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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES INDUCED BY RHIZOBACTERIA IN OIL PALM SEEDLINGS SUBMITTED TO WATER DEFICIT

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Abstract: The oil palm (*Elaeis guineensis* Jacq.) is an important oilseed crop for the whole world. However, water deficiency can directly affect the development of seedlings in nurseries. The use of plant growth-promoting rhizobacteria is a potential strategy to induce drought tolerance and reduce plant mortality in the field. The objective of the study was to evaluate the ability to maintain photosynthetic performance and relieve oxidative stress in oil palm seedlings inoculated with rhizobacteria and subjected to water deficit. The experiment was carried out in a greenhouse in a completely randomized design. The treatments consisted of 8 seedlings inoculated with Bacillus amyloliquefaciens (UFRAB01) and 8 non-inoculated seedlings (control) of oil palm evaluated at two times: before and after the imposition of water deficit. The inoculation increased height and diameter of the stem in relation to the control in irrigated seedlings and in water deficit. Leaf water potential was affected only by water deficit with an increase in modulus of 180%. Inoculation managed to maintain higher mean values of net photosynthesis and carboxylation efficiency compared to non-inoculated seedlings. In the condition of water deficit, the levels of MDA and activity of the CAT enzyme, however the activity of the APX enzyme increased in the inoculated seedlings. Therefore, the water deficit drastically reduced the photosynthetic performance of all oil palm seedlings, and the inoculation of UFRAB01 mitigated the damage to the photosynthetic apparatus to maintain the highest averages of net photosynthesis in relation to the non-inoculated seedlings. Under field conditions, UFRAB01 inoculation can induce greater tolerance to moderate drought and decrease seedling mortality.

Keywords: Liquid photosynthesis, Biostimulation, Dry.

INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) stands out as one of the most cultivated oilseed crops in the world due to the high consumption of oil from its fruits in the food, pharmaceutical and cosmetic industries (HOMMA; VIEIRA, 2012). However, in non-irrigated nurseries or with inadequate irrigation, the production of seedlings with lower quality and low vigor limits the availability of seedlings for renewal and expansion of commercial plantations.

Water deficit acts as a limiting factor for plant growth, as it affects water relations and causes changes in physiological and biochemical processes (BARBOSA et al. 2017). Plants decrease stomatal opening to reduce water loss through transpiration, however, increase resistance to CO2 entry in leaves and cause reductions in net photosynthesis (FLEXAS et al. 2012). The smaller stomatal opening decreases the concentration of mesophilic CO2 and impairs the carboxylation activity of the rubisco enzyme (STEFFEN, 1991), which stops consuming ATP and NADPH generated in photochemical reactions and allows free electrons to react with free molecular oxygen. to form reactive oxygen species (ROS). The accumulation of ROS causes several damages to the cell, as they react with molecules of DNA, RNA, proteins and membrane lipids, resulting in oxidative stress (HUNG et al., 2005).

A strategy with potential to induce tolerance to water stress may be the use of plant growth promoting rhizobacteria (RPCP), as they stimulate root growth to improve the efficiency of water and nutrient absorption, as observed in açaí seedlings inoculated with RPCP (CASTRO et al. 2019). Other studies (FORNI et al. 2016, GAGNÉ-BOURQUE et al. 2016) report that plants inoculated with BPCPP are more tolerant to water deficit because they manage to mitigate the effects of oxidative stress caused by ROS through the activation of antioxidant enzymes, such as catalase (CAT) and ascorbate peroxidase (APX).

Knowing that the water deficit alters the biochemical processes, which impairs the quality of the seedlings, the study seeks to test the hypothesis that the inoculation of RPCP mitigates the effects of oxidative stress and, consequently, increases the tolerance to the water deficit in cactus pear seedlings. oil. The aim of the study was to evaluate the ability to maintain photosynthetic performance and relieve oxidative stress in oil palm seedlings inoculated with rhizobacteria and subjected to water deficit.

