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Morphophysiology of soursop under salt stress and H_2O_2 application in the pre-flowering phase[1]

Morfofisiologia de gravioleira sob estresse salino e aplicação de H_2O_2 na fase de pré-floração

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HIGHLIGHTS:

Increase in the electrical conductivity of water above 0.8 dS m^{-1} negatively affects the growth of soursop plants.
Stomatal conductance is reduced when the electrical conductivity of irrigation water is above 0.8 dS m^{-1}.
Application of 30 μM of H_2O_2 increases electrolyte leakage in the leaf blade.

ABSTRACT: In the semi-arid region of the Brazilian Northeast, water sources generally have high levels of salts, standing out as one of the abiotic stresses that restrict the growth and development of plants. In this context, the objective of the present study was to evaluate the effects of foliar applications of hydrogen peroxide on the morphophysiology of soursop under salt stress in the pre-flowering phase. The experiment was carried out in a greenhouse, using a randomized block design and a 4 × 4 factorial arrangement, with four values of electrical conductivity of the irrigation water - ECw (0.8, 1.6, 2.4, and 3.2 dS m^{-1}) and four concentrations of hydrogen peroxide – H_2O_2 (0, 10, 20, and 30 μM), with three replicates. An increase in water electrical conductivity from 0.8 dS m^{-1} reduced stomatal conductance, CO_2 assimilation rate, instantaneous carboxylation efficiency, and leaf water saturation deficit. It inhibited the growth of soursop plants at 370 days after transplanting. Hydrogen peroxide at concentrations of up to 10 μM increased leaf transpiration and water use efficiency of soursop plants irrigated with 1.8 dS m^{-1} water in the pre-flowering phase.

Key words: *Annona muricata* L., acclimatization, salinity, reactive oxygen species

RESUMO: Na região semiárida do Nordeste brasileiro as fontes hídricas geralmente, possuem elevados teores de saís, destacando-se como um dos estresses abióticos que restringe o crescimento e desenvolvimento das plantas. Neste contexto, o presente estudo objetivou-se, avaliar os efeitos de aplicações foliares de peróxido de hidrogênio na morfofisiologia de gravioleira sob estresse salino na fase de pré-floração. O experimento foi conduzido em casa de vegetação, utilizando-se o delineamento de blocos casualizados e arranjo fatorial 4 × 4, sendo quatro valores de condutividade elétrica da água de irrigação - CEa (0,8; 1,6; 2,4 e 3,2 dS m^{-1}) e quatro concentrações de peróxido de hidrogênio – H_2O_2 (0; 10; 20 e 30 μM), com três repetições. O aumento da condutividade elétrica da água a partir de 0,8 dS m^{-1} diminuiu a condutância estomática, a taxa de assimilação de CO_2, a eficiência instantânea de carboxilação, o déficit de saturação hídrica foliar e inibiu o crescimento das plantas de graviola aos 370 dias após o transplantio. O peróxido de hidrogênio na concentração de até 10 μM aumentou a transpiração foliar e a eficiência no uso da água de plantas de gravioleira irrigadas com água de 1,8 dS m^{-1} na fase de pré-floração.

Palavras-chave: *Annona muricata* L., aclimatação, salinidade, espécie reativa de oxigênio

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Introduction

Soursop (*Annona muricata* L.) adapts well to the conditions of cultivation in the Brazilian semi-arid region due to its edaphoclimatic characteristics. However, in this region, water sources are often artesian wells that have high concentrations of soluble salts, which may limit production (Lima et al., 2022).

Salt stress can reduce water availability to plants, leading to a reduction in the osmotic potential of the soil solution, inducing stomatal closure and a decrease in transpiration and CO_2 assimilation rate, besides causing enzyme inactivation, pigment degradation, and lipid peroxidation of membranes (Ramos et al., 2022).

Studies have highlighted the deleterious effects of salinity on fruit crops (Veloso et al., 2022; Capitulino et al., 2022). Veloso et al. (2022), when evaluating gas exchange and growth of soursop under salt stress (ECw from 0.7 to 3.7 dS m^{-1}), verified that this crop is sensivel to the salinity of the irrigation water from 0.7 dS m^{-1}, which compromised the gas exchange, absolute growth rates of plant height and stem diameter, and relative growth rate of plant height in soursop at 120 days after transplanting.

Among the strategies used to attenuate salt stress in plants, hydrogen peroxide (H_2O_2) application stands out. H_2O_2 is a reactive oxygen species and functions as a signaling molecule to mediate stress responses (Li et al., 2016). Silva et al. (2019c), in a study with soursop Morada Nova under irrigation using water with electrical conductivity of up to 3.5 dS m^{-1} and exogenous application of H_2O_2 (0 to 100 μM) via seed imbibition and foliar spraying, observed that the application of 25 and 50 μM attenuated the effects of salt stress on stomatal conductance, CO_2 assimilation rate, and chlorophyll a content.

In this context, the objective of the present study was to evaluate the effects of foliar applications of hydrogen peroxide on the morphophysiology of soursop under salt stress in the pre-flowering phase.

Material and Methods

The experiment was carried out during April 2020 and May 2021, in an arch-type greenhouse, covered with a 150-micron low-density polyethylene, belonging to the Unidade Acadêmica de Engenharia Agrícola - UAEA of the Universidade Federal de Campina Grande - UFCG, in Campina Grande, Paraiba, Brazil, at the geographical coordinates 7°15'18" S latitude, 35°52'28" W longitude, and an average altitude of 550 m. Daily air temperature (maximum and minimum) and relative air humidity data measured inside the greenhouse are presented in Figure 1.

The treatments consisted of four levels of electrical conductivity of irrigation water - ECw (0.8, 1.6, 2.4, and 3.2 dS m^{-1}) and four concentrations of hydrogen peroxide - H_2O_2 (0, 10, 20, and 30 μM), in a 4 × 4 factorial arrangement, distributed in randomized blocks, with three replicates and one plant per plot. The concentrations of H_2O_2 and the electrical conductivity values of water were defined based, respectively, on the studies conducted by Veloso et al. (2020) and Silva et al. (2019b).

