









## H<sub>2</sub>O<sub>2</sub> as attenuator of salt stress on the physiology and growth of hydroponic cherry tomato

## H<sub>2</sub>O<sub>2</sub> como atenuante do estresse salino na fisiologia e crescimento de tomate cereja hidropônico

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**ABSTRACT** - In arid and semi-arid regions, agricultural production is challenging due to the scarcity of water for irrigation, so brackish water is commonly used. However, the use of these waters negatively affects the growth and development of crops. In this context, it is essential to look for strategies to mitigate the effects of salt stress on plants. The objective of this study was to evaluate the effects of foliar application of H<sub>2</sub>O<sub>2</sub> on gas exchange, photosynthetic pigments, photochemical efficiency, and growth of cherry tomato plants in hydroponic cultivation with saline nutrient solution. The experiment was carried out in a greenhouse in Pombal-PB, using a Nutrient Film Technique (NFT) hydroponic system. Treatments were distributed in a split-plot scheme, in which the levels of electrical conductivity of the nutrient solution - ECns (2.1, 2.8, 3.5, and 4.2 dS m<sup>-1</sup>) were considered the plots and the five concentrations of H<sub>2</sub>O<sub>2</sub> (0, 12, 24, 36, and 48 µM) were considered the subplots, with six replicates and two plants per plot. ECns from 2.1 dS m<sup>-1</sup> reduced gas exchange, photochemical efficiency, photosynthetic pigments, relative water content, and growth of cherry tomato. H<sub>2</sub>O<sub>2</sub> at concentrations of 36 and 48 µM associated with saline nutrient solution of 2.1 dS m<sup>-1</sup> stimulated plant height, growth, and chlorophyll *b* synthesis, respectively. Hydrogen peroxide alone did not affect gas exchange, chlorophyll fluorescence, photosynthetic pigments, and growth of cherry tomato.

**Keywords:** *Solanum lycopersicum*. Acclimatization. Nutrient solution.

**Conflict of interest:** The authors declare no conflict of interest related to the publication of this manuscript.



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**RESUMO** - Em regiões áridas e semiáridas, a produção agrícola é desafiadora devido à escassez de água para irrigação, sendo comum o uso de água salobra. No entanto, o uso dessas águas afeta negativamente o crescimento e o desenvolvimento das culturas. Neste contexto, é essencial a busca por estratégias para amenizar os efeitos do estresse salino nas plantas. O objetivo desta pesquisa foi avaliar efeitos da aplicação foliar de H<sub>2</sub>O<sub>2</sub> nas trocas gasosas, nos pigmentos fotossintéticos, na eficiência fotoquímica e no crescimento de plantas de tomate cereja em cultivo hidropônico com solução nutritiva salina. O experimento foi desenvolvido em casa de vegetação em Pombal-PB, utilizando o sistema hidropônico tipo técnica de fluxo laminar de nutriente. Os tratamentos foram distribuídos em esquema de parcelas subdivididas, onde os níveis de condutividade elétrica da solução nutritiva - ECsn (2,1; 2,8; 3,5 e 4,2 dS m<sup>-1</sup>) foram considerados as parcelas e as cinco concentrações de H<sub>2</sub>O<sub>2</sub> (0, 12, 24, 36 e 48 µM) como subparcelas, com seis repetições e duas plantas por parcela. A ECsn a partir de 2,1 dS m<sup>-1</sup> reduziu as trocas gasosas, a eficiência fotoquímica, os pigmentos fotossintéticos, o conteúdo relativo de água e o crescimento do tomate cereja. O H<sub>2</sub>O<sub>2</sub> nas concentrações de 36 e 48 µM associadas à solução nutritiva salina, de 2,1 dS m<sup>-1</sup> estimulou o crescimento em altura de plantas e a síntese de clorofila *b*, respectivamente. O H<sub>2</sub>O<sub>2</sub> de forma isolada não afetou as trocas gasosas, a florescência da clorofila, os pigmentos fotossintéticos e o crescimento do tomate cereja.

**Palavras-chave:** *Solanum lycopersicum*. Acimação. Solução nutritiva.

### INTRODUCTION

Cherry tomato (*Solanum lycopersicum* L.) var. Cerasiforme is a table variety that stands out for its sweet flavor and smaller fruit size (GONÇALVES et al., 2018). It is a vegetable of great economic and social importance in Brazil, being cultivated in several regions of the country (FRANCA; LEITÃO; CAMPECHE, 2017). Although the national production of cherry tomato is lower compared to other tomato varieties among the main groups, the added value of cherry fruits is offset by their great acceptance among consumers and high commercial value of the product (CASTAÑEDA et al., 2020).

Despite the economic and social potential of this vegetable crop in the semi-arid region of Northeast Brazil, in this region the quality of water sources used in irrigation is a limiting factor for crop production, since rainfall is poorly distributed in space and time and temperatures lead to a gradual reduction in water availability, both in quality and quantity (PINHEIRO et al., 2022), with common occurrence of water sources with moderate to high salt levels, due to the climate imbalance.

Water salinity can cause changes in plant physiology, significantly reducing yield due to osmotic stress, which causes changes in osmotic homeostasis and ionic toxicity, especially in plants sensitive to salt stress (SUN et

al., 2020). The restriction in the absorption of water and nutrients limits the growth and development of plants, with negative effects on yield. Excessive accumulation of toxic ions, especially sodium and chloride, damages the cytoplasm, affecting biochemical and photosynthetic functions (TAVARES FILHO et al., 2020).

Vegetable production through hydroponic cultivation is a sustainable strategy, as it allows the control of production factors, especially water and nutrient management, in addition to enabling the use of brackish water, allowing year-round production in a greenhouse (OLIVEIRA et al., 2022). In hydroponic cultivation, as there is no soil involved, only the osmotic potential of the solution affects growth and the matric potential present in conventional cultivation is not considered. For this reason, this system is suitable to reduce the socioeconomic effects caused by the lack of water in arid regions (COSTA et al., 2020).

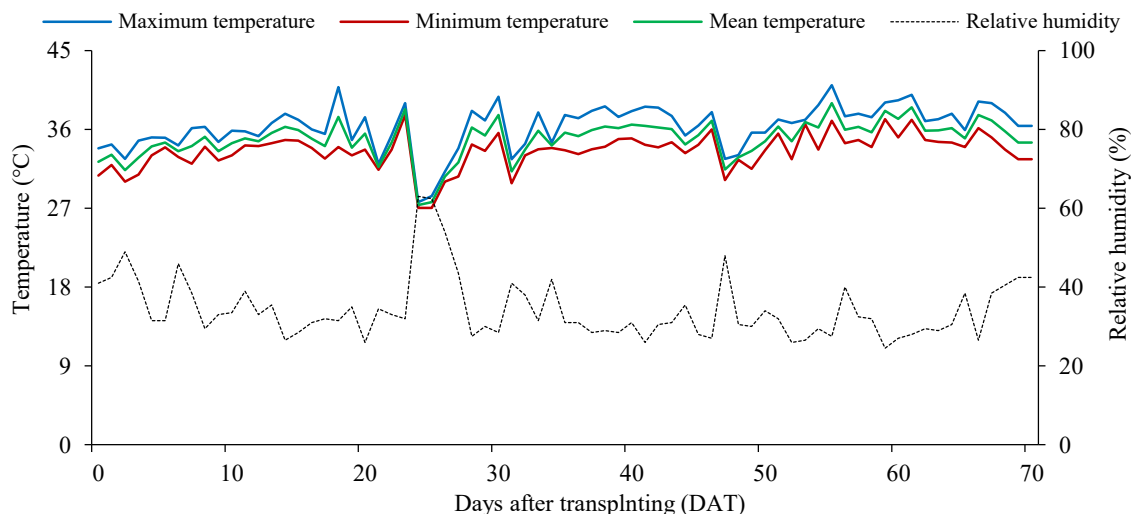
In addition, it is necessary to look for alternatives to reduce the deleterious effects of salinity, and one example is foliar application of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), as observed in studies with zucchini (DANTAS et al., 2022a) and mini watermelon (SILVA et al., 2022a). H<sub>2</sub>O<sub>2</sub> is a reactive oxygen species that plays a fundamental role in the acclimatization of plants to salt stress (SILVA et al., 2021), contributing to the production of antioxidant enzymes, proteins, and compounds capable of regulating various mechanisms under stress conditions, improving water and nutrient absorption and photosynthetic efficiency, contributing to the maintenance of plant ionic and redox homeostasis (CARVALHO et al., 2011). Dantas et al. (2022b), in a study with zucchini in a hydroponic

system, observed that foliar application of H<sub>2</sub>O<sub>2</sub> reduced the effects of salt stress on stomatal conductance and internal CO<sub>2</sub> concentration. In another study, these authors also found that foliar spraying of H<sub>2</sub>O<sub>2</sub> at concentrations of 60 and 40 μM associated with nutrient solution with electrical conductivity of 2.1 dS m<sup>-1</sup> promoted increments in stem diameter and root length of zucchini plants (DANTAS et al., 2021).

