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Mineral composition and physiology of soursop under salt stress and application of hydrogen peroxide

Composição mineral e fisiologia da gravioleira sob estresse salino e aplicação de peróxido de hidrogênio

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Highlights

Salt stress reduces pigment biosynthesis and quantum efficiency in plants.
Water salinity increases the sodium and chlorine contents.
The H_2O_2 concentration of 30 µM increases salt stress on soursop leaf NPK content.

Abstract

The soursop plant adapts well to the edaphoclimatic conditions of the semi-arid region of northeastern Brazil. However, the presence of waters with high salt concentrations stands out as limiting factor for the expansion of the cultivation of this fruit crop. Therefore, identifying strategies to enable fruit production is crucial. This study aimed to assess the mineral composition in leaf tissues and the physiology of soursop cv. Morada Nova plants under saline water irrigation and foliar application of hydrogen peroxide during the pre-flowering phase. The research was conducted in a greenhouse in Campina Grande, PB, Brazil, employing a randomized block design in a 4 × 4 factorial arrangement. This arrangement included

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four levels of irrigation water electrical conductivity (ECw: 0.8, 1.6, 2.4, and 3.2 dS m^{-1}) and four hydrogen peroxide (H_2O_2) concentrations (0, 10, 20, and 30 µM), with three replications, totaling 48 experimental plots with one plant each. Saline water irrigation adversely influenced the biosynthesis of chlorophyll *a* and *b*, initial and variable fluorescence, and the quantum efficiency of photosystem II in soursop, 370 days post-transplanting. Hydrogen peroxide concentration of 30 µM exacerbated the effect of salt stress on the leaf nitrogen, phosphorus, and potassium contents. However, concentrations of 15, 12, and 9 µM, respectively, enhanced the N, P, and K levels in the leaf tissues of soursop cv. Morada Nova, 780 days post-transplanting. Nutrient accumulation in the leaves of soursop cv. Morada Nova during the pre-flowering phase occurs in the following descending order: P>N>K>S>Cl>Na.
Key words: *Annona muricata* L. Plant nutrition. Salt stress.

Resumo

A gravioleira adapta-se bem às condições edafoclimáticas da região semiárida do nordeste brasileiro, no entanto a ocorrência de águas com concentrações elevadas de sais, destaca-se como um fator limitante para a expansão do cultivo desta frutífera. Dessa forma, buscar estratégias para viabilizar a produção de frutíferas é de grande importância. Assim, objetivou-se com esta pesquisa avaliar a composição mineral nos tecidos foliares e a fisiologia de gravioleira cv. Morada Nova cultivada sob irrigação com águas salinas e aplicação foliar de peróxido de hidrogênio na fase de pré-floração. A pesquisa foi conduzida sob condições de casa de vegetação em Campina Grande - PB, utilizando-se o delineamento em blocos casualizados, em esquema fatorial 4 × 4, sendo quatro níveis 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, totalizando 48 parcelas experimentais e uma planta por parcela. A irrigação com águas salinas afetou negativamente a biossíntese de clorofila *a* e *b*, a fluorescência inicial, variável e a eficiência quântica do fotossistema II da gravioleira, aos 370 dias após o transplantio. A concentração de peróxido de hidrogênio de 30 µM intensificou o efeito de estresse salino sobre os teores foliares de nitrogênio, fósforo e potássio. Já as concentrações de 15, 12 e 9 µM, respectivamente, aumentaram os teores de N, P e K nos tecidos foliares de gravioleira cv. Morada Nova, aos 780 dias após o transplantio. O acúmulo de nutrientes nas folhas de gravioleira cv. Morada Nova na fase de pré-floração segue a seguinte ordem decrescente: P>N>K>S>Cl>Na.
Palavras-chave: *Annona muricata* L. Nutrição vegetal. Estresse salino.