Soursop seedlings Morada Nova were obtained from a commercial nursery accredited by the Registry of Seeds and Seedlings, in the District of São Gonçalo, Sousa-PB, Brazil, produced in polyethylene bags with dimensions of 10 × 20 cm. The soursop Morada Nova was chosen because it is the most used by producers, composing most commercial orchards in Brazil.

The experiment was conducted using plastic pots adapted as drainage lysimeters, with a capacity of 200 L, filled with a 1.0-kg layer of crushed stone followed by 230 kg of soil classified as Neossolo Regolítico (Entisol) of clay loam texture, collected in the 0-30 cm layer, from the municipality of Riachão do Bacamarte – PB (7°15'34" S latitude, 35°40'1" W longitude, and average altitude of 192 m), whose physical and chemical attributes (Table 1) were determined according to Teixeira et al. (2017).

The irrigation waters were prepared by dissolving NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ salts, in the equivalent proportion of 7:2:1, respectively, in local-supply water (ECw = 0.38 dS m^{-1}). This proportion is commonly found in sources of water used for irrigation in small properties in the Northeast. The irrigation waters were prepared considering the relationship between ECw and salt concentration (Richards, 1954), according to Eq 1:

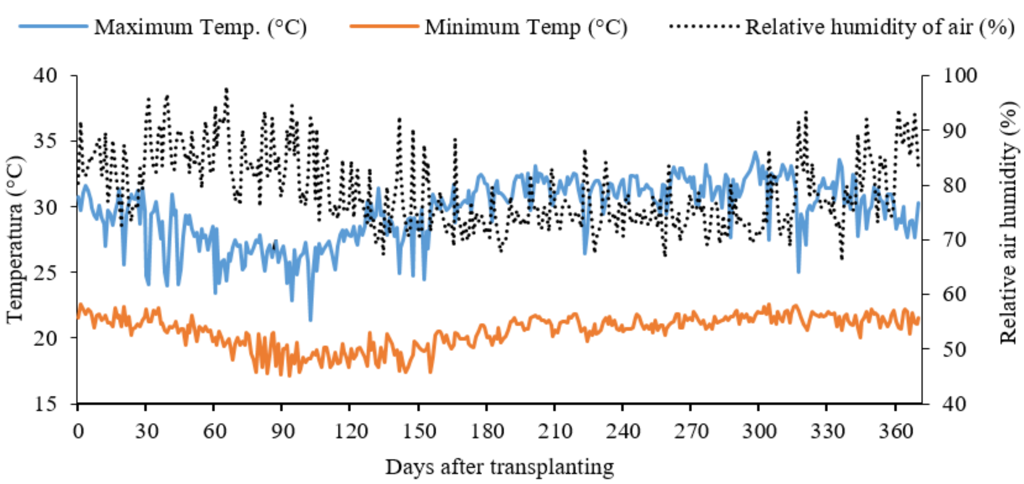


Figure 1. Air temperature (maximum and minimum) and average relative air humidity observed in the internal area of the greenhouse during the experimental period

Table 1. Chemical and physical attributes of the soil, in the 0-30 cm layer, used in the experiment, before the application of the treatments

Chemical characteristics								
pH H_2O 1:2.5	OM ($g\ dm^{-3}$)	P ($mg\ dm^{-3}$)	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+
			($cmol_c\ kg^{-1}$)					
6.5	8.1	79	0.24	0.51	14.9	5.4	0	0.9

Chemical characteristics				Physical characteristics				
EC_{se} ($dS\ m^{-1}$)	CEC ($cmol_c\ kg^{-1}$)	SAR_{se} ($(mmol\ L^{-1})^{0.5}$)	ESP (%)	Particle-size fraction ($g\ kg^{-1}$)			Moisture ($dag\ kg^{-1}$)	
				Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
2.15	21.94	0.16	2.3	572.7	100.7	326.6	25.91	12.96

OM - Organic Matter: Walkley-Black Wet Digestion; Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc at pH 7.0; Al^{3+} and H^+ extracted with 0.5 M CaOAc at pH 7.0; ESP - Exchangeable sodium percentage; EC_{se} - Electrical conductivity of soil saturation extract; CEC - Cation exchange capacity; SARse - Sodium adsorption ratio of the soil saturation extract; [1]- Field capacity; [2] - Wilting point

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

where:

Q - quantity of salts to be added ($mmol_c\ L^{-1}$); and,

ECw - electrical conductivity of water ($dS\ m^{-1}$).

At 80 days after transplanting, irrigation with saline water began, applying water in each lysimeter according to treatment in order to maintain soil moisture close to the field capacity, and the volume to be applied was determined based on the water requirement of the plants, estimated by the water balance, as shown in Eq 2:

$$VI = \frac{(Va - Vd)}{(1 - LF)} \quad (2)$$

where:

VI - volume of water to be applied in the next irrigation event (mL);

Va - volume applied in the previous irrigation event (mL);

Vd - volume drained after the last irrigation event (mL); and,

LF - leaching fraction of 0.10, applied every 30 days to avoid excessive accumulation of salts.

Hydrogen peroxide concentrations were obtained by dilution in distilled water and checked using a spectrophotometer at an absorbance wavelength of 240 nm.

The applications of H_2O_2 started at 30 days after transplanting the seedlings to the lysimeters and continued at intervals of 30 days, spraying the abaxial and adaxial sides of the leaves, so as to fully wet them, using a backpack sprayer, with applications performed between 17:00 and 18:00 hours. To decrease the surface tension of the drops on the leaf surface, the Wil fix adjuvant was used at a concentration of 0.5 mL L^{-1} of solution. The sprayer used was from Jacto - Jacto XP® with a capacity of 12 L, with working pressure (maximum) of 88 psi (6 bar), and JD 12P nozzle, and the average volume applied per plant was 375 mL.

Fertilization with nitrogen, phosphorus, and potassium was performed according to the recommendation of Cavalcante et al. (2008) for the soursop crop, applying 100 g of nitrogen, 60 g of P_2O_5, and 40 g of K_2O per plant per year, divided into 24 portions and applied at 15-day intervals. The nitrogen, phosphorus, and potassium sources used were ammonium sulfate (21% N), monoammonium phosphate (61% P_2O_5, 12% N), and potassium chloride (60% K_2O), respectively.

