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Hydrogen peroxide to mitigate the effects of salt stress in the mini watermelon under hydroponic cultivation[1]

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ABSTRACT - The use of substances that can mitigate the harmful effects of salt stress is a promising alternative for the use of saline water, especially in semi-arid regions. The aim of this study was to evaluate the effect of different concentrations of hydrogen peroxide in mitigating salt stress in the mini watermelon grown in a hydroponic system. The study was carried out in a greenhouse, in an area belonging to the Federal University of Campina Grande, Pombal, Paraíba. The experimental design was completely randomised using a split-plot factorial scheme with four levels of electrical conductivity for the nutrient solution—ECns (2.1, 3.1, 4.1, and 5.1 dS m^{-1}) considered the plots, and four concentrations of hydrogen peroxide—H_2O_2 (0, 20, 40, and 60 μM) the subplots, with five replications. The foliar application of hydrogen peroxide in concentrations of between 17 and 20 µM mitigated the effects of salt stress on stomatal conductance, the rate of CO_2 assimilation, carboxylation efficiency, and carotenoid content in the mini watermelon up to an ECns of 5.1 dS m^{-1}. Foliar application of hydrogen peroxide in concentrations greater than 20 µM intensified the effects of salt stress on gas exchange and the synthesis of photosynthetic pigments in the mini watermelon. Furthermore, there was an increase in electrolyte leakage in the leaf blade.

Key words: *Citrullus lanatus*. Salinity. Protected environment. Elicitor.

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INTRODUCTION

Fruit farming is an activity of great importance for the agricultural sector in Brazil (SILVA *et al*., 2019a), especially in the northeast of the country. The production of watermelon (*Citrullus lanatus* L.) has increased in recent years; in 2021 Brazil produced 2,141,970 tons of watermelon, in a planted area of 91,922 hectares, with the northeast being the primary producer, responsible for 37.45% (802.192 thousand tonnes) of domestic production, with a harvested area of 38,972 hectares (IBGE, 2021).

Water salinity is one of the main factors to limit plant growth and productivity in the region. Much of the northeast of Brazil has a semi-arid climate, characterised by high rates of evapotranspiration and low rainfall, resulting in water scarcity for most of the year (SILVA *et al*., 2019b). In addition, water sources available for irrigation generally have a high salt concentration (SOARES *et al*., 2018).

An excess of salts in the water can compromise the metabolic and biochemical activities of plants, affecting their productive potential due to a decrease in stomatal conductance, inhibition of photosynthesis, reduction in protein synthesis, impairment of enzyme activities, and degradation of photosynthetic pigments (LIANG *et al*., 2018). Furthermore, an imbalance between the production and removal of reactive oxygen species (ROS) caused by salt stress, leads to the peroxidation of membrane lipids and, consequently, to electrolyte leakage in the leaf blade, which can cause cell death (HATAMI *et al*., 2018).

Hydroponic systems have become an alternative to conventional cultivation, affording greater water use efficiency and fewer risks associated with the use of saline water (COSTA *et al*., 2020). Plants grown in hydroponic systems tend to be more tolerant to salt stress, considering that in such a system, the soil matric potential is non-existent (OLIVEIRA *et al*., 2023).

Given the growing need to use brackish water in agriculture, research to enable the use of such water sources has become essential. In this respect, hydrogen peroxide stands out as a promising alternative, capable of mitigating the harmful effects of salt stress (SILVA *et al*., 2021a).

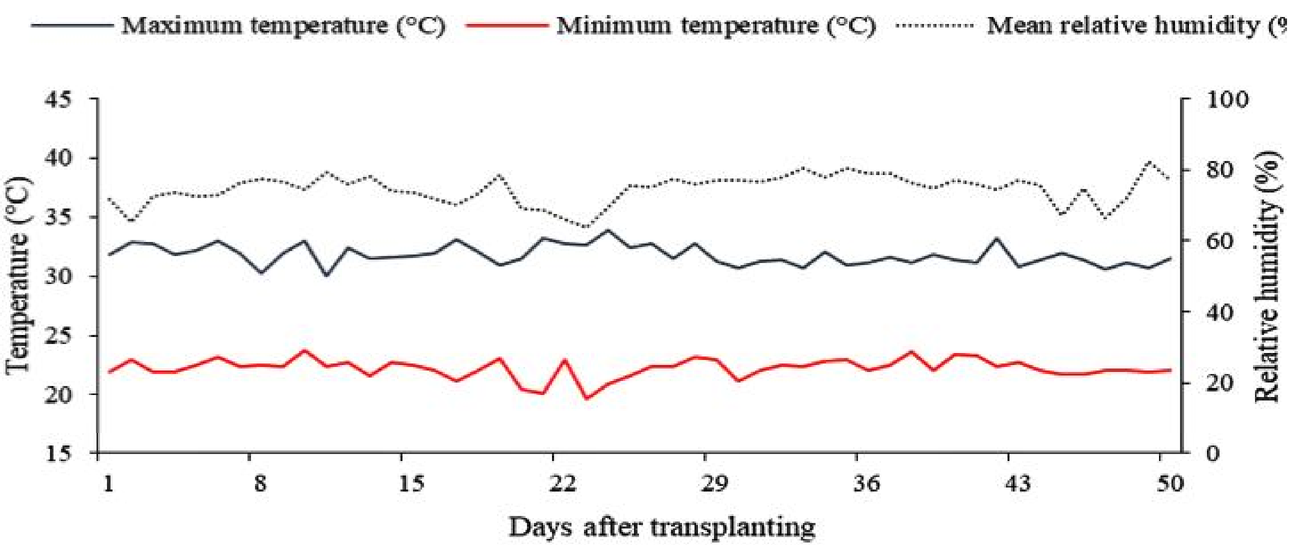
Hydrogen peroxide is a reactive oxygen species that performs the function of hormone signalling, controlled by its production and elimination. At low concentrations, hydrogen peroxide acts to regulate biological processes, increasing the absorption of water and nutrients by plants, reducing the absorption of Na^+ and Cl^-, and favouring plant tolerance to salt stress (FAROUK; QADOS, 2018; SILVA; AZEVEDO NETO; GHEYI, 2019).

However, information concerning its use in cultivating the mini watermelon in a hydroponic system is scarce. Therefore, the aim of this study was to evaluate the effect of different concentrations of hydrogen peroxide in mitigating salt stress in the mini watermelon grown in a hydroponic system.

MATERIAL AND METHODS

The study was carried out from March to May 2021 in a protected environment (greenhouse) at the Centre of Agri-Food Science and Technology (CCTA) of the Federal University of Campina Grande (UFCG), in Pombal, Paraíba, located at 6°46’13’’ S and 37°48’6’’ W, at a mean altitude of 184 m. Temperature data (maximum and minimum) and mean relative humidity of the air at the experimental site are shown in Figure 1.

