plants inoculated with the bacteria 1 and Mix in relation to the control of 50.0 and 25.0%, respectively. When analyzing the plants irrigated



Means followed by the same letter do not differ by Tukey test ($p \geq 0.05$).

Figure 3. Root tissue length of corn (*Zea mays*) plants, hybrid variety AG 1051 from SEMINIS® inoculated with bacteria for induction of salinity resistance - Juazeiro, BA, Brazil, 2022.

with 4.5 dS m^{-1}, there was an expressive response of the root system length in the presence of bacterium 1. Which can be explained by mechanisms that some rhizospheric bacteria have to synthesize phytohormones, as an example indoleacetic acid (IAA) (Bashan et al., 2004), which is the main auxin found in plant tissues, responsible for inducing root and root hair growth, water and nutrient uptake, as well as tolerance to low soil moisture conditions (Angulo et al., 2014).

Among plant growth regulators, indoleacetic acid (IAA) is the most common natural auxin found in plants (Sousa & Sousa, 2020). This phytormone functions as a key regulator for many aspects of plant growth and development including plant cell division, extension, and differentiation; stimulates seed and tuber germination; increases the rate of xylem and root development; initiates lateral and adventitious root formation; mediates responses to light, gravity, and flowering; affects photosynthesis, pigment formation, biosynthesis of various metabolites, and resistance to stressful conditions.

Several genera of bacteria, including *Bacillus*, can produce volatile organic compounds. 2,3-butanediol and Acetoin are examples of better-known synthesized substances that are responsible for significant improvements in plant root growth (Batista, 2017).

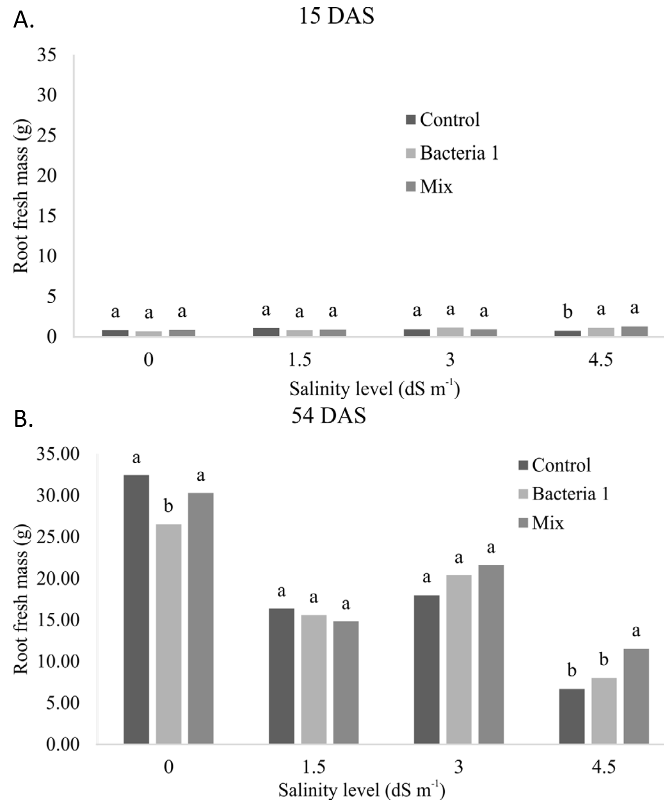
For the bacterial mix (Figure 3), there was a 25.0% reduction in root system length when irrigated with 4.5 dS m^{-1} water compared to the control treatment and T1. This result can be explained because some bacteria under severe stress can lead to overproduction of reactive oxygen species (Liang et al., 2018).

Root mass is also an important evaluative parameter for understanding the data. As can be seen (Figure 4A and 4B), significant interaction occurred between the factors analyzed. At 15 DAS, there was a variation among the treatments, with no difference between the control, 1.5 and 3.0 dS m^{-1} (Figure 4A). However, when irrigation with brackish water (4.5 dS m^{-1}) was considered, there was a significant difference with the presence of bacterium 1 and the mix, increasing the fresh matter by 24.0 and 28.0%, respectively, relative to the control treatment (Figure 4A).

The reduction in root length is an expected result for high salinity levels, as high salt levels lead to a decrease in soil water potential when dissolved ions come in contact with the roots, causing an adjustment to occur through a decrease in soil osmotic potential (Hamad et al., 2015).

PGPB are bacteria that can be endophytic, free-living in the soil or rhizobacteria that colonize the rhizosphere known to offer several benefits to agriculture, among them tolerance to biotic and abiotic stresses (Ramakrishna et al., 2019). According to Dias et al. (2022), microorganisms associated with native plants of arid and semiarid regions may present advantages due to their adaptations to withstand water deficiency, promoting cell protection mechanisms and benefiting the plant when inoculated.

