

## CATIONIC NATURE OF WATER AND HYDROGEN PEROXIDE ON THE FORMATION OF PASSION FRUIT SEEDLINGS<sup>1</sup>

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**ABSTRACT** - This study was carried out with the objective of evaluating the water relations, photosynthetic pigments and growth of passion fruit cv. BRS Rubi do Cerrado, as a function of the cationic nature of irrigation water and exogenous application of hydrogen peroxide. The experiment was carried out under greenhouse conditions in Pombal – PB, Brazil. The experimental design was randomized blocks, in a 6 × 4 factorial scheme, corresponding to six cationic nature of water - CNW (S<sub>1</sub> - Control; S<sub>2</sub> - Na<sup>+</sup>; S<sub>3</sub> - Ca<sup>2+</sup>; S<sub>4</sub> - Na<sup>+</sup>+Ca<sup>2+</sup>; S<sub>5</sub> - Mg<sup>2+</sup> and S<sub>6</sub> - Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup>) and four concentrations of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> (0, 20, 40 and 60 μM), distributed in a randomized block design with four replicates. Plants in the control treatment (S<sub>1</sub>) were irrigated using water with electrical conductivity (EC<sub>w</sub>) of 0.3 dS m<sup>-1</sup>, while those of the other treatments (S<sub>2</sub>; S<sub>3</sub>; S<sub>4</sub>; S<sub>5</sub> and S<sub>6</sub>) were subjected to EC<sub>w</sub> of 3.0 dS m<sup>-1</sup>, prepared with different cation(s). Application of 60 μM of H<sub>2</sub>O<sub>2</sub> reduced the percentage of intercellular electrolyte leakage in plants irrigated with water of calcic composition. Salinity of water composed of sodium, sodium+calcium and sodium+calcium+magnesium, and H<sub>2</sub>O<sub>2</sub> concentrations of 40 and 60 μM resulted in lower leaf water potential. The biomass accumulation of passion fruit was more sensitive to the variation of the electrical conductivity of the water. Regardless of the cationic nature, the use of water with electrical conductivity of 3.0 dS m<sup>-1</sup> produced passion fruit seedlings with a Dickson quality index higher than 0.2, considered acceptable.

**Keywords:** *Passiflora edulis* Sims. Salt stress. Acclimatization.

## NATUREZA CATIONICA DA ÁGUA E PERÓXIDO DE HIDROGÊNIO NA FORMAÇÃO DE MUDAS DE MARACUJEIRO

**RESUMO** - Realizou-se esta pesquisa com o objetivo de avaliar as relações hídricas, os pigmentos fotossintéticos e o crescimento do maracujazeiro cv. BRS Rubi do Cerrado, em função da natureza cationica da água de irrigação e aplicação exógena de peróxido de hidrogênio. O experimento foi desenvolvido em condições de casa de vegetação em Pombal - PB. O delineamento experimental foi o de blocos casualizados, em esquema fatorial 6 × 4, sendo seis natureza cationica da água - NCA (S<sub>1</sub> - Testemunha; S<sub>2</sub> - Na<sup>+</sup>; S<sub>3</sub> - Ca<sup>2+</sup>; S<sub>4</sub> - Na<sup>+</sup>+Ca<sup>2+</sup>; S<sub>5</sub> - Mg<sup>2+</sup> e S<sub>6</sub> - Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup>) e quatro concentrações de peróxido de hidrogênio – H<sub>2</sub>O<sub>2</sub> (0, 20, 40 e 60 μM), distribuídos em delineamento de blocos ao acaso com quatro repetições. As plantas do tratamento testemunha (S<sub>1</sub>) foram irrigadas com água de condutividade elétrica (CEa) de 0,3 dS m<sup>-1</sup>, e os demais tratamentos (S<sub>2</sub>; S<sub>3</sub>; S<sub>4</sub>; S<sub>5</sub> e S<sub>6</sub>) foram submetidos à CEa de 3,0 dS m<sup>-1</sup>, preparada com diferente(s) cátion(s). Aplicação de 60 μM de H<sub>2</sub>O<sub>2</sub> diminuiu a percentagem de extravasamento de eletrólitos nas plantas irrigadas com água de composição cálcica. A salinidade da água constituída de sódio, sódio+cálcio e sódio+cálcio+magnésio e concentrações de 40 e 60 μM de H<sub>2</sub>O<sub>2</sub> resultou em menor potencial hídrico foliar. O acúmulo de fitomassas do maracujazeiro foi mais sensível à variação da condutividade elétrica da água. Independente da natureza cationica, o uso de água com condutividade elétrica de 3,0 dS m<sup>-1</sup> produziu mudas de maracujazeiro com índice de qualidade de Dickson superior a 0,2 considerado aceitável.

**Palavras- chave:** *Passiflora edulis* Sims. Estresse salino. Aclimação.

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## INTRODUCTION

Fruit growing is one of the segments in the Brazilian economy that has stood out in recent years and continues in full evolution with regard to both the production of fresh fruits and the industrialization of juices and nectars. It is considered one of the most dynamic activities, being responsible for the generation of employment and income, becoming an important mechanism for rural development, with possibilities for the development of the fruit processing agroindustry, favoring the expansion of fruit production centers in Brazil (SANTOS et al., 2017).

Among the fruit crops of greatest economic and social importance in the Northeast, passion fruit (*Passiflora edulis* Sims) stands out as the third juice most produced by the agroindustry (ZACHARIAS; FALEIRO; ALMEIDA, 2020), with high aggregated value and great acceptance both fresh and as concentrated pulp, which can be used for the processing of juices, sweets, ice cream, nectars and liqueurs, and can be marketed both in the domestic market and for export (BRANDÃO et al., 2020).

The Brazilian production of passion fruit in the 2019 season was 593,429 tons in a planted area of 41,800 hectares. Bahia stands out as the main producer at the national level with 168,457 tons, Ceará in second place with 145,102 tons, and Minas Gerais occupies third place with 44,934 tons (IBGE, 2019). Despite the good yield indicators, in the Northeast region, its production depends on the use of irrigation, due to the water scarcity caused by the climate imbalance. In this region, most groundwater (wells) and surface water (small and medium-sized reservoirs and ponds) have high salt levels, besides showing variability in ionic composition (LIMA et al., 2019).

The salinity of water, depending on its cationic nature, can cause varying degrees of stress on plants (LIMA et al., 2019), especially physiological changes, such as interruption of membranes, ionic imbalance, reduction in the capacity to detoxify reactive oxygen species and reduction in photosynthetic activity (AHANGER et al., 2017). Excess salts in water and/or soil induce ionic stress in plants and cause changes in fundamental processes such as growth, photosynthesis, protein synthesis, lipid metabolism, and biosynthesis of photosynthetic pigments (GUPTA et al., 2021).

Among the alternatives that can be used to attenuate the stress caused by salinity on plants, the exogenous application of hydrogen peroxide -  $H_2O_2$  stands out (VELOSO et al., 2018; SILVA et al., 2019a; SILVA et al., 2020).  $H_2O_2$  can act as a key regulator in modulating the defense response of plants to salt stress, as its electrochemical characteristics enable it to cross membranes and

spread between cell compartments, which facilitates its signaling function (SILVA et al., 2020). At low concentrations,  $H_2O_2$  regulates metabolism, cooperating with other hormones and signaling molecules, and confers tolerance to stress (SMIRNOFF; RNAUD, 2019).

