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RESEARCH ARTICLE

Biochemical and enzymatic alterations of watermelon associated with irrigation management and inoculation with Rhizobacteria

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Abstract

Water stress has caused major losses in the agricultural productivity of crops, inducing the search for alternatives for sustainable cultivation. In this context, the objective of this study was to evaluate the tolerance of watermelon under water stress, inoculated with bacterial strains of the genus *Bacillus* spp., regarding the biochemical and enzymatic variables in the flowering stage. A randomized block design was adopted in a split-plot 4x4 factorial scheme, with plots consisting of four levels of soil water availability (40%, 60%, 80% and 100% of field capacity - FC) and subplots consisting of four inoculations (Negative Control (NC); XX6.9 bacteria; P6.2 bacteria; MIX – co-inoculation of XX6.9 and P6.2 bacteria), with five replicates. XX6.9 bacteria and NC were the treatments most affected by severe water stress, since at the soil water availability (SWA) level of 40% FC they showed high contents of the oxidative marker (MDA) and proline. Although the inoculation with XX6.9 bacteria promoted a higher content of osmoregulators such as proteins, total soluble sugars and reducing sugars, it was not enough to attenuate the effects of water deficit. On the other hand, treatments with P6.2 bacteria and MIX of bacteria showed reduced levels of MDA at the SWA level of 40% FC, accompanied by high enzymatic activity of POD and CAT, which may contribute to the tolerance of the watermelon crop to water stress.

Keywords: Plant growth promotion; reactive oxygen species; semi-arid region; oxidative stress; hydric stress.

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1. Introduction

The water stress is a persistent challenge for global food production, and its intensity is expected to expand in the imminent future (Dubey et al., 2021). In this context, the search for food security for the growing world population alerts us to the need to produce more food sustainably (Martínez-Valderrama et al., 2023). Some works have been described in relation to plant stress associated with water deficits in field conditions, among them, for watermelon cultivation we can mention (Olotu et al., 2024; Mohan et al., 2024; Silva et al., 2024;).

In regions where water is the limiting factor for crop production, maximizing water productivity through deficit irrigation is often more economically profitable for a farmer than maximizing yield (Kamer et al., 2022). Thus, the incorporation of growth-promoting rhizobacteria into production systems has become a promising strategy to minimize limiting factors in crops, such as water stress (Bhadrecha et al., 2023).

Adverse effects of drought on crops include reduced growth and a drastic increase in oxidative stress markers, such as hydrogen peroxide and lipid peroxidation. However, inoculation with species of the genus *Bacillus* has been shown to have a protective effect on stabilization of oxidative status (Maslennikova et al., 2021).

The mitigation of water stress with bacterial strains occurs through the increased production of enzymes, such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and proline. In addition, bacterial strains exhibit plant growth-promoting characteristics, which include phosphate production, siderophore synthesis, and increased hormone levels of gibberellin and auxin (Yadav et al., 2022).

Rhizobacteria are also responsible for forming biofilm, which is formed by multicellular aggregates that adhere to root surface through the production of substances, such as polysaccharides, proteins, and DNA (Vieira et al., 2021).

Due to the numerous benefits promoted by the inoculation of bacteria in plants, the possibility of applying these microorganisms in seeds as potential inoculants in the near future is considered. However, plant-microorganism associations need to be perfected to improve drought tolerance in cultivated plants, given the complexity of plant-microorganism and microorganism-microorganism interactions and their dependence on environmental conditions.

Thus, the objective of this study was to evaluate the tolerance of watermelon [(*Citrullus lanatus* (Thunb.) Matsum & Nakai] under water stress, inoculated with bacterial strains of the genus *Bacillus* spp., regarding the biochemical and enzymatic variables in the flowering stage.

2. Methodology

The experiment was conducted from Sept. to Nov. 2021 in a greenhouse (with 40% Chromatinet® shade net) in the municipality of Juazeiro, BA, Brazil, with geographic coordinates: 09º 24′ 50″ S latitude, 40º 30′ 10″ W longitude and 368 m altitude. This site has striking characteristics related to low average annual rainfall and high evapotranspiration rates.