METHODOLOGY

PLANT GROWTH

Oil palm seeds (Compacta x Ghana genotype) were sown in plastic trays containing 2.5 L of crushed coconut fiber substrate (Golden mix). At 30 days after germination, the seedlings that had two expanded leaves and a height close to 12 cm were transplanted into plastic bags (15 x 25 cm, length x height) containing substrate composed of 60% Oxisol and 40% organic matter like. The pH and concentrations of macro and micronutrients in the substrate were adjusted as recommended for oil palm (SILVA CRAVO et al. 2007).

Cultivation was carried out in a greenhouse at the Universidade Federal Rural da Amazônia in Belém, PA, which has AMI-type climatic characteristics according to the Koppen-Geiger classification. During the experimental period, the environmental conditions of air temperature, relative humidity, air vapor pressure deficit and incident radiation were monitored. The seedlings were irrigated daily to replace the water lost through evapotranspiration and maintain soil moisture close to 100% field capacity (KLAR et al., 1966).

INOCULATION OF RHIZOBACTERIA

The rhizobacterium UFRAB01 used in the study was isolated from commercial oil palm plantations and identified as Bacillus amyloliquefaciens (GenBank MK967809) by Lima et al. 2020. UFRAB01 is stored and preserved in the microorganism collection of the Plant Protection Laboratory of '' Universidade Federal Rural da Amazônia", Belém, PA, Brazil.

UFRAB01 was cultured in solid medium 523 (KADO and HESKETT 1970) for 48 h at 28°C. Bacterial suspensions were prepared with distilled and sterilized water, and the concentration was adjusted in a spectrophotometer to A540 = 0.1 (108 CFU). The roots were sectioned to standardize the root length at seven centimeters and, before being transplanted into plastic bags with the substrate, they were immersed in 500 mL of each bacterial suspension for 20 min. The non-inoculated seedlings (control) were immersed in distilled and sterilized water.

IMPOSITION OF WATER DEFICIT

Six months after the inoculation of UFRAB01, the oil palm seedlings, a group formed by 8 inoculated seedlings and another group formed by 8 seedlings not inoculated with UFRAB01, were subjected to complete suspension of irrigation. Evaluations of biometry, gas exchange, water potential and SPAD and leaf samples for biochemical analysis were performed at the final time (28 days after the imposition of water deficit).

LEAF WATER POTENTIAL

Simultaneously with the gas exchanges, the leaf water potential (Ψw) was measured in the third expanded and physiologically mature leaf, from the apex to the base, with a Scholander-type pressure pump (m 670, Pms Instrument Co., Albany, USA) as per described by PINHEIRO et al. (2008).

PHYSIOLOGICAL ANALYSIS: GAS EXCHANGE AND TOTAL CHLOROPHYLL CONTENT

Gas exchange analyzes were carried out on the third fully expanded and physiologically mature leaf, from the apex to the base, 2 months after inoculation of the UFRAB01 rhizobacterium in the oil palm seedlings. Net CO2 assimilation (A), stomatal conductance to water vapor (gs), intercellular CO2 concentration (Ci) and transpiration rate (E) were measured between 10:00 and 12:00 (determined by diurnal gas exchange curve) using a portable open-flow gas exchange system (LI-6400XT, LI-COR, Lincoln, NE) at an external CO2 concentration of 400 μmol mol-1 air and PAR of 1000 μmol photons m−2 s− 1. Blue light availability was adjusted to 10% of PAR to optimize stomatal opening. Relative total chlorophyll content was determined using a portable total chlorophyll meter (SPAD 502-plus, Konica Minolta, Osaka, Japan).

BIOMETRIC ASSESSMENTS

Biometric evaluations were: height (cm), number of leaves and stem diameter (mm). The measurements of the collar diameter were made with the aid of a digital caliper (precision of 0.01 mm) and the height was measured using a measuring tape (distance between the collar and the apex of the tallest leaf). The total number of leaves was obtained by directly counting the emitted, expanded and expanding leaves (BENINCASA, 1998).