Silva et al. (2019a), in a study evaluating the induction of tolerance to salt stress in soursop seedlings using  $H_2O_2$ , observed that the deleterious effects of water salinity on gas exchange and growth of soursop were mitigated by the exogenous application of  $H_2O_2$ . In another study, Silva et al. (2020) concluded that foliar spraying of  $H_2O_2$  increased the tolerance of sunflower plants to salt stress, mainly due to the balance of ionic homeostasis and redox. In this context, the objective of this study was to evaluate the water relations, photosynthetic pigments, and growth of passion fruit cv. BRS Rubi do Cerrado as a function of the cationic nature of irrigation water and exogenous application of  $H_2O_2$ .

## MATERIAL AND METHODS

The experiment was carried out between November 2019 and February 2020 in a protected environment (greenhouse) at the Center of Science and Agri-Food Technology - CCTA of the Federal University of Campina Grande - UFCG, located in the municipality of Pombal, Paraíba, PB, Brazil, with the geographic coordinates 6°47'20" latitude and 37°48'01" longitude, at an altitude of 194 m.

The treatments were arranged in a  $6 \times 4$  factorial scheme, corresponding to six cationic nature of the water - CNW ( $S_1$  - Control;  $S_2$  -  $Na^+$ ;  $S_3$  -  $Ca^{2+}$ ;  $S_4$  -  $Na^+Ca^{2+}$ ;  $S_5$  -  $Mg^{2+}$ , and  $S_6$  -  $Na^+Ca^{2+}Mg^{2+}$ ) and four concentrations of hydrogen peroxide -  $H_2O_2$  (0, 20, 40, and 60  $\mu M$ ), distributed in a randomized block design with four replicates, and the plot consisted of two plants, totaling 192 experimental units.

It should be pointed out that the plants of the control treatment ( $S_1$ ) were irrigated using water with electrical conductivity (ECw) of 0.3  $dS\ m^{-1}$ , while the other types of water ( $S_2$ ;  $S_3$ ;  $S_4$ ;  $S_5$ , and  $S_6$ ) were maintained with ECw of 3.0  $dS\ m^{-1}$ .  $Na^+Ca^{2+}$  and  $Na^+Ca^{2+}Mg^{2+}$  waters were prepared using equivalent ratios of 1:1 for Na:Ca and 7:2:1 for Na:Ca:Mg, respectively.

Seeds of cv. BRS Rubi do Cerrado passion fruit were used in this study. It is a cultivar that has fruits with red or purplish peel weighing 120 to 300 g (average of 170 g), with a soluble solids content of 14° Brix and juice yield around 35%, resistance to the main diseases of passion fruit (viral diseases, bacteriosis, anthracnose, and scab) and high levels of yield, which are the most important characteristics of this cultivar (EMBRAPA, 2012).

Passion fruit seedlings were grown using polyethylene bags with dimensions of 15 x 30 cm, filled with a ratio of 2:1:1 (on a volume basis) of *Neossolo Regolítico* (Psamments) with sandy loam texture, sand, and organic matter (well-decomposed bovine manure), coming from the rural area of the municipality of São Domingos, PB, at a depth of

0-20 cm. The bags were distributed equidistantly, supported on benches at 0.80 m height from the ground. The physical and chemical characteristics of the soil obtained according to the methodology proposed by Teixeira et al. (2017) are presented in Table 1.

**Table 1.** Chemical and physical characteristics of the soil used in the experiment, before the application of the treatments.

Chemical characteristics								
pH H <sub>2</sub> O)	OM	P	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H <sup>+</sup>
(1:2.5)	g kg <sup>-1</sup>	(mg kg <sup>-1</sup> )	cmol <sub>c</sub> kg <sup>-1</sup>					
5.58	2.93	39.20	0.23	1.64	9.07	2.78	0.00	8.61
Chemical characteristics				Physical characteristics				
EC <sub>se</sub>	CEC	SAR <sub>se</sub>	ESP	Size fraction (g kg <sup>-1</sup> )			Water content (dag kg <sup>-1</sup> )	
(dS m <sup>-1</sup> )	cmol <sub>c</sub> kg <sup>-1</sup>	(mmol L <sup>-1</sup> ) <sup>0.5</sup>	%	Sand	Silt	Clay	33.42 kPa <sup>1</sup>	1519.5 kPa <sup>2</sup>
2.15	22.33	0.67	7.34	572.70	100.70	326.60	25.91	12.96

pH – Hydrogen potential, O.M. - Organic Matter: Walkley-Black Wet Digestion; Ca<sup>2+</sup> and Mg<sup>2+</sup> extracted with 1 M KCl at pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted with 1 M NH<sub>4</sub>OAc at pH 7.0; Al<sup>3+</sup>+H<sup>+</sup> extracted with 0.5 M CaOAc at pH 7.0; EC<sub>se</sub> - Electrical conductivity of saturation extract; CEC - Cation exchange capacity; SAR<sub>se</sub> - Sodium adsorption ratio of the saturation extract; ESP - Exchangeable sodium percentage; <sup>1,2</sup>referring to field capacity and permanent wilting point

The irrigation waters with the different cationic natures were obtained by the addition of Na<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> salts in chloride form, according to the pre-established treatments, using water from the local supply system (Pombal-PB), whose quantity was determined considering the relationship between EC<sub>w</sub> and the concentration of salts (RICHARDS, 1954), as shown in Equation 1:

$$C \text{ (mmol}_c \text{ L}^{-1}) = 10 \times EC_w \text{ (dS m}^{-1}) \quad (1)$$

where:

C = Concentration of salts to be added (mmol<sub>c</sub> L<sup>-1</sup>);  
 EC<sub>w</sub> = Electrical conductivity of water (dS m<sup>-1</sup>).

Hydrogen peroxide concentrations were established according to a study conducted by Silva et al. (2019b). The solutions with desired concentrations were prepared by diluting H<sub>2</sub>O<sub>2</sub> in distilled water and, soon after preparation, they were stored in a container in dark ambient. H<sub>2</sub>O<sub>2</sub> applications were performed every two weeks manually at 17:00 h, by spraying the abaxial and adaxial sides of the leaves, to fully wet them, using a sprayer.

Before sowing, the volume of water required for the soil to reach field capacity was determined. After the soil moisture was increased to field capacity, sowing was carried out by placing two passion fruit seeds per bag, two centimeters deep and distributed equidistantly. Ten days after sowing (DAS), thinning was performed to maintain only one

plant per bag.

After sowing, irrigation was performed daily at 17:00 h, applying in each bag the volume corresponding to that obtained by the water balance, and the volume of water to be applied to the plants was determined using Equation 2:

$$VI = \frac{(V_a - V_d)}{(1 - LF)} \quad (2)$$

where:

VI = Volume of water to be used in the irrigation event (mL); V<sub>a</sub> = volume applied in the previous irrigation event (mL); V<sub>d</sub> = Volume of water drained (mL) and LF = leaching fraction of 0.2.

The fertilizations were carried out as top-dressing, according to the recommendation of fertilization for pot experiments, contained in Novais, Neves and Barros (1991), applying 100 and 300 mg kg<sup>-1</sup> of soil of nitrogen and phosphorus (P<sub>2</sub>O<sub>5</sub>), respectively, in the form of urea and monoammonium phosphate (MAP), via irrigation water, at 15 and 30 days after sowing (DAS). Potassium fertilization was split into 2 portions applied via fertigation (18 and 36 DAS), at 10-day intervals and each bag received 150 mg of K<sub>2</sub>O kg<sup>-1</sup> of soil, using potassium chloride as a source. To meet the requirement for micronutrients, foliar sprayings were performed with a solution containing 1.5 g L<sup>-1</sup> of Ubyfol® [(N (15%); P<sub>2</sub>O<sub>5</sub> (15%); K<sub>2</sub>O (15%); Ca (1%); Mg (1.4%); S (2.7%); Zn (0.5%); B (0.05%); Fe (0.5%); Mn (0.05%); Cu (0.5%);

Mo (0.02%)] at 10, 20, 30 and 40 DAS.

