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Native bacteria from the caatinga biome mitigate the effects of drought on melon (Cucumis melo L.)

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Abstract

Plant growth-promoting bacteria (PGPB) from drylands are promising biological resources to mitigate the negative effects induced by water deficit. The aim of this study was to evaluate the effects of bacteria native from the Caatinga biome on the initial growth of melon plants subjected to water deficit. Nine bacteria (M1.1, T11.2, PH5.2, T11.1, T2.1, T1.1, M7.1, XX6.9 and XX6.6) isolated from Caatinga soils were tested in two varieties of melons (Cantaloupe and Yellow) cultivated under two water availability scenarios (50% irrigation and 100% irrigation of the crop evapotranspiration - ETc). In the control treatment, no inoculation was performed. The effects of the treatments on shoot length (SL), shoot dry mass (SDM), root length (RL) and root dry mass (RDM) were evaluated. In the scenario without water deficit (100% ETc replacement), the isolates PH5.2, T2.2, M7.1, XX6.9 promoted ($p<0.05$) the root and shoot biomasses in the Cantaloupe variety, while in the Yellow variety, growth promotion was sporadic, with three isolates (M1.1, M7.1 and XX6.9) promoting at least one parameter evaluated. In the scenario with a water deficit (50% ETc replacement), isolates T1.1 and XX6.9 promoted the total biomasses (SDM and RDM) of the Cantaloupe and Yellow varieties, respectively. All isolates stimulated RL in the Cantaloupe variety. Bacteria isolated from the Caatinga promote growth and reduce the effects of water deficit in melon and thus are potential inoculants to enhance production in the early stages of melon cultivation in semiarid regions.

Keywords: growth promotion, rhizobacteria, semiarid, water deficit

Introduction

The stress caused by low water availability is considered an important constraint for agricultural productivity and efficiency worldwide (Sarazin et al., 2017). Changes in air temperature and precipitation levels are extending water scarcity periods and resulting in drier years, especially in arid and semiarid regions of the planet. These changes may further reduce water availability and increase the risk of severe droughts and more extensive and frequent droughts, which are the main limiting factors of yields in these regions (Ali et al., 2017).

The techniques currently applied to mitigate the negative effects of drought are, for the most part, dependent on technologically advanced resources and, for this reason, are difficult to apply in practice in the field (Niu et al., 2018). An alternative that has gained prominence in the agricultural sector is the use of microorganisms that induce tolerance to water deficit and help crops to improve water use.

Inoculation of plant growth-promoting bacteria (PGPB) is an approach that is already used in the agricultural sector for different purposes. The interaction between PGPB and plants may also reduce the negative effects of abiotic and biotic stresses, thus increasing crop productivity (Amna et al., 2020; Danish et al., 2020).

Among the mechanisms that help plants inoculated with PGPB under water deficit conditions are changes in the root system, osmoregulation and osmoprotection, biosynthesis of phytohormones, antioxidant enzymes and extracellular polysaccharides, in addition to transcriptional regulation of genes responsive to water stress (Jochum et al., 2019). The interaction between plants and microorganisms allows the bacteria to use one or more of these mechanisms to maintain crop productivity in times of limited water availability.

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The melon (*Cucumis melo* L.) has stood out in semiarid regions because it is a highly profitable crop with rapid economic return. Brazil ranks 11th among the largest melon producers (Kill et al., 2016), with the northeastern region of the country being responsible for approximately 93% of the fruit production. In addition to having an impact on the national economy, the crop is an important promoter of diversification of agricultural activity in producing areas (Silva et al., 2014), playing a socioeconomic role in ensuring income for farmers, especially those engaged in rainfed cultivation.

Studies have shown that melon crops are affected by limited water availability (Faria et al., 2015; Simões et al., 2018). However, studies on the use of microorganisms and their effects on crops subjected to water deficit are scarce. Thus, the aim of this study was to evaluate the effect of bacteria native from the Caatinga biome on the initial development of melon plants subjected to water deficit.

Material and Metods

The study was conducted at the Department of Technology and Social Sciences (DTSS) of the State University of Bahia (SUB) in the city of Juazeiro (latitude: 09° 24' 50'' S; longitude: 40° 30° 10' W; elevation: 368 m) in a greenhouse with 50% shading. The climate of the region is BSh type, according to the Köppen classification, and is characterized by being semiarid and hot. The average annual precipitation is 540 mm, and the average temperature is 27 °C.