At 54 DAS (Figure 4B), it is observed that the inoculation of seeds with the mix, in the absence of salinity, promoted a gain in mass compared to bacteria 1, but equal to that observed in the control. When irrigated with saline water, regardless of concentration, the plants significantly reduced root fresh mass. Plants irrigated with 1.5 and 3.0 dS m^{-1}, although there was a reduction in root mass, there was no difference between the treatments applied, however, using 4.5 dS m^{-1}, an increase in root mass by a significant 58% compared to the control treatment using the bacteria mix could be observed.



Means followed by the same letter do not differ by Tukey test (p ≥ 0.05).

Figure 4. Root fresh mass of corn (*Zea mays*) plants, hybrid variety AG 1051 from SEMINIS® inoculated with bacteria for induction of salinity resistance, at 15 (A) and 54 (B) days after sowing (DAS) - Juazeiro, BA, Brazil, 2022.

In evaluating the tolerance of *Bacillus* inoculated corn to salinity, Ferreira et al. (2018) reported that saline conditions negatively affected plant dry mass in both non-inoculated and inoculated plants, with average reductions of 85.0 and 95.0% for aerial part and root, respectively, at the highest salinity level. A similar result was observed at 54 DAS (Figure 4B). However, the same author reports that in the treatments without salinity (control) and with higher salt concentration, inoculation of *B. subtilis* promoted greater aboveground and root dry mass than treatments without *B. subtilis*. This result also corroborates with the result presented in Figure 4B, at 54 DAS, in which the bacterial mix was superior to the others with respect to dry matter (DM) accumulation of the root system.

Indole-3-acetic acid (IAA) production by rhizobacteria is a very common feature (Bahi et al., 2016). Increased IAA in the rhizosphere promotes root growth and therefore indirectly increases nutrient uptake capacity (Ahemad & Kibret, 2014). Bacteria such as *Azospirillum* spp., *Pseudomonas* spp., and *Bacillus thuringiensis* are examples of IAA producing PGPB

(Santos et al., 2022). Results of work conducted by Alcantara et al. (2021), report the increment in root dry mass in *Azospirillum* inoculated maize, suggesting a response of the favoring of phytohormone synthesis performed by PGPB, increasing the total root length, surface area and root volume and total length of fine roots of the maize crop (Calvo et al., 2017).

Analyzing the variable of total soluble sugars (TSS) in root tissue, a significant interaction was observed between the factors bacteria and salinity (Figure 5A). Bacteria 1 with river water showed a significant reduction of 29% in TSS levels compared to the control, but at 1.5 and 3.0 dS m^{-1} there was an increase of 28.0 and 44.0%, respectively, when compared to the river water treatment. As for the treatment with the mix of bacteria, there is a significant increase only in the water containing 3 dS m^{-1}, of approximately 66% compared to the control.

The TSS values obtained in root tissue at 1.5 dS m^{-1} increased with bacterium 1. The mix obtained better values at the 3.0 dS m^{-1} concentration. However, no significant difference in TSS content was observed in plants irrigated with 4.5 dS m^{-1}.

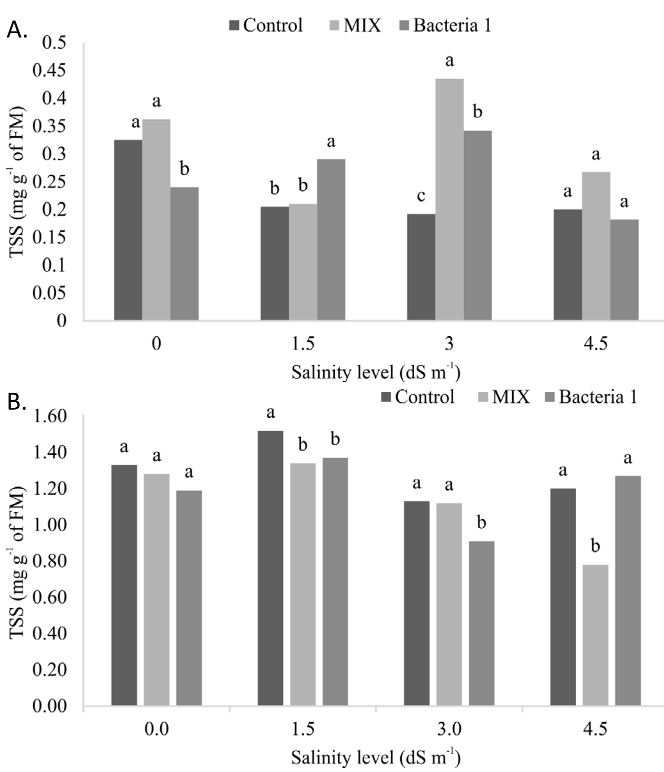
A high concentration of salts can lead to an imbalance in the interaction of carbohydrates, polysaccharides, proteins, and oil percentage from decreased photosynthetic rate, osmotic stress, ionic and nutritional imbalance, and can reduce or increase the levels of this solute throughout the plant (Farooq et al., 2019).