In view of the above, the objective of this study was to evaluate the effects of foliar application of H<sub>2</sub>O<sub>2</sub> as an attenuator of salt stress effects on leaf gas exchange, photosynthetic pigments, and growth of cherry tomato in an NFT hydroponic system.

## MATERIAL AND METHODS

The experiment was carried out from October 2022 to February 2023, under conditions of arched greenhouse, with a 150-micron low-density polyethylene cover, at the Center of Sciences and Agri-food Technology - CCTA, belonging to the Federal University of Campina Grande - UFCG, in Pombal, PB, Brazil, located at the geographic coordinates 6° 46' 8" S, 37° 48' 06" W, average altitude 193 m. According to Köppen's classification, the municipality of Pombal has a climate classified as hot and humid semi-arid, AW' (ALVARES et al., 2013). Data on temperature (maximum and minimum) and relative humidity were collected using a digital thermohygrometer inside the greenhouse and are presented in Figure 1.



**Figure 1.** Average daily temperature data (maximum, mean, and minimum) and average relative humidity of the air during the experimental period.

The experimental design was completely randomized, in a split-plot scheme, with four levels of electrical conductivity of the nutrient solution – ECNs (2.1, 2.8, 3.5, and 4.2 dS m<sup>-1</sup>) as plots and the concentrations of hydrogen peroxide – H<sub>2</sub>O<sub>2</sub> (0, 12, 24, 36, and 48 μM) as subplots. Each plot contained two plants and the experiment was conducted with six replicates. ECNs levels were defined based on a study conducted by Silva et al. (2022a), while H<sub>2</sub>O<sub>2</sub> concentrations

were established based on the study conducted by Silva et al. (2022b).

The nutrient solution used was that recommended by Hoagland and Arnon (1950), whose composition and nutrient concentrations are shown in Table 1. The solution was prepared in local water supply (0.3 dS m<sup>-1</sup>) and had an electrical conductivity of 2.1 dS m<sup>-1</sup> after preparation.

**Table 1.** Chemical composition of the nutrient solution recommended by Hoagland and Arnon (1950), used in the hydroponic cultivation of orange cherry tomatoes.

Nutrients	Fertilizers	Quantity (g 1000L <sup>-1</sup> )
P/K	KH <sub>2</sub> PO <sub>4</sub>	136.09
K/N	KNO <sub>3</sub>	101.10
Ca /N	Ca (NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	236.15
Mg	MgSO <sub>4</sub> .4H <sub>2</sub> O	246.49
B	H <sub>3</sub> BO <sub>3</sub>	3.10
Mn	MnSO <sub>4</sub> .4H <sub>2</sub> O	1.70
Zn	ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.22
Cu	CuSO <sub>4</sub>	0.75
Mo	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> . 4H <sub>2</sub> O	1.25
	EDTA – Na	13.9
Fe	FeSO <sub>4</sub>	13.9

The saline nutrient solutions were prepared so as to have an equivalent ratio of 7:2:1 referring to Na:Ca:Mg, respectively, from the salts NaCl, CaCl<sub>2</sub>.2H<sub>2</sub>O, and MgCl<sub>2</sub>.6H<sub>2</sub>O, a ratio that prevails in water sources used for irrigation in small farms in the Northeast, considering the relationship between EC<sub>w</sub> and salt concentration (RICHARDS, 1954), according to Equation 1, to prepare waters in the laboratory:

$$Q \approx 10 \times EC_w \quad (1)$$

where:

Q = Sum of cations (mmol<sub>c</sub> L<sup>-1</sup>); and

EC<sub>w</sub> = Desired electrical conductivity after discounting the EC<sub>w</sub> of the water from the municipal supply system (dS m<sup>-1</sup>).

Seedlings were produced using Blueline<sup>®</sup> orange cherry tomato seeds (Santo Antônio de Posse/SP, Brazil). This cultivar has a cycle of approximately 90 days, a very productive plant, tasty fruits, and orange color.

Orange cherry tomato seeds were sown in 80 mL plastic cups containing coconut fiber substrate, one seed per cup. Prior to sowing, the coconut fiber was sanitized and washed with hypochlorite (2 to 2.5%). From germination to the appearance of the second pair of true leaves, nutrient solution at a 50% concentration of the recommendation of Hoagland and Arnon (1950) was applied. Then, the plants were removed from the coconut fiber and inserted directly into the hydroponic profiles at 30 days after sowing (DAS), using a vertical staking with nylon string to keep the stem erect.

The hydroponic system used was Nutrient Film Technique (NFT), made from PVC pipes with 100 mm diameter and 6 m length. The system was composed of four subsystems, spaced 0.80 m apart, each with three channels separated by 0.40 m. In the channels, the spacing was 0.50 m between plants and 1.0 m between treatments.

The hydroponic system channels were installed at 0.60

m height, with 4% slope to assist in the nutrient solution flow. At the lowest level of each bench of the hydroponic system, a 150 L polyethylene recipient was installed to collect and conduct the nutrient solution back to the channels. The nutrient solution was pumped to the head of the channels by a 35 W pump, with an average flow rate of 3.0 L min<sup>-1</sup>. Nutrient solution circulation was programmed by a timer, with an intermittent flow of 15 min during the day and 45 min at night, according to the needs of the crop.

To ensure that the EC<sub>n</sub>s and pH values were in accordance with the established treatments, the nutrient solution was monitored daily and completely renewed every 12 days. When necessary, EC<sub>n</sub>s was adjusted with the addition of public-supply water with EC<sub>w</sub> = 0.3 dS m<sup>-1</sup>, while pH was regulated by the addition of 0.1 M KOH or HCl solution.

H<sub>2</sub>O<sub>2</sub> concentrations were obtained by diluting H<sub>2</sub>O<sub>2</sub> in deionized water at each application event. Applications were carried out manually with a spray bottle, so as to fully wet the leaves (abaxial and adaxial sides). A non-ionic adhesive spreader was added to break the surface tension and increase absorption by the leaves. Applications were carried out from 5:00 p.m., applying an average of 19 mL per plant in total, at an interval of 12 days, totaling 3 applications. A cardboard structure was used to avoid the drift of the treatments among the plants. Plants were monitored and phytosanitary practices were carried out when necessary.

H<sub>2</sub>O<sub>2</sub> application began 10 days after inserting the plants in the hydroponic profiles. At 72 hours after the H<sub>2</sub>O<sub>2</sub> concentrations were applied, the saline nutrient solutions began to be applied, according to the established treatments.

At 64 days after transplanting (DAT), leaf gas exchange was evaluated by determining stomatal conductance - *g<sub>s</sub>* (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), transpiration - *E* (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), CO<sub>2</sub> assimilation rate - *A* (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), internal CO<sub>2</sub> concentration - *C<sub>i</sub>* (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), instantaneous water use efficiency - *WUE* [(μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>], instantaneous carboxylation efficiency - *CE<sub>i</sub>* [(μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>], intrinsic water use efficiency - *WUE<sub>i</sub>* [(μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (μmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>].

Gas exchange was determined with a portable carbon dioxide analyzer (IRGA), LCPro+ Portable Photosynthesis System<sup>®</sup> (ADC BioScientific Limited, UK), on leaves located in the middle third of the plants under ambient temperature conditions, maintaining the atmospheric CO<sub>2</sub> concentration and using an artificial radiation source of 1,200 μmol m<sup>-2</sup> s<sup>-1</sup>, established through the light-photosynthesis response curve (FERNANDES et al., 2021).