The monthly climatic data, obtained from the automatic weather station, installed at the institution where the study was carried out, are shown in Figure 1.

A randomized block design was adopted, in a split-plot 4x4 factorial scheme, with plots consisting of four levels of soil water availability (40; 60; 80 and 100% of field capacity - FC) and subplots consisting of four types of seed inoculation (Negative Control (NC) - No bacteria; XX6.9 bacteria; P6.2 bacteria; and MIX (corresponding to the co-inoculation of XX6.9 bacteria and P6.2 bacteria)), with five replicates.

Black polyethylene pots with capacity for 21 L were used for cultivation. The pots were arranged at spacing of 2 m between rows and 0.5 m between pots in each row. A layer of crushed stone (2.5 kg) was initially put into the pots and then a fine-mesh screen. The pots were filled with soil, always maintaining a constant mass of 21.5 kg of soil for standardization purposes.

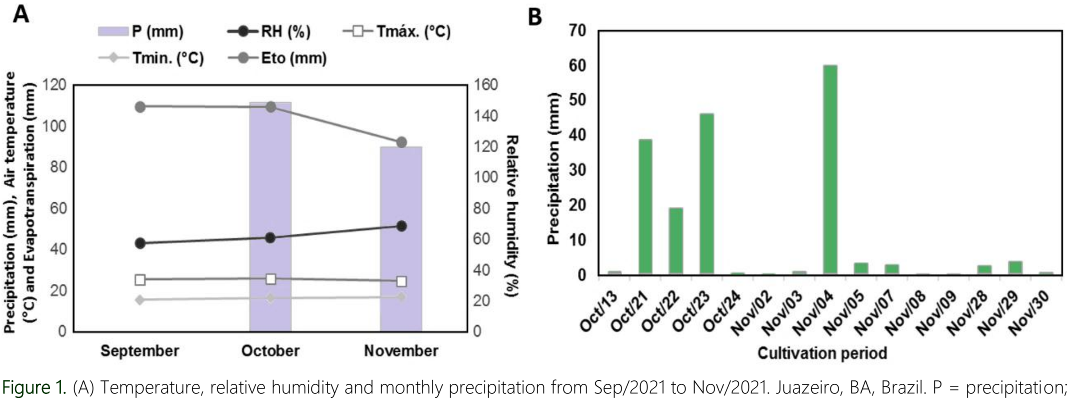

Figure 1. (A) Temperature, relative humidity and monthly precipitation from Sep/2021 to Nov/2021. Juazeiro, BA, Brazil. P = precipitation; ETo = Evapotranspiration; Tmax = Maximum air temperature; Tmin = Minimum air temperature; RH = Relative humidity and (B) Daily precipitation (mm) during the cultivation period. Juazeiro, BA, Brazil, 2024.

Table 1
Chemical attributes of the soil used in the study

	pH			EC ($dS \cdot m^{-1}$)					
	$CaCl_2$	KCl	H_2O	Sat. Ext.	V (%)	$100 \cdot Na^+/T$	P ($mg \cdot dm^{-3}$)	m (%)	OM ($g.kg^{-1}$)
S1	5.58	6.14	6.58	0.026	96.92	0.82	5.05	0	9.86

Sorption Complex ($cmol_c/dm^3$ of ADFE)								
	Ca^{+2}	Mg^{+2}	K^+	Na^+	SB	Al^{+3}	$H^+ + Al^{+3}$	T
S1	2.30	2.02	0.37	0.04	4.58	0	0.15	4.88

Sat. Ext.: Saturated extract; SB: Sum of bases; T: Cation exchange capacity; V: Base saturation percentage; m: Aluminum saturation percentage; OM: Organic matter; P: Phosphorus content; S1 = Samples 1.

The soil used in the study was classified as Fluvic Neosol (EMBRAPA, 2013), and samples were taken to the Soil, Water and Limestone Analysis Laboratory - LASAC, where its chemical characterization was carried out, whose results are shown in Table 1.