Micronutrient applications were performed every 15 days using a Dripsol® Micro solution at a concentration of 1.0 g L^{-1}, composed of Mg (1.1%), Zn (4.2%), B (0.85%), Fe (3.4%), Mn (3.2%), Cu (0.5%), and Mo (0.05%), on the adaxial and abaxial sides of the leaves, using a backpack sprayer. During the experiment, cultural practices such as formative pruning, weeding, soil scarification, and phytosanitary control were performed as needed.

Formative pruning was carried out to leave the plant with a single stem, the first branch at 50 cm height from the soil surface, and three well-located branches at different heights, symmetrically distributed in a spiral pattern. These, called primary branches, formed the base structure of the crown and were pruned when they reached 30 cm in length, to stimulate the sprouting of secondary branches and control lateral growth.

Gas exchange, physiological indices, and growth of soursop cv. Morada Nova were evaluated at 370 days after transplanting (DAT). Gas exchange was evaluated based on the CO_2 assimilation rate - A ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$), transpiration - E ($mmol\ H_2O\ m^{-2}\ s^{-1}$), stomatal conductance - gs ($mol\ H_2O\ m^{-2}\ s^{-1}$), and internal CO_2 concentration - Ci ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$), in leaves of the middle third of the plants, using a portable infrared gas analyzer - IRGA (Infrared Gas Analyser, LCpro-SD model, from ADC BioScientific, UK). The A/gs and A/Ci ratios were used to calculate instantaneous water use efficiency – WUEi [($\mu mol\ CO_2\ m^{-2}\ s^{-1}$) ($mmol\ H_2O\ m^{-2}\ s^{-1}$)$^{-1}$] and instantaneous carboxylation efficiency - CEi [($\mu mol\ m^{-2}\ s^{-1}$) ($\mu mol\ mol^{-1}$)$^{-1}$], respectively. Readings were performed between 7:00 and 10:00 a.m., on the third fully expanded leaf counted from the apical bud, under natural conditions of air temperature and CO_2 concentration using an artificial radiation source of 1,200 $\mu mol\ m^{-2}\ s^{-1}$, established through the photosynthetic light-response curve.

Percentage electrolyte leakage (% EL) was expressed as the percentage of initial electrical conductivity relative to the electrical conductivity after treatment for 90 min at 90 ºC: [(Xi/Xf) × 100] (Sousa et al., 2017).

To determine the water saturation deficit (WSD), two leaves from the middle third of the main branch were collected to obtain four discs of 12 mm in diameter from each leaf. Immediately after collection, the discs were weighed, avoiding moisture loss to obtain the fresh mass (FM); then, these samples were placed in a beaker, immersed in 50 mL of distilled water and stored for 24 hours. After this period, excess water was removed from the discs with paper towels to obtain the turgid mass (TM) of the sample, which was then dried in an oven at ≈ 65 ± 3 °C until reaching constant weight to obtain the dry mass (DM) of the samples. WSD was determined according to Lima et al. (2015), by Eq 3:

$$WSD = \frac{TM - FM}{TM - DM} \times 100 \quad (3)$$

where:

WSD - water saturation deficit (%);
FM - leaf fresh mass (g);
TM - turgid mass (g); and,
DM - dry mass (g).

For growth analyses, the following parameters were measured: crown height (H_{Crown}), stem diameter (SD), and crown diameter (D_{Crown}). The D_{Crown} was determined by the average crown diameter in the row (DR) and interrow (DIR) directions. Crown volume (V_{Crown}) and vegetative vigor index (VVI) were obtained according to Eqs. 4 and 5, respectively, following the methodology of Portella et al. (2016):

$$V_{Crown} = \frac{\pi \times H_{Crown} \times DR \times DIR}{6} \quad (4)$$

$$VVI = \frac{\left[H_{Crown} + D_{Crown} + (SD + 10)\right]}{100} \quad (5)$$

where:

V_{Crown} - crown volume (m^3);
VVI - vegetative vigor index;
H_{Crown} - crown height (m);
DR - crown diameter in the row direction (m);
DIR - crown diameter in the interrow direction (m); and,
SD - stem diameter (mm).

The data were subjected to the distribution normality test (Shapiro-Wilk) at $p \leq 0.05$. Subsequently, the analysis of variance and linear and quadratic regression analyses were performed using the statistical program SISVAR-ESAL version 5.7 (Ferreira, 2019). In cases of significance of the interaction between factors, SigmaPlot software v.12.5 was used to create the response surfaces.

Results and Discussion

There was a significant effect (Table 2) of the interaction between electrical conductivity and H_2O_2 concentrations on transpiration (E), internal CO_2 concentration (Ci), and instantaneous water use efficiency (WUEi). The effect of electrical conductivity was significant for stomatal conductance (gs), CO_2 assimilation rate (A), and instantaneous carboxylation efficiency (CEi). H_2O_2 concentrations alone did not influence any of the variables analyzed.

The increase in the electrical conductivity of the water negatively affected the stomatal conductance (gs) of soursop cv. Morada Nova (Figure 2A); plants grown under ECw of 3.2 dS m^{-1} showed a reduction of 40.57% (0.0396 mol H_2O m^{-2} s^{-1}) in gs compared to plants irrigated using water with electrical conductivity of 0.8 dS m^{-1}. Stomatal closure in plants is a way to prevent water loss to the atmosphere, maintaining water potential in leaves and avoiding dehydration of guard cells, since the accumulation of salts in the soil results in a decrease in osmotic potential near the roots, causing limitations in water absorption (Dias et al., 2019).

The results found in this study for gs are in agreement with those obtained by Veloso et al. (2022), who, in an assay with soursop cv. Morada Nova (ECw from 0.7 to 3.7 dS m^{-1}), found a reduction of 37.93% in plants subjected to the highest ECw compared to those that received the lowest ECw.