The experiment was conducted between September and October 2020, with a duration of thirty-five days between planting and final evaluation. Two varieties of melon were used in the experiment: Cantaloupe v. gold and yellow v. Gladial. The treatments consisted of inoculation with nine bacteria (M1.1, T11.2, PH5.2, T11.1, T2.1, T1.1, M7.1, XX6.9 and XX6.6.) and the control without inoculation. The nine bacteria used in this study were previously isolated from plants native to the Caatinga and compose the microbial culture collection of the Laboratory of Microbial Ecology and Biotechnology of the Semiarid Region of SUB. The bacteria selected for the study showed prior efficiency in promoting growth and inducing drought tolerance *in vitro* (data not shown). The experimental design used was a completely randomized design with five replications, totaling 50 experimental units. The irrigation depth was established as a function of the crop evapotranspiration (ETc) determined daily. ETc was determined using the lysimetry weighing method to quantify the daily water loss. Of the total evapotranspired water, 100% was replaced for the plants growing in the scenario without water deficit, and 50% was replaced for the plants growing in the scenario with water deficit. The plants were subjected to this water regime for 35 days.

For inoculation, bacterial suspensions were prepared with the aforementioned isolates and adjusted to a concentration of $1x10^9$ CFU mL^{-1} in a spectrophotometer at OD 600 nm. Melon seeds were germinated on germitest paper for 4 days to ensure planting uniformity and then planted in 300 ml containers containing specific substrate for seedlings. One day after planting, 2 ml of the inoculum for each treatment was applied to each container. The negative control consisted of applying the same volume of sterile distilled water.

The analyses were performed 35 days after inoculation. To analyze the initial growth of the melon, the following parameters were evaluated for the two varieties: root length (cm), root dry mass (g/pl), shoot length (cm) and shoot dry mass (g/pl). A graduated ruler was used to measure the plant height and root length, and a digital caliper was used to determine the stem diameter. The seedlings were separated into shoots and roots to determine the dry mass. The samples were placed in paper bags, which were then transferred to a drying oven with forced circulation at 60 °C for 72 hours and then weighed on an analytical balance. Statistical analyses were performed using Sisvar v 5.6 software (Ferreira, 2019). The data were subjected to a test of normality (Shapiro Wilks) before analysis of variance. When significance was observed, Dunnett's test was applied. All tests were performed at a 5% probability level.

Results and discussion

Inoculation with bacteria isolated from the Caatinga biome promoted ($p<0.05$) melon growth in the initial phase of plant development in both water availability scenarios, but the response was dependent on the variety of melon (**Figure 1** and **Figure 2**).

When the plants were grown in an environment without water deficit (100% W), three isolates (M7.1, XX6.9 and XX6.6) promoted ($p <0.05$) root growth in the Cantaloupe variety (Figure 1). On the other hand, there was no significant increase ($p>0.05$) in the root growth of inoculated plants of the yellow variety. No isolate promoted the increase in the aerial part of the two varieties grown in an environment with water sufficiency.Under water deficit conditions (50% W), there was an increase ($p<0.05$) in root length in both varieties. In this scenario, all isolates tested promoted root growth of the Cantaloupe variety, and two isolates (T1.1 and XX6.9) promoted root growth of the yellow variety (Figure 1). In the Cantaloupe