BIOCHEMICAL ANALYSIS: LIPID PEROXIDATION

Lipid peroxidation was measured by malonic aldehyde (MDA) levels, extracted as described by Cakmak and Horst (1991). Frozen plant tissue samples (20 mg) were crushed in eppendorf tubes and homogenized in 250 μL of 0.1% trichloroacetic acid (w/v) and the homogenate centrifuged at 15,000

x g, for 15 min, at 4°C. The supernatant was collected and 150 µL of 0.5% thiobarbituric acid (TBA) (prepared in 20% trichloroacetic acid) was added to a 50 µL aliquot. The tubes were vortexed and incubated at 90°C for 20 min. The reaction was stopped by immersing the tubes in an ice bath and the mixture was clarified by centrifugation at 13,000 x g, for 4 min, at 4°C. The absorbance (ABS) of the samples was determined in a spectrophotometer (Multiskan GO 3.2) at 532 and 600 nm, where 532 nm represents the maximum absorption of the MDA-TBA complex and the unspecific ABS (600 nm) discounted. The MDA molar extinction coefficient (155 mM-1 cm-1) was used for the calculations and the results expressed in nmol MDA g-1 of fresh matter (FM).

BIOCHEMICAL ANALYSIS: ANTIOXIDANT ENZYMES

Ascorbate peroxidase (APX, EC 1.11.1.11) and catalase (CAT, EC 1.11.1.6) were extracted from 20 mg of fresh mass ground in 200 µL of extraction medium containing 50 mM TFK (pH 7,0); 2 mM EDTA; 0.001% (v/v) Triton X-100; 14 mM 2-Mercaptoethanol; 20 mM ascorbate and 0.001 g PVP. After centrifugation at 15,000 x g for 15 min. at 4°C, the supernatant was collected for enzymatic analyses. APX activity was accompanied by a decrease in ABS at 290 nm in a reaction medium consisting of 50 mM TFK (pH 7.0); 0.1 mM H2O2; 0.5 mM of ascorbate and 3 µL of enzymatic extract (NAKANO AND ASADA, 1981). For calculations, 1 unit (U) of APX is the amount of enzyme capable of oxidizing 1 µmol of ascorbate min-1. The CAT reaction medium consisted of 50 mM TFK (pH 7.0) and 12.5 mM H2O2; and the reaction was started by adding 3 µL of extract. The CAT activity was determined by monitoring the ABS decrease at 240 nm (HAVIR AND MCHALE, 1987) and for the calculations it will be considered that 1 U of CAT is the amount of enzyme capable of oxidizing 1 µmol of H2O2 min-1. The protein dosages for calculating the enzymatic activities of SOD, APX and CAT were performed according to Bradford (1976).

STATISTICAL ANALYSIS

The experiment was carried out in a completely randomized design. The treatments consisted of 8 seedlings inoculated with UFRAB01 and 8 non-inoculated seedlings (control) of 6-month-old oil palm, evaluated at two times: before and after the imposition of water deficit. The data were submitted to the variance homogeneity test and, when significant, the means of the treatments were compared by Student's t test ($P \leq 0,05$).

RESULTS

In the irrigation condition, the seedlings inoculated with UFRAB01 increased by 25% the height and by 16% the diameter of the stem in relation to the non-inoculated seedlings (control). These increases were maintained when the seedlings were subjected to water deficit, being 26% for height and 16% for stem diameter in relation to the control (Figure 1A and 1B). The number of leaves and total chlorophylls were not affected by UFRAB01 inoculation nor by the imposition of water deficit (Figure 1C and 1D).

There were no significant differences between UFRAB01 and control under full irrigation condition for the evaluated physiological parameters (Figure 2). However, in the imposition of water deficit, all physiological parameters decreased in inoculated and control seedlings. For this water deficit condition, seedlings inoculated with UFRAB01 increased net CO2 assimilation by 58% and carboxylation efficiency by 67% compared to control seedlings (Figure 2A and 2F).