Photosynthetic pigments were quantified according to the methodology of Arnon (1949). From 1 g of fresh matter of the third leaf, homogenized in 4 mL of 80% acetone, after 72 hours hermetically sealed in the dark, the concentrations of chlorophyll and carotenoids in the solutions were determined using a spectrophotometer (Thermo Scientific<sup>®</sup>, Genesys 20 model) at the absorbance wavelength (ABS) (470, 646, and 663 nm), using Equations 2, 3, 4 and 5. The values obtained for chlorophyll *a*, chlorophyll *b* and carotenoids were expressed in mg g<sup>-1</sup> of FM (fresh matter).

$$(\text{Chl } a) = 12.21 \times \text{ABS}_{663} - 2.81 \times \text{ABS}_{646} \quad (2)$$

$$(\text{Chl } b) = 20.13 \times \text{ABS}_{646} - 5.03 \times \text{ABS}_{663} \quad (3)$$

$$(\text{Chl } T) = 17.3 \times \text{ABS}_{646} + 7.18 \times \text{ABS}_{663} \quad (4)$$

$$(\text{Car}) = \frac{(1000 \times \text{ABS}_{470} - 1.82 \times \text{Chl } a - 85.02 \times \text{Chl } b)}{198} \quad (5)$$

Chlorophyll *a* fluorescence in the dark phase was measured in fully expanded leaves of the middle third and pre-adapted to the dark for 30 min, using a pulse-modulated fluorometer (Sciences Inc.- OS-30p model, Hudson, USA). For this purpose, clips were placed on the leaves for 30 min before the readings to adapt them to the dark, and the initial fluorescence (F<sub>0</sub>), maximum fluorescence (F<sub>m</sub>), variable fluorescence (F<sub>v</sub>), F<sub>v</sub>/F<sub>0</sub> ratio, and quantum efficiency of photosystem II (F<sub>v</sub>/F<sub>m</sub>) were measured.

To determine the relative water content in the leaves (RWC), four leaf discs (113 mm<sup>2</sup>) were collected and weighed on an analytical balance to obtain fresh mass (FM), turgid mass (TM) after 24 h submerged in distilled water, and dry mass (DM) after 48 h of drying in a forced air ventilation oven at 60 °C. RWC was determined according to the methodology of Weatherley (1950), using Equation 6.

$$\text{RWC} = \frac{\text{FM} - \text{DM}}{\text{TM} - \text{DM}} \times 100 \quad (6)$$

Where:

RWC - Relative water content (%);

FM - leaf fresh mass (g);

TM - leaf turgid mass (g); and

DM - leaf dry mass (g).

Plant height was determined at 5 cm from the

hydroponic profile to the insertion of the apical meristem, measured with a graduated ruler. Stem diameter was measured at 5 cm from the hydroponic profile with a digital caliper. Number of leaves was obtained by counting the leaves, considering those with a minimum length of 5 cm. At the end of the orange cherry tomato cycle (70 DAT), the plants were removed from the hydroponic profiles, separated into different parts, placed in paper bags and dried in a forced air circulation oven at temperature of 65 °C for 72 h; subsequently, the material was weighed to obtain dry mass of leaves (LDM), stem (SDM), and root (RDM), by weighing on a scale with a precision of 0.01 g.

The data obtained were subjected to the normality test (Shapiro & Wilk), and later analysis of variance was performed by the F test at p ≤ 0.05 probability level; when significant, polynomial regression analysis was performed for the salinity levels of the nutrient solution and concentrations of hydrogen peroxide, using the statistical software SISVAR – ESAL (FERREIRA, 2019). SigmaPlot<sup>®</sup> software was used to construct the response surface curves when there was a significant effect of the interaction between factors (ECNs × H<sub>2</sub>O<sub>2</sub>).

## RESULTS AND DISCUSSION

There were significant effects of the interaction between saline nutrient solution and hydrogen peroxide (ECNs × H<sub>2</sub>O<sub>2</sub>) on the stomatal conductance (*g<sub>s</sub>*), internal CO<sub>2</sub> concentration (*C<sub>i</sub>*) and intrinsic water use efficiency (*WUE<sub>i</sub>*) of tomato plants (Table 2). ECNs significantly affected transpiration (*E*), CO<sub>2</sub> assimilation rate (*A*), and instantaneous carboxylation efficiency (*CE<sub>i</sub>*). On the other hand, H<sub>2</sub>O<sub>2</sub> concentrations did not significantly affect any of the variables analyzed.

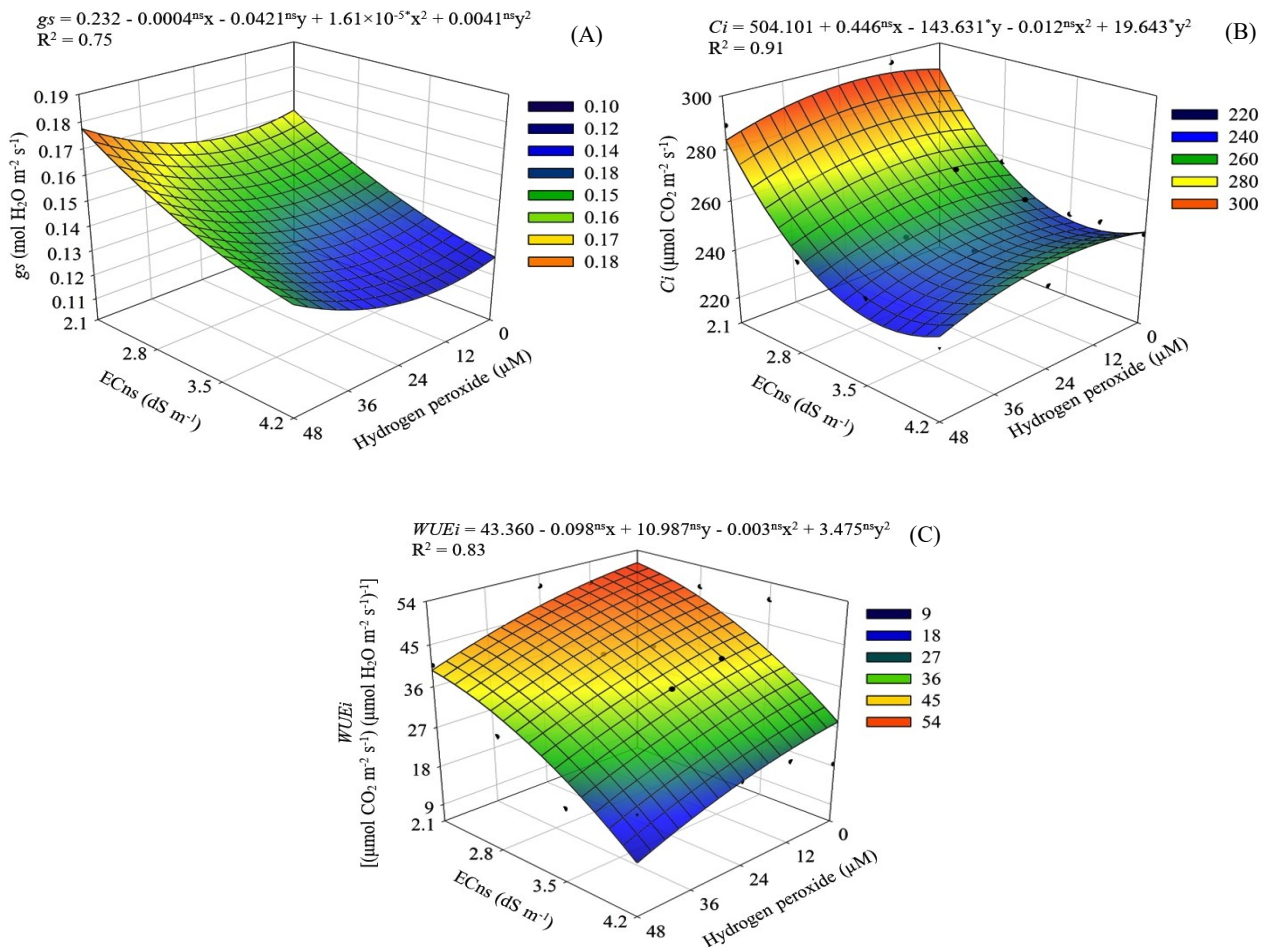
The stomatal conductance of orange cherry tomatoes decreased with the increase in ECNs, regardless of H<sub>2</sub>O<sub>2</sub> concentration (Figure 2A). However, it was observed that foliar application of H<sub>2</sub>O<sub>2</sub> at concentration of 48 μM was able to reduce the effects of salt stress, with maximum estimated value of 0.179 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in the *g<sub>s</sub>* of plants subjected to ECNs of 2.1 dS m<sup>-1</sup>, corresponding to an increase of 10.49% (0.017 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) compared to plants subjected to the same ECNs level (2.1 dS m<sup>-1</sup>) and without H<sub>2</sub>O<sub>2</sub> application. It is worth pointing out that in the present study the application of H<sub>2</sub>O<sub>2</sub> at concentration greater than 36 μM intensified the deleterious effects of salt stress on the stomatal conductance of orange cherry tomato plants.