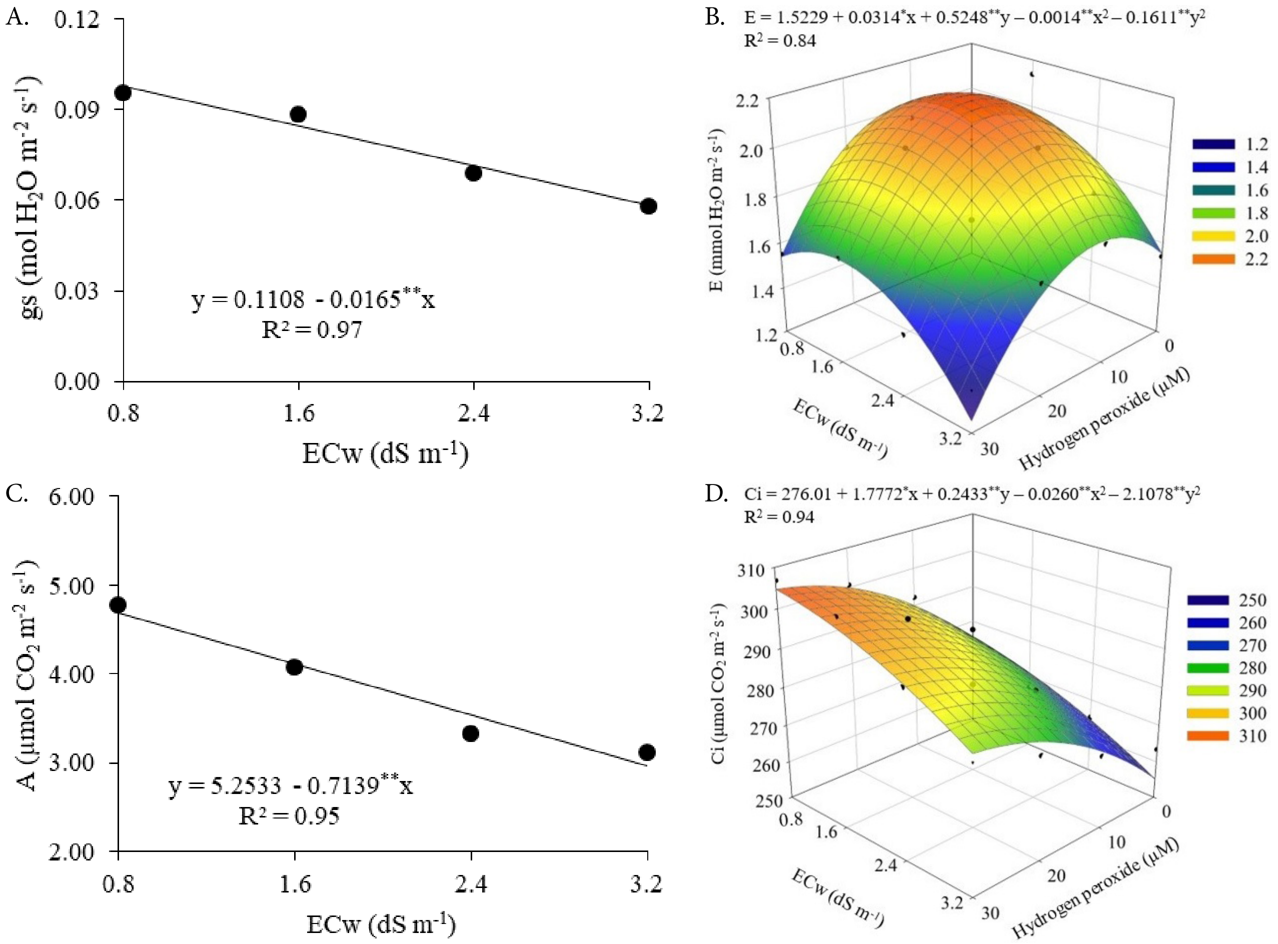
The transpiration (E) (Figure 2B) of soursop plants irrigated with water of 1.6 dS m^{-1} and subjected to a concentration of 10 μM stood out with the highest value (2.13 mmol H_2O m^{-2} s^{-1}), corresponding to an increase of 9.02% (0.18 mmol H_2O m^{-2} s^{-1}) compared to plants irrigated with the same ECw, but without application of H_2O_2 (0 μM). On the other hand, H_2O_2 concentrations above 10 μM associated with increased ECw caused a reduction in E, with the lowest value (1.23 mmol H_2O m^{-2} s^{-1}) obtained in plants subjected to 30 μM of H_2O_2 and irrigated with water of 3.2 dS m^{-1}.

A different result was found by Veloso et al. (2020), who studied the physiological changes and growth of soursop

Table 2. Summary of the analysis of variance for stomatal conductance (gs), transpiration (E), CO_2 assimilation rate (A), internal CO_2 concentration (Ci), instantaneous carboxylation efficiency (CEi), and instantaneous water use efficiency (WUEi) of soursop cv. Morada Nova, at 370 days after transplanting, as a function of electrical conductivity of irrigation water and subjected to foliar application of hydrogen peroxide

Source of variation	DF	Mean squares					
		gs	E	A	Ci	CEi	WUEi
Electrical conductivity of water (ECw)	4	0.00035**	0.4650**	4.640**	753.17ns	0.000870**	0.432ns
Linear regression	1	0.00081**	0.6710ns	13.37**	1199.3ns	0.000028**	0.692ns
Quadratic regression	1	0.00010ns	0.0030**	0.083ns	1050.0ns	0.000112ns	0.585ns
Hydrogen peroxide (H_2O_2)	4	0.00034ns	0.5300**	11.83ns	435.76ns	0.000190ns	1.474**
Linear regression	1	0.22273ns	0.6712**	23.51ns	193.50ns	0.000375ns	2.387**
Quadratic regression	1	0.00008ns	0.0003ns	0.393ns	905.67ns	0.000020ns	0.589ns
Interaction (ECw × H_2O_2)	16	0.00080ns	0.2120**	9.450ns	3984.1**	0.000177ns	2.422**
Blocks	3	0.00010ns	0.2128ns	0.248ns	154.06ns	0.000060ns	0.250ns
Residual	30	0.000149	0.0550	0.156	171.27	0.000060	0.134
CV (%)		15.65	17.18	10.57	4.78	10.26	16.81

ns and ** respectively not significant and significant at $p \leq 0.01$ by F test. DF - Degrees of freedom; CV - Coefficient of variation



X and Y – ECw and concentration of H_2O_2, respectively (Fig. B and D); * and ** respectively, significant at $p \leq 0.05$ and significant at $p \leq 0.01$ by F test