Figure 1 - Air temperature (maximum and minimum) and mean relative humidity of the air inside the greenhouse during the experimental period



Seeds of the mini watermelon ‘Sugar Baby’ from Agristar® were used in the study. This cultivar has a cycle of approximately 90 days, with vigorous and highly productive plants, and adapts well to the various growing areas in Brazil. It produces sweet and refreshing fruit, with a diameter of between 15 and 20 cm (AGRISTAR, 2023).

The treatments were distributed in a completely randomised split-plot design, with the levels of electrical conductivity of the nutrient solution - ECns (2.1, 3.1, 4.1, and 5.1 dS m^{-1}) considered the plots, and four concentrations of hydrogen peroxide – H_2O_2 (0, 20, 40, and 60 μM) the subplots, with five replications. The H_2O_2 solutions were applied via foliar spray. Due to the lack of research with H_2O_2 in vegetable crops, the concentrations used in this study were based on study carried out with the soursop (*Annona muricata* L.) (VELOSO *et al*., 2020), while the salinity levels of the nutrient solution were established based on research carried out by Dantas *et al*. (2021) with the courgette (*Cucurbita pepo* L).

The hydroponic system employed Laminar Nutrient Flow Technique - NFT was made from polyvinyl chloride (PVC) pipe, 100 mm in diameter and six metres in length, and comprised four subsystems spaced 0.80 m apart, each subsystem containing three channels (replications) spaced 0.40 m apart, with a spacing of 0.50 m between plants and 1.0 m between the subsystems.

The channels were supported on trestles at a height of 0.60 m with a 4% slope to drain the nutrient solution. At the lowest end of each bench a 150 L polyethylene box was placed to collect and recirculate the nutrient solution to the channels. The nutrient solution was injected into the hydroponic channels using a 35W pump with a flow rate of 3 L min^{-1}, and circulated by timer, for an intermittent flow of 30 minutes every hour. The nutrient solution proposed by Hoagland and Arnon (1950) was used, whose nutrient composition resulted in an electrical conductivity of 2.1 dS m^{-1} and is shown in Table 1.

Sowing was carried out in polyethylene containers with a capacity of 50 mL, containing vegetable sponges (collected from a cucurbit of genus *Luffa*) arranged in trays. Before sowing, the sponges were sanitised with hypochlorite (2% to 2.5%), washed and air-dried. During the germination phase until the appearance of the first true leaf (on average ten days after sowing), a nutrient solution at a concentration of 50% of that recommended by Hoagland and Arnon (1950) was used. Following emergence of the first true leaf, the vegetable sponges were removed, and the seedlings inserted into the hydroponic profiles. The nutrient solution was then used at full concentration (100%) according to each treatment.

The saline solutions were obtained by adding sodium chloride (NaCl), calcium chloride ($CaCl_2.2H_2O$), and magnesium chloride ($MgCl_2.6H_2O$) salts, in the equivalent proportion of 7:2:1, to a nutrient solution prepared using water taken from the municipal supply of the city of Pombal (ECw = 0.3 dS m^{-1}). This saline solution contains Na, Ca, and Mg in proportions commonly found in water used for irrigation in the semi-arid region of northeastern Brazil.

The solution was completely replaced every eight days; however, the electrical conductivity and pH were monitored daily, and whenever necessary, the solution was adjusted by adding water from the municipal supply, always keeping the ECns in accordance with the established treatments, and the pH between 5.5 and 6.5 by adding 0.1 M KOH or HCl. The ECns and pH were monitored using a bench conductivity meter (MB11, MS Techonopon®) and digital pH meter (COMBO5, AKSO®).

Table 1 - Chemical composition of the nutrients in the general nutrient solution recommended by Hoagland and Arnon (1950), used in hydroponic cultivation of the mini watermelon

Nutrient	Fertilizer	Quantity (g 1000 L^{-1})
P/K	KH_2PO_4	136.09
K/N	KNO_3	101.10
Ca /N	$Ca (NO_3)_2.4H_2O$	236.15
Mg	$MgSO_4.4H_2O$	246.49
B	H_3BO_3	3.10
Mn	$MnSO_4.4H_2O$	1.70
Zn	$ZnSO_4.7H_2O$	0.22
Cu	$CuSO_4$	0.75
Mo	$(NH_4)_6M_{07}O_{24}. 4H_2O$	1.25
	EDTA – Na	13.9
Fe	$FeSO_4$	13.9

The plants were trained vertically, and phytosanitary management was carried out preventively to control the possible appearance of pests—aphids (*Aphis gossypii*), whitefly (*Aleyrodidae*), cutworm (*Agrotis ipsilon*), melon worm (*Diaphania hyalinata*), and curcubit beetle (*Diabrotica speciosa*)—by the application of chemical pesticides based on chlorfenapyr and cypermethrin. The solutions were prepared using 1 g $10L^{-1}$ and 2.5 mL $10L^{-1}$, respectively.

The hydrogen peroxide concentrations were obtained by diluting H_2O_2 – 30% in deionised water. These were subsequently stored in plastic containers lined with aluminium foil, and kept in a low-temperature environment (<12 ºC).

After transplanting, the plants were treated with a foliar application of hydrogen peroxide as per each treatment. The application was carried out manually using a spray bottle at dusk to ensure complete wetting of the leaves on both sides (abaxial and adaxial) at 10-day intervals, with the first application 72 hours prior to treatment with the saline nutrient solution and continuing for a total of three applications. While spraying the H_2O_2, a plastic curtain was used to prevent the product from drifting between the plants of the different treatments.

The following traits were evaluated 50 days after transplanting (DAT): relative water content; electrolyte leakage in the leaf blade; gas exchange—stomatal conductance (*gs*), transpiration (*E*), rate of CO_2 assimilation (*A*), and internal CO_2 concentration (*Ci*); instantaneous water use efficiency (*WUEi*) (*A/E*); instantaneous carboxylation efficiency (*CEi*) (*A/Ci*); and photosynthetic pigments—chlorophyll *a*, chlorophyll *b* and carotenoids. The fresh weight, and polar and equatorial diameter of the mini watermelon fruit were also measured.