Reduction in stomatal conductance is a strategy to reduce water loss and thus the absorption of water and salts from the soil solution, without affecting photosynthetic activity (DIAS et al., 2019). The positive effects of H<sub>2</sub>O<sub>2</sub> on the stomatal conductance of tomato plants up to the concentration of 36 μM may have been due to defense mechanisms that induce signaling and activation of the antioxidant enzyme system and thus attenuate the detrimental effects of salt stress (CARVALHO et al., 2011).

**Table 2.** Summary of the analysis of variance for stomatal conductance (*gs*), transpiration (*E*), CO<sub>2</sub> assimilation rate (*A*), internal CO<sub>2</sub> concentration (*Ci*), instantaneous water use efficiency (*WUE*), instantaneous carboxylation efficiency (*CEi*), and intrinsic water use efficiency (*WUEi*) of cherry tomato plants cultivated with saline nutrient solution (ECNs) and application of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in a hydroponic system at 65 days after transplanting.

Sources of Variation	DF	Mean squares						
		<i>gs</i>	<i>E</i>	<i>A</i>	<i>Ci</i>	<i>WUE</i>	<i>CEi</i>	<i>WUEi</i>
Saline nutrient solution (ECNs)	3	0.0092*	1.623*	22.57**	6719.12**	0.799 <sup>ns</sup>	0.00012**	1387.0**
Linear regression	1	0.0096**	4.06**	57.80**	14967.2**	2.36 <sup>ns</sup>	0.00035**	38.72**
Quadratic regression	1	0.00023*	0.60*	9.93 <sup>ns</sup>	4593.7**	0.00 <sup>ns</sup>	0.00073 <sup>ns</sup>	83.66**
Residual 1	6	0.0025	0.060	0.409	31.06	0.016	0.00015	5.44
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	4	0.0071*	0.672 <sup>ns</sup>	1.077 <sup>ns</sup>	645.27*	0.309 <sup>ns</sup>	0.00018 <sup>ns</sup>	568.3**
Linear regression	1	0.0018*	1.65 <sup>ns</sup>	2.67*	1044.3**	0.91 <sup>ns</sup>	0.00013 <sup>ns</sup>	38.07*
Quadratic regression	1	0.009*	0.05 <sup>ns</sup>	0.71 <sup>ns</sup>	656.09**	0.01 <sup>ns</sup>	0.00010*	1191.7**
Interaction (ECNs × H <sub>2</sub> O <sub>2</sub> )	12	0.0029*	0.179 <sup>ns</sup>	0.858 <sup>ns</sup>	175.14*	0.039 <sup>ns</sup>	0.00014 <sup>ns</sup>	118.84**
Residual 2	34	0.0066	0.270	1.091	96.42	0.021	0.00011	37.85
CV 1(%)		5.57	6.56	10.57	2.17	7.89	14.17	5.89
CV 2(%)		5.25	13.92	17.25	3.82	8.90	14.90	15.77

ns, \*, \*\*, respectively not significant, significant at  $p \leq 0.05$  and  $p \leq 0.01$ . DF: Degrees of freedom, CV: Coefficient of variation.



X and Y - Concentration of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> and electrical conductivity of the nutrient solution - ECNs, respectively; \* - Significant at  $p \leq 0.05$  by the F test, respectively; <sup>ns</sup> not significant.

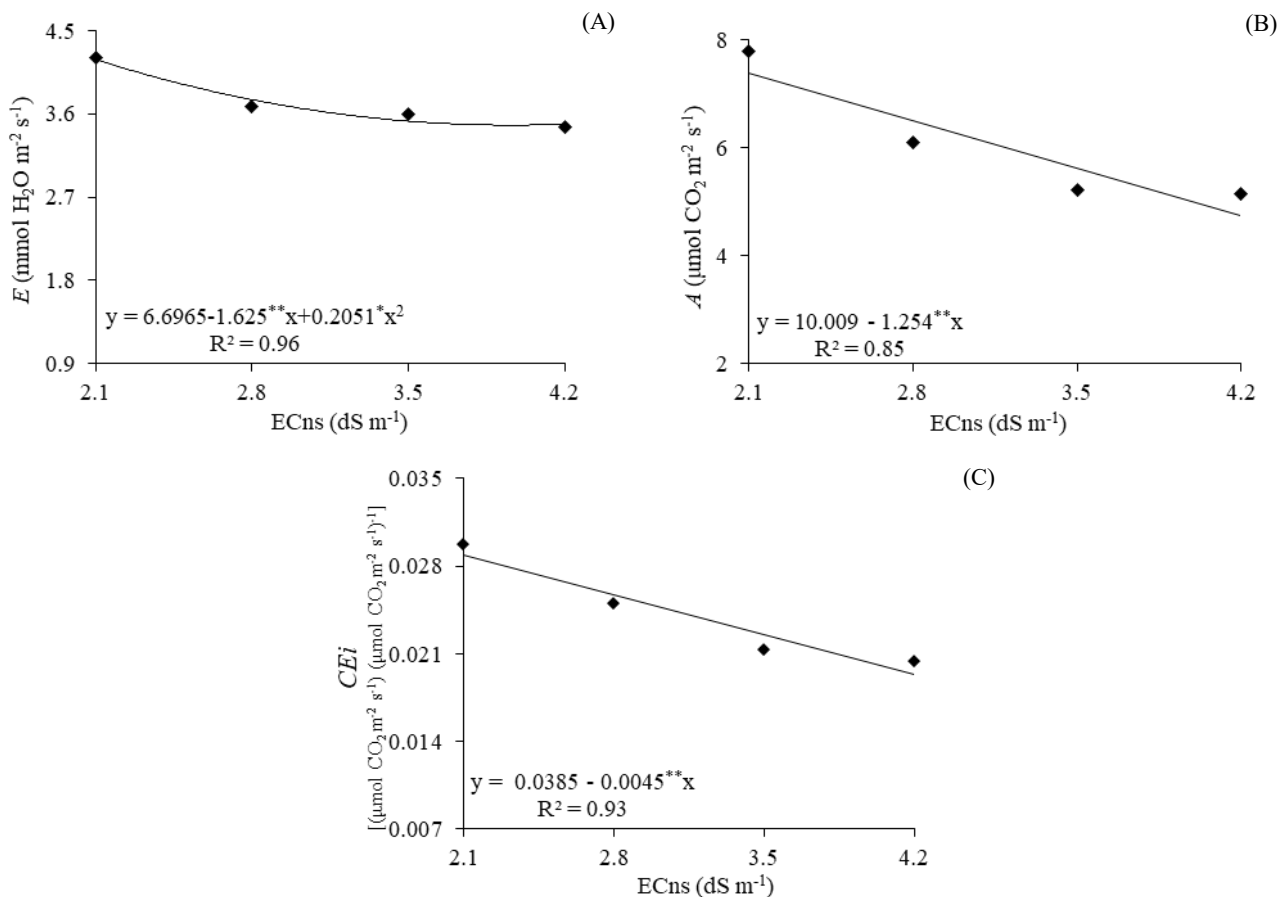
**Figure 2.** Stomatal conductance (*gs*), internal CO<sub>2</sub> concentration (*Ci*) and intrinsic water use efficiency (*WUEi*) of orange cherry tomato plants, as a function of the interaction between saline nutrient solution – ECNs and concentrations of hydrogen peroxide – H<sub>2</sub>O<sub>2</sub> in a hydroponic system, at 65 days after transplanting.

Regarding the internal CO<sub>2</sub> concentration (Figure 2B), plants cultivated under ECns of 2.1 dS m<sup>-1</sup> obtained the highest estimated value (293.24 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) when subjected to H<sub>2</sub>O<sub>2</sub> concentration of 19 μM. On the other hand, the lowest value of *C<sub>i</sub>* (235.34 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) was observed in plants subjected to H<sub>2</sub>O<sub>2</sub> concentration of 48 μM and ECns of 3.7 dS m<sup>-1</sup>. Foliar application of H<sub>2</sub>O<sub>2</sub> in plants under salt stress can contribute to the activation of the defense system through the activity of antioxidant enzymes, such as superoxide dismutase, catalase, and ascorbate peroxidase, resulting in the reduction of the deleterious effects on leaf gas exchange (CARVALHO et al., 2011). During photosynthesis, CO<sub>2</sub> fixed in the mesophyll cell is consumed in the synthesis of sugars, which results in a reduction (DIAS et al., 2019). Dantas et al. (2022a), in zucchini cultivated with ECns 2.1 to 6.6 dS m<sup>-1</sup> in an NFT hydroponic system, also found that the internal CO<sub>2</sub> concentration decreased with the increase in irrigation water salinity and the lowest value was obtained in plants subjected to ECns of 6.6 dS m<sup>-1</sup>.