Figure 2. Stomatal conductance - gs (A) and CO_2 assimilation rate - A (C) as a function of electrical conductivity of irrigation water – ECw; and transpiration - E (B) and internal CO_2 concentration - Ci (D) of soursop cv. Morada Nova, at 370 days after transplanting, as a function of the interaction between ECw and hydrogen peroxide - H_2O_2

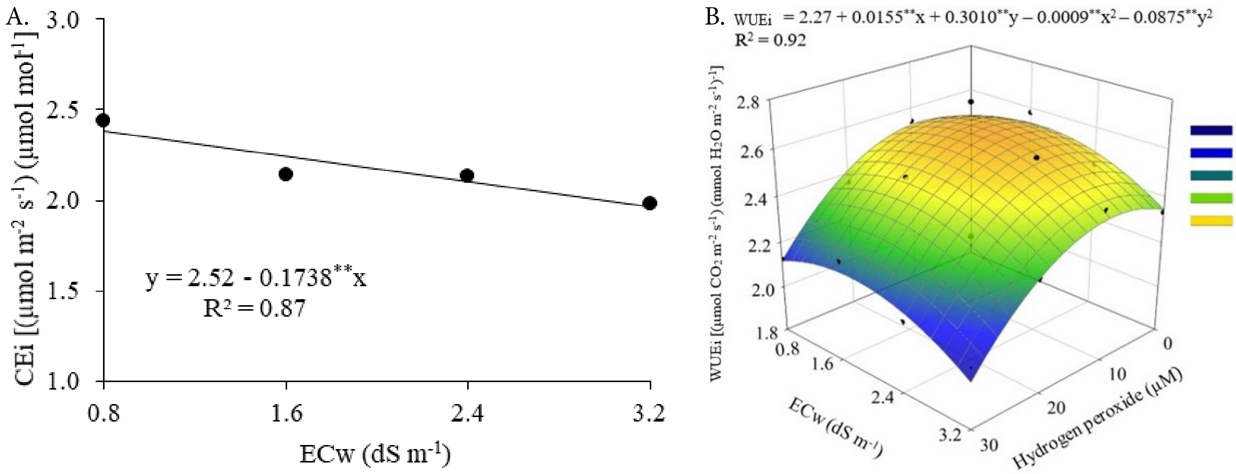
cv. Morada Nova cultivated with saline waters and H_2O_2 in the post-grafting phase and concluded that the H_2O_2 concentration of 20 μM mitigated the effects of salinity on transpiration, in addition to promoting the biosynthesis of photosynthetic pigments and reducing cell damage, at 150 days after transplanting. The divergence between the results obtained may be related to the form of propagation of the crop and the time of evaluation.

Under salt stress conditions, the plant tends to suffer limitations in water absorption due to the reduction of its osmotic potential. However, to ensure water absorption and keep cells turgid, the plant tends to reduce its transpiration flow and adjust osmotically (Silva et al., 2019b). Thus, a reduction in E may have occurred due to the partial closure of the stomata (Figure 2A), affecting the capacity for water absorption by the root system in soursop plants.

CO_2 assimilation rate (A) was negatively affected by the increase in electrical conductivity of irrigation water (Figure 2C), with a reduction of 13.59% per unit increment in ECw, i.e., a decrease of 36.59% (1.71 μmol CO_2 m^{-2} s^{-1}) in plants subjected to ECw of 3.2 dS m^{-1} compared to those irrigated with ECw of 0.8 dS m^{-1}. Plants irrigated with ECw of 0.8 dS m^{-1} had the highest value of A (4.68 μmol CO_2 m^{-2} s^{-1}), while the lowest value (2.97 μmol CO_2 m^{-2} s^{-1}) was observed in plants cultivated with water of highest salinity level (3.2 dS m^{-1}). A decrease in CO_2 assimilation rate in plants cultivated under salinity has also been observed in soursop (Silva et al., 2019c), West Indian cherry (Dias et al., 2019), and passion fruit (Andrade et al., 2022).

Regarding the effect of ECw on the internal carbon concentration (Ci) (Figure 2D), the highest Ci value (304.77 μmol CO_2 m^{-2} s^{-1}) was observed in plants irrigated with 0.8 dS m^{-1} water and subjected to foliar application of H_2O_2 at a concentration of 30 μM, corresponding to an increase of 10.88% (29.92 μmol CO_2 m^{-2} s^{-1}) compared to plants irrigated with the same ECw, but without application of H_2O_2 (0 μM). Despite the beneficial effect of hydrogen peroxide at the concentration of 30 μM, it can be observed that Ci decreased with the increase in the electrical conductivity of irrigation water. Silva et al. (2019a) observed that the exogenous application of 25 μM H_2O_2 was able to mitigate the effects of salinity on passion fruit plants, favoring gas exchange and plant growth.

For instantaneous carboxylation efficiency (CEi), the regression equation (Figure 3A) indicated a decrease of 6.90% per unit increment in ECw. When comparing the CEi of plants subjected to ECw of 3.2 dS m^{-1} to that of plants that received the lowest salinity level (0.8 dS m^{-1}), a reduction of 17.65% was observed. The increase in electrical conductivity levels of irrigation water results in accumulation of salts in the soil solution and restricts the uptake of water and



X and Y – ECw and concentration of H_2O_2, respectively (Fig. B); * and ** respectively, significant at $p \leq 0.05$ and significant at $p \leq 0.01$ by F test

Figure 3. Instantaneous carboxylation efficiency - CEi (A) as a function of electrical conductivity of irrigation water - ECw and instantaneous water use efficiency - WUEi (B) of soursop plants cv. Morada Nova, at 370 days after transplanting, as a function of the interaction between ECw and hydrogen peroxide

nutrients by plants. Thus, to avoid water loss, plants partially close their stomata and, consequently, the entry of CO_2 into the substomatal chamber is restricted, compromising the instantaneous carboxylation efficiency (Pinheiro et al., 2022).

Similar results were found by Capitulino et al. (2022), who studied gas exchange and growth of soursop under salt stress (ECw ranging from 0.6 to 3.0 dS m^{-1}) and methods of H_2O_2 application and found a reduction of 24.32% in CEi, when plants were irrigated with the highest salinity level compared to those irrigated with ECw of 0.6 dS m^{-1}.