Before differentiating the soil water availability (SWA) levels, inoculated and non-inoculated seeds were placed to germinate in the soil, and the pots were irrigated so as to maintain SWA at field capacity (SWA level of 100% FC) until obtaining a seedling with the first true leaf, a period that extended to 17 days after sowing (DAS). From the appearance of the true leaf until the end of the watermelon cycle (72 DAS), the volume of water applied began to be differentiated to maintain the SWA of these treatments.

Irrigation management was carried out using a TDR100 model from Campbell (Time Domain Reflectometry) equipment. Four different probes were selected, one for each type of inoculation. After reading, the treatments were manually irrigated using a graduated cylinder. In the period of SWA differentiation, irrigation was based on replacing the volume of water consumed to the levels evaluated in the study, aiming to maintain the limits established in the treatments.

Two strains of the genus *Bacillus* spp., named XX6.9 and P6.2, from the collection of the Microbial Biotechnology Laboratory, were used. In previous studies conducted by the Laboratory's research group, the strains have shown efficiency in environments with water restrictions for the maize crop, and plant growth-promoting potential in monocots. Both bacterial strains were previously isolated from the rhizosphere of cacti native to the Caatinga (Dias et al., 2022; Silva et al., 2023).

The bacterial isolates were cultured for 48h at 28 ± 2 °C. For the microbiolization of the seed, xanthan gum solution was added to the cultures, after the concentrations had been individually adjusted by spectrophotometry model UV-1600 from Pró-Análise (OD 540 nm). Subsequently, the seeds of watermelon var. Crimson Sweet were immersed in the suspension for 40 min.

The seeds used as control were immersed in xanthan gum solution to be subjected to the same procedure as the other treatments. After this period, the seeds were left to dry on paper towels at room temperature.

Three seeds per pot were sown at 3 cm depth, in order to obtain one plant per pot after thinning.

The biochemical variables total soluble sugars (TSS) (Yemm & Willis, 1954), reducing sugars (RS) (Miller, 1959), total proteins (Pt) (Bradford, 1976) and proline (Pr) (Bates et al., 1973), as well as the enzymatic variables catalase (CAT) (Peixoto, 1999) and peroxidase (POD) (Teisseire & Guy, 2000), and the oxidative stress marker, lipid peroxidation (MDA) (Heath & Packer, 1968), were measured at fruiting (72 DAS).

To determine the enzymatic activity, the plant material was collected, wrapped in aluminum foil, properly identified, and frozen in liquid nitrogen (±196 °C) to immediately paralyze all metabolic reactions. Subsequently, the samples were stored in a freezer for further analysis of enzyme activity.

The data were subjected to analysis of variance (ANOVA) with F test at 5% and 1% significance levels. When significant, the means were compared by regression analysis (soil water availability) and Scott-Knott test (inoculation of bacteria), using SISVAR software version 4.0 (Ferreira, 2011).

3. Results and discussion

In the analysis of variance, soil water availability (SWA) levels caused significant effects on all enzymatic and biochemical variables. For the bacterial inoculation, all enzymatic and biochemical variables were influenced, except for reducing sugars (RS). In addition, significant interaction was observed between SWA and bacterial inoculations for antioxidant enzymes and biochemical factors.

Regarding lipid peroxidation (MDA) (Figure 2) as a function of SWA, the regression curves of the evaluated treatments followed a quadratic behavior, except for the treatment P6.2, which showed a linear and decreasing response.

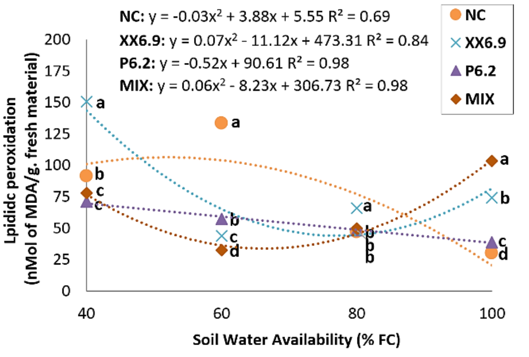

Figure 2. Lipid peroxidation of watermelon inoculated (XX6.9, P6.2 and MIX) or not (NC) subjected to soil water availability levels, at 72 DAS. Means followed by identical letters do not differ from each other by the Scott-Knott test at 5% significance level. Juazeiro, BA, 2024.