For the intrinsic water use efficiency of orange cherry tomato (Figure 2C), plants cultivated under ECns of 2.1 dS m<sup>-1</sup> obtained the highest estimated value, 51.11 [(μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (μmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>], when subjected to H<sub>2</sub>O<sub>2</sub>

concentration of 0 μM. The lowest value of 16.59 [(μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (μmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>] was found at ECns of 4.2 dS m<sup>-1</sup> at a H<sub>2</sub>O<sub>2</sub> concentration of 48 μM, with a reduction of 68.16% when compared to the highest value of intrinsic water use efficiency. The reduction in intrinsic water use efficiency may be related to osmotic stress and absorption of toxic ions, since, under stressful conditions, such as drought or salinity, the decrease in water loss through stomatal closure also restricts the entry of CO<sub>2</sub> (SÁ et al., 2019).

Transpiration was reduced with the increase in the electrical conductivity of the nutrient solution (ECns), with the maximum estimated value of 4.19 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> obtained in plants under saline nutrient solution of 2.1 dS m<sup>-1</sup>, while those subjected to ECns of 4.2 dS m<sup>-1</sup> expressed the lowest *E* (3.49 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) (Figure 3A). Stomatal closure resulted in lower transpiration, a strategy used by plants to reduce water loss as a result of lower absorption of water and nutrients. However, this mechanism can be beneficial under salt stress conditions, as transpiration creates a negative pressure of water in the aerial part that can be balanced through greater absorption of water by the roots, forming a mechanism of tolerance to salt stress (LIMA et al., 2017).



\*, \*\* - Significant at  $p \leq 0.05$  and  $\leq 0.01$  by the F test, respectively.

**Figure 3.** Transpiration - *E* (A), CO<sub>2</sub> assimilation rate - *A* (B), and instantaneous carboxylation efficiency - *CEi* (C) of orange cherry tomato plants, as a function of salinity levels of nutrient solution - ECns in a hydroponic system, at 65 days after transplanting.

Due to the reductions in stomatal conductance (Figure 2A) and transpiration (Figure 3A), when the plants were subjected to different levels of salinity of the nutrient solution, the CO<sub>2</sub> assimilation rate was compromised, showing a decrease of 12.53% per unit increment in ECns (Figure 3B). Nutrient solution salinity may have induced an imbalance in the photosynthesis process, since tomato plants have C3 photosynthetic metabolism and need to keep their stomata open for longer time to fix CO<sub>2</sub> by the RuBisCO enzyme in the Calvin cycle (GUIMARÃES et al., 2019). Roque et al. (2022) evaluated cherry tomatoes grown in pots under irrigation with saline water and found a decrease in CO<sub>2</sub> assimilation rate with the increase in the salt concentration in the irrigation water, when the plants were irrigated with high-salinity water (4.3 dS m<sup>-1</sup>), compared to those grown with low-salinity water (0.3 dS m<sup>-1</sup>).

Instantaneous carboxylation efficiency (Figure 3C) decreased linearly with the increase in the salinity levels of

the nutrient solution, by 11.69% per unit increment in ECns levels. In relative terms, there was a reduction of 32.53% in the CEi of plants cultivated under ECns of 4.2 dS m<sup>-1</sup> compared to those subjected to the lowest salinity level (2.1 dS m<sup>-1</sup>). Instantaneous carboxylation efficiency (CEi) is directly linked to CO<sub>2</sub> assimilation rate and intracellular CO<sub>2</sub> concentration; moreover, as salt stress becomes more severe, the dehydration of mesophyll cells inhibits photosynthesis, mesophyll metabolism is impaired and, consequently, carboxylation efficiency is decreased (JACINTO JUNIOR et al., 2019).

The salinity levels of the nutrient solution significantly influenced the maximum (F<sub>m</sub>) and the variable fluorescence (F<sub>v</sub>) of tomato at 65 days after sowing. The concentrations of hydrogen peroxide and the interaction between the factors (ECns × H<sub>2</sub>O<sub>2</sub>) did not significantly affect any of the variables measured at 65 days after transplanting (Table 3).

**Table 3.** Summary of the analysis of variance for initial fluorescence (F<sub>0</sub>), maximum fluorescence (F<sub>m</sub>), variable fluorescence (F<sub>v</sub>), quantum efficiency of photosystem II (F<sub>v</sub>/F<sub>m</sub>), and maximum efficiency of the photochemical process in photosystem II in the dark phase (F<sub>v</sub>/F<sub>0</sub>) of tomato cultivated with saline nutrient solution (ECns) and concentrations of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in a hydroponic system, at 64 days after transplanting.

Sources of Variation	DF	Mean squares				
		F <sub>0</sub>	F <sub>m</sub>	F <sub>v</sub>	F <sub>v</sub> /F <sub>m</sub>	F <sub>v</sub> /F <sub>0</sub>
Saline nutrient solution (ECns)	3	2206.7 <sup>ns</sup>	3883.3 <sup>**</sup>	2117.6 <sup>**</sup>	0.004 <sup>ns</sup>	0.109 <sup>ns</sup>
Linear regression	1	5401.7 <sup>ns</sup>	11445.3 <sup>**</sup>	6311.2 <sup>**</sup>	0.014 <sup>**</sup>	0.3181 <sup>ns</sup>
Quadratic regression	1	62.0 <sup>ns</sup>	120.4 <sup>ns</sup>	19.26 <sup>ns</sup>	0.000 <sup>ns</sup>	0.001 <sup>ns</sup>
Residual 1	6	89.76	101.1	14.57	0.000	0.058
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	4	26.94 <sup>ns</sup>	743.8 <sup>ns</sup>	524.41 <sup>ns</sup>	0.002 <sup>ns</sup>	1.995 <sup>ns</sup>
Linear regression	12	16.13 <sup>ns</sup>	800.8 <sup>ns</sup>	73.63 <sup>ns</sup>	0.003 <sup>ns</sup>	5.106 <sup>ns</sup>
Quadratic regression	1	66.88 <sup>ns</sup>	1030.0 <sup>ns</sup>	257.52 <sup>ns</sup>	0.004 <sup>ns</sup>	0.991 <sup>ns</sup>
Interaction (ECns × H <sub>2</sub> O <sub>2</sub> )	12	407.85 <sup>ns</sup>	262.5 <sup>ns</sup>	24.07 <sup>ns</sup>	0.000 <sup>ns</sup>	0.055 <sup>ns</sup>
Residual 2	34	47.39	136.30	575.40	0.000	0.042
CV 1(%)		6.87	1.79	0.86	1.93	7.51
CV 2(%)		4.99	2.08	5.43	2.84	6.44

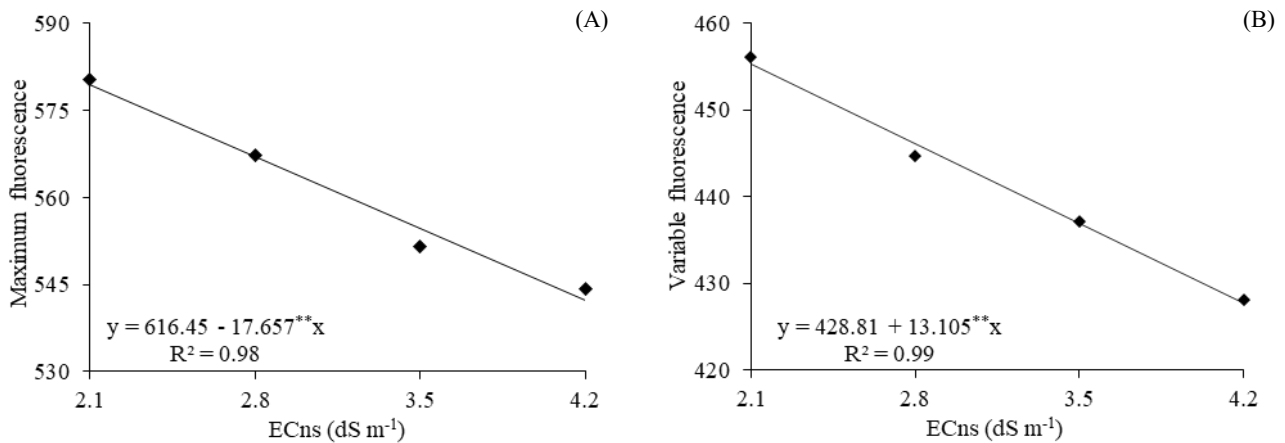
ns, and \*\* respectively, not significant and, significant at p ≤ 0.01. DF: Degrees of freedom, CV: Coefficient of variation.