To determine the relative water content (RWC), two leaves were removed from the middle third of the main branch to obtain five discs, 12 mm diameter, from each leaf. The discs were weighed immediately after perfuration to avoid any loss of moisture and to determine the fresh weight (FW); the samples were then placed in a beaker, immersed in 50 mL of distilled water and stored for 90 minutes. After this period, excess water was removed from the discs with a paper towel to obtain the turgid weight (TW) of the samples, which were then oven-dried at ≈ 65 ± 3 ºC to constant weight to obtain the dry weight (DW). The RWC was determined as per Lima *et al.* (2015) using Equation 1:

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100 \qquad (1)$$

where:

RWC – relative water content (%);

FW – fresh weight (g);

TW – turgid weight (g); and

DW – dry weight (g).

Electrolyte leakage in the leaf blade (EL) was determined using a copper perforator to obtain five leaf discs per experimental unit, each with an area of 1.54 cm^2, which were washed and placed in an Erlenmeyer® flask containing 50 mL of distilled water. After being covered with aluminium foil, the Erlenmeyer® flasks were stored at 25 °C for 24 hours and the initial electrical conductivity of the medium (Xi) was then measured using a benchtop conductivity meter (MB11, DW Techonopon®). The Erlenmeyer® flasks were then oven-dried (SL100/336, SOLAB®) at 90 °C for 120 minutes and, once the contents had cooled, the final electrical conductivity (Xf) was measured. Electrolyte leakage in the leaf blade was expressed as the percentage of initial electrical conductivity relative to the electrical conductivity following the 120-minute treatment at 90 ºC (SCOTTI-CAMPOS *et al*., 2013).

Gas exchange was measured in the third leaf counting from the apex of the main branch of the plant, at an irradiation intensity of 1,200 µmol photons m^{-2} s^{-1} and air flow of 200 mL min^{-1}, using the LCPro+ portable photosynthesis measurement system from ADC BioScientific Ltda.

The photosynthetic pigment content was quantified as per Arnon (1949), using plant extracts from disc samples taken from the third completely expanded leaf from the apex. For each sample, 6.0 mL of 80% acetone P.A. was used. The chlorophyll and carotenoid concentration in the solutions was determined using a spectrophotometer at an absorbance wavelength (ABS) of 470, 647, and 663 nm, using Equations 2, 3 and 4:

$$Chlorophyll \cdot a \cdot (Chl \cdot a) = (12.25 \times ABS_{663}) - (2.79 \times ABS_{647}) \qquad (2)$$

$$Chlorophyll \cdot b \cdot (Chl \cdot b) = (21.5 \times ABS_{647}) - (5.10 \times ABS_{663}) \qquad (3)$$

$$Carotenoids(Car) = ((1000 \times ABS_{470}) - (1.82 \times Chl \cdot a) - (85.02 \times Chl \cdot b))/198 \qquad (4)$$

The values obtained for the levels of chlorophyll *a*, chlorophyll *b* and carotenoids in the leaves were expressed in mg g^{-1} fresh matter.

The following traits were analysed at harvest: fruit fresh weight (FFW), polar (PD) and equatorial diameter (ED) of the mini watermelon fruit. The FFW was determined on a precision balance of 0.01 g; the PD and ED of the fruit were obtained with a tape measure and expressed in centimetres. The time of harvest was determined as when the tendrils closest to the peduncle began to dry (approximately 50 days after insertion into the hydroponic profiles).

The data were subjected to the Shapiro-Wilk normality test. Analysis of variance was then carried out at 0.05 probability, and whenever significant, linear and quadratic regression analysis was performed using the SISVAR-ESAL statistical software (FERREIRA, 2019). The choice of regression model (linear or quadratic) was made using the coefficient of determination (R^2). In the case of a significant interaction between the factors, the TableCurve 3D software was used to create the response surfaces.

RESULT AND DISCUSSION

The interaction between the salinity levels of the nutrient solution and the hydrogen peroxide concentration had a significant effect ($p \leq 0.05$) on electrolyte leakage in the leaf blade only (Table 2). The salinity levels of the nutrient solution had a significant effect ($p \leq 0.01$) on %EL and RWC. The hydrogen peroxide concentrations had no significant effect on any of the variables under analysis.

Increase in the electrical conductivity of the nutrient solution resulted in an increase in electrolyte leakage in the leaf blade, regardless of the hydrogen peroxide concentration (Figure 2A), with the lowest % EL (32.10%) found in plants subjected to an ECns of 2.1 dS m^{-1} and grown at a concentration of 0 µM H_2O_2. It can also be seen that the increase in H_2O_2 concentration intensified the harmful effects on the % EL, the plants grown at an ECns of 2.1 dS m^{-1} and sprayed with a concentration of 60 µM H_2O_2 obtained a % EL of 50.35%, i.e. an increase of 56.85% compared to plants grown at the same level of ECns but with no H_2O_2.

The increase in electrolyte leakage in the leaf blade may be related to the excess of salts in the nutrient solution, which favours production of reactive oxygen species (ROS), causing oxidative stress (BAGHERI; GHOLAMI; BANINASAB, 2019). Furthermore, hydrogen peroxide in high concentrations can quickly diffuse through the subcellular membrane, increasing the production of malondialdehyde, which results in oxidative damage to the cell membrane (FAROOQ *et al*., 2017). It is worth noting, however, that the cell membrane is only considered damaged when electrolyte leakage exceeds 50% (SULLIVAN, 1971).

The increase in ECns had a negative effect on the relative water content of the leaves (Figure 2B), with a reduction of 3.67% per unit increase in the ECns. Plants subjected to an ECns of 5.1 dS m^{-1} showed a reduction in RWC of 9.05% compared to those grown at an ECns of 2.1 dS m^{-1}. The reduction in RWC results in a loss of turgor in the plant tissue, since salinity causes osmotic stress, making it difficult to absorb and translocate water from the nutrient solution to the plant, which affects its growth and metabolism (MENDONÇA *et al*., 2022).

Similar results were found in research conducted by Soares *et al*. (2023), who evaluated the effect of the salinity of the nutrient solution (ECns ranging from 2.1 to 5.4 dS m^{-1}) on the physiology and production of the ‘Gaucho’ melon (*Cucumis melo* L.) grown in a hydroponic system, and found a reduction in the relative water content due to an increase in the electrical conductivity of the nutrient solution, obtaining the lowest RWC (75.66%) in plants grown at an ECns of 5.4 dS m^{-1}.