At 60 days after sowing (DAS), the following parameters were evaluated: leaf water potential ( $\Psi_w$ ), percentage of intercellular electrolyte leakage (% IEL), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) content, as well as the growth-related variables: plant height (PH), stem diameter (SD), leaf dry biomass (LDB), stem dry biomass (SDB), root dry biomass (RDB), and total dry biomass (TDB). The water potential ( $\Psi_w$ ) was analyzed with the aid of a Scholander pressure bomb. To determine the  $\Psi_w$ , fully expanded leaves in the position between the 3<sup>rd</sup> and 5<sup>th</sup> from the apex, with good phytosanitary conditions, were collected. To make the measurement, the chamber was pressurized with compressed gas until the exudation of liquid by the xylem, when the applied pressure reading was recorded. The negative value corresponded to the organ's  $\Psi_w$  (TURNER, 1981). The readings were performed between 6:00 and 7:00 a.m.

The percentage of intercellular electrolyte leakage was obtained according to Scotti-Campos et al. (2013), as shown in Equation 3:

$$\% \text{ IEL} = \frac{C_i}{C_f} \times 100 \quad (3)$$

where:

% IEL = percentage of intercellular electrolyte leakage;

$C_i$  = initial electrical conductivity ( $\text{dS m}^{-1}$ );

$C_f$  = final electrical conductivity ( $\text{dS m}^{-1}$ ).

Chlorophyll and carotenoid contents were quantified by spectrophotometer at absorbance wavelength (ABS) of 470, 646, and 663 nm, according to the methodology of Arnon (1949), using Equations. 4, 5, and 6:

$$\text{Chl } a = 12.21 \text{ ABS}_{663} - 2.81 \text{ ABS}_{646} \quad (4)$$

$$\text{Chl } b = 20.13 \text{ ABS}_{646} - 5.03 \text{ ABS}_{663} \quad (5)$$

$$\text{Car} = (1000 \text{ ABS}_{470} - 1.82 \text{ Chl } a - 85.02 \text{ Chl } b) / 198 \quad (6)$$

where:

Chl *a* = Chlorophyll *a*; Chl *b* = Chlorophyll *b*; and Car = Total Carotenoids. The values obtained for chlorophyll *a*, chlorophyll *b*, and carotenoids contents in the leaves were expressed in  $\text{mg g}^{-1}$  of fresh matter ( $\text{mg g}^{-1}$  FM).

To determine biomass accumulation, the plants were cut close to the soil surface and separated into leaves, stem, and roots. Subsequently, the different parts (leaves, stem, and roots) were packed in paper bags and dried in a forced-air circulation oven, at a temperature of 65 °C, until reaching constant weight. Then, the material was

weighed to obtain the values expressed in gram (g), for leaf biomass (LDB), stem biomass (SDB), root biomass (RDB), whose sum resulted in the total dry biomass (TDB) of the plant.

The quality of passion fruit seedlings was determined using the Dickson Quality Index - DQI (DICKSON; LEAF; HOSNER, 1960), as shown in Equation 7:

$$\text{DQI} = \frac{(\text{TDB})}{(\text{PH}/\text{SD}) + (\text{SDB}/\text{RDB})} \quad (7)$$

where:

DQI = Dickson quality index, PH = plant height (cm), SD = stem diameter (mm), TDB = total dry biomass ( $\text{g plant}^{-1}$ ), SDB = shoot dry biomass ( $\text{g plant}^{-1}$ ) and RDB = root dry biomass ( $\text{g plant}^{-1}$ ).

The obtained data were evaluated by analysis of variance by the F test. In cases of significance, Tukey test ( $p < 0.05$ ) was performed for the cationic nature of irrigation water, and analysis of linear and quadratic polynomial regression ( $p < 0.05$ ) was performed for hydrogen peroxide concentrations, using the statistical program SISVAR-ESAL version 5.6 (FERREIRA, 2019).

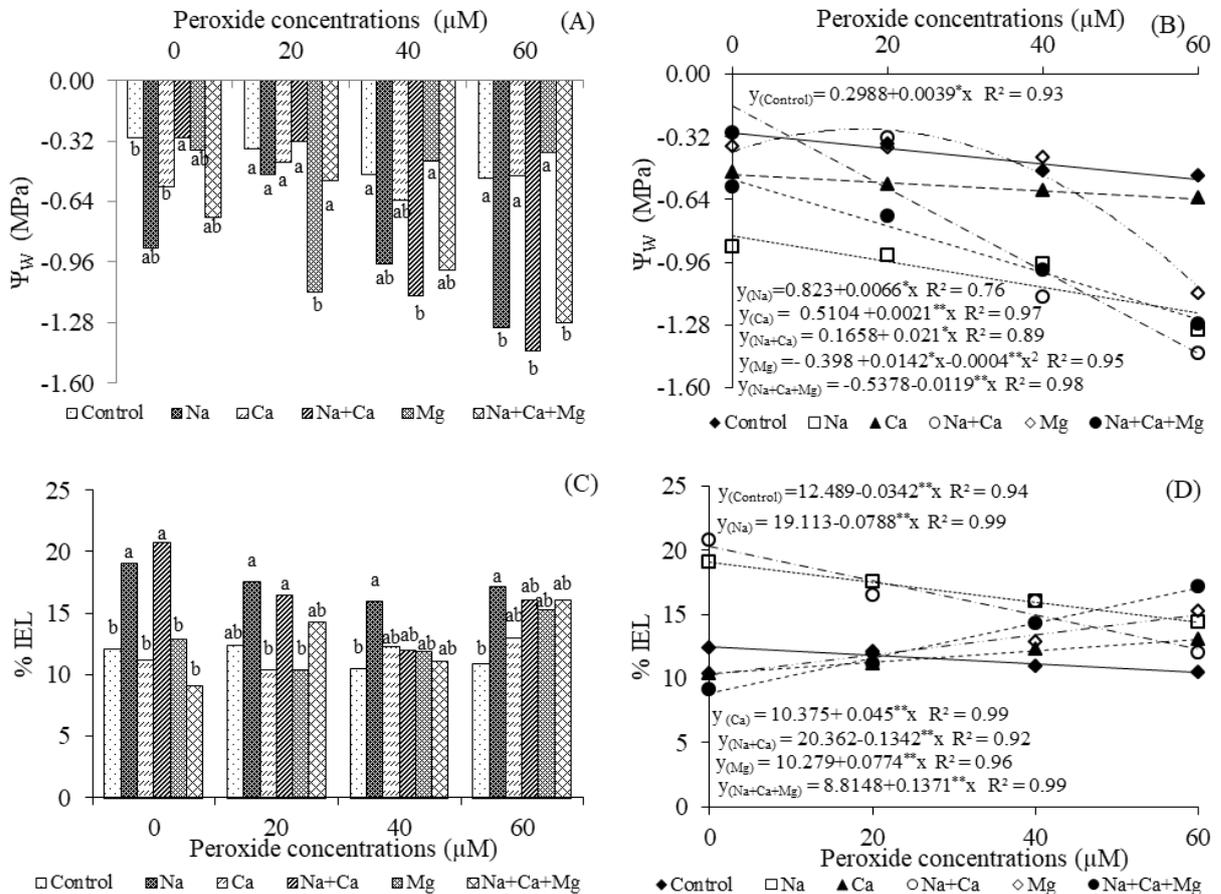
## RESULTS AND DISCUSSION

The interaction between the factors ( $\text{CNW} \times \text{H}_2\text{O}_2$ ) significantly influenced the water potential ( $\Psi_w$ ), percentage of intercellular electrolyte leakage (% IEL), chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) contents of passion fruit cv. BRS Rubi do Cerrado, at 60 DAS. The source of variation of cationic nature of the water significantly affected the water potential, percentage of intercellular electrolyte leakage and chlorophyll *a*, chlorophyll *b*, and Car contents of passion fruit.  $\text{H}_2\text{O}_2$  concentrations caused significant effect on  $\Psi_w$ , % IEL and Chl *a* of passion fruit.