The maximum fluorescence (F<sub>m</sub>) of orange cherry tomato plants decreased linearly with the increase in the electrical conductivity of the saline nutrient solution, by 2.86% per unit increment in ECns (Figure 4A). In relative terms, there was a 6.4% decrease in the F<sub>m</sub> of plants subjected to ECns of 4.2 dS m<sup>-1</sup> compared to those cultivated with a saline nutrient solution of 2.1 dS m<sup>-1</sup>.

The decrease in F<sub>m</sub> is caused by the inactivation of PSII in the thylakoid membranes, which in turn results from the reduction of quinone, which affects the flow of electrons between photosystems. This situation may have had a negative impact on the photochemical activity of the leaves, as a high F<sub>m</sub> affects the transfer of energy for the formation of the reducing agent NADPH, ATP, and reduced ferredoxin, promoting a greater capacity for CO<sub>2</sub> assimilation in the biochemical phase of photosynthesis (DIAS et al., 2021). Similar results were found by Mendonça et al. (2022), in a study evaluating the effects of saline nutrient solution under similar conditions on okra plants, in which maximum fluorescence decreased by 4.13% per unit increment in ECns.

The variable fluorescence (F<sub>v</sub>) of cherry tomato plants decreased with the increase in the electrical conductivity of the nutrient solution (Figure 4B), with a reduction of 3.05% per unit increment in ECns. Variable fluorescence is related to the ability of the plant to transfer the energy of the electrons released by the pigment molecules for the production of NADPH, ATP, and reduced ferredoxin, so the reduction in F<sub>v</sub> due to the increase in salinity indicates that the photosynthetic apparatus was damaged by salt stress, consequently causing negative effects on the photosynthetic process (DIAS et al., 2019).

There was a significant effect of the interaction between saline nutrient solution and hydrogen peroxide (ECns × H<sub>2</sub>O<sub>2</sub>) concentrations on the contents of chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) of cherry tomato plants (Table 4). The salinity levels of the nutrient solution (ECns) caused a significant effect on the relative water content (RWC) of orange cherry tomato plants at 65 days after transplanting.



\*\* - Significant at  $p \leq 0.01$  by the F test.

**Figure 4.** Maximum fluorescence (A) and variable fluorescence (B) of cherry tomato plants, as a function of the salinity levels of the nutrient solution - ECNs in a hydroponic system, at 65 days after transplanting.

**Table 4.** Summary of the analysis of variance for the contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), chlorophyll *a/b* (Chl *a/b*), total chlorophyll (Chl *T*), carotenoids (Car), and relative water content (RWC) of cherry tomato plants, cultivated with saline nutrient solution (ECNs) and concentrations of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in a hydroponic system, at 65 days after transplanting.

Sources of Variation	DF	Mean squares					
		Chl <i>a</i>	Chl <i>b</i>	Chl <i>a/b</i>	Chl <i>T</i>	Car	RWC
Saline nutrient solution (ECNs)	3	0.008**	0.004**	0.730 <sup>ns</sup>	0.028 <sup>ns</sup>	0.00073 <sup>ns</sup>	387.52**
Linear regression	1	0.023**	0.012**	2.14**	0.085**	0.00027*	986.2**
Quadratic regression	1	0.001**	0.000 <sup>ns</sup>	0.028 <sup>ns</sup>	0.00001 <sup>ns</sup>	0.00013 <sup>ns</sup>	154.2**
Residual 1	6	0.000	0.000	0.019	0.001	0.00029	4.69
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	4	0.003**	0.003**	0.391 <sup>ns</sup>	0.0036 <sup>ns</sup>	0.00018 <sup>ns</sup>	20.57 <sup>ns</sup>
Linear regression	1	0.000 <sup>ns</sup>	0.002**	0.448 <sup>ns</sup>	0.007 <sup>ns</sup>	0.00012 <sup>ns</sup>	0.198 <sup>ns</sup>
Quadratic regression	1	0.000*	0.000**	0.781 <sup>ns</sup>	0.00001 <sup>ns</sup>	0.00010 <sup>ns</sup>	80.20**
Interaction (ECNs × H <sub>2</sub> O <sub>2</sub> )	12	0.001**	0.000*	0.051 <sup>ns</sup>	0.002 <sup>ns</sup>	0.00024 <sup>ns</sup>	14.10 <sup>ns</sup>
Residual 2	34	0.000	0.000	0.029	0.001	0.00052	15.88
CV 1(%)		3.59	6.76	6.22	11.72	6.10	3.80
CV 2(%)		8.81	10.30	7.59	12.08	9.58	6.62

<sup>ns</sup>, \* and \*\* respectively, not significant, significant at  $p \leq 0.05$  and  $p \leq 0.01$ . DF: Degrees of freedom, CV: Coefficient of variation.

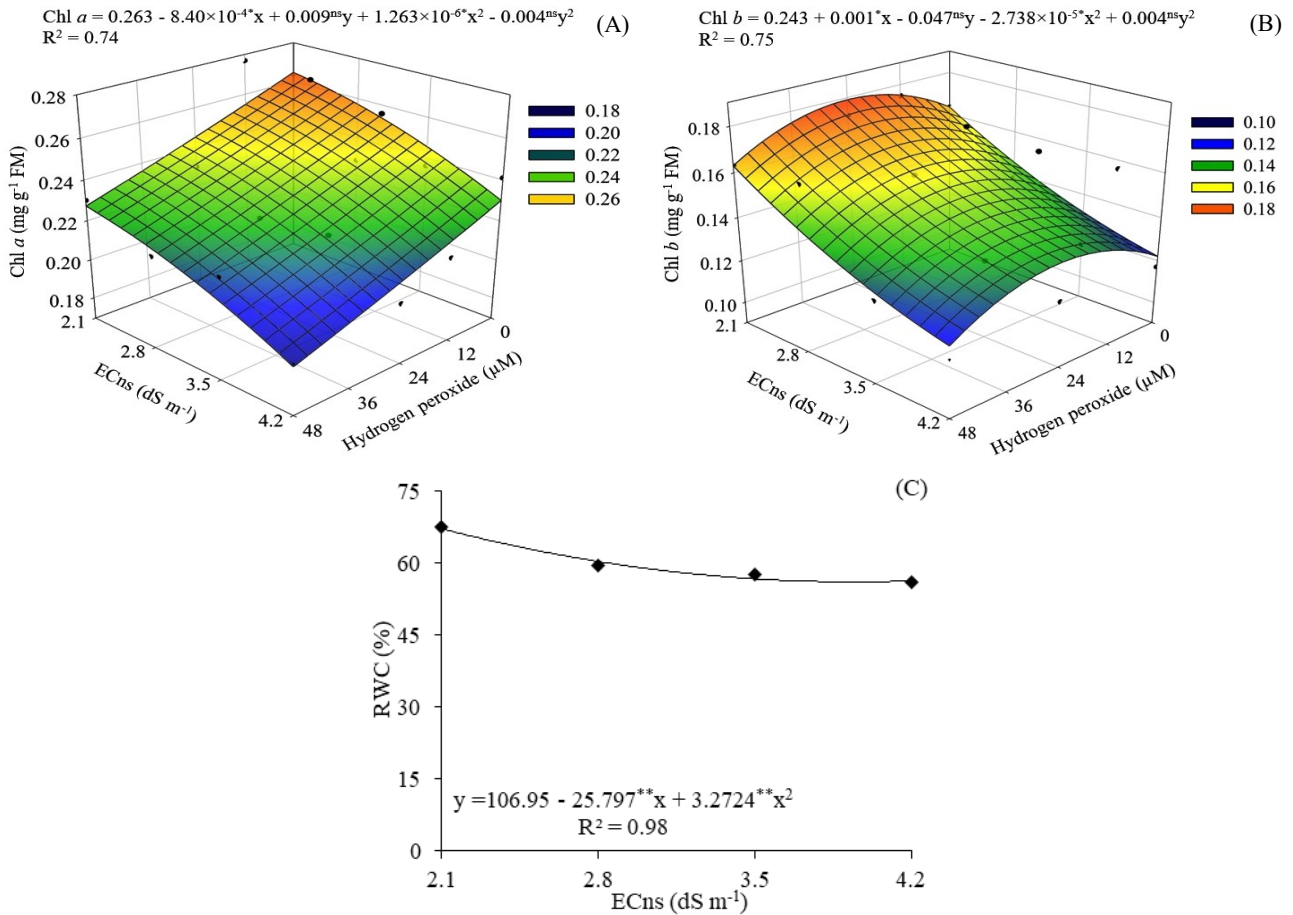
Under the highest salinity level of the nutrient solution (4.2 dS m<sup>-1</sup>), H<sub>2</sub>O<sub>2</sub> application at a concentration of 48 μM significantly reduced the chlorophyll *a* contents, with a minimum value of 0.193 mg g<sup>-1</sup> FM (Figure 5A). On the other hand, under the lowest salinity of the nutrient solution (2.1 dS m<sup>-1</sup>) and without foliar application of H<sub>2</sub>O<sub>2</sub>, higher contents of Chl *a* (0.264 mg g<sup>-1</sup> FM) were observed. For chlorophyll *b* contents, plants subjected to ECNs of 2.1 dS m<sup>-1</sup> and H<sub>2</sub>O<sub>2</sub> at a concentration of 18 μM obtained the highest value (0.171 mg g<sup>-1</sup> FM) (Figure 5B). The lowest contents of Chl *b* (0.101 mg g<sup>-1</sup>) were observed in plants cultivated under ECNs of 4.2 dS m<sup>-1</sup> and 48 μM of H<sub>2</sub>O<sub>2</sub>.