The highest lipid peroxidation in Negative Control (NC) (106.56 nMol MDA g fresh matter^{-1}) occurred with SWA level of 52% FC, with a subsequent reduction with increasing SWA, reaching 30.24 nMol MDA g fresh matter^{-1}, with 100% field capacity (FC) (Figure 2).

The content of MDA, the end product of lipid peroxidation, is used to determine the extent of oxidative damage. If the MDA content increased, it means that reactive oxygen species (ROS) were generated, leading to membrane disturbance, which can cause oxidative stress to proteins, nucleic acids, lipids, and the electron transport chain (Ashagari et al., 2020; Alkahtani et al., 2021a). When analyzing the MDA content between the treatments as a function of SWA alone (Figure 2), it was found that the lowest value at SWA of 40% FC was obtained with the P6.2 treatment, while the lowest value at SWA of 60% FC was obtained with the MIX treatment, decreasing almost 22 and 76%, respectively, compared to NC.

This is not the first time that inoculation in Cucurbitaceae species has contributed to reduction of MDA under water stress. Alkahtani et al. (2021a) explain that the better performance with inoculation is due to the increase in phenols and greater enzymatic activity, which protect proteins and lipids from oxidative stress, neutralizing ROS, reducing the formation of MDA, and preventing the cell membrane from being damaged.

On the other hand, the XX6.9 and NC treatments had higher MDA content than the other treatments at the respective SWA levels (40 and 60% FC) (Figure 2). Abbasi et al. (2020) also observed the same behavior with bacterial treatments. However, the parameters of vegetative growth were not affected by water stress. Therefore, it is important to evaluate other parameters to determine the efficacy of the inoculation.

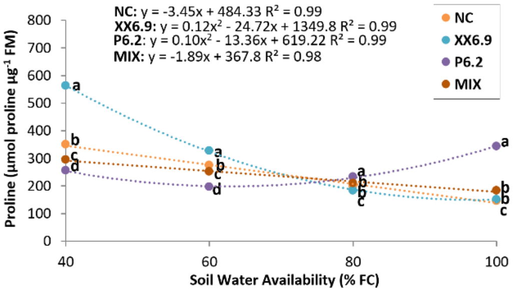

Figure 3. Proline in the leaf tissue of watermelon inoculated (XX6.9, P6.2 and MIX) or not (NC) subjected to soil water availability levels, at 72 DAS. FC – Field capacity. Means followed by identical letters do not differ from each other by the Scott-Knott test at 5% significance level. Juazeiro, BA, 2024.

Figure 3 shows the behavior of proline as a function of SWA. For the treatments with XX6.9 bacteria, MIX and NC, the regression showed an increase in proline as SWA decreased (Figure 3). In addition, the increase in proline was more pronounced with the XX6.9 treatment (Figure 3), whose mean was 563.67 µmol proline μg^{-1} FM at the SWA level of 40% FC, with an increase of 61% compared to NC. Lu et al. (2021) demonstrated that the mean of the XX6.9 treatment was even higher than that observed in the watermelon crop without inoculation at -50 kPa, for which the means ranged from 150 to 200 µmol proline μg^{-1} FM. The increase in proline constitutes an instantaneous response to water stress, as this organic acid acts as an antioxidant and a signaling molecule, preventing the action of ROS. It acts as an osmotic regulator, reducing the osmotic potential of the cell and, in the case of root cells, allows water uptake even in harsh environments (Cui et al., 2021).

Plants are already equipped with an antioxidant defense that includes SOD (superoxide dismutase) and APX (ascorbate peroxidase), in addition to CAT and POD. However, under severe and prolonged conditions, the system is not sufficient to prevent damage. Therefore, inoculation with *Bacillus* would have a positive effect on inducing enzymes, alleviating oxidative damage (Anee et al., 2019; Latef et al., 2020), as observed in the present study.