Based on the analysis of the interaction between factors ( $\text{CNW} \times \text{H}_2\text{O}_2$ ) for leaf water potential (Figure 1A), it can be observed that in the absence of leaf application of  $\text{H}_2\text{O}_2$  (0  $\mu\text{M}$ ) passionfruit plants irrigated with water containing  $\text{Na}^{++}\text{Ca}^{2+}$  ( $\text{S}_4$ ) stood out with the highest value of  $\Psi_w$  (-0.297 MPa) compared to the  $\text{S}_2$  treatment. When plants received foliar application of 20  $\mu\text{M}$  and were irrigated with low-ECw water ( $\text{S}_1$ ) and water containing sodium ( $\text{S}_2$ ), calcium ( $\text{S}_3$ ), sodium+calcium ( $\text{S}_4$ ) and sodium+calcium+magnesium ( $\text{S}_6$ ) in their composition, they had the most significant values in terms of  $\Psi_w$  (-0.357; -0.492; -0.432; -0.322 and -0.527 MPa) in comparison to those subjected to salinity of water of magnesian nature ( $\text{S}_5$ ). Passion

fruit plants subjected to H<sub>2</sub>O<sub>2</sub> concentrations of 40 and 60 μM obtained the highest Ψ<sub>w</sub> levels when irrigated using water of low EC<sub>w</sub> (S<sub>1</sub>) and with cationic composition of Ca<sup>2+</sup> (S<sub>3</sub>) and Mg<sup>2+</sup> (S<sub>5</sub>).

Thus, the beneficial role of H<sub>2</sub>O<sub>2</sub> in maintaining the leaf Ψ<sub>w</sub> gradient of passion fruit plants with the soil solution is evident.



Means followed by different letters show significant difference between treatments by Tukey test (p < 0.05). **Figure 1.** Water potential - Ψ<sub>w</sub> (A and B) and percentage of intercellular electrolyte leakage -% IEL (C and D) in leaf tissues of passion fruit plants cv. BRS Rubi do Cerrado as a function of the interaction between the cationic nature of water and hydrogen peroxide concentrations (H<sub>2</sub>O<sub>2</sub>), at 60 days after sowing.

Regarding the effects of H<sub>2</sub>O<sub>2</sub> concentrations on each cationic nature of water (Figure 1B), it is observed that plants subjected to the treatments S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub> and S<sub>6</sub> showed a linear reduction in leaf Ψ<sub>w</sub>, which decreased by 26.10, 16.03, 8.22, 253.31 and 44.25% with each 20 μM increase in H<sub>2</sub>O<sub>2</sub> concentration. On the other hand, plants irrigated with water containing magnesium showed a quadratic behavior (Figure 1B), with the lowest value (-0.986 MPa) estimated when using 60 μM of H<sub>2</sub>O<sub>2</sub>. The reduction in leaf water potential in plants grown with water containing Na<sup>+</sup>, Na<sup>+</sup>+Ca<sup>2+</sup> and Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup> is possibly associated with the accumulation of ions in the vacuole and/or the synthesis of compatible solutes in the cytosol. Reduction of Ψ<sub>w</sub> results in stomatal closure, which prevents the transport of water vapor and CO<sub>2</sub>, decreasing photosynthesis and transpiration (HNILÍČKOVÁ et al., 2017).

The percentage of intercellular electrolyte

leakage in leaf tissues (Figure 1C) of plants cultivated under water with cationic nature of water containing Na<sup>+</sup>, Na<sup>+</sup>+Ca<sup>2+</sup> without exogenous application of H<sub>2</sub>O<sub>2</sub> (0 μM) was statistically higher than that found in plants that were subjected to the control treatment (S<sub>1</sub>) and the other cationic nature of the water (Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup>). When the H<sub>2</sub>O<sub>2</sub> concentration of 20 μM was used (Figure 1C), plants cultivated with water prepared with Na<sup>+</sup> and Na<sup>+</sup>+Ca<sup>2+</sup> obtained the highest %D, differing significantly from those that were irrigated with water containing Ca<sup>2+</sup> and Mg<sup>2+</sup>. On the other hand, in plants that received 40 μM of H<sub>2</sub>O<sub>2</sub>, the highest % IEL was verified when they were irrigated using water prepared with Na<sup>+</sup>, but this treatment was statistically superior only to the control (S<sub>1</sub>). A similar situation was also observed in plants that received H<sub>2</sub>O<sub>2</sub> concentration of 60 μM. Lima et al. (2020a) in a study with West Indian cherry cv. BRS 366 Jaburu under irrigation with saline waters

(ECw of 0.8 and 4.5 dS m<sup>-1</sup>) prepared with the 7:2:1 proportion of NaCl, CaCl<sub>2</sub>.2H<sub>2</sub>O, and MgCl<sub>2</sub>.6H<sub>2</sub>O, found an increase in the percentage of cell damage in leaf tissues when plants were irrigated with the highest salinity level.

When analyzing the H<sub>2</sub>O<sub>2</sub> concentrations considering each cationic nature of water (Figure 1D), it was verified that plants subjected to the treatments S<sub>1</sub>, S<sub>2</sub>, and S<sub>4</sub> showed a linear reduction in % IEL as the H<sub>2</sub>O<sub>2</sub> concentration increased, with decreases of 5.47, 8.24, and 13.18% with each 20 μM increase in H<sub>2</sub>O<sub>2</sub>. On the other hand, plants cultivated with water composed of Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup> showed increments of 8.67, 15.05 and 31.10%, respectively, in the percentage of intercellular electrolyte leakage with an increase of 20 μM in H<sub>2</sub>O<sub>2</sub> concentrations. The increase in percentage of intercellular electrolyte leakage of leaf tissues stands out as a mechanism that prevents tissue desiccation due to the reduction of osmotic potential and consequently leaf water potential (DUARTE; SOUZA, 2016). The percentage of intercellular electrolyte leakage is a consequence of excess oxygen free radicals resulting from cell membrane alteration produced by oxidation of acids in lipid bilayer, a process known as lipid peroxidation (CARRASCO-RÍOS; PINTO, 2014).