The results obtained can be attributed to the synthesis of reactive oxygen species, which is favored by the water deficit caused by salt stress, hindering the metabolism of

plants, through the oxidation of photosynthetic pigments, including chlorophyll *b* (SILVA et al., 2016). According to Nóbrega et al. (2020), excessive presence of salts in plant tissues can affect the synthesis of chlorophyll *a* and *b*, due to their degradation by the activation of the enzyme chlorophyllase, which reduces photosynthesis and the production of pigmentation proteins.

For the relative water content - RWC (Figure 5C), it was observed that cherry tomato plants cultivated under ECNs of 2.1 dS m<sup>-1</sup> obtained the maximum estimated value of 67.48%. In contrast, the minimum RWC value was 56.06% at the ECNs of 4.2 dS m<sup>-1</sup>. The reduction in water content in the leaf blade reflects the action of the osmotic effects due to the high concentration of salts, which restricts the absorption of water and nutrients by the plants.





X and Y - Concentration of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> and electrical conductivity of the nutrient solution - ECNs, respectively; \*, \*\* - Significant at p ≤ 0.05 and ≤ 0.01 by the F test, respectively; <sup>ns</sup> not significant.

**Figure 5.** Chlorophyll *a* - Chl *a* (A) and chlorophyll *b* - Chl *b* (B) contents of cherry tomato plants in a hydroponic cultivation, as a function of the interaction between saline nutrient solution - ECNs and concentrations of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> and relative water content - RWC in the leaf blade (C), as a function of ECNs levels, at 65 days after transplanting.

There was a significant effect of the salinity levels of the nutrient solution on the stem diameter (SD), stem dry mass (SDM), and root dry mass (RDM) of orange cherry tomato plants. The interaction between factors (ECNs × H<sub>2</sub>O<sub>2</sub>) significantly influenced the height of tomato plants. On the other hand, H<sub>2</sub>O<sub>2</sub> concentrations did not significantly affect any of the variables evaluated at 70 days after transplanting (Table 5).

The height of orange cherry tomato plants decreased with the increase in the electrical conductivity of the nutrient solution (Figure 6). However, foliar application of H<sub>2</sub>O<sub>2</sub> at a concentration of 48 μM was able to reduce the effects of salt stress, with the maximum estimated value of 81.56 cm in PH obtained at ECNs of 2.1 dS m<sup>-1</sup>, corresponding to an increase of 2.53% (2.06 cm) compared to plants cultivated with the same ECNs level (2.1 dS m<sup>-1</sup>) and without application of H<sub>2</sub>O<sub>2</sub>. Plants that grow under salt stress may have a reduction in their water uptake, affecting their development due to osmotic and ionic effects that alter the photosynthetic rate and

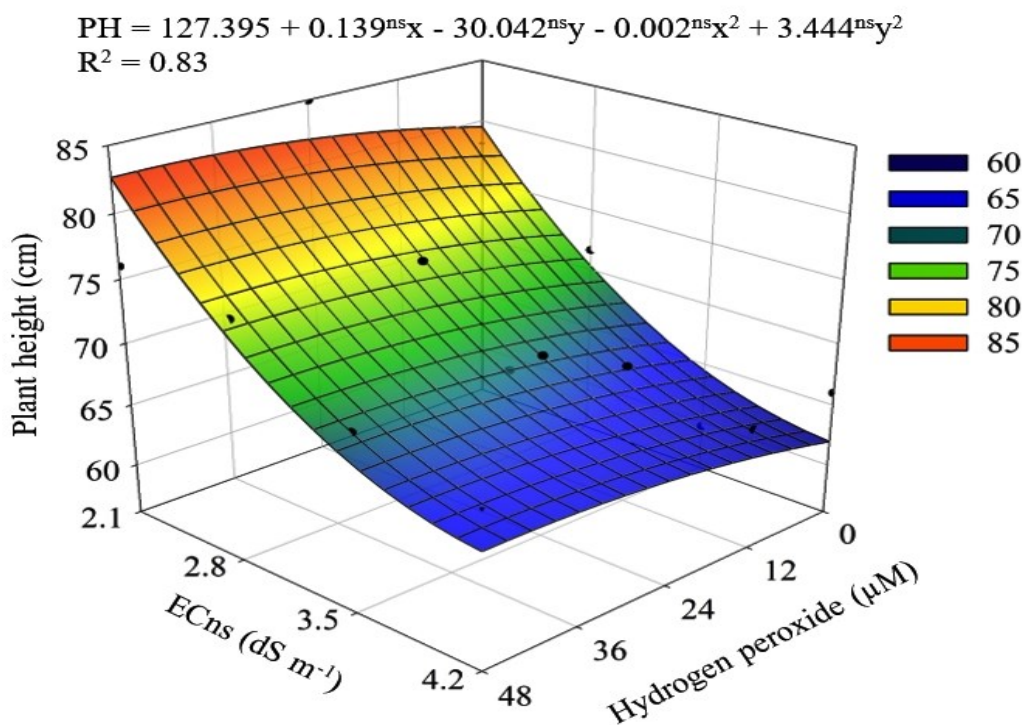
metabolism of plants (LIMA et al., 2020). However, in this study, the beneficial effect obtained by the foliar application of H<sub>2</sub>O<sub>2</sub> at concentration of 36 μM may be related to its signaling role, contributing to the production of proteins and carbohydrates, in addition to boosting the plant defense system, detoxifying reactive oxygen species and aiding plant growth (SILVA et al., 2022b).

The stem diameter of cherry tomato plants decreased linearly with the increase in the salinity levels of the nutrient solution (Figure 7A), by 9.3% per unit increment in ECNs. When comparing the SD of plants subjected to ECNs of 4.2 dS m<sup>-1</sup> to the value of those that received the lowest salinity level of the nutrient solution (2.1 dS m<sup>-1</sup>), a reduction of 24.04% was observed. This decrease in stem diameter growth may be linked to the increase in reactive oxygen species that cause various biochemical disturbances and physiological changes, such as decreased stomatal opening, which imposes limitations on plant growth (DANTAS et al., 2021).

**Table 5.** Summary of the analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL), dry mass of leaves (LDM), stem (SDM), and roots (RDM) of cherry tomato plants, cultivated with saline nutrient solution (ECNs) and concentrations of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in a hydroponic system, at 70 days after transplanting.