Introduction

The soursop (*Annona muricata* L.), a member of the family Annonaceae, is primarily cultivated for its fruit pulp, which is utilized in making juice, nectar, and various derivatives (Watanabe et al., 2014). Added to this, the fruit is renowned for its nutritional and medicinal properties (Gajalakshmi et al., 2012; Sandoval Paixão et al., 2021).

In the semi-arid regions of Brazil, agricultural areas often encounter springs with high salt concentrations, particularly those underground, leading to inferior water quality and constraints on agricultural use (Nobre et al., 2012; Dias et al., 2019; Veloso

et al., 2023). An excess of salts near the root zone can induce nutritional imbalances, with elevated sodium levels in soil solution compromising the uptake of essential ions such as Ca^{2+}, Mg^{2+}, and K^{+} by plants (Cruz et al., 2018).

Veloso et al. (2020) observed physiological and growth alterations in soursop plants irrigated with saline water. The authors noted that an increase in irrigation water salinity from 1.6 dS m^{-1} affected stomatal conductance, transpiration rates, and intercellular CO_2 levels. While there are studies on salt stress impacts on soursop, research focusing on its mineral composition remains scarce and incipient.

It is important to consider that the extent of salt stress effects on plants varies with species, genotype, exposure duration, irrigation and fertilization practices, developmental stage, climatic conditions, and the stress tolerance mechanisms of the plant. These mechanisms include maintaining ionic and osmotic balance and eliminating reactive oxygen species (Soares et al., 2018; A. A. R. Silva et al., 2021).

An alternative to mitigate the adverse effects of salt stress in plants involves the use of elicitors such as hydrogen peroxide (H_2O_2) (T. A. Shalaby et al., 2021). As a reactive oxygen species (ROS), when generated and accumulated in plants under stress conditions, H_2O_2 can cause oxidative damage to the photosynthetic machinery, biological macromolecules, and cell membranes (Zheng et al., 2009; Kaya et al., 2023). However, at low concentrations, ROS can positively influence plant metabolism, signaling pathways, and triggering defense responses to both biotic and abiotic stresses (Asghar et al., 2021; O. A. E. Shalaby et al., 2023).

Hydrogen peroxide has been demonstrated to alleviate salt stress effects in various plant species, including soursop (Veloso et al., 2020), sour passion fruit (Andrade et al., 2022), and guava (J. T. A. Ferreira et al., 2023).

Therefore, the objective of this study was to analyze the mineral composition of the leaf tissues and physiological parameters of soursop cv. Morada Nova plants in response to saline water irrigation and foliar application of hydrogen peroxide during the pre-flowering stage.

Material and Methods

The experiment was conducted from April 2020 to May 2022 in a greenhouse at the Agricultural Engineering Academic Unit (UAEA) of the Federal University of Campina Grande (UFCG), located in Campina Grande, Paraíba, Brazil. The location is situated at 7°15'18'' S latitude and 35°52'28'' W longitude, with an average elevation of 550 m. Figure 1 illustrates the observed maximum and minimum temperatures, along with relative humidity levels inside the greenhouse.