The instantaneous water use efficiency (WUEi) (Figure 3B) of soursop plants irrigated with 1.8 dS m^{-1} water and subjected to a concentration of 10 μM stood out with the highest value (2.59[(μmol CO_2 m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$]), corresponding to an increase of 2.37% ([0.065 (μmol CO_2 m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$]), compared to plants irrigated with the same ECw, but without application of H_2O_2 (0 μM). In turn, hydrogen peroxide is related to the regulation of several mechanisms under conditions of abiotic and biotic stresses, so the beneficial effect of H_2O_2 at low concentrations may be associated with its role as a molecular signaling agent, regulating several pathways, including responses to salt stress (Baxter et al., 2014).

There was a significant effect ($p \leq 0.01$) of water electrical conductivity levels on water saturation deficit (WSD), percentage of intercellular electrolyte leakage (% EL), and stem diameter (SD) of soursop plants (Table 3). Hydrogen peroxide concentrations influenced WSD, % EL, and SD. There was no effect of the interaction between salinity levels (SL) and hydrogen peroxide concentrations for the analyzed variables of soursop plants cv. Morada Nova at 370 days after transplanting.

The percentage of electrolyte leakage of soursop cv. Morada Nova increased linearly by 9.09% per unit increase in ECw (Figure 4A). According to the regression equation, % EL increased by 20.35% in soursop plants subjected to ECw of 3.2 dS m^{-1} compared to those under 0.8 dS m^{-1}.

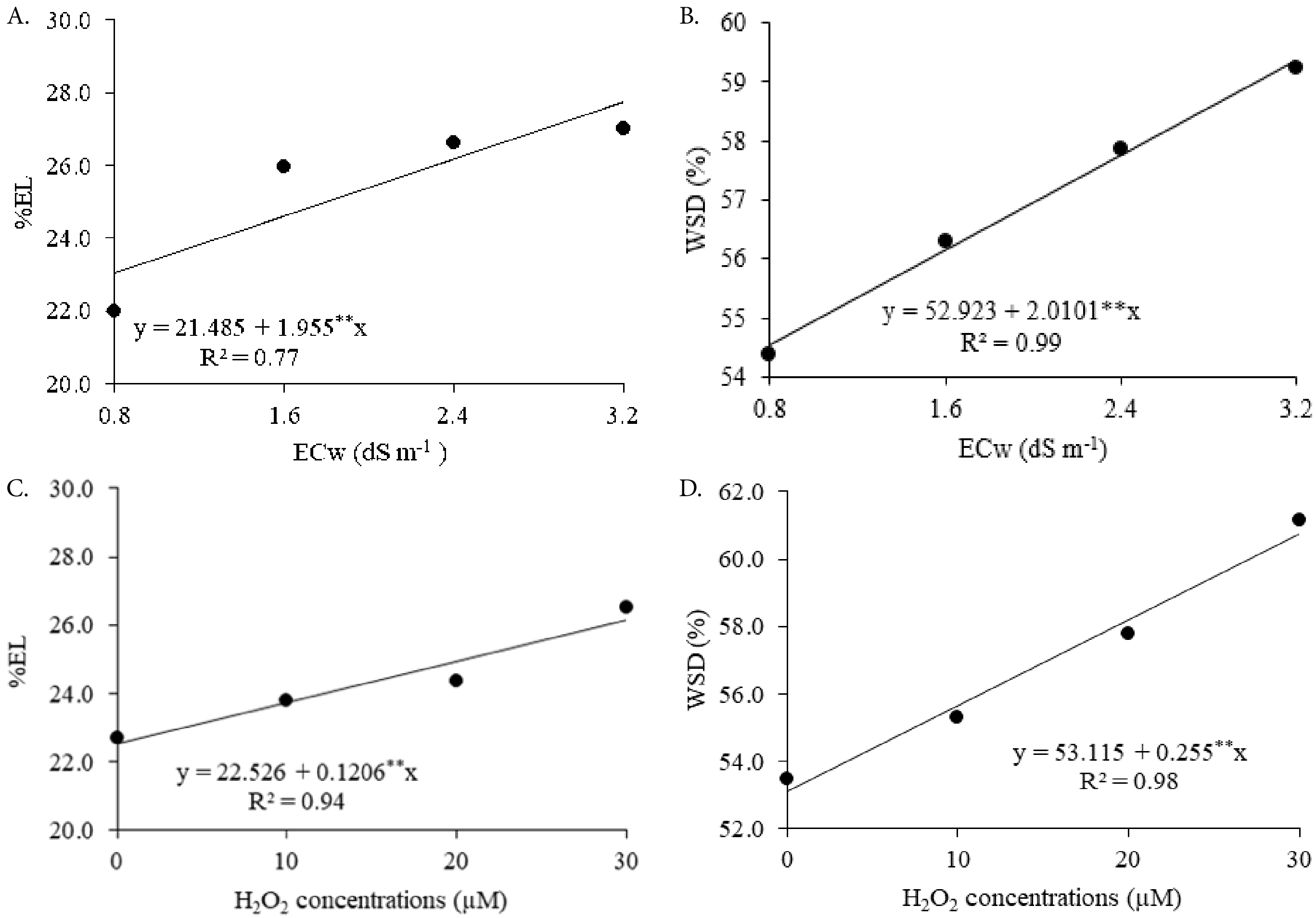
The increase in the percentage of electrolyte leakage observed in soursop plants irrigated with water of the highest salinity level (3.2 dS m^{-1}) may have occurred because the increase in the concentration of salts in water can alter the nutritional balance and limit the availability of nutrients to plants, including Ca, an element that is essential for cell wall formation, thus generating an increase in the percentage of electrolyte leakage under conditions of high salinity (Wanderley et al., 2020).