Table 2 - Summary of the analysis of variance for electrolyte leakage (%EL) and relative water content (RWC) of the mini watermelon ‘Sugar Baby’ grown with saline nutrient solution and foliar application of hydrogen peroxide in a hydroponic system, 50 days after transplanting

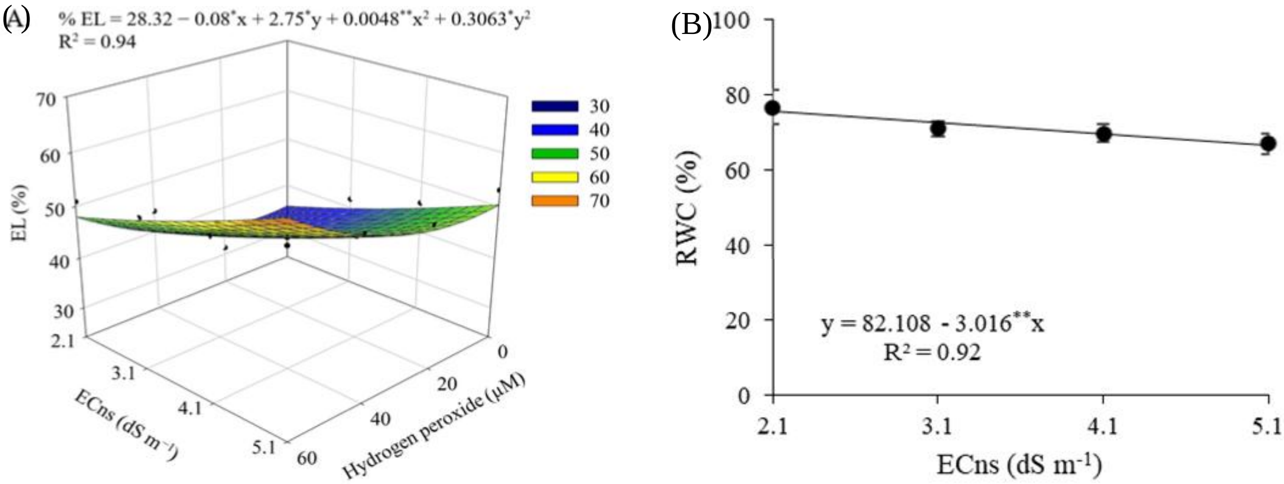
Source of variation	DF	Mean squares	
		%EL	RWC
Saline nutrient solution (ECns)	3	1107.13**	390.75**
Linear regression	1	4284.97**	905.31**
Quadratic regression	1	737.54^{ns}	169.76^{ns}
Residual 1	12	77.57	19.03
Hydrogen peroxide (H_2O_2)	3	315.1^{ns}	72.37^{ns}
Linear regression	1	330.36^{ns}	16.37^{ns}
Quadratic regression	1	0.02^{ns}	137.23^{ns}
Interaction (ECns × H_2O_2)	9	320.59*	29.24^{ns}
Residual 2	48	174.72	45.04
CV1 (%)		19.58	6.11
CV2 (%)		25.39	9.40

^{ns}, * and ** respectively, not significant, significant at $p < 0.05$, and significant at $p \leq 0.01$. DF: degree of freedom; CV: coefficient of variation

There was a significant effect ($p \leq 0.01$) of the interaction between the salinity levels of the nutrient solution and the hydrogen peroxide concentration on stomatal conductance, rate of CO_2 assimilation, and instantaneous carboxylation efficiency (Table 3). The salinity of the nutrient solution had a significant effect on all the gas exchange variables except *E*. The hydrogen peroxide concentration significantly affected *Ci*, *E* and *CEi*.

Increasing the electrical conductivity of the nutrient solution resulted in a linear increase in the internal CO_2 concentration (Figure 3A), with a rise of 6.19% for each unit increase in ECns. The plants subjected to an ECns of 5.1 dS m^{-1} showed an increase of 16.43% (28.48 µmol CO_2 m^{-2} s^{-1}) compared to those grown at an ECns of 2.1 dS m^{-1}. The increase in internal concentration in the substomatal chamber in plants grown

Figure 2 - Electrolyte leakage (%EL) in the leaf blade (A) of the mini watermelon 'Sugar Baby' as a function of the interaction between the electrical conductivity of the nutrient solution – ECns and the hydrogen peroxide concentration, with relative water content – RWC (B) as a function of the ECns, 50 days after transplanting



X and Y – H_2O_2 concentration and ECns, respectively; * and ** significant at $p \leq 0.05$ and 0.01 respectively. The vertical bars represent the standard error(n = 5)

Table 3 - Summary of the analysis of variance for internal CO_2 concentration (*Ci*), stomatal conductance (*gs*), transpiration (*E*), rate of CO_2 assimilation (*A*), instantaneous carboxylation efficiency (*CEi*), and instantaneous water use efficiency (*WUEi*) in the mini watermelon 'Sugar Baby' grown with saline nutrient solution and foliar application of hydrogen peroxide in a hydroponic system, 50 days after transplanting

Source of variation	DF	Mean Ssuares					
		Ci	gs	E	A	CEi	WUEi
Saline nutrient solution (ECns)	3	2653.1**	0.07**	1.1^{ns}	78.8*	44.4 x 10-4*	2.8**
Linear regression	1	4075.5**	0.19**	2.9^{ns}	167.5**	13.3 x 10-4*	3.7**
Quadratic regression	1	663.1^{ns}	0.01^{ns}	0.2^{ns}	1.1^{ns}	10.5 x 10-4^{ns}	2.6*
Residual 1	12	677.84	0.02	1.26	29.85	1.90 x 10-3	1.0
Hydrogen peroxide (H_2O_2)	3	1573.4*	0.01^{ns}	1.7*	30.7^{ns}	47.6 x 10-4*	1.1^{ns}
Linear regression	1	1419.6^{ns}	0.01^{ns}	1.5^{ns}	48.6^{ns}	87.4 x 10-4*	1.8^{ns}
Quadratic regression	1	3052.6*	0.01^{ns}	3.6**	7.9^{ns}	30.0 x 10-4^{ns}	0.9^{ns}
Interaction (ECns × H_2O_2)	9	841.5^{ns}	0.03**	0.2^{ns}	65.9**	45.3 x 10-4**	0.8^{ns}
Residual 2	48	458.48	0.01	0.30	19.11	1.40 x 10-3	0.4
CV1 (%)		14.07	17.79	12.47	16.36	15.08	11.10
CV2 (%)		11.57	18.28	21.04	20.44	19.00	18.67

^{ns,*} and ** respectively, not significant, significant at $p \leq 0.05$, and significant at $p \leq 0.01$. DF: degree of freedom; CV: coefficient of variation under salt stress is an indication of deterioration of the photosynthetic apparatus, considering that the damage caused to the structures responsible for CO_2 fixation is due not only to stomatal factors, but also non-stomatal factors, a result of salts accumulating in the leaves and interfering with enzyme activity (HUSSAIN *et al*., 2012; SILVA *et al*., 2021b).