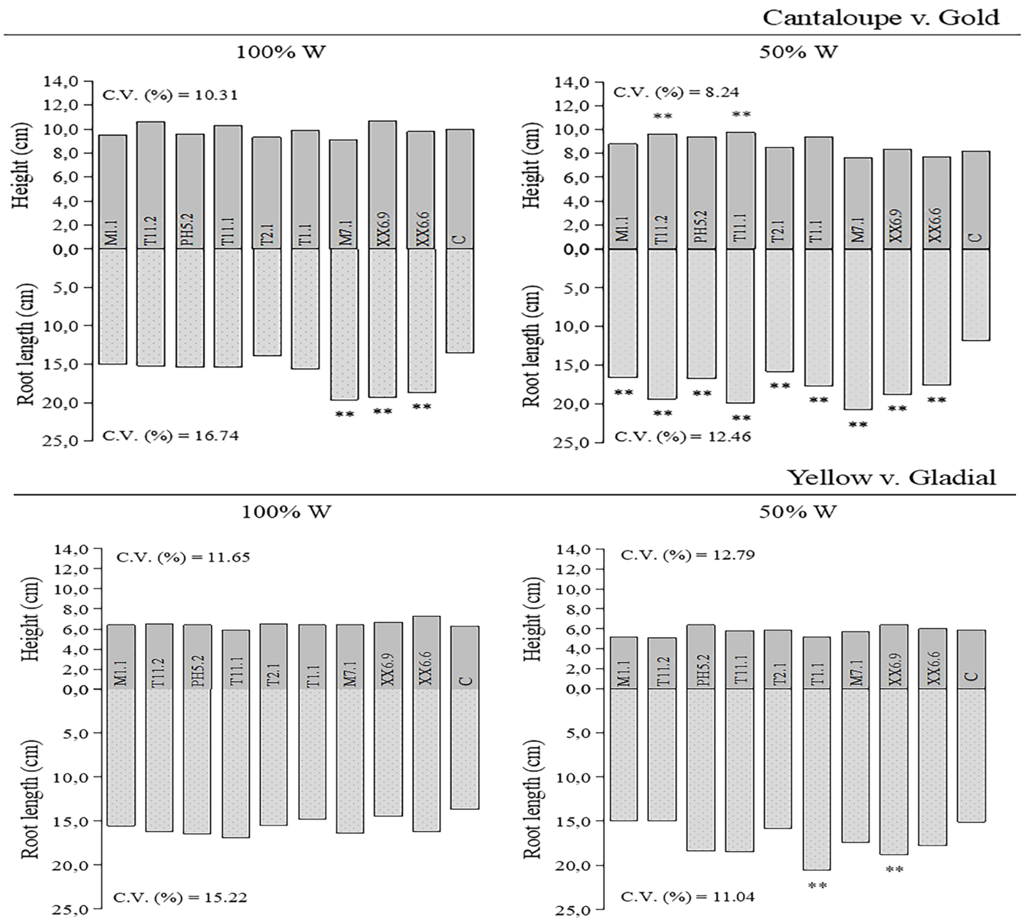

Figure 1: Height (cm) and root length (cm) of Cantaloupe and Yellow melon plants inoculated with bacteria and subjected to two water stress scenarios (100% and 50% W). W = water. ** differs from the control (C) at the 1% probability level according to Dunnett's test. C = Noninoculated control. CV = coefficient of variation.

variety, the increase in root length ranged from 25% to 43%, while in the yellow variety, the increase was 33% (XX6.9) and 37% (T1.1). Under the water deficit scenario, the plants of the Cantaloupe variety inoculated with isolates T11.2 and T11.1 were taller ($p<0.05$) than those in the control (C). There was no significant increase in plant height of the yellow variety inoculated with the isolates.

These results show that the bacteria tested have the potential to promote the initial development of melon plants under both water conditions, providing an advantage in drought and/or dry spells, which have been intensified due to climate change.

Water deficit is known to be a limiting factor that causes negative changes in the morphology, physiology and anatomy of agricultural crops, affecting their development and growth, mainly by the formation of reactive oxygen species, inhibition of processes related to photosynthesis and gas exchange, and intensification of protein denaturation (Shen et al., 2017). In plants under water-limited conditions, cell turgor is reduced and ethylene synthesis undergoes significant changes, resulting in lower cell expansion, leading to a lower growth rate. The maintenance of growth even under water deficit indicates that the plant has mechanisms that ensure its development under these adverse conditions (Morales et al., 2015).

Adaptations to reduce the damage caused by drought involve morphological changes in the plant, for example, the expansion of the root system to expand the area of exploitation by the plant, aiming to maximize the absorption of water and nutrients. Plants with longer roots tend to withstand lean periods more efficiently than plants with less developed roots (Ilyas et al., 2021).

A well-structured root system is a key agronomic trait in horticultural crops that directly affects yield and tolerance to abiotic stresses and increases nutrient absorption and assimilation (Koevoets et al., 2016). Ensuring a more developed and vigorous root system during the early stages of melon cultivation, allowing for a greater ability to explore the soil, is a fundamental condition to ensure maximum productivity (Jung & McCouch, 2013).