When analyzing peroxidase (POD) as a function of SWA, it was observed that the treatments were influenced by the SWA levels, and the regression showed a linear trend, except for the P6.2 treatment, whose response was quadratic (Figure 4).

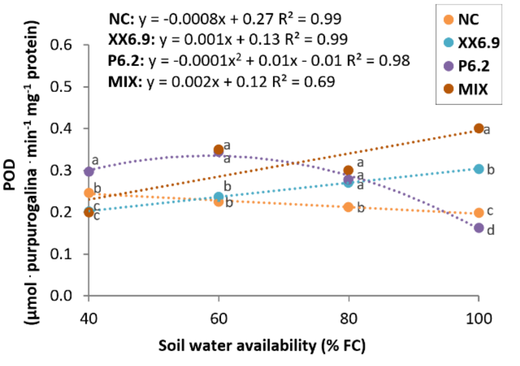

Figure 4. Peroxidase (POD) of watermelon inoculated (XX6.9, P6.2 and MIX) or not (MIX) subjected to soil water availability levels, at 72 DAS. Means followed by identical letters do not differ from each other by the Scott-Knott test at 5% significance level. Juazeiro, BA, 2024.

The P6.2 treatment proved to be superior in water-restricted environments compared to the other treatments, with maximum POD (0.34 µmol purpurogallin min^{-1} mg^{-1} protein) at the SWA level of 59.5% (Figure 4). This result was lower than that obtained in cantaloupe melon (*Cucumis melo* L.) inoculated with a mix (*Pseudomonas*, *Azotobacter* and *Enterobacter cloacae*), which averaged 1.1 µmol purpurogallin min^{-1} mg^{-1} protein at 50% FC (Zahedyan et al., 2022).

In relation to the test of means for POD, when analyzing the applied treatments at each SWA level separately (Figure 4), it was observed that the P6.2 treatment showed a higher POD activity at the SWA level of 40% FC. On the other hand, at the SWA levels of 60 and 80% FC, the P6.2 and MIX treatments did not show significant difference, but POD activity was higher at both SWA levels. This is compatible with what was expected for the treatments, since both reduced MDA contents at the aforementioned SWA levels (P6.2 and MIX at SWA levels of 40 and 60% FC, respectively) (Figure 2).

The increase in enzyme activity when accompanied by a reduction in MDA content leads to a decrease in oxidative stress under water-stressed conditions (La et al., 2019). Bikdeloo et al. (2021) state that low MDA contents in 'Crimson Sweet' watermelon and the greater induction of the enzyme peroxidase are indicative of its better ability to cope with oxidative stress.

When analyzing the influence of SWA as a function of the treatments with bacteria on catalase activity, the treatments NC, P6.2 and MIX showed a quadratic behavior (Figure 5).

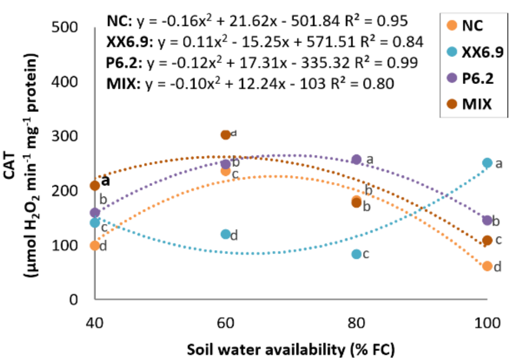

Figure 5. Catalase (CAT) of watermelon inoculated (XX6.9, P6.2 and MIX) or not (NC) subjected to soil water availability levels, at 72 DAS. Means followed by identical letters do not differ from each other by the Scott-Knott test at 5% significance level. Juazeiro, BA, 2024.