The application of hydrogen peroxide at a concentration of 17 µM resulted in a reduction in *Ci* in the mini watermelon (Figure 3B), where the lowest value (177.9 µmol CO_2 m^{-2} s^{-1}) stood out. On the other hand, concentrations greater than 17 µM resulted in an increase in *Ci*, with the highest value (197.4 µmol CO_2 m^{-2} s^{-1}) recorded in plants that received a concentration of 60 µM H_2O_2. This result may be related to the inhibition of RuBisCO activity by oxidative stress, which prevents the conversion of absorbed CO_2 into photoassimilates and consequently increases the *Ci*.

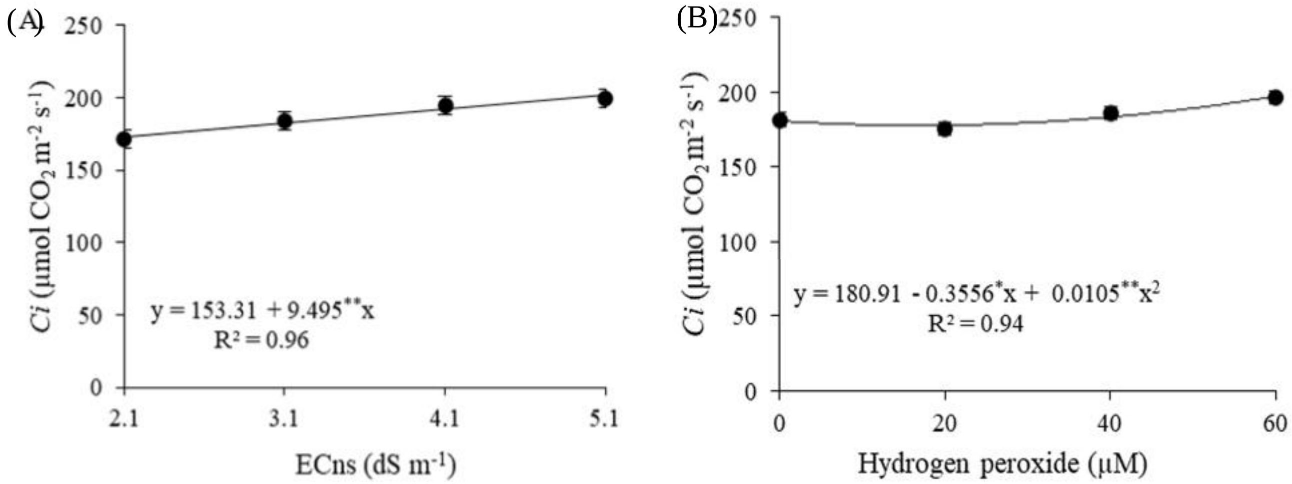
For stomatal conductance and the rate of CO_2 assimilation (Figure 4A and B), plants subjected to a concentration of 20 µM and grown at an ECns of 2.1 dS m^{-1} achieved the highest *gs* (0.53 mol H_2O m^{-2} s^{-1}), while those grown at an ECns of 2.7 dS m^{-1} recorded a higher value for *A* (30.96 µmol CO_2 m^{-2} s^{-1}). However, when hydrogen peroxide was sprayed in concentrations greater than 20 µM the harmful effects of salinity were intensified, resulting in the lowest values for *gs* (0.26 mol H_2O m^{-2} s^{-1}) and *A* (20.83 µmol CO_2 m^{-2} s^{-1}) in plants sprayed with a concentration of 60 µM and grown at an ECns of 5.1 dS m^{-1}.

The beneficial effect of hydrogen peroxide on the rate of CO_2 assimilation is possibly related to its role in improving root and leaf growth, thereby increasing the water absorption capacity (DENG *et al*., 2012). In addition, the application of hydrogen peroxide favours the absorption of nutrients that are important for the photosynthetic process, including N, P, and K (FAROUK; QADOS, 2018). Nitrogen is a constituent of amino acids and soluble sugars which aid in the osmotic adjustment of plants to salinity (ASHRAF *et al*., 2018). Potassium is involved in the translocation and maintenance of the water balance, and participates in various biochemical and physiological functions, such as osmoregulation and reducing the excessive absorption of ions such as Na^+ (MEENA *et al*., 2022). Whereas phosphorus makes up part of the structural composition, and is responsible for storing and transferring energy during the photosynthetic process (AQUINO *et al*., 2021).

The H_2O_2 concentration affected transpiration in the mini watermelon (Figure 4C). Plants subjected to a concentration of 20.87 µM stood out with a higher value for *E* (5.16 mmol H_2O m^{-2} s^{-1}), corresponding to an increase of 3.49% (0.174 mmol H_2O m^{-2} s^{-1}) compared to plants grown at a concentration of 0 µM. It was found that concentrations greater than 20.87 µM caused a reduction in transpiration, with the lowest estimated value of 4.55 mmol H_2O m^{-2} s^{-1} recorded in plants that received 60 µM H_2O_2.