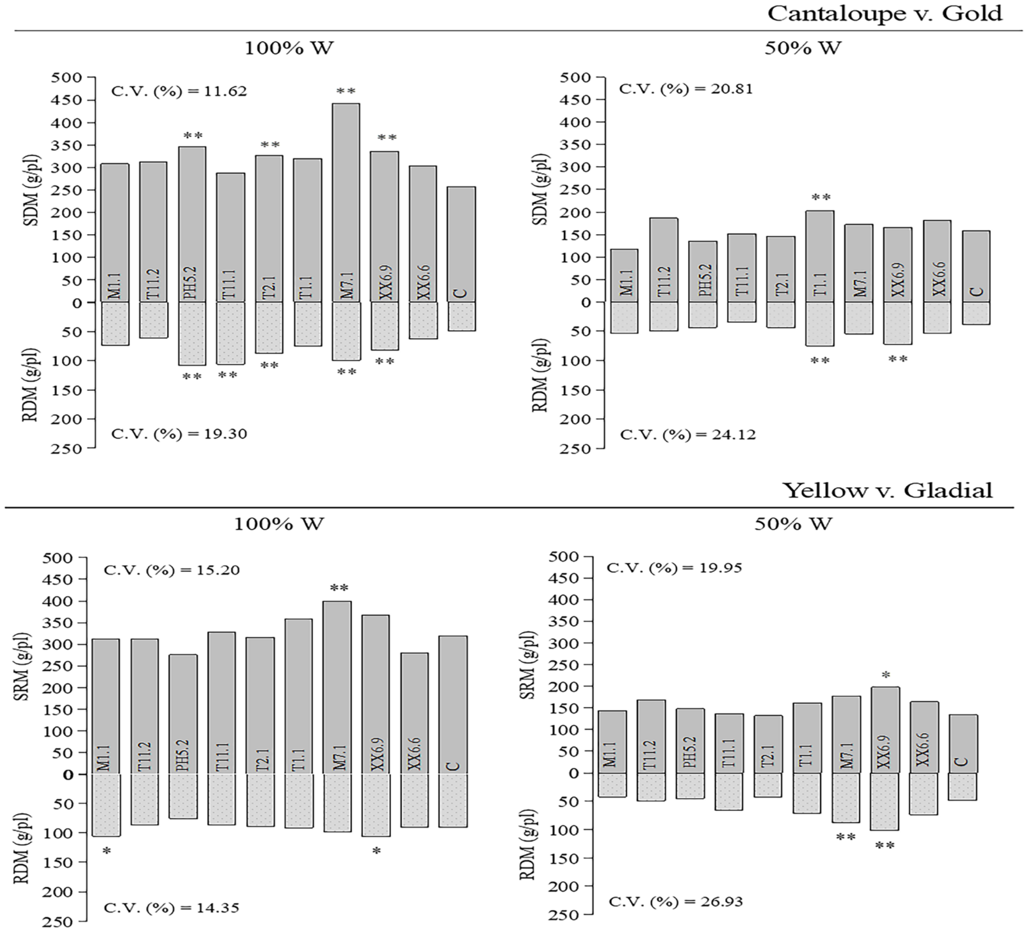

Figure 2: Dry mass of the aerial (SDM) and root (RDM) biomasses of Cantaloupe and Yellow melon plants inoculated with bacteria and subjected to two water stress scenarios (100% and 50% W). W = water. * and ** differ from the control (C) at 5% and 1% probability according to Dunnett's test, respectively. C = Noninoculated control. CV = Coefficient of variation.

One of the pathways responsible for the growth of the root system and consequent tolerance to biotic and abiotic stress and increased productivity is auxins (Blinkov et al., 2014; Numan et al., 2018). The ability of different microorganisms to produce such phytohormones and promote the growth and productivity of different crops is already widely cited (Kudoyarova et al., 2019) and represents a pathway of much interest to the agricultural sector. The bacterial isolates used in this study were able to produce auxins at concentrations ranging from 1.4 to 21.7 µg mL^{-1} (data not shown), which may explain the results found.

In the present study, the increase in the height of the inoculated plants indicates that under water scarcity conditions, the interaction between the microorganism and the melon acted to maintain the processes of plant growth, demonstrating the presence of mechanisms of induction of tolerance to drought and the potential of these isolates to support the morphophysiology of the plant in situations with reduced water supply.