Absence of exogenous application of H<sub>2</sub>O<sub>2</sub> and the concentration of 40 μM did not significantly influence the synthesis of chlorophyll *a* in plants (Figure 2A), regardless of the cationic nature of water. However, when comparing the Chl *a* contents of plants that received 20 μM of H<sub>2</sub>O<sub>2</sub>, better results were obtained with those that received water with low ECw (Control) and prepared with Ca<sup>2+</sup>, but they did not differ significantly from the treatments S<sub>2</sub> (Na<sup>+</sup>) and S<sub>4</sub> (Na<sup>+</sup>+Ca<sup>2+</sup>). Plants that received the highest concentration of H<sub>2</sub>O<sub>2</sub> (60 μM) obtained the lowest Chl *a* content when they were cultivated with water composed of Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup>. H<sub>2</sub>O<sub>2</sub> concentrations of 20 and 60 μM stimulated the synthesis of chlorophyll *a* in plants grown under S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, and S<sub>4</sub>.

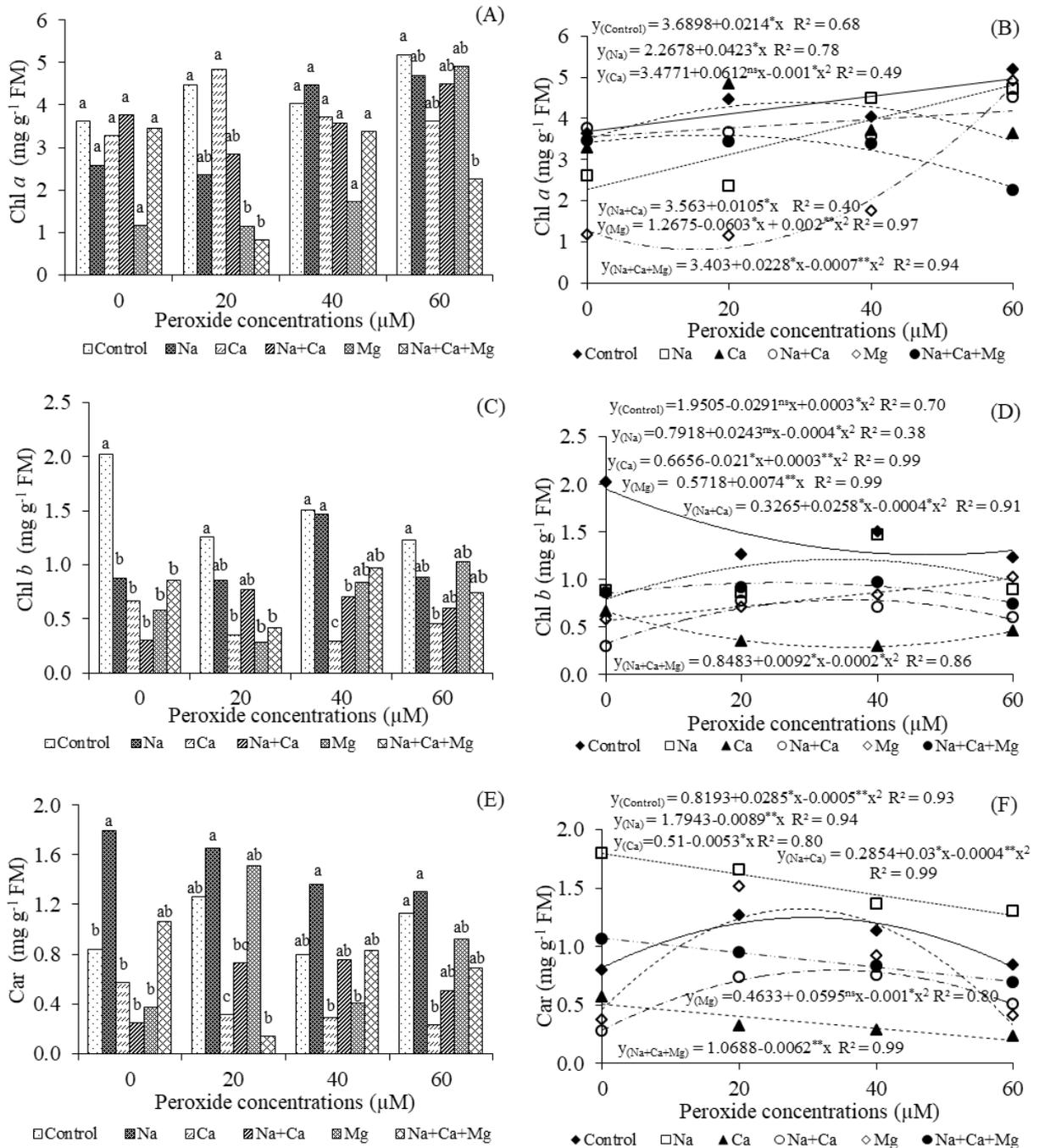
For chlorophyll *a* contents (Figure 2B), there were linear increments of 11.59, 37.30 and 5.89% for each 20 μM increase of H<sub>2</sub>O<sub>2</sub> in plants subjected to irrigation with water of low salinity (S<sub>1</sub>) and composed of Na<sup>+</sup> and Na<sup>+</sup>+Ca<sup>2+</sup>. In plants irrigated with water composed of Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup>, the highest levels of Chl *a* (4.41; 4.85 and 3.58 mg g<sup>-1</sup> FM) were obtained with the estimated H<sub>2</sub>O<sub>2</sub> concentrations of 31, 60 and 16 μM, respectively. The exposure of plants to moderate stresses or signaling metabolites such as H<sub>2</sub>O<sub>2</sub> can promote metabolic signaling in the cell and activation of antioxidant enzymes, such as superoxide dismutase, catalase, guaiacol peroxidase and ascorbate peroxidase; consequently, there is a

decrease in oxidative stress on plants (CAVERZAN; CASASSOLA; BRAMMER, 2016). Silva et al. (2019b) in a study to evaluate the photosynthetic pigments of soursop seedlings cv. 'Morada Nova' irrigated with saline water and subjected to H<sub>2</sub>O<sub>2</sub> application by seed immersion and foliar spraying, verified that plants under the highest salinity level (3.5 dS m<sup>-1</sup>) and subjected to 50 μM of H<sub>2</sub>O<sub>2</sub> obtained the highest averages of Chl *a*.

It was verified that, in the absence of H<sub>2</sub>O<sub>2</sub> application (0 μM), plants irrigated with water of lowest salinity level (S<sub>1</sub>) obtained the highest Chl *b* content (Figure 2C), being statistically superior to those cultivated with water composed of Na<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>+Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup>. When using H<sub>2</sub>O<sub>2</sub> concentrations of 20 μM, there was an increase in Chl *b* synthesis in plants subjected to the control treatment (S<sub>1</sub>) compared to those grown under S<sub>3</sub>, S<sub>5</sub> and S<sub>6</sub>. On the other hand, for plants that received 40 μM of H<sub>2</sub>O<sub>2</sub> and irrigation with water of low salinity (S<sub>1</sub>) and sodic nature (S<sub>2</sub>), there was a significant difference in Chl *b* contents in comparison to those subjected to the treatments S<sub>3</sub> and S<sub>4</sub>. Under the highest concentration of H<sub>2</sub>O<sub>2</sub> (60 μM), it can be observed that the Chl *b* contents of plants grown under low salinity (S<sub>1</sub>) differed significantly only from the contents of those that received water prepared with Ca<sup>2+</sup> (S<sub>3</sub>).

Thus, it is verified that H<sub>2</sub>O<sub>2</sub> concentrations from 20, 40 and 60 μM mitigate the effect of salt stress on plants irrigated with water of sodic nature. The decrease in chlorophyll *b* synthesis in plants irrigated with water composed of Ca<sup>2+</sup> may be a strategy of protection and/or acclimatization in which the reduction in energy expenditure, carbon skeletons and nutrients necessary for chlorophyll synthesis may favor other physiological processes associated with the attenuation of salt stress (RHEIN et al., 2015).