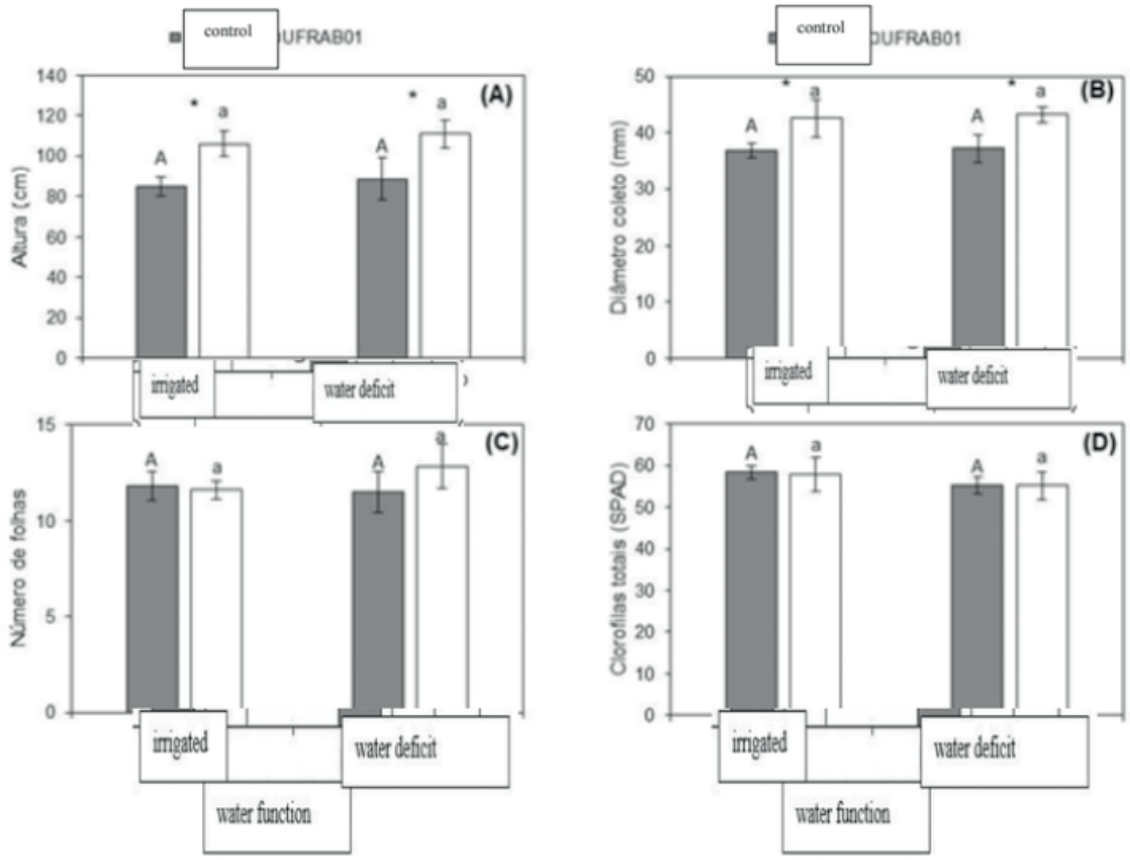

Figure 1. Height (A), stem diameter (B), number of leaves (C) and total chlorophylls (D) in oil palm seedlings inoculated with UFRAB01 and subjected to water deficit.

Columns represent means of 8 SD replicates. Equal capital letters indicate non-significant differences between irrigated control means and water deficit. Equal lower case letters indicate non-significant differences between irrigated UFRAB01 means and water deficit, according to the paired Student's t test ($P<0.05$). The asterisk indicates significant differences between control and UFRAB01 for the same water regime, according to the unpaired Student t-test ($P<0.05$).

Leaf water potential increased with the imposition of water deficit. However, there were no differences between control seedlings and seedlings inoculated with UFRAB01 (Figure 3). Although there are differences, these values indicate the magnitude of the water deficit that the oil palm seedlings were subjected to, on average -2.6 Mpa.

For biochemical markers, malonic aldehyde (MDA) levels and catalase antioxidant enzyme activity showed no significant difference between UFRAB01 and control (Figure 4). However, the enzyme ascorbate peroxidase was highlighted in the inoculated seedlings, showing an increase of 45% in relation to the control (Figure 5).