Four isolates (PH5.2, T2.1, M7.1 and XX6.9) stimulated the production and accumulation of phytomass in the aerial part of the melon plants (Figure 2). Of these, PH5.2, T2.1, M7.1 and XX6.9 increased the aerial part of the plants of the Cantaloupe variety and isolate M7.1 of the yellow variety. Under the optimal water supply condition, the isolates enhanced the growth of the Cantaloupe variety to a greater extent, with an increase of up to 41% for the treatment inoculated with the isolate M7.1, than that of the yellow variety, with an increase of 20% when inoculated with the same isolate. It is important to consider that the treatment with isolate XX6.9 performed well in both water regimes and for both crop varieties. This result is fundamental because both under water deficit and under ideal irrigation conditions, the isolate will enhance root and aerial biomass.

Azospirillum brasilensis, *Pseudomonas fluorescens* and *Rhizobium tropici* were reported to have the ability to induce plant growth promotion by increasing the dry biomass of both shoots and roots (Sá et al., 2019). This ability is related to biological nitrogen fixation, phosphate solubilization and production of phytohormones. Although the exact mechanism for the increase in biomass was not revealed in this study, two of these characteristics were confirmed in the isolates tested (auxin production and asymbiotic nitrogen fixation), in addition to the ability of the M7.1 and XX6.9 isolates to produce abundant exopolysaccharides (EPS) (data not shown).

Studies on melons inoculated with PGPB are still scarce, but (Islam et al., 2016) observed growth promotion in melon seedlings inoculated with different PGPB, especially the isolate *B. subtilis* PPB8, which increased the dry mass of both roots and shoots by 100 and 128.5%, respectively, compared to the noninoculated control. We found very important results concerning the ability of the isolates PH5.2, T2.1, M7.1 and XX6.9 to increase both root and shoot biomass under ideal irrigation conditions, revealing that they induce mechanisms that are of great importance for horticulture. This result was repeated for isolates T1.1 and XX6.9 under water deficit conditions.

The interaction between plants and microorganisms can result in the synthesis or inhibition of different plant hormones to attenuate the negative effects of the reduction in the photosynthetic area due to a lack of water. At times of reduced water demand, bacteria from the rhizosphere and phyllosphere increase the production of abscisic acid (ABA), which accelerates stomatal closure (Devarajan et al., 2021, Yuzikhin et al., 2021), keeping the plant physiologically active for longer periods. With this mechanism, plants are able to optimize water use, using less water to fix the CO_2 molecule, which is essential for accumulating biomass even in times of water shortage.

Another important observation is the greater efficiency of the isolates in promoting growth for the Cantaloupe variety. The efficiency of use of a particular microorganism or consortium of microorganisms is closely linked to its ability to colonize (interact with) the root system of the crop. Different plant species attract microorganisms by exuding molecules through the root. Cucumber plants, for example, can secrete citric acid and attract *Bacillus amyloliquefaciens* communities, whereas banana plants can secrete fumaric acid and stimulate root colonization by *Bacillus subtilis* (Zhang et al., 2014). The same seems to be expected for different genotypes of the same species. (Weinert et al., 2011) showed a dependence relationship between the operating taxonomic unit (OTU) portion detected and three different potato cultivars. Cultivar-dependent effects on OTU richness were also observed by (Peiffer et al., 2013) based on the analysis of 27 maize cultivars.

Considering the current agricultural situation, where there is a great demand for biological inputs driven by consumer pressure for healthier and more sustainable products, together with the market crisis that has impacted the prices of inputs such as fertilizers and pesticides and climate change-induced heat and droughts, there is an urgent race to develop new technologies that can meet the demand. Thus, the selection and use of microorganisms with adaptations that can stimulate the development of different crops in different climate scenarios will be a trend for commercial biological products in the coming years.

Conclusion

Bacteria isolated from dry lands of the Caatinga promote melon growth in the early stage of crop development, under both ideal water supply and water deficit conditions. The isolates XX6.9 and M7.1 were the most efficient in promoting the growth of the melon varieties in the scenario without water deficit. In the water deficit scenario, isolates T1.1 and XX6.9 promoted the total biomass of the Cantaloupe and Yellow varieties, respectively. The results point to potential studies aimed at the development of an inoculant to support production in the early stages of the culture of melons grown in semiarid regions.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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