When analyzing the TSS content in leaf tissue (Figure 5B) it can be seen that the values obtained from plants irrigated with river water (control) had no difference between the treatments. It is important to note that when in the presence of 1.5 dS m^{-1}, the values obtained with the bacteria 1 and mix were lower than those reported for the control, however, they remained similar to those observed when the plants were irrigated with river water (control).

The reduction in values was observed with irrigation of 3.0 dS m^{-1}. For the values obtained with 4.5 dS m^{-1}, there was a recovery in TSS content in the presence of bacterium 1, adjusting its metabolism to this salt stress, because the values did not differ statistically from the negative control. The increase in TSS concentration may be related to the induction of an adjustment aimed at cellular osmotic balance (Villa et al., 2019). One possibility for this to happen, would be through direct mechanisms used by some bacteria producing phytormones, enzymes, and nutrient availability (Tiwari et al., 2019).

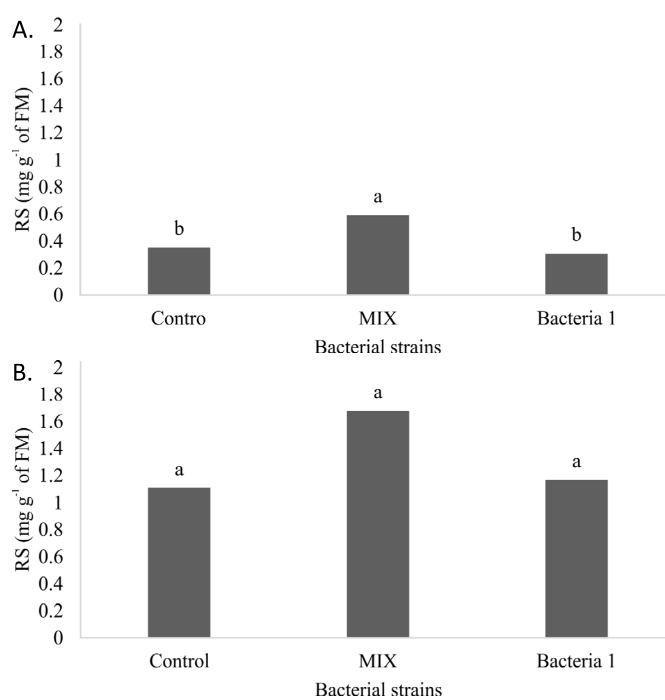
The content of reducing sugars (RS) at 54 DAS in root and leaf tissue (Figure 6A and 6B), respectively, in corn plants obtained from bacterially inoculated seeds, show that the bacterial mix promoted a higher concentration of RS in the root tissue and that there was no difference in the content obtained of this carbohydrate in the leaf tissue.

Total soluble sugars (TSS) are mostly made up of hexoses and small-chain sugars, comprising all the soluble sugars stored in the tissues, among which sucrose stands out as being



Means followed by the same letter do not differ by Tukey test (p ≥ 0.05).

Figure 5. Content of total soluble sugars (TSS) present in root (A) and leaf tissue (B) of corn (*Zea mays*) plants, hybrid variety AG 1051 from SEMINIS® inoculated with bacteria for induction of resistance to salinity at different levels, at 54 days after sowing (DAS) - Juazeiro, BA, Brazil, 2022.



Means followed by the same letter do not differ by Tukey test (p ≥ 0.05).

Figure 6. Content of reducing sugars (RS) present in root (A) and leaf tissue (B) of corn (*Zea mays*) plants, hybrid variety AG 1051 from SEMINIS® inoculated with bacteria for induction of salinity resistance at 54 days after sowing (DAS) - Juazeiro, BA, Brazil, 2022.