The water saturation deficit of soursop cv. Morada Nova increased linearly in response to the increase in irrigation water salinity (Figure 4B), with an increase of 3.79% per unit

Table 3. Summary of the analysis of variance for the percentage of electrolyte leakage (% EL), water saturation deficit (WSD), crown diameter (D_{Crown}), crown volume (V_{Crown}), crown height (H_{Crown}), stem diameter (SD), and vegetative vigor index (VVI) of soursop cv. Morada Nova, at 370 days after transplanting, as a function of electrical conductivity of irrigation water and subjected to foliar application of hydrogen peroxide

Source of variation	DF	Mean squares						
		%EL	WSD	D_{Crown}	V_{Crown}	H_{Crown}	SD	VVI
Electrical conductivity of water (ECw)	4	77.82^{**}	52.0^{**}	516337.1^{**}	0.00652^{**}	0.0396^{**}	18.73^{**}	0.000008^{ns}
Linear regression	1	135.78^{**}	155.0^{**}	792858.4^{**}	0.01247^{ns}	0.1131^{ns}	2.367^{*}	0.000008^{ns}
Quadratic regression	1	39.271^{ns}	0.92^{ns}	701340.9^{ns}	0.00285^{**}	0.0411^{**}	52.16^{ns}	0.000015^{ns}
Hydrogen peroxide (H_2O_2)	4	31.166^{*}	132.0^{**}	1059816^{ns}	0.00242^{ns}	0.0046^{ns}	17.57^{**}	0.00014^{ns}
Linear regression	1	35.711^{**}	390.0^{**}	202695.8^{ns}	0.00392^{ns}	0.0001^{ns}	5.011^{ns}	0.000007^{ns}
Quadratic regression	1	31.793^{ns}	7.27^{ns}	1930325.1^{ns}	0.00226^{ns}	0.0009^{ns}	41.21^{**}	0.000033^{ns}
Interaction (ECw × H_2O_2)	16	39.195^{ns}	1.46^{ns}	0.183802^{ns}	0.00268^{**}	0.0181^{*}	15.20^{**}	0.00006^{ns}
Blocks	3	0.9045^{ns}	2.62^{ns}	134214^{ns}	0.00607^{ns}	0.0038^{ns}	1.322^{ns}	0.00001^{ns}
Residual	30	0.7527	0.31	24338	0.00082	0.0049	1.5708	0.00001
CV (%)		3.56	6.57	8.94	7.35	4.43	3.61	2.74

^{ns}, * and **, respectively not significant, significant at $p \leq 0.05$ and significant at $p \leq 0.01$ by F test. DF - Degrees of freedom; CV - Coefficient of variation



** significant at $p \leq 0.01$ by F test

Figure 4. Percentage of electrolyte leakage - % EL (A) and water saturation deficit - WSD (B) of soursop cv. Morada Nova as a function of electrical conductivity of irrigation water - ECw and % EL (C) and WSD (D) , as a function of hydrogen peroxide concentrations, at 370 days after transplanting

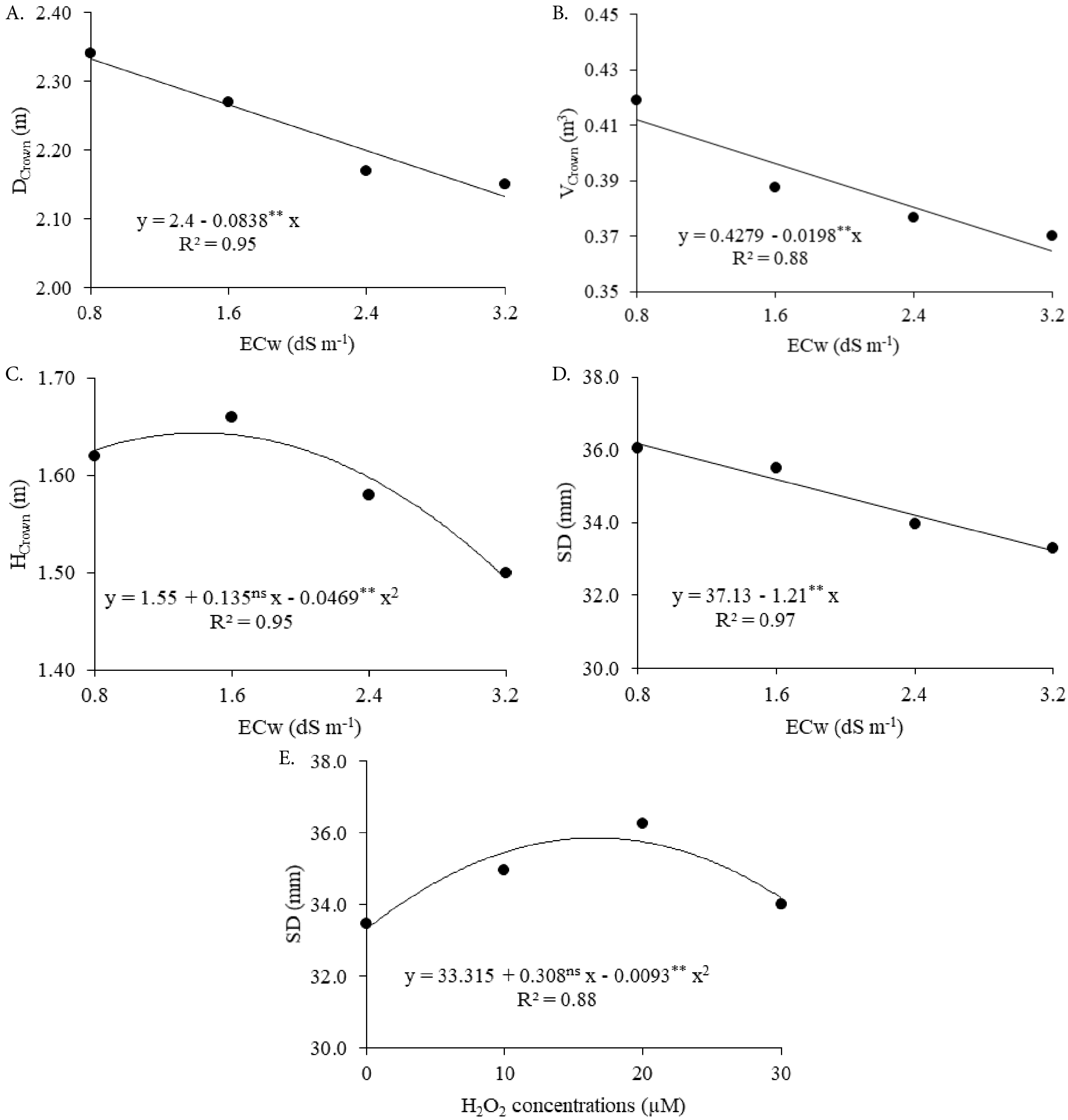
increment of ECw. When comparing plants irrigated with 3.2 dS m^{-1} water to those cultivated under the lowest ECw level (0.8 dS m^{-1}), an increase in WSD of 8.84% is verified. An increase in WSD in the leaf blade reflects the plant's difficulty in absorbing water due to a decrease in the osmotic potential in the root zone caused by the accumulation of salts in the soil solution. In addition, the increase in water saturation deficit results in a decrease in turgor pressure and inhibits cell expansion and elongation (Silva et al., 2019b).