DISCUSSION

The present study revealed the physiological and biochemical mechanisms altered by the inoculation of *Bacillus amyloliquefaciens* (UFRAB01) in oil palm seedlings subjected to water deficit. In seedlings of other palm trees, such as the açaí palm submitted to water deficit (CASTRO et al. 2019), the inoculation of *Bacillus* sp. alleviated negative effects on photosynthetic performance up to 50% field capacity. For Mohammadi et al. (2016) the inoculation of rhizobacteria can contribute to increase drought tolerance, as the inoculated plants can activate the defense enzymatic system, produce osmoregulatory solutes to maintain water potential and alter the balance of hormones to promote root and plant growth. aerial (Fan et al. 2015).

In the present study, UFRAB01 inoculation induced an increase in plant height and stem diameter under stress conditions. The shoot growth, as well as the collar diameter, can be attributed to the ability of UFRAB01 to induce the biosynthesis of some hormones, such as gibberellins and cytokinins, which regulate height increase, leaf expansion and chlorophyll synthesis, as observed in seedlings of coconut palms (CARDOSO et al. 2021) inoculated with rhizobacteria where the height and diameter of the collar increased by 26% and 30%, respectively.

Under conditions of water abundance, net photosynthesis (A) was not altered by UFRAB01 inoculation. However, under water deficit, seedlings inoculated with UFRAB01 were able to maintain higher mean values of net photosynthesis compared to control seedlings. Probably, the activity of rubisco was modulated to assimilate CO2 with greater efficiency, as observed in the higher average values of A/Ci in the inoculated seedlings and under water deficit. These results suggest that UFRAB01 does not necessarily need to increase stomatal conductance (gs), intercellular carbon concentration (Ci) or transpiration (E) to increase net photosynthesis, since under water deficit other processes that involve better integrity of the rubisco seem to be a priority to increase CO2 carboxylation efficiency and maintain net photosynthesis at higher levels than non-inoculated plants, as observed by Timmusk et al. (2014) in wheat plants inoculated with biostimulants and subjected to water deficit.

Knowing that MDA levels were not altered under water deficit conditions, the inoculation of UFRAB01 was not able to alleviate the damage caused by lipid peroxidation, since the activation of greater APX activity was not enough to reduce the action of reactive oxygen species in -2.7 Mpa of drought. Similar results were found with different Palma palm oil hybrids, noting that there were no evident signs of lipid peroxidation in stressed plants of BRS Manicoré up to -3.9Mpa (SILVA et al., 2017).

Probably, other defense systems such as the non-enzymatic one, which involve the biosynthesis of antioxidant compounds: carotenoids, violaxanthins, xanthophylls and chlorophylls, may have been activated by