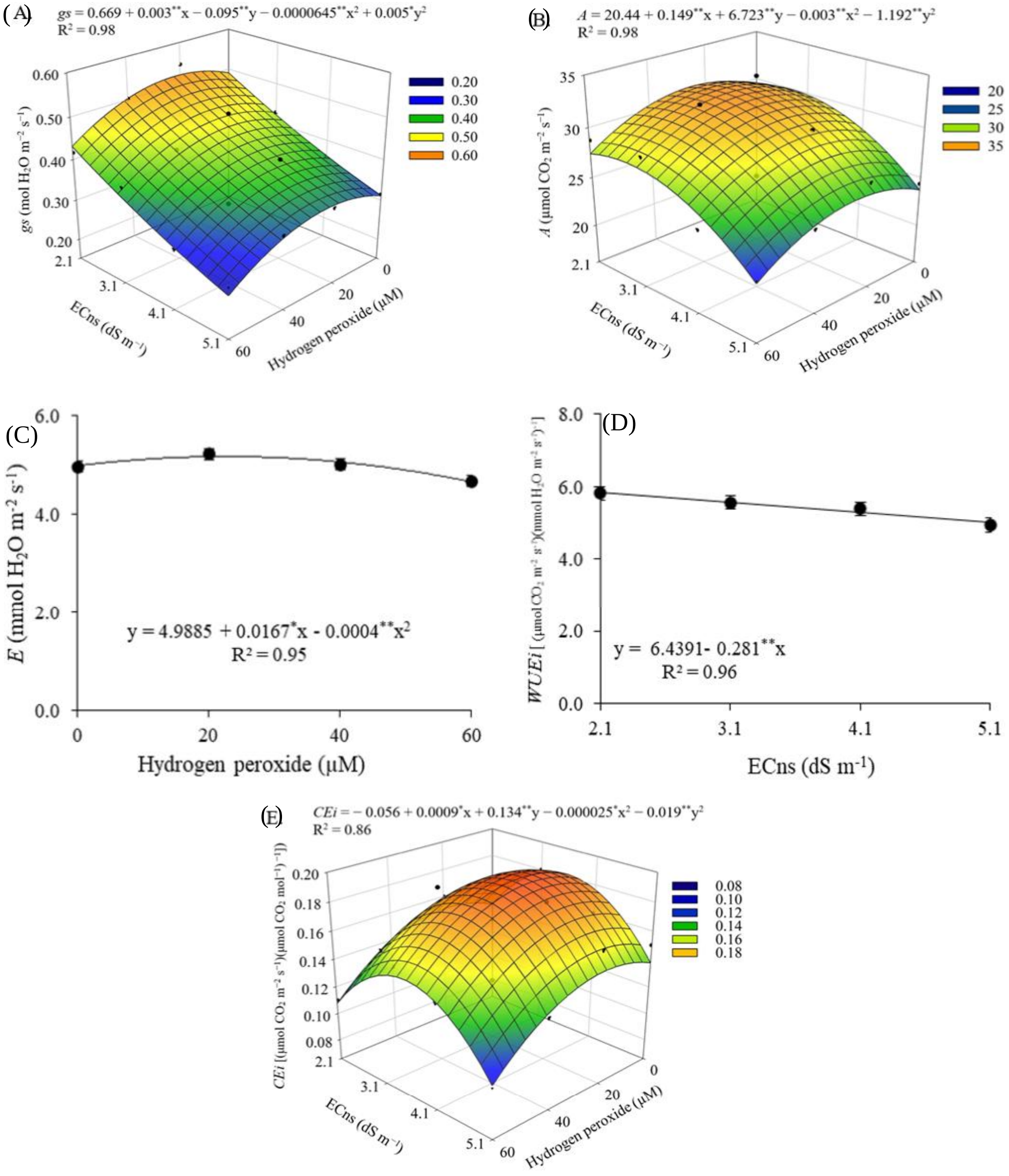
The increased transpiration in plants subjected to a concentration of 20.87 µM may contribute to maintaining water absorption and ensuring leaf turgor, allowing the plant to exchange gases with the environment (VELOSO *et al*., 2022). As seen in this study, the concentration of 20 µM H_2O_2 also favoured an increase in stomatal conductance (Figure 4A) and rate of assimilation (Figure 4B), promoting an increase in these variables compared to the control treatment (0 µM).

Figure 3 - Internal CO_2 concentration - *Ci* in the mini watermelon 'Sugar Baby' as a function of the saline nutrient solution - ECns (A) and hydrogen peroxide concentration (B), at 50 days after transplanting



* and ** significant at p ≤ 0.05 and 0.01 respectively. The vertical bars represent the standard error(n = 5)

Figure 4 - Stomatal conductance - *gs* (A), rate of CO_2 assimilation - *A* (B), and instantaneous carboxylation efficiency - *CEi* (E) in the mini watermelon 'Sugar Baby' as a function of the interaction between the electrical conductivity of the nutrient solution – ECns and the hydrogen peroxide concentration, and transpiration - *E* (C) as a function of the H_2O_2 concentration, and water use efficiency - *WUEi* (D) as a function of the ECns of the nutrient solution, 50 days after transplanting



X and Y – H_2O_2 and ECns concentration, respectively; * and ** significant at $p \leq 0.05$ and 0.01, respectively. The vertical bars represent the standard error(n = 5)

Instantaneous water use efficiency in the mini watermelon decreased linearly with the increases in ECns levels, with a reduction of 4.36% per unit increase in ECns (Figure 4D). Plants grown in the nutrient solution with the highest salinity (5.1 dS m^{-1}) showed a reduction of 14.42% (0.84 (μmol CO_2 m^{-2} s^{-1})(mmol H_2O m^{-2} s^{-1}) $^{-1}$), compared to those subjected to an ECns of 2.1 dS m^{-1}. It should be noted that in this study, the increase in salinity of the nutrient solution reduced the relative water content (Figure 2B), which may have been due to the water deficit induced by the osmotic effect promoting a reduction in the water absorbed by the plants. Furthermore, the rate of CO_2 assimilation (Figure 4B) was also compromised by the increase in ECns. All of these factors may have contributed to reduce the instantaneous water use efficiency.

When studying the interaction of the salinity of the nutrient solution and hydrogen peroxide concentrations on the *CEi* (Figure 4E), it was found that the plants sprayed with a concentration of 20 μM H_2O_2 and grown at an ECns of 3.5 dS m^{-1} stood out with the highest *CEi* (0.190 (μmol CO_2 m^{-2} s^{-1})(μmol CO_2 m^{-2} s^{-1})$^{-1}$), corresponding to an increase of 5.55% (0.01 (μmol CO_2 m^{-2} s^{-1})(μmol CO_2 m^{-2} s^{-1})$^{-1}$) compared to plants grown at the same level of ECns (3.5 dS m^{-1}) but subjected to a concentration of 0 μM H_2O_2. According to Baxter, Mittler, and Suzuki (2014), the beneficial effect of hydrogen peroxide in low concentrations may be related to its role as a signalling molecule, regulating several pathways, including the response to salt stress.

The interaction between the saline nutrient solution (ECns) and the hydrogen peroxide concentrations only had a significant effect ($p \leq 0.01$) on the chlorophyll *a* and carotenoids of the mini watermelon (Table 4). The saline levels of the nutrient solution significantly affected each of the variables except Chl *a* and Chl *b*. The hydrogen peroxide concentrations had a significant effect on the carotenoid content.

The increase in hydrogen peroxide concentration intensified the harmful effects of the salinity of the nutrient solution on the chlorophyll *a* content of the mini watermelon (Figure 5A); the plants grown at an ECns of 2.1 dS m^{-1} and with no H_2O_2 (0 μM) stood out with a higher value for Chl *a* (0.124 mg g^{-1} FW), corresponding to an increase of 66.49% (0.049 mg g^{-1} FW) in relation to the plants grown at an ECns of 5.1 dS m^{-1} and with no H_2O_2 (0 μM). On the other hand, the lowest value for Chl *a* (0.045 mg g^{-1} FW) was obtained in plants sprayed with a concentration of 60 μM and grown at an ECns of 5.1 dS m^{-1}.

The reduction in chlorophyll *a* synthesis seen in the plants grown at an ECns of 5.1 dS m^{-1} and with no H_2O_2 (0 μM) may be related to a reduction in the absorption of ions such as Mg^{2+} and Fe^{2+} which are involved in the formation of chlorophyll and which have limited absorption due to competition with Na^+ and Cl^- (SOARES *et al*., 2021). Excess salts can alter the structure of organelles, pigment levels, and the activities of enzymes involved in the photosynthetic process (SHAHVERDI; OMIDI; TABATABAEI, 2019).