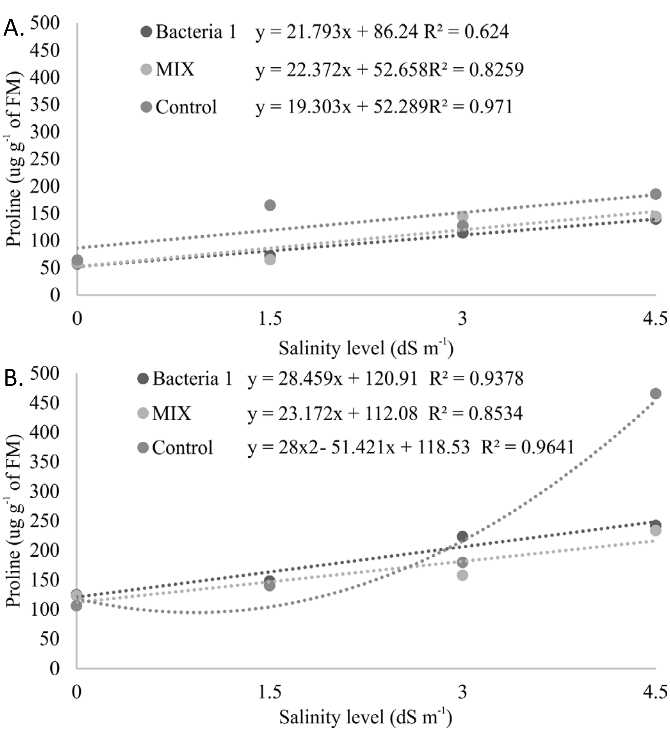
in the greatest quantity. The balance between TSS and RS shows the availability of the carbohydrate to be translocated. The greater the difference between TSS and RS, the higher the sucrose contents (Mesquita et al., 2018).

The bacteria may have been able to synthesize compatible solutes to aid in the mobilization of soil nutrients being taken up by the roots in those treatments involving the mix. Indolacetic acid (IAA) is one of the substances that some bacteria secrete and thereby help mitigate the effects of stress by adjusting physiological processes in plant cells such as photosynthesis (Radhakrishnan & Baek, 2017).

The levels of the amino acid proline (Figure 7A) in the leaf tissue of bacterially inoculated plants increased linearly with increasing salt concentration. However, with irrigation at 4.5 dS m^{-1} in plants maintained as a negative control, this value practically doubled, causing the response to present a quadratic adjusted model. The values observed in the root system (Figure 7B) maintained a repeating linear behavior, and standing out again with irrigation of 4.5 dS m^{-1} in plants kept under negative control.

According to Ferrreira et al. (2018), at the highest NaCl concentration (200 mM), proline concentration increased in non-inoculated plants, and *B. subtilis* inoculation promoted a reduction in proline concentration. This result corroborates with the data obtained in this study.

Some authors have reported that the increase in proline content may be associated with the water content in the leaves, as there is a strong correlation between leaf water



Means followed by the same letter do not differ by Tukey test ($p \geq 0.05$).

Figure 7. Content of proline in leaf tissue (A) and root system (B) in corn (*Zea mays*) plants, hybrid variety AG 1051 from SEMINIS® inoculated with bacteria to induce salinity resistance at different levels (0, 1.5, 3.0, and 4.5 dS m^{-1}) at 54 days after sowing (DAS) - Juazeiro, BA, Brazil, 2022.

potential and proline concentration (Nadeem et al., 2010), indicating that proline is important in maintaining water in the leaves and consequently, maintaining photosynthetic activity by absorbing water and nutrients from the soil.

The amino acid proline is one of the adaptive strategies for osmotic adjustment, playing an important role in osmoregulation, allowing the plant to have a greater tolerance to biotic and abiotic factors. This adaptation occurs through a reduction in osmotic potential and increased cell turgidity, improving water uptake (Silveira et al., 2016).

According to Maia & Tanaka (2007), one of the most common and best documented responses is the ability of some species to osmotically adjust their cells. This happens through the active accumulation of sugars, organic acids, amino acids, or ions in the cytosol to decrease the osmotic potential and consequently maintain the water potential and turgor of their cells near the optimum level.

As a result, the accumulation of proline did not differ between the treatments evaluated (Control, mix, and bacteria 1) at salt concentrations with irrigation of 1.5 and 3.0 dS m^{-1}, with only a high content of proline in leaf tissue (negative control) when irrigated with 4.5 dS m^{-1}. Ferreira et al. (2018) pointing out that the accumulation of proline in plants confers protection against salinity and water stress, however, in plants not inoculated with bacteria.

Conclusion

The treatments containing the bacterium (XX6.9) and the mix (XX6.9 + T1.1 and T11.1) promoted gains in root system growth and fresh mass accumulation at the beginning of cultivation and reducing its loss at 54 DAS. There was also a gain in carbohydrate accumulation, as well as promotion of osmotic adjustment of the cells with the accumulation of the amino acid proline. Thus, the benefit of inoculating the seeds with bacteria and reducing the losses caused by saline stress is evident.

Compliance with Ethical Standards

Author contributions: Conceptualization: ACM, AFJS; Data curation: DNSR, ATRF; Formal analysis: DNSR, ATRF; Funding acquisition: ACM; Investigation: DNSR, ATRF; Methodology: DNSR, ATRF; Project administration: ACM, AFJS; Resources: ACM, AFJS; Supervision: ACM.

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