NC, P6.2 and MIX treatments showed maximum CAT activity (226.24; 264.99; 262.41 μmol H_2O_2 min^{-1} mg^{-1} protein, respectively) at the respective SWA levels of 67, 69 and 60% FC (Figure 5). It was observed that the MIX treatment induced higher activity compared to NC at a lower SWA. When analyzing the means of CAT in the applied treatments as a function of SWA alone (Figure 5), it can be seen that the MIX increased CAT by 22% compared to NC at the SWA level of 60% FC.

When employing a bacterial mix, one must consider the metabolism of each strain and that inoculation affects the growth of the coexisting strains, leading to interactions, which can be neutral, positive (cooperation, synergism, commensalism) or negative (amensalism, competition, antagonism). Therefore, compatibility between strains it is fundamental (Moreno-Galván et al., 2020; Thomloudi et al., 2019), which explains the good performance of MIX in relation to CAT.

Another characteristic to consider is the sampling origin of the strains. Ideally, the site should come from native plants that grew in arid environments and co-evolved with their microbiota, contributing for the strains to be adapted, tolerant, with long survival and persistence in diverse environments (Gamalero & Glick, 2022), a condition consistent with the materials used in this study.

In addition to the strain-to-strain interaction, another factor is the compatibility between the strains and the watermelon crop. Stress minimization depends on a specific plant-strain interaction, and this influence becomes evident through enzymatic activity and lipid peroxidation (Azeem et al., 2022; Pinski et al., 2019).

The means of CAT in the applied treatments as a function of SWA alone (Figure 5) show that CAT activity with the MIX treatment was also higher at the SWA level of 40% FC (208.92 μmol H_2O_2 min^{-1} mg^{-1} protein). This result is associated with the fact that CAT is located in all ROS-producing compartments and works as a fine regulator converting H_2O_2 into $H_2O+½O_2$. Therefore, the higher the CAT activity, the more efficient the system and the greater its capacity to decompose H_2O_2 (Jayaraj & Beevy, 2021; Sood et al., 2020).

About total protein (Figure 6), a decreasing linear regression can be observed for the NC and XX6.9 treatments, i.e., they increase protein content with the reduction of SWA.

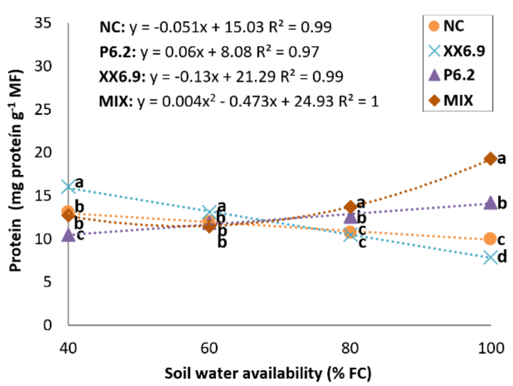

Figure 6. Total proteins of the watermelon crop inoculated (XX6.9, P6.2 and MIX) or not (NC) subjected to soil water availability levels, at 72 DAS. Means followed by identical letters do not differ from each other by the Scott-Knott test at 5% significance level. Juazeiro, BA, 2024.

NC obtained an average protein content of 13.04 mg g^{-1} with SWA of 40% FC (Figure 6), consistent with that obtained in yellow melon cv. Gold Mine, which showed increase in proteins under 50% FC (16.94 mg g^{-1}), with a subsequent decrease under 100% FC, revealing a constitutive characteristic of the variety, which may justify, in part, a drought tolerance strategy (Cruz et al., 2022). XX6.9 bacteria also increased protein content at SWA levels from 100 to 40% FC, from 7.88 to 16.01 mg g^{-1}, respectively (Figure 6), and led to a protein production higher than that obtained with NC at the SWA range from 40 to 60% FC.

Protein synthesis is induced by bacteria in order to adjust the osmotic potential, ensure the normal functioning of plant physiological processes, and inhibit protein degradation (Mendoza-Labrador et al., 2021; Prgomet et al., 2020; Sheteiwy et al., 2021). Thus, it is also interesting to note that the XX6.9 treatment increased protein content between SWA levels of 40 and 60% FC, as well as proline content (Figure 6). This is an indication that the treatment with XX6.9 has a greater capacity to synthesize these solutes under water restriction.