Table 4 - Summary of the analysis of variance for the levels of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoids (Car), fresh fruit weight (FFW), polar diameter (PD), and equatorial diameter (ED) of the mini watermelon 'Sugar Baby' grown with saline nutrient solution and foliar application of hydrogen peroxide in a hydroponic system, 50 days after transplanting

Source of variation	DF	Mean squares					
		Chl a1	Chl b1	Car	FFW	PD	ED
Saline nutrient solution (ECns)	3	4.2 x 10^{-3ns}	0.03^{ns}	0.86**	340179.6**	139.4**	196.4**
Linear regression	1	2.2 x 10^{-3ns}	0.06^{ns}	2.53**	768919.5**	218.7**	345.1**
Quadratic regression	1	5.4 x 10^{-3ns}	0.04^{ns}	0.03^{ns}	180980.6*	165.4*	182.5*
Residual 1	12	1.9 x 10^{-3}	5.1x10^{-3}	0.05	33342.7	340.5	2.23
Hydrogen peroxide (H_2O_2)	3	5.6 x 10^{-3ns}	0.04^{ns}	0.44**	11213.9^{ns}	31.4^{ns}	43.1^{ns}
Linear regression	1	7.6 x 10^{-3ns}	0.07^{ns}	0.48*	8316.8^{ns}	34.1^{ns}	21.7^{ns}
Quadratic regression	1	7.7 x 10^{-3ns}	0.02^{ns}	0.70**	9882.7^{ns}	45.4^{ns}	92.1^{ns}
Interaction (CEsn × H_2O_2)	9	1.2 x 10^{-2**}	0.05^{ns}	0.29**	23767.1^{ns}	51.9^{ns}	37.9^{ns}
Residual 2	48	3.5 x 10^{-3}	6.1x10^{-3}	0.07	25718.59	275.5	2.25
CV1 (%)		9.24	11.01	14.69	16.85	19.82	11.28
CV2 (%)		10.89	13.57	17.51	22.37	22.04	11.42

$^{ns, *}$ and ** respectively, not significant, significant at $p \leq 0.05$, and significant at $p \leq 0.01$. DF: degree of freedom; CV: coefficient of variation.[1] Data transformed into $\sqrt{x+1}$

According to Zhang *et al*. (2020), high concentrations of reactive oxygen species such as H_2O_2 can induce oxidative stress, causing lipid peroxidation, damage to cellular integrity (Figure 2A), and degradation of the photosynthetic pigments (Figure 5A and B), which can then result in cellular senescence.

Up to a concentration of 20 µM, spraying with hydrogen peroxide promoted an increase in the carotenoid content, regardless of the electrical conductivity of the nutrient solution (Figure 5B). The plants subjected to a concentration of 20 µM and grown at an ECns of 3.5 dS m^{-1} obtained a higher value for Car (1.21 mg g^{-1} FW). Comparing in relative terms the Car of plants grown in water of 3.5 dS m^{-1} and subjected to a H_2O_2 concentration of 20 µM to plants grown at the same saline level but with no H_2O_2 (0 µM), there was an increase of 12.04% (0.13 mg g^{-1} FW), indicating that the hydrogen peroxide was effective in acclimatising the mini watermelon to salt stress.

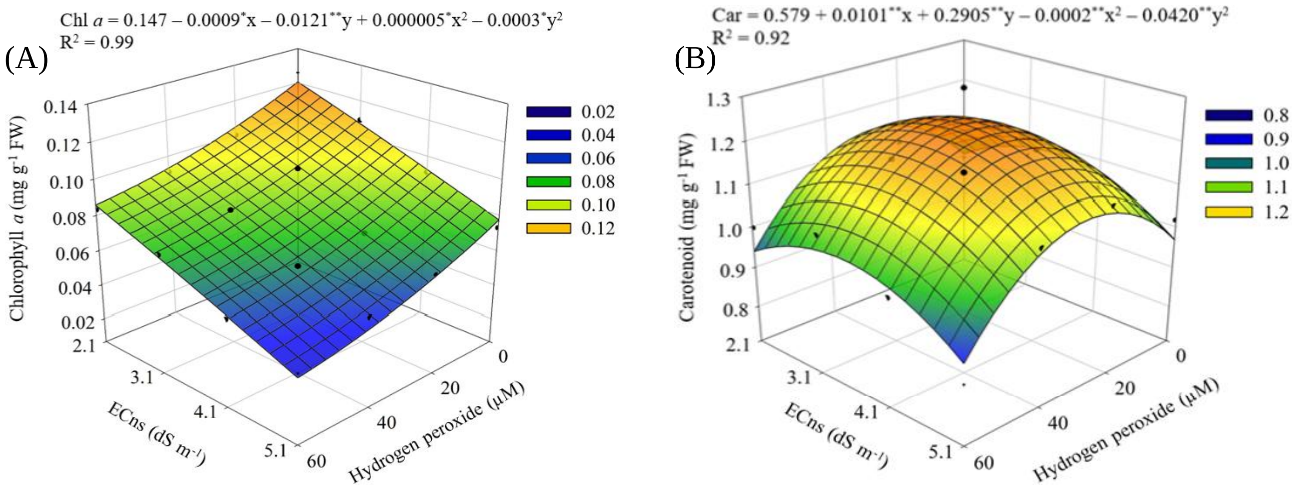
Similar results were found in research conducted by Silva *et al*. (2019c), who evaluated the effect of hydrogen peroxide (0 to 100 µM) on gas exchange and photosynthetic pigments in seedlings of the soursop (*Annona muricata* L.) under salt stress (ECw = 0.7 to 3.5 dS m^{-1}) and found that the application of hydrogen peroxide at a concentration of 25 µM resulted in an increase in the carotenoid content, obtaining the maximum value for Car (1.86 mg g^{-1} FW) in plants irrigated with an ECw of 3.5 dS m^{-1}. Carotenoids are pigments that can have a photoprotective effect on the photochemical apparatus, and this increase in carotenoids is possibly a defence mechanism to prevent photo-oxidative damage to the chlorophyll molecules (RAVEN; EVERT; EICHHORN, 2007).

The increase in salinity of the nutrient solution had a negative effect on the fresh weight of the mini watermelon fruit (Figure 6A), with a reduction of 10.7% per unit increase in the ECns. Plants grown under the highest salinity (5.1 dS m^{-1}) showed a reduction of 41.2% (240.14 g) in FFW compared to those grown at an ECns of 2.1 dS m^{-1}. This reduction in fruit fresh weight may be related to the low rate of CO_2 assimilation seen in plants grown at the highest levels of ECns (Figure 4B).