For the H_2O_2 concentrations, there was an increase in % EL (Figure 4C) of the order of 16.06% in soursop plants subjected to H_2O_2 application at the concentration of 30 µM compared to those that were not subjected to H_2O_2 application. Hydrogen peroxide is the most stable reactive oxygen species in cells, but at high concentrations, it can spread rapidly through the cell membrane, resulting in oxidative damage (Farooq et al., 2017), which may have occurred in the soursop plants cv. Morada Nova.

Hydrogen peroxide concentrations influenced the WSD of soursop (Figure 4D). Plants subjected to a concentration of 30 µM stood out with the highest WSD value (60.76%), with an increase of 14.40% compared to plants that were not subjected to H_2O_2 (0 µM). A similar result was found by Silva et al. (2022), who studied the effect of hydrogen peroxide in the attenuation of salt stress on physiological indicators and growth of soursop in the seedling formation phase and observed an increase in WSD in plants subjected to the H_2O_2 concentration of 30 µM.

Dito & Gadallah (2019) state that low H_2O_2 concentrations can favor photosynthetic activity by improving the antioxidant metabolism of plants. On the other hand, at high concentrations, H_2O_2 can intensify the harmful effects of salt stress, causing changes in plant metabolism due to the restriction of photosynthetic activity, which may have occurred in the present study, thus causing an increase in the WSD of soursop plants.

For the crown diameter (D_{Crown}) and crown volume (V_{Crown}) (Figures 5A and B) of soursop plants, there were reductions of 3.49 and 4.63% per unit increment in ECw, respectively. When comparing the D_{Crown} and V_{Crown} of plants irrigated with water of the highest salinity (3.2 dS m^{-1}) with the values of those cultivated with the lowest ECw (0.8 dS m^{-1}), reductions of 8.84 and 13.27% were observed, respectively. Inhibition of growth in D_{Crown} and V_{Crown} may be associated with the displacement of Ca^{2+} from cell wall binding sites due to Na^+ accumulation, reducing pectin reticulation and consequently affecting cell elongation and division (Byrt et al., 2018). Veloso et al. (2020) observed a reduction in the growth of soursop plants subjected to salinity and attributed it to the decrease in water availability or excessive accumulation of Na^+ and Cl^- in plant tissues.



ns, **, respectively not significant and significant at $p \leq 0.01$

Figure 5. Crown diameter - D_{Crown} (A), crown volume - V_{Crown} (B), crown height - H_{Crown} (C), stem diameter - SD (D) of soursop cv. Morada Nova, as a function of electrical conductivity of water (ECw) and stem diameter - SD (E) as a function of concentrations of hydrogen peroxide - H_2O_2, at 370 days after transplanting

The results obtained in the present study are consistent with those reported by Lacerda et al. (2022), who studied guava cv. 'Paluma' under irrigation with saline water (ECw between 0.6 and 3.2 dS m^{-1}) and observed that irrigation with water of 3.2 dS m^{-1} reduces crown diameter, crown volume, and vegetative vigor index of guava cv. Paluma, at 390 days after transplanting.

It can be observed that the estimated ECw of 1.44 dS m^{-1} led to the highest value of H_{Crown} in soursop (1.65 m) (Figure 5C). In turn, the minimum value of H_{Crown} was obtained at the highest ECw level (3.2 dS m^{-1}) (1.50 m). Reductions in plant growth may be the result of restriction in the absorption of water and nutrients, which may occur due to the partial closure of the stomata, which contributes to lower uptake of CO_2 from the external environment, causing limitations in the production of photoassimilates and, consequently, a reduction in the vegetative organs of the plant (Carvalho et al., 2020).

The stem diameter (SD) of soursop cv. Morada Nova (Figure 5D) decreased linearly under irrigation with saline water by 3.26% per unit increment in ECw, with a decrease of 8.76% in the SD of plants subjected to ECw of 3.2 dS m^{-1} compared to those irrigated with ECw of 0.8 dS m^{-1}. Reduction of SD in plants was also observed by Xavier et al. (2022), in a study conducted with 'Paluma' guava under salt stress (ECw ranging from 0.6 to 4.2 dS m^{-1}). The inhibition of plant growth is related to changes in soil water potential caused by excess salts, which restrict water absorption, decreasing turgor pressure and plant cell activity by inhibiting cell expansion and elongation (Lima et al., 2020a; Lima et al., 2020b).

H_2O_2 concentrations showed a quadratic effect on the SD of soursop cv. Morada Nova (Figure 5E), with the maximum estimated value of 35.86 mm obtained at the estimated concentration of 17 µM, while the lowest value was 33.31 mm, observed in plants that did not receive foliar application of H_2O_2 (0 µM). Veloso et al. (2020), while evaluating the physiological changes and growth of soursop cultivated with saline waters and H_2O_2 in the post-grafting phase, observed that the exogenous application of 20 µM of H_2O_2 reduces the harmful effects of salinity on the rootstock and scion stem diameters of soursop plants irrigated with water of 1.6 dS m^{-1}.

Conclusions

1. Increase in electrical conductivity of irrigation water from 0.8 dS m^{-1} compromises stomatal conductance, CO_2 assimilation rate, and instantaneous carboxylation efficiency and inhibits the growth of soursop plants at 370 days after transplanting.

2. Hydrogen peroxide up to a concentration of 10 µM increases leaf transpiration and water use efficiency of soursop plants irrigated with 1.8 dS m^{-1} water in the pre-flowering phase.

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