Proteins and proline are osmotic regulators, which maintain water content and protect cells, and because proline is made up of hydrophilic and hydrophobic sections, it can affect protein solubility and prevent albumin abnormality. Studies have already shown the relationship between proline and the surface of proteins, increasing stability and preventing modifications in their structure (Alkahtani et al., 2021b). Thus, as the XX6.9 treatment had a higher proline content, this may also have been a determining factor for protein maintenance.

For total soluble sugars as a function of SWA levels, regression analysis showed that the mathematical model that best fitted to the XX6.9 treatment was the decreasing linear model (Figure 7).

The XX6.9 treatment induced a higher production of TSS at SWA levels with water restriction (Figure 7). Regarding the interference of treatments in TSS as a function of the SWA analyzed individually (Figure 7), it can be seen that the XX6.9 treatment increased TSS by 149; 304 and 91% compared to NC at SWA levels of 40; 60 and 80% FC, respectively.

TSS accumulation is a strategy to withstand damage to cell organelles, proteins, and membranes and increase the stability of cell membranes to provide a stable environment for photosynthetic reaction, act on osmotic balance, and allow plants to absorb water from the soil (Lu et al., 2020; Vieira et al., 2020). Although the P6.2 and MIX treatments did not increase TSS values above the XX6.9 treatment, they were superior to NC at the SWA range from 60 to 80% FC (Figure 7). This response demonstrates that inoculation, regardless of inoculant, increases TSS in water-limiting environments.

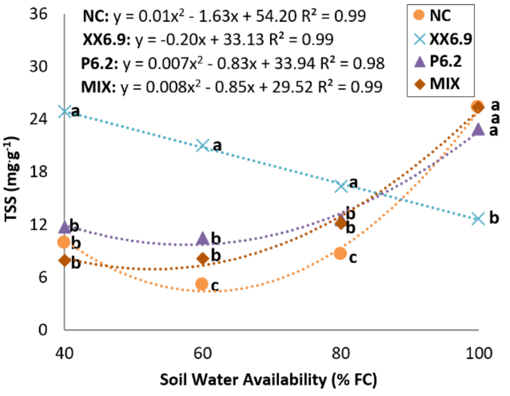

Figure 7. Total soluble sugars (TSS) of watermelon inoculated (XX6.9, P6.2 and MIX) or not (NC) subjected to soil water availability levels, at 72 DAS. Means followed by identical letters do not differ from each other by the Scott-Knott test at 5% significance level. Juazeiro, BA, 2024.

In this context, it should be noted that one of the impacts of water stress is the impairment of physiological activities, such as water absorption, cell division, CO_2 assimilation, and reduction in the supply of assimilates, including sucrose (Alkahtani et al., 2021a). However, this was not the case for plants inoculated with the bacteria.

When analyzing reducing sugars (RS) as a function of SWA levels, it was observed that the quadratic model was the one that best fitted to the data (Figure 8).

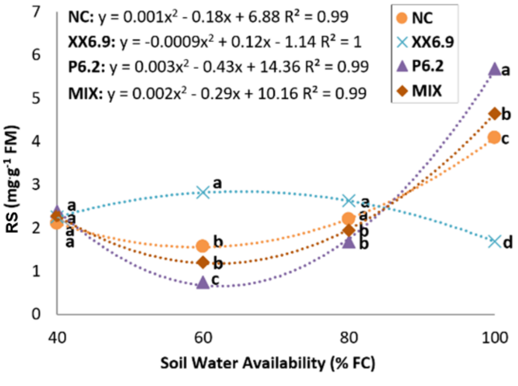

Figure 8. Reducing sugars (RS) of watermelon inoculated (XX6.9, P6.2 and MIX) or not (NC), subjected to soil water availability levels, at 72 DAS. Means followed by identical letters do not differ from each other by the Scott-Knott test at 5% significance level. Juazeiro, BA, 2024.