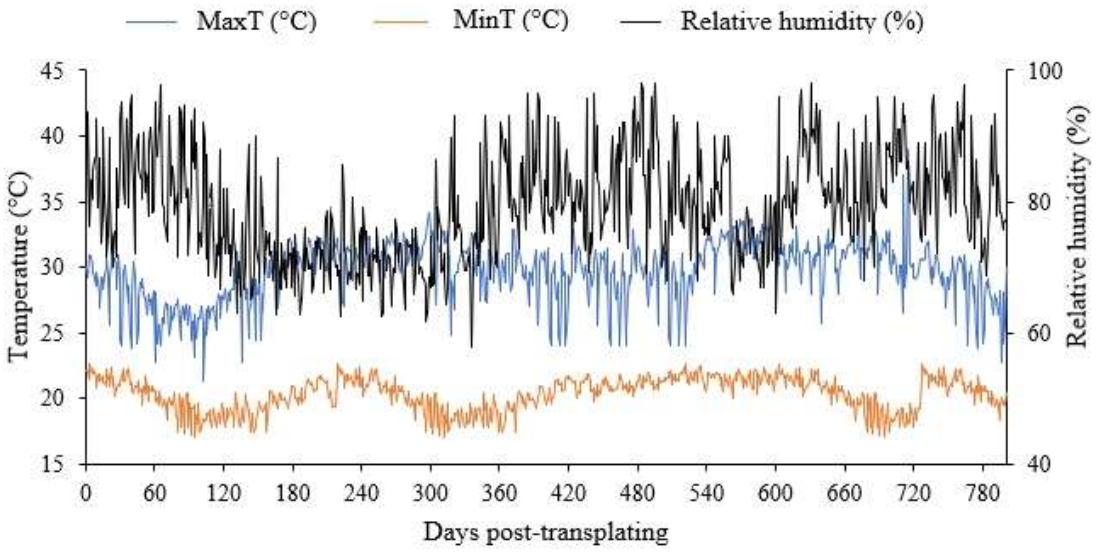

Figure 1. Air temperature (maximum and minimum) and average relative humidity inside the greenhouse during the experimental period.

The experimental design consisted of a 4 × 4 factorial arrangement, combining four levels of irrigation water electrical conductivity (ECw: 0.8, 1.6, 2.4, and 3.2 dS m^{-1}) with four hydrogen peroxide concentrations (H_2O_2: 0, 10, 20, and 30 µM). These were distributed across randomized blocks, each with three replicates, resulting in a total of 48 experimental plots, with one plant allocated to each plot. The selection of ECw levels was based on prior research by A. A. R. Silva et al. (2019a), and the chosen H_2O_2 concentrations were based on a study by Veloso et al. (2020).

Seedlings of soursop cv. Morada Nova were sourced from a commercial nursery accredited in the Seeds and Seedlings Registry, located in the district of São Gonçalo, Sousa - PB, Brazil. These seedlings were grown in polyethylene bags measuring 10 × 20 cm. The choice of the Morada Nova cultivar was based on its widespread use in commercial orchards in Brazil, its productive potential, and the size of its fruits, which can reach up to 15 kg (São José et al., 2014).

For the experiment, plastic pots (capacity of 211.5 dm^3) adapted as drainage lysimeters were employed, with dimensions of 0.75 m deep and an area of 0.2115 m^2. These pots were filled with an initial layer of 1.0 kg of crushed stone, followed by 230 kg of soil classified as a Regosol (*Psamments*) with a clay-loam texture. The soil was collected at a depth of 0-30 cm from the municipality of Riachão do Bacamarte - PB. The chemical and physical-hydraulic characteristics of the soil (Table 1) were determined in accordance with the methods described by Teixeira et al. (2017).

Table 1
Chemical and physical-hydraulic characteristics of the soil (0-30 cm depth) used in the experiment prior to administration of treatments

Physicochemical characteristics								
pH H_2O	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+
1:2.5	g dm^{-3}	mg dm^{-3}	..... cmolc kg^{-1}					
6.5	8.1	79	0.24	0.51	14.9	5.4	0	0.9
.....Chemical characteristics.....			..... Physical-hydraulic characteristics.....					
EC_{se}	CEC	SAR_{se}	ESP	Particle fraction (g kg^{-1})			Moisture (dag kg^{-1})	
dS m^{-1}	$cmol_c$ kg^{-1}	(mmol $L^{-1})^{0.5}$	%	Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
2.15	16.54	0.16	3.08	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, pH 7.0; Na^+ and K^+ extracted using 1 M NH_4OAc, pH 7.0; Al^{3+} and H^+ extracted with 0.5 M CaOAc, pH 7.0; EC_{se} - electrical conductivity of the saturation extract; CEC - cation-exchange capacity; SAR_{se} - sodium adsorption ratio of the saturation extract; ESP - exchangeable sodium percentage.