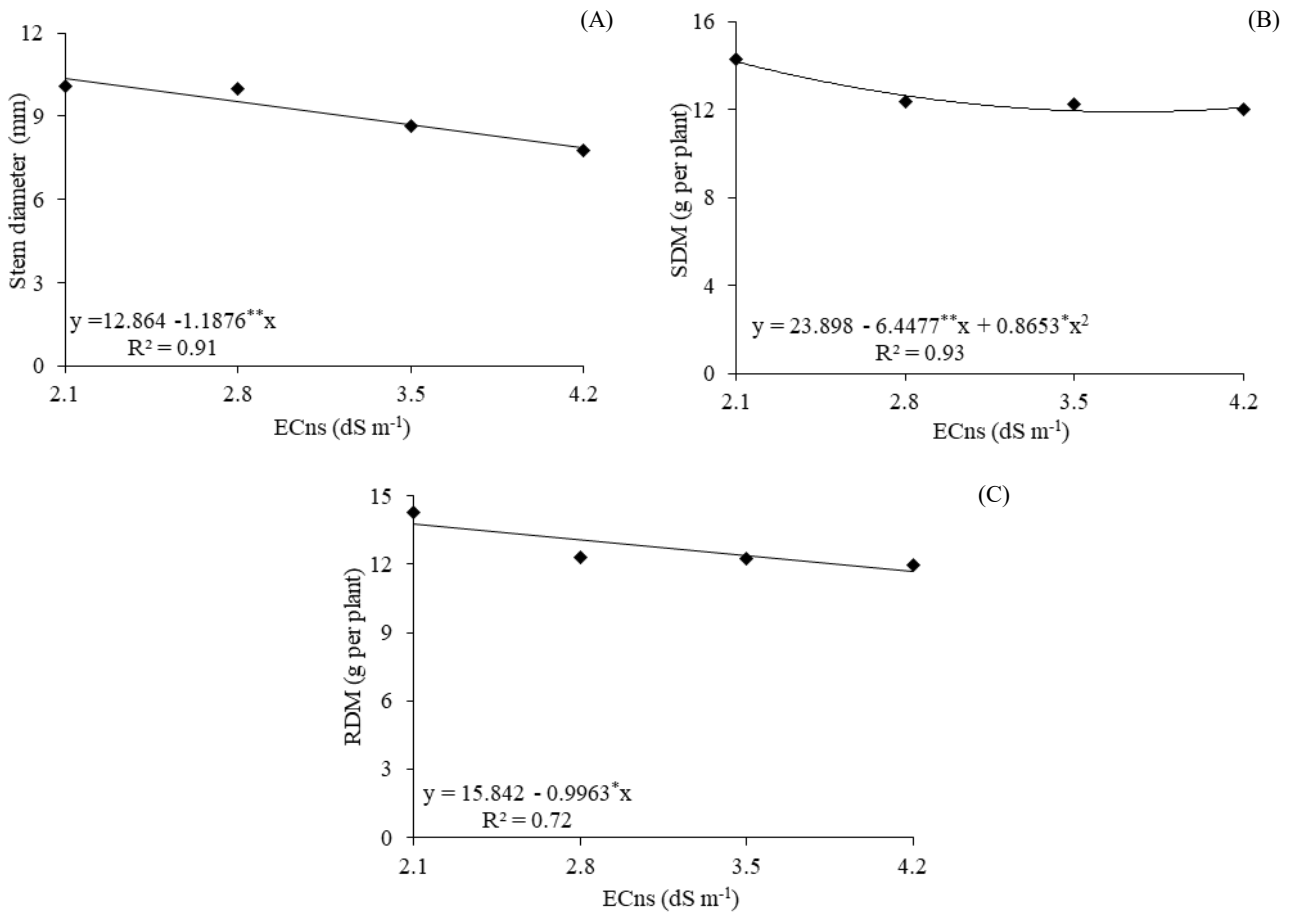
Sources of Variation	DF	Mean squares					
		PH	SD	NL	LDM	SDM	RDM
Saline nutrient solution (ECNs)	3	1014.8**	18.82*	60.977 <sup>ns</sup>	10.675 <sup>ns</sup>	16.770*	6.46*
Linear regression	6	2914.0**	51.79**	0.000 <sup>ns</sup>	14.822 <sup>ns</sup>	36.45**	11.46*
Quadratic regression	1	120.41 <sup>ns</sup>	2.59 <sup>ns</sup>	117.60 <sup>ns</sup>	5.787 <sup>ns</sup>	10.79*	0.277 <sup>ns</sup>
Residual 1	1	20.62	0.978	16.09	2.691	1.662	0.61
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	4	36.54 <sup>ns</sup>	0.857 <sup>ns</sup>	22.566 <sup>ns</sup>	3.294 <sup>ns</sup>	3.149 <sup>ns</sup>	1.41 <sup>ns</sup>
Linear regression	1	0.208 <sup>ns</sup>	0.001 <sup>ns</sup>	0.033 <sup>ns</sup>	1.961 <sup>ns</sup>	0.741 <sup>ns</sup>	5.24 <sup>ns</sup>
Quadratic regression	1	118.33 <sup>ns</sup>	1.537 <sup>ns</sup>	14.880 <sup>ns</sup>	7.978 <sup>ns</sup>	7.86 <sup>ns</sup>	0.08 <sup>ns</sup>
Interaction (ECNs × H <sub>2</sub> O <sub>2</sub> )	12	88.87*	1.632 <sup>ns</sup>	17.144 <sup>ns</sup>	8.194 <sup>ns</sup>	3.358 <sup>ns</sup>	1.42 <sup>ns</sup>
Residual 2	34	27.00	0.525	6.395	3.205	0.926	0.57
CV 1(%)		6.47	10.84	11.85	9.72	10.15	11.07
CV 2(%)		7.40	7.94	7.47	10.60	7.58	10.63

DF - degrees of freedom; CV (%) - coefficient of variation; \* \*\* significant at p ≤ 0.05 and p ≤ 0.01, respectively <sup>ns</sup> not significant.



X and Y - Concentration of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> and electrical conductivity of the nutrient solution - ECNs, respectively; <sup>ns</sup> not significant.

**Figure 6.** Plant height of cherry tomato as a function of the interaction between the salinity levels of the nutrient solution (ECNs) and concentrations of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), at 70 days after transplanting.



\*, \*\* - Significant at  $p \leq 0.05$  and  $\leq 0.01$  by the F test, respectively.

**Figure 7.** Stem diameter - SD (A), stem dry mass - SDM (B), and root dry mass - RDM (C) of cherry tomato plants, as a function of the salinity levels of the nutrient solution - ECns, in a hydroponic system, at 70 days after transplanting.

For stem dry mass (Figure 7B), it was observed that plants under ECns of 2.1 dS m<sup>-1</sup> reached the maximum estimated value of 14.17 g per plant, while those subjected to ECns of 4.2 dS m<sup>-1</sup> expressed the lowest SDM accumulation (11.93 g per plant). The reduction in biomass accumulation may be related to the decrease in the photosynthetic rate of plants. Excess of salts can impair the physiological aspects of the plant, causing ionic, osmotic, hormonal, and nutritional changes, consequently leading to suboptimal growth (ROQUE et al., 2022). In a study conducted by Silva et al. (2022a), with cherry tomatoes grown under salt stress in pots, these authors found that plants irrigated with low-salinity water (0.6 dS m<sup>-1</sup>) had a 10.72 g higher SDM compared to plants irrigated using water with ECw of 2.6 dS m<sup>-1</sup>.

As for the root dry mass of cherry tomato plants (Figure 7C), there was a decreasing linear behavior with the increase in nutrient solution salinity, with a reduction of 6.29% per unit increment in ECns. Plants subjected to 4.2 dS m<sup>-1</sup> had their RDM reduced by 15.21% (2.09 g per plant) compared to those under ECns of 2.1 dS m<sup>-1</sup>. The reduction of root dry mass is a result of lower root growth.

The effect of salinity on the root system is due to the direct contact between the roots and the salts in the environment, which slows growth and evapotranspiration

rates (LIMA et al., 2014). This reduction may be related to the deleterious effects caused by salt stress, as the high concentrations of sodium salts negatively affect the physiological aspects of the plant, promoting ionic, osmotic, hormonal, and nutritional changes, thus causing reductions in growth and, consequently, in biomass accumulation (SÁ et al., 2019). Mendonça et al. (2022), when evaluating okra plants subjected to salt stress, found a reduction in root dry mass accumulation of 9.11% per unit increment in ECns.

## CONCLUSIONS

Nutrient solution salinity from 2.1 dS m<sup>-1</sup> negatively affects leaf gas exchange, photochemical efficiency, photosynthetic pigments, relative water content, and growth of cherry tomato at 65 days after transplanting. Foliar application of hydrogen peroxide at concentrations of 36 and 48 μM associated with nutrient solution salinity of 2.1 dS m<sup>-1</sup> stimulates growth in plant height and the synthesis of chlorophyll *b*, respectively, in cherry tomato plants. Hydrogen peroxide alone did not affect gas exchange, chlorophyll fluorescence, photosynthetic pigments, and growth of the orange cherry tomato at 65 days after transplanting.

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