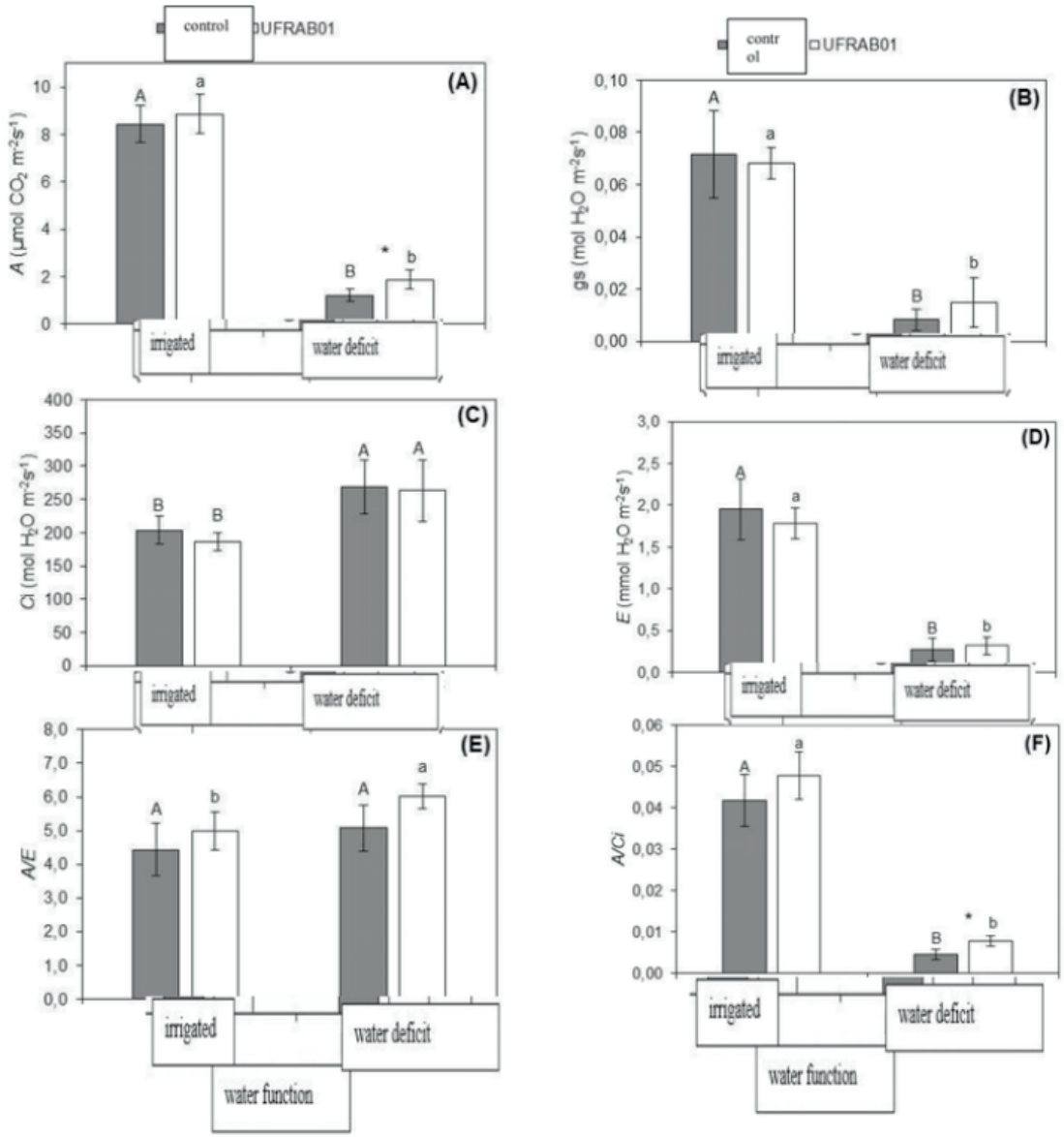

Figure 2. [A] - Net CO2 assimilation (A), [gs] – stomatal conductance (B), [Ci] – intercellular CO2 concentration (C), [E] transpiration rate (D), [A/Ci] - efficiency of water use water (E) and [A/Ci] - carboxylation efficiency (F) in oil palm seedlings inoculated with UFRAB01 and subjected to water deficit.

Columns represent means of 8 SD replicates. Equal capital letters indicate non-significant differences between irrigated control means and water deficit. Equal lower case letters indicate non-significant differences between irrigated UFRAB01 means and water deficit, according to the paired Student's t test ($P<0.05$). The asterisk indicates significant differences between control and UFRAB01 for the same water regime, according to the unpaired Student t-test ($P<0,05$).