The irrigation water used in the experiment was prepared with varying levels of electrical conductivity by dissolving salts of NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ in the equivalent proportion of 7:2:1, respectively, in the local supply water (ECw = 0.38 dS m-1). This proportion of salts reflects the common composition of water sources used for irrigation in small farms in the Northeast (Medeiros, 1992). The ratio between ECw and salt concentration was considered when preparing the irrigation water (Richards, 1954), according to Eq. 1:

$$A = 10 \times ECw \quad (1)$$

where A - amount of salts to be added ($mmol_c$ L^{-1}); ECw - water electrical conductivity (dS m^{-1}).

Hydrogen peroxide solution in different concentrations were prepared by diluting reagent grade H_2O_2 in distilled water, followed by calibration using a spectrophotometer at an absorbance wavelength of 240 nm. Foliar applications commenced 45 days post-transplanting (DPT) of the seedlings into the lysimeters and were conducted at 30-day intervals. To ensure thorough wetting of the leaves, a knapsack sprayer equipped with a 1 cm adjustable conical metal nozzle, operating at a pressure of 2.07 MPa and a flow rate of 1.1 L min^{-1}, was utilized to spray both the abaxial and adaxial surfaces of the leaves. Spraying was carried out between 17h00 and 18h00, with approximately 400 mL of H_2O_2 solution applied per plant. Additionally, to mitigate solution drift between treatments, a plastic canvas curtain was positioned around each plant during the application of the hydrogen peroxide solution.

At 60 days DPT, irrigation with saline water commenced, with water applied to each lysimeter according to the designated treatment to maintain soil moisture near maximum retention capacity of soil, using the water balance principales. The amount

of water to be administered was determined based on the water needs of the plants. Water was applied manually daily, at 17h00. The volume of water to be applied in each irrigation event (VI) was calculated by Eq. 2:

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

where VI - volume of water to be applied in the irrigation event (mL); Vp - volume applied in the previous irrigation event (mL); Vd - volume drained (mL); LF - leaching fraction, set at 0.10, applied every 30 days to prevent excessive salt accumulation in the root zone.

NPK fertilization commenced at 15 DPT following the recommendations of F. J. de A. Cavalcante et al. (2008). This involved applying 40 g of N, 60 g of K_2O, and 40 g of P_2O_5 per plant annually. The fertilization application was divided into 24 installments, administered every 15 days. Urea (45% N), potassium sulfate (50% K_2O and 17% sulfur), and monoammonium phosphate (12% N and 54% P_2O5) served as sources of nitrogen, potassium, and phosphorus, respectively.

Micronutrients were applied foliarly, starting at 60 DPT. A solution of Dripsol® Micro, at a concentration of 2.5 g L^{-1}, was applied every two weeks. This product comprised N (15%), P_2O_5 (15%), K_2O (15%), Ca (1%), Mg (1.4%), S (2.7%), Zn (0.5%), B (0.05%), Fe (0.5%), Mn (0.05%), Cu (0.5%), and Mo (0.02%).

Pruning (training) was executed when the plant reached a height of 60 cm, involving the removal of the apical meristem. Three well-distributed and equidistant branches were selected from the emerging shoots. These branches were subsequently pruned upon reaching a length of 40 cm (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 1999). Throughout the experiment, continuous monitoring was conducted to detect pests and diseases. Immediate measures were taken upon detection of incidence, and appropriate pesticides were employed to eradicate pests and diseases.

Chlorophyll *a* fluorescence was measured at 370 DPT using a modulated pulse fluorometer, model OS5p from Opti Science, employing the Fv/Fm protocol. This protocol allowed the determination of fluorescence induction variables: initial (F_0), maximum (Fm), variable (Fv = Fm – F_0) fluorescence, and quantum efficiency of photosystem II (Fv/Fm) (Sá et al., 2015). To perform the protocol, the leaves were dark-adapted for 30 min using a clip from the equipment to ensure oxidation of all acceptors, i.e., with open reaction centers.