Regarding the effects of foliar application of H<sub>2</sub>O<sub>2</sub> on each cationic composition of the water (Figure 2D), it is observed that the chlorophyll *b* contents of plants subjected to the treatments S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>4</sub>, and S<sub>6</sub> were described by the quadratic model, with estimated maximum values of 1.95, 1.16, 0.66, 0.74, and 0.95 mg g<sup>-1</sup> FM at H<sub>2</sub>O<sub>2</sub> concentrations of 0, 31, 0, 32, and 24 μM. Contrary to what was observed for the other cationic natures (Figure 2D), plants irrigated using water prepared with magnesium (S<sub>5</sub>) showed a linear increase in Chl *b* contents, equal to 25.88% for each 20 μM increase of H<sub>2</sub>O<sub>2</sub>. Lima et al. (2020b), evaluating the photosynthetic pigments of passion fruit cv. BRS Rubi do Cerrado as a function of irrigation water salinity, obtained by the 7:2:1 ratio of NaCl, CaCl<sub>2</sub>.2H<sub>2</sub>O and MgCl<sub>2</sub>.6H<sub>2</sub>O, concluded that the ECw reduced chlorophyll *b* contents at 60 DAS.



Means followed by different letters show significant difference between treatments by Tukey test ( $p < 0.05$ ). **Figure 2.** Chlorophyll *a* - Chl *a* (A and B), chlorophyll *b* - Chl *b* (C and D) and carotenoids - Car (E and F) of passion fruit plants cv. BRS Rubi do Cerrado, as a function of the interaction between the cationic nature of water and hydrogen peroxide concentrations (H<sub>2</sub>O<sub>2</sub>), at 60 days after sowing.

According to the means comparison test (Figure 2E), plants under irrigation with water of sodic nature and in the absence of H<sub>2</sub>O<sub>2</sub> application (0 μM) obtained the highest Car contents. When using the H<sub>2</sub>O<sub>2</sub> concentration of 20 μM, plants irrigated with water of low salinity (S<sub>1</sub>) and composed of Na<sup>+</sup> and Mg<sup>2+</sup> had Car contents statistically higher than those of plants that were grown under salinity of water with Ca<sup>2+</sup>, Na<sup>+</sup>+Ca<sup>2+</sup>

and Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup>. It was verified (Figure 2E) that the Car contents of plants subjected to 40 μM of H<sub>2</sub>O<sub>2</sub> and to the water composition of the treatments Control, Na<sup>+</sup>, Na<sup>+</sup>+Ca<sup>2+</sup> and Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup> differed significantly from those irrigated using water prepared with Ca<sup>2+</sup> and Mg<sup>2+</sup>. For plants grown under foliar application of 60 μM of H<sub>2</sub>O<sub>2</sub>, the highest Car contents were obtained when they were irrigated with water from the treatments S<sub>1</sub>, S<sub>2</sub>, S<sub>4</sub>, S<sub>5</sub>

and S<sub>6</sub> compared to those that received S<sub>3</sub>. In general, it was verified that plants irrigated with sodic water obtained the highest carotenoids contents; however, there was a tendency of reduction in this pigment with the increase in H<sub>2</sub>O<sub>2</sub> concentration.

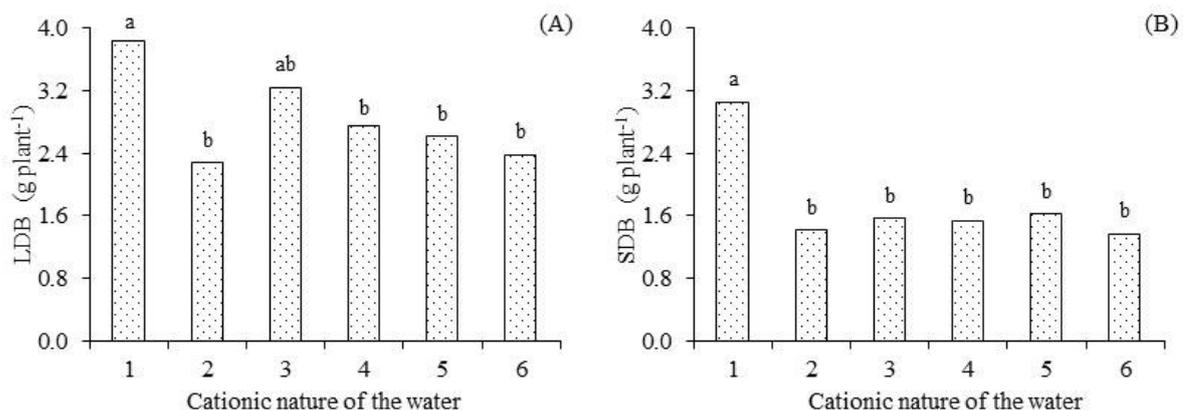
The carotenoids contents of plants subjected to irrigation using water prepared with Na<sup>+</sup>, Ca<sup>2+</sup> and Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup> decreased sharply with the increase in H<sub>2</sub>O<sub>2</sub> concentrations (Figure 2F), with reductions of 9.92, 20.78, and 11.60% respectively, for each 20 μM increase of H<sub>2</sub>O<sub>2</sub>. Plants grown under irrigation with water of low salinity (S<sub>1</sub>) and composed of Na<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> obtained the highest Car values (1.22, 0.84 and 1.34 mg g<sup>-1</sup> FM) when they received foliar application with estimated concentrations of 29, 38, and 30 μM of H<sub>2</sub>O<sub>2</sub>. The synthesis of carotenoids is extremely important in plant metabolism, because they are antioxidants and play a role as a free radical eliminator, besides increasing the capacity to reduce the damage caused by reactive oxygen species (ABDALLAH; ABDELGAWAD; EL-BASSIOUNY, 2016). Lima et al. (2018), in a study evaluating the photosynthetic pigments of West Indian cherry cv. BRS 366 Jaburu as a function of irrigation with saline water (EC<sub>w</sub> of 0.8 and 3.8 dS m<sup>-1</sup>) obtained from the dissolution of NaCl, CaCl<sub>2</sub>.2H<sub>2</sub>O and MgCl<sub>2</sub>.6H<sub>2</sub>O, concluded that irrigation with high-salinity water stimulated the biosynthesis of carotenoids.

There was a significant effect of the interaction between factors (CNW × H<sub>2</sub>O<sub>2</sub>) on the root dry biomass (RDB) and total dry biomass

(TDB) of passion fruit plants, at 60 DAS. The cationic nature of the water significantly affected the variables LDB, SDB, RDB, TDB, and DQI. The H<sub>2</sub>O<sub>2</sub> concentrations applied exogenously did not significantly influence any variable of passion fruit evaluated at 60 DAS.

For the leaf dry biomass of passion fruit (Figure 3A), in the treatment with application of low-salinity water (Control), it was statistically superior to that of plants subjected to irrigation with water from the other treatments (Na<sup>+</sup>, Na<sup>+</sup>+Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup>); however, passion fruit plants, when irrigated with water of cationic nature consisting of Na<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>+Ca<sup>2+</sup>, Mg<sup>2+</sup> and Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup> showed no significant difference between them.