A reduction in fresh fruit weight was also observed in a study conducted by Ó *et al*. (2020), who evaluated the effect of the salinity of the nutrient solution (ECns ranging from 2.5 to 6.5 dS m^{-1}) on the production and quality of the mini watermelon 'Sugar Baby' and found a 19.2% reduction in the FFW of plants irrigated at the highest level (6.5 dS m^{-1}) compared to plants grown at a salinity of 2.5 dS m^{-1}.

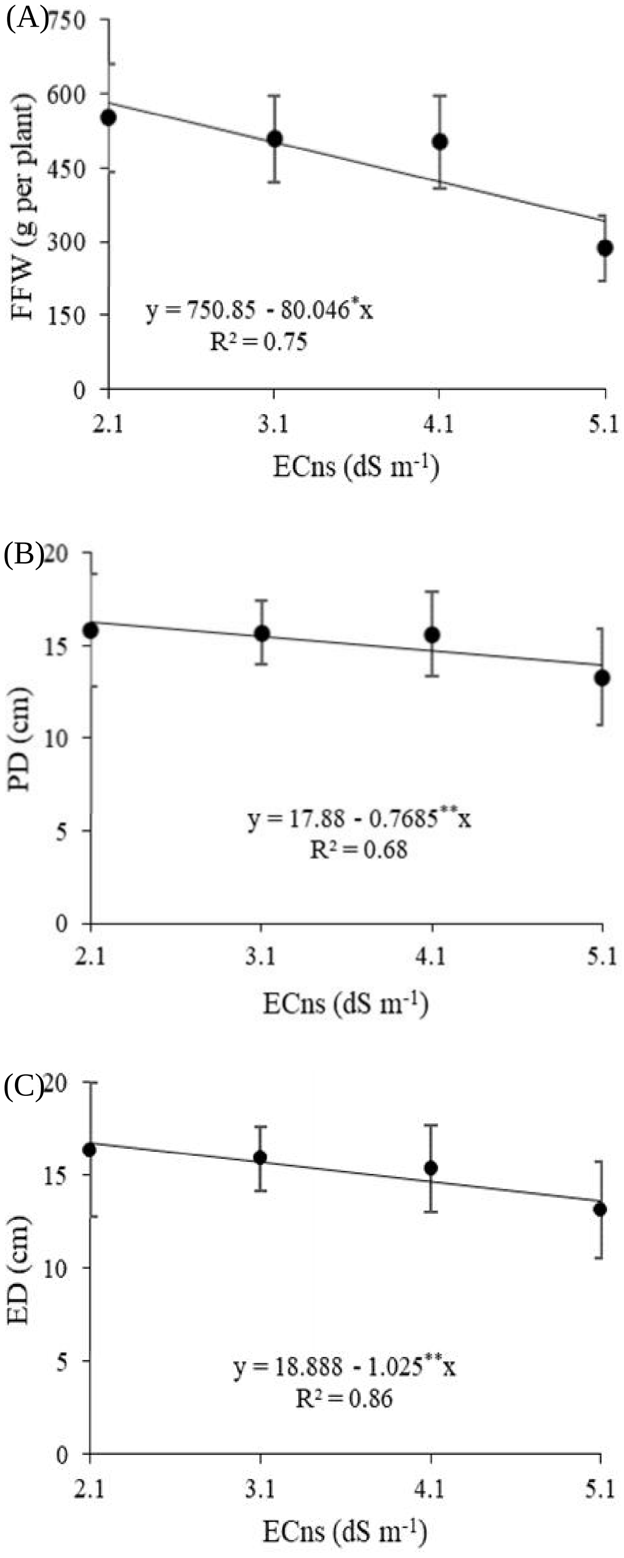
Increasing the salinity of the nutrient solution also reduced the polar and equatorial diameter of the mini watermelon fruit (Figure 6B and C). The plants subjected to an ECns of 2.1 dS m^{-1} achieved greater values for PD (16.27 cm) and ED (16.74 cm). When compared in relative terms, the PD and ED of plants grown at an ECns of 5.1 dS m^{-1} in relation to those that received an ECns of 2.1 dS m^{-1} showed a reduction of 14.2% (2.31 cm) in PD and 18.34% (3.07 cm) in ED. Despite the reduction in fruit diameter due to the harmful effect of salinity, the shape/appearance of the fruit was not affected, as the PD/ED ratio was always close to 1, giving the fruit a spherical shape (SILVA *et al*., 2022).

Figure 5 - Chlorophyll *a* - Chl *a* (A) and carotenoid - Car (B) content of the mini watermelon 'Sugar Baby' as a function of the interaction between the electrical conductivity of the nutrient solution – ECns and the hydrogen peroxide concentration, 50 days after



X and Y – H_2O_2 concentration and ECns, respectively; * and ** significant at $p \leq 0.05$ and 0.01 respectively

Figure 6 - Fresh fruit weight - FFW (A), polar diameter - PD (B), and equatorial diameter - ED (C) of mini watermelon fruit as a function of saline nutrient solution – ECns



* and ** significant at $p \leq 0.05$ and 0.01, respectively. The vertical bars represent the standard error(n = 5)

The reduction in fresh weight and polar and equatorial diameters of the mini watermelon fruit (Figure 6) due to the increase in salinity of the nutrient solution may be related to the decrease in osmotic potential restricting the absorption of water and nutrients, and even resulting in cell damage caused by oxidative stress in the plant (OLIVEIRA *et al*., 2014). It should be noted that in this study, the increase in salinity of the nutrient solution increased electrolyte leakage in the leaf blade, and reduced the relative water content, the synthesis of chlorophyll *a*, and gas exchange in the mini watermelon, possibly reflecting in reductions in FFW, PD, and ED. Salinity causes changes in plant physiology in response to such factors as osmotic stress, ion toxicity, and nutritional imbalance (NEGRÃO; SCHMÖCKEL; TESTER, 2017).

CONCLUSIONS

1. Foliar application of hydrogen peroxide in concentrations of between 17 and 20 µM mitigates the effects of salt stress on stomatal conductance, the rate of CO_2 assimilation, instantaneous carboxylation efficiency, and carotenoid content in the mini watermelon ‘Sugar Baby’ up to an electrical conductivity of the nutrient solution of 5.1 dS m^{-1};
2. Foliar application of hydrogen peroxide in concentrations greater than 20 µM intensifies the effects of salt stress on gas exchange, the synthesis of photosynthetic pigments, and electrolyte leakage in the leaf blade in the mini watermelon, 50 days after transplanting.

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