As observed in the RS content (Figure 8), the XX6.9 treatment also showed higher RS at the SWA level of 60% FC, more limiting than the other treatments. The highest RS content (3.08 mg g^{-1} FM) was obtained at 68% of the soil water retention capacity.

Vieira et al. (2020) also observed quadratic behavior in melon cv. 'Piel de sapo' under 50; 75; 100 and 125% ETc, with maximum RS (6.22 mg g^{-1} FM) at 50% ETc.

Table 2 shows the difference between TSS and RS, determined by the decrease in the TSS variable by RS in inoculated and non-inoculated treatments.

Table 2
Difference between total soluble sugars (TSS) and reducing sugars (RS) in watermelon crop

	TSS - RS			
	40%	60%	80%	100%
NC	7.86	3.62	6.36	21.23
XX6.9	22.61	18.16	13.70	10.99
P6.2	9.36	9.74	10.87	17.22
MIX	5.69	6.97	10.21	20.72

The XX6.9 treatment was responsible for the highest synthesis of TSS under SWA levels of 40; 60 and 80% FC, showing its capacity to synthesize total soluble sugars (Table 2).

In summary, the P6.2 and MIX treatments induced the activation of enzymes under low water availability, while the XX6.9 treatment was responsible for the synthesis of osmoprotectants (proline, TSS, RS and total proteins). As observed for XX6.9, the treatments with *Bacillus endophyticus*, *Bacillus altitudinis* and *Bacillus megaterium* also synthesized more osmolytes instead of activating antioxidant enzymes (Devarajan et al., 2021).

When plants reach a severe stress level, the accumulation of solutes has a high energy cost, and the solutes tend to be degraded, leading the plant to use other mechanisms to survive (Santos et al., 2020), such as the enzyme system, so the XX6.9 treatment may not have been efficient in this study. In addition, it was observed that bacterial treatments induced more POD than CAT, because this enzyme represents a better defense. CAT plays an overall role in tolerance by minimizing H_2O_2 accumulation, while POD, in addition to draining H_2O_2, participates in lignin synthesis. Lignin is responsible for increased structural rigidity, durability of tissues, water transport in xylem vessels, physical barrier against pathogens, and complexation of carbohydrates and proteins (Bezerra et al., 2020).

4. Conclusions

The results show that the XX6.9 bacteria and NC treatments were the most affected by severe water stress, since at the SWA level of 40% FC they showed high contents of the oxidative marker (MDA) and proline. Although inoculation with XX6.9 bacteria promoted a higher content of osmoregulators such as proteins, TSS and RS, this treatment was not sufficient to attenuate the effects of water deficit. On the other hand, the treatments with P6.2 bacteria and MIX of bacteria showed reduced levels of MDA at the soil water availability level of 40% FC, accompanied by the high enzymatic activity of POD and CAT, contributing to the tolerance of the watermelon crop to water stress.

Future studies could further investigate the long-term impact of these treatments under field conditions and explore their effectiveness in different soil types and climatic regions. Furthermore, unraveling the molecular mechanisms underlying the interaction between these bacteria and the plant's response to stress can provide deeper knowledge for the development of effective agricultural practices.

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Authors' Contributions
Conceptualization: Simões W. L., Mesquita A. C. Data curation: Simões W. L., Mesquita A. C., Araújo, M. G. Formal analysis: Simões W. L., Mesquita A. C., Araújo M. G. Funding acquisition: Simões W. L., Mesquita A. C. Methodology: Simões W. L., Mesquita A. C., Araújo M. G., Carvalho R. N., Felix A. T. R., Silva J. S. Project administration: Simões W. L., Mesquita A. C. Supervision: Mesquita A. C. Writing-original draft: Simões W. L., Mesquita A. C., Araújo M. G. Writing-review & editing: Simões W. L., Mesquita A. C., Araújo M. G., Carvalho R. N., Felix A. T. R., Silva J. S.

Policy on Conflict of Interest
There are no potential personal, commercial, political, academic or financial conflicts of interest. There is no use of artificial intelligence.

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