The decrease in leaf dry biomass accumulation is the result of the osmotic effect caused by the excess of salts in the soil solution, which reduced the water potential and restricted the absorption of water and nutrients by plants. Ultimately, it causes changes in the photosynthetic rate and energy diversion for the activation and maintenance of metabolic activity and membrane integrity, as well as the synthesis of organic solutes for osmoregulation and/or protection of macromolecules (SILVA JÚNIOR et al., 2012). Souza et al. (2017), in a study with guava crop under salinity (EC<sub>w</sub> ranging from 0.3 to 3.5 dS m<sup>-1</sup>) of water composed of Na:Ca:Mg in the equivalent proportion of 7:2:1, respectively, observed reductions in shoot dry biomass accumulation, equal to 4.49% per unit increment in EC<sub>w</sub>.



1 - Control; 2 - Na<sup>+</sup>; 3 - Ca<sup>2+</sup>; 4 - Na<sup>+</sup> + Ca<sup>2+</sup>; 5 - Mg<sup>2+</sup>; 6 - Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup>. Means followed by different letters show significant difference between treatments by Tukey test ( $p < 0.05$ ).

**Figure 3.** Leaf dry biomass - LDB (A) and stem dry biomass - SDB (B) of passion fruit plants cv. BRS Rubi do Cerrado as a function of the cationic nature of the water, at 60 days after sowing.

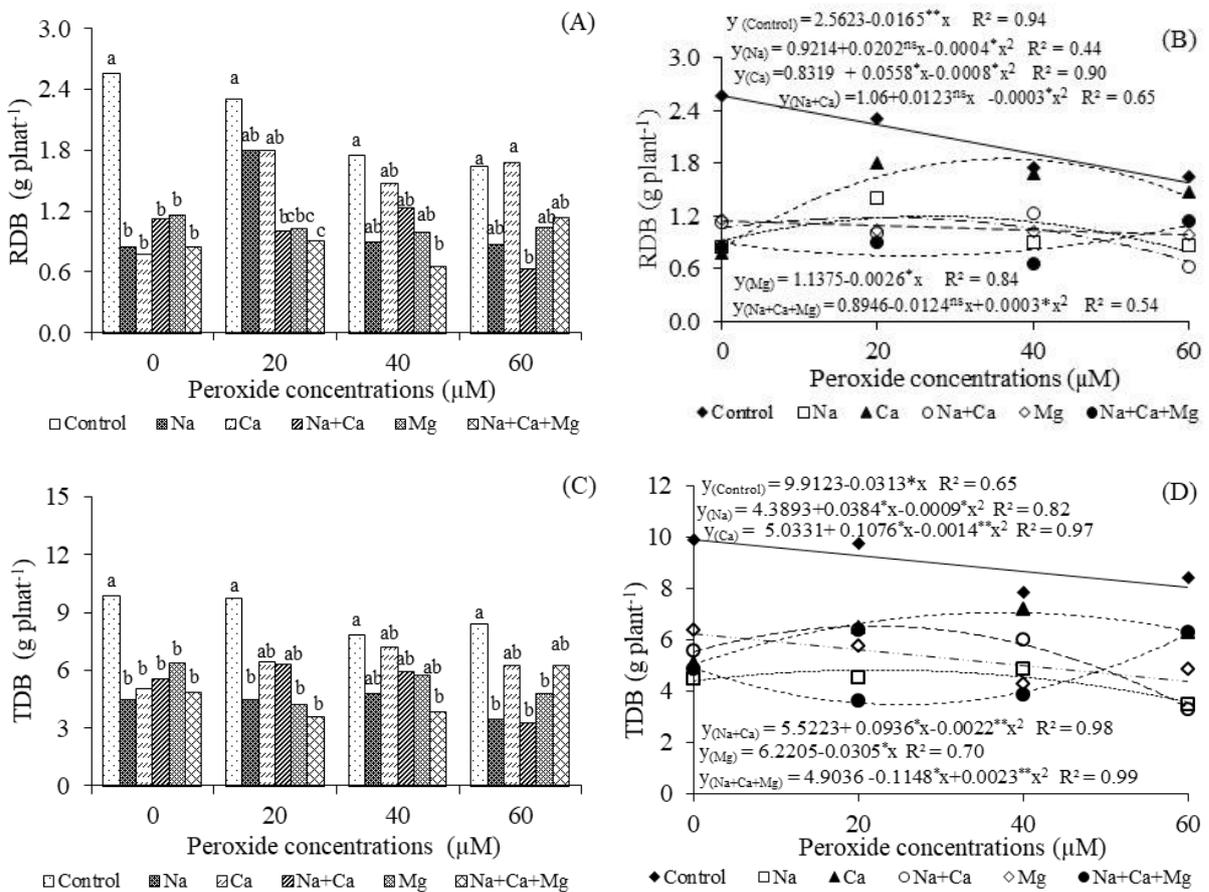
The stem dry biomass of plants irrigated with low-salinity water (S<sub>1</sub>) was statistically higher than that of plants that were under irrigation with water of distinct cationic nature (Figure 3B); despite this, when analyzing only the cationic nature of the water,

the effect occurred in a similar way. The reduction in biomass accumulation by passion fruit plants is also related to the osmotic effect caused by the high concentrations of salts in the root zone, promoting changes in ionic and osmotic homeostasis, thus

causing a reduction in growth and, consequently, in biomass accumulation (SÁ et al., 2019). Veloso et al. (2019), when evaluating the growth of ‘Morada Nova’ soursop plants irrigated with saline water (ECw ranging from 0.3 to 3.7 dS m<sup>-1</sup> and with Na:Ca:Mg in the equivalent proportion of 7:2:1), also observed a reduction in the accumulation of stem dry biomass as water salinity levels increased.

The root dry biomass of passion fruit (Figure 4A) was negatively affected by the cationic nature of irrigation water when subjected to the absence of hydrogen peroxide (0 μM), with the highest RDB obtained in plants cultivated under the lowest level of electrical conductivity of water (Control). The

foliar application of 20 μM of H<sub>2</sub>O<sub>2</sub> led to the highest values of RDB in plants subjected to the treatments S<sub>1</sub>, S<sub>2</sub> and S<sub>3</sub>. When using an H<sub>2</sub>O<sub>2</sub> concentration of 40 μM, the lowest accumulation of root dry biomass was found in plants grown with S<sub>6</sub>. On the other hand, plants that received a concentration of 60 μM showed higher RDB when they were subjected to the treatments S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, S<sub>5</sub> and S<sub>6</sub>. Reactive oxygen species, such as H<sub>2</sub>O<sub>2</sub>, play a fundamental role in several biological mechanisms, such as plant growth under stress conditions (SADDHE et al., 2018). At low concentrations, H<sub>2</sub>O<sub>2</sub> can induce antioxidative defense systems, which allow plants to withstand different types of stress (TERZI et al., 2014).



Means followed by different letters show significant difference between treatments by Tukey test (p < 0.05). **Figure 4.** Root dry biomass - RDB (A and B) and total dry biomass - TDB (C and D) of passion fruit plants cv. BRS Rubi do Cerrado as a function of the interaction between the cationic nature of the water (CNW) and hydrogen peroxide concentrations - H<sub>2</sub>O<sub>2</sub>, as a function of CNW, at 60 days after sowing.