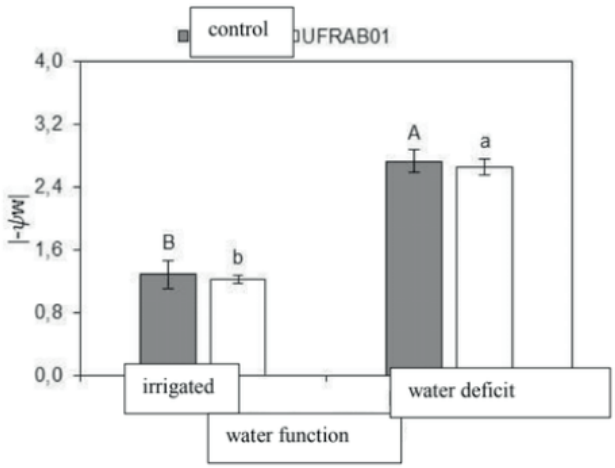

Figure 3. Leaf water potential (ψ_w) in oil palm seedlings inoculated with UFRAB01 and subjected to water deficit. The columns represent the means of 8 repetitions: ± DP. Equal capital letters indicate non-significant differences between irrigated control means and water deficit. Equal lower case letters indicate non-significant differences between irrigated UFRAB01 means and water deficit, according to the paired Student's t test (P<0.05).

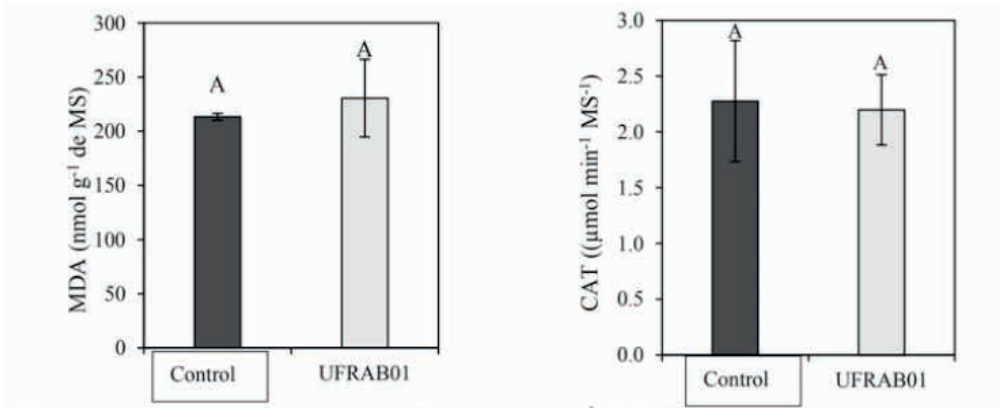

Figure 4. MDA(A) and Catalase (B) levels in oil palm seedlings inoculated with UFRAB01 and subjected to water deficit. The columns represent the means of 8 repetitions: ± DP. Equal capital letters indicate non-significant differences between means according to the paired Student t-test (P<0,05).

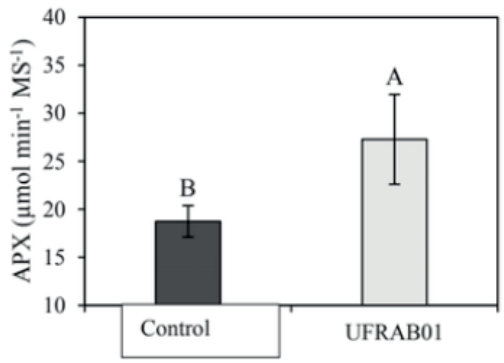

Figure 5. Ascorbate peroxidase levels in oil palm seedlings inoculated with UFRAB01 and subjected to water deficit. Columns represent means of 8 SD replicates. Different capital letters indicate significant differences between UFRAB01 and control means, according to the paired Student t-test (P<0.05).

the inoculation of UFRAB01 to mitigate the oxidative damage caused by water deficit. These compounds can act as dissipators of surplus energy at the level of photosystems to divert electrons and prevent the excessive production of reactive oxygen species that cause cellular damage in addition to membrane lipids (CARVALHO et al., 2019).

CONCLUSION

The water deficit drastically impairs the photosynthetic performance of all oil palm seedlings, and the inoculation of UFRAB01 mitigated the damage to the photosynthetic apparatus to maintain the highest averages of net photosynthesis in relation to non-inoculated seedlings. Probably, non-enzymatic defense mechanisms were activated to try to mitigate the oxidative damage in the photosynthetic apparatus and, more forceful responses of UFRAB01 inoculation may have occurred at less severe levels of water deficit.

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