When analyzing the effects of H<sub>2</sub>O<sub>2</sub> concentrations on each cationic nature of the water (Figure 4B), it was verified that plants cultivated with water of low ECw (S<sub>1</sub>) and magnesium composition (S<sub>5</sub>) showed a linear reduction in RDB, equal to 12.87 and 4.57% for each 20 μM increase of H<sub>2</sub>O<sub>2</sub>. On the other hand, the highest RDB accumulations of plants irrigated with water prepared with Na<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>+Ca<sup>2+</sup> and Na<sup>+</sup>+Ca<sup>2+</sup>+Mg<sup>2+</sup>

(1.176, 1.805, 1.18 and 1.231 g plant<sup>-1</sup>) were obtained under estimated H<sub>2</sub>O<sub>2</sub> concentrations of 29, 35, 21 and 60 μM, respectively. The excess of salts in water and/or soil, regardless of the nature of cations, hampers the entry of water in plant cells due to the decrease in osmotic potential and causes changes in photosynthetic capacity and, as a consequence, plant growth is inhibited.

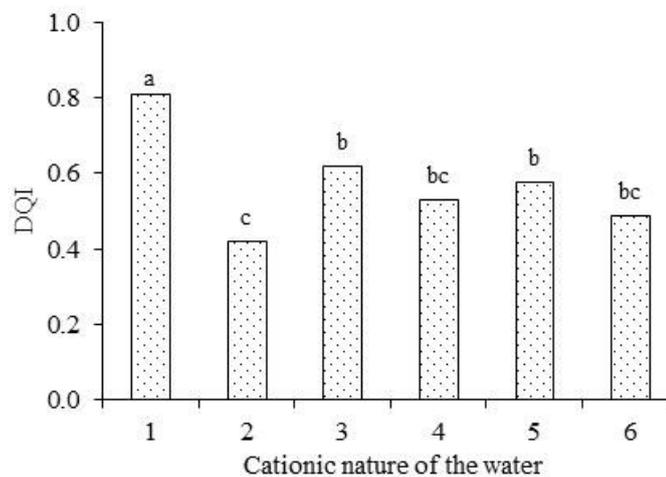
As observed for RDB (Figure 4A), the

absence of foliar application of  $\text{H}_2\text{O}_2$  and the  $\text{H}_2\text{O}_2$  concentration of 20  $\mu\text{M}$ , passion fruit plants irrigated with water of lowest ECw ( $\text{S}_1$ ) obtained the highest total dry biomass accumulation (Figure 4C), differing statistically from those subjected to the different cationic nature of irrigation water ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Na}^+\text{+Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Na}^+\text{+Ca}^{2+}\text{+Mg}^{2+}$ ). Plants subjected to foliar application of  $\text{H}_2\text{O}_2$  of 20  $\mu\text{M}$  and irrigated with the treatments  $\text{S}_2$ ,  $\text{S}_5$  and  $\text{S}_6$  differed significantly only from those that received low-salinity water ( $\text{S}_1$ ). When using 40  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$ , the lowest accumulation of TDB was obtained in plants irrigated with water composed of  $\text{Na}^+\text{+Ca}^{2+}\text{+Mg}^{2+}$ . Foliar application of 60  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$  intensified the effect of salt stress on plants cultivated with water composed of  $\text{Na}^+$ ,  $\text{Na}^+\text{+Ca}^{2+}$ , and  $\text{Mg}^{2+}$ . The reduction in RDB accumulation in plants grown under low salinity ( $\text{S}_1$ ) and in the absence of foliar application of  $\text{H}_2\text{O}_2$  is related to the variation in the level of electrical conductivity of water, as there were no significant differences between the cationic natures of the water, which may also be associated with restriction in the absorption of water and nutrients, as explained earlier.

In the analysis of the  $\text{H}_2\text{O}_2$  concentrations considering each cationic nature of the water (Figure 4D), a linear decrease was observed in the TDB of plants irrigated with water of low salinity ( $\text{S}_1$ ) and

composed of magnesium ( $\text{S}_5$ ), with decreases of 6.31 and 9.80% for each 20  $\mu\text{M}$  increase of  $\text{H}_2\text{O}_2$ . When using waters composed of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Na}^+\text{+Ca}^{2+}$  and  $\text{Na}^+\text{+Ca}^{2+}\text{+Mg}^{2+}$ , the highest TDB values (4.79; 7.10; 6.51 and 6.29  $\text{g plant}^{-1}$ ) were estimated at  $\text{H}_2\text{O}_2$  concentrations of 22, 39, 23 and 60  $\mu\text{M}$ , respectively. It can be inferred from the TDB data (Figure 4D) that the mitigating effect of  $\text{H}_2\text{O}_2$  during the formation of passion fruit seedlings depends on its concentration, level of electrical conductivity and nature of the cation present in the irrigation water.

For the Dickson Quality Index of passion fruit seedlings (Figure 5), the highest values (0.81, 0.62 and 0.57) were obtained in plants irrigated with low-salinity water (Control) and for treatments with waters composed of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , respectively. However, when comparing the DQI of plants subjected irrigation with water containing  $\text{Ca}^{2+}$ ,  $\text{Na}^+\text{+Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Na}^+\text{+Ca}^{2+}\text{+Mg}^{2+}$ , there was no significant difference between them. The lowest DQI (0.42) was verified in plants subjected to salinity of water with sodic composition. Despite the reduction in the DQI of plants grown under salinity of water with sodic nature, it is noted that the value obtained (0.42) characterizes a seedling of acceptable quality for field transplanting, considering that seedlings with DQI greater than 0.2 are considered of good quality (DICKSON; LEAF; HOSNER, 1960).



1 - Control; 2 -  $\text{Na}^+$ ; 3 -  $\text{Ca}^{2+}$ ; 4 -  $\text{Na}^+ + \text{Ca}^{2+}$ ; 5 -  $\text{Mg}^{2+}$ ; 6 -  $\text{Na}^+\text{+Ca}^{2+}\text{+Mg}^{2+}$ . Means followed by different letters show significant difference between treatments by Tukey test ( $p < 0.05$ ).

**Figure 5.** Dickson Quality Index - DQI of passion fruit plants cv. BRS Rubi do Cerrado as a function of the cationic nature of the water, at 60 days after sowing.

According to Oliveira et al. (2013), DQI is an important morphological characteristic used to determine the quality and rusticity of the seedlings, because it expresses the growth capacity and balance in the distribution of biomass under stress conditions. Veloso et al. (2018), when evaluating the quality of soursoop seedlings cv. 'Morada Nova', irrigated with

waters of different salinity levels (ECw from 0.3 to 3.5  $\text{dS m}^{-1}$ ) prepared with salts of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$  in the proportion of 7:2:1 also found that plants under ECw up to 3.5  $\text{dS m}^{-1}$  had DQI higher than 0.2, is characterized as seedlings of good quality for field transplanting.

## CONCLUSIONS

Exogenous application of 60  $\mu\text{M}$  of hydrogen peroxide reduces the percentage of intercellular electrolyte leakage in leaf tissues of passion fruit plants irrigated with water of calcic composition at 60 days after sowing.

The salinity of water composed of sodium, sodium+calcium, and sodium+calcium+magnesium results in lower leaf water potential in passion fruit plants subjected to 40 and 60  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$ .

Foliar application of  $\text{H}_2\text{O}_2$  promotes an increase in carotenoids content in passion fruit plants cultivated with water of sodic nature, regardless of the concentration.

The biomass accumulation of passion fruit cv. BRS Rubi do Cerrado is more sensitive to the variation in the electrical conductivity of water compared to the cationic nature of the water.

Regardless of the cationic nature, the use of water with electrical conductivity of 3.0  $\text{dS m}^{-1}$  produces passion fruit seedlings with a Dickson quality index above the acceptable limit.

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