On the same day, photosynthetic pigments (chlorophyll *a* - Chl *a*; chlorophyll *b* - Chl *b*; chlorophyll *total* - Chl *t*; and carotenoids) were quantified according to Arnon (1949). Plant extracts were prepared from samples of blade discs taken from the third mature leaf from the apex. The chlorophyll and carotenoid contents in the solutions were determined using a spectrophotometer at absorbance wavelengths (ABS) of 470, 647, and 663, employing Eqs. 3, 4, 5, and 6:

Chlorophyll *a* (Chl *a*) = (12.21 × ABS_{663}) - (2.81 × ABS_{647}) ..... (3)

Chlorophyll *b* (Chl *b*) = (20.13 × ABS_{647}) - (5.03 × ABS_{663}) ..... (4)

Carotenoids = [(1000 × ABS_{470}) - (1.82 × Chl *a*) - (85.02 × Chl *b*)] /198 ..... (5)

Chlorophyll *total* (Chl *t*) = (17.3ABS_{647} + 7.18ABS_{663}) ..... (6)

The values for chlorophyll a, b, total and carotenoid contents in the leaves were expressed in mg g-1 of fresh weight (FW).

The mineral composition (N, K, P, Na, Cl, and S) was assessed at 780 DPT, coinciding with the pre-flowering phase of the soursop plants. Leaves were collected from the middle portion of the plant canopy for this purpose. After collection, the plant samples underwent washing with distilled water followed by drying in a forced-air oven at 65 °C until a constant weight was achieved. Subsequently, the dried samples were weighed, ground, and subjected to chemical analysis according to the methodology recommended by Silva (1999).

To determine the foliar nitrogen (N) content in the leaves, hydrochloric acid (HCℓ) at a concentration of 0.01 mol L□¹ was utilized, while for phosphorus (P), potassium (K), sulfur (S), and sodium (Na), 1 mol L□¹ HCℓ was used. Chlorine (Cl) analysis was conducted using 1 mol L□¹ nitric acid (HNO3). All analyses were conducted at the Soil and Plant Nutrition Laboratory (LSNP) at CCTA/UFCG. The Na/K and N/P ratios in the leaves of soursop cv. Morada Nova were determined following the methodology proposed by Tedesco et al. (1985).

The collected data underwent a normality distribution test (Shapiro-Wilk test). Subsequently, analysis of variance was performed at a significance level of 0.05. In cases where a significant effect was observed from individual factors, regression analysis was carried out using the SISVAR-ESAL v.5.6 statistical software (D. F. Ferreira, 2019). In the event of a significant interaction between factors, response surfaces were generated using SigmaPlot v.12.5 software.

Results and Discussion

An interaction effect (Table 2) was observed between irrigation water salinity levels and H2O2 concentrations on the leaf contents of phosphorus (P), potassium (K), and the N/P and Na/K ratio of soursop plants at 780 DPT. The irrigation water salinity levels significantly influenced the sulfur (S), chlorine (Cl), and sodium (Na) contents in the plant leaves. Additionally, hydrogen peroxide concentrations significantly affected soursop leaf nitrogen (N) levels.

Table 2
Summary of analysis of variance of N, P, K, S, Na and Cl, and N/P and Na/K ratio in the leaves of soursop cv. Morada Nova plants irrigated with saline water and subjected to foliar application of hydrogen peroxide, 780 days post-transplanting

Source of variation	DF	Mean squares							
		N	P	K	S	Na	Cl	N/P	Na/K
Salt level (SL)	4	194.5^{ns}	142.9**	81.60**	0.257**	13.18**	0.2575**	0.0047**	0.2215**
Linear regression	1	8.97^{ns}	259.7**	57.77**	0.346**	38.80**	0.6816**	0.0079^{ns}	0.5827*
Quadratic regression	1	258.0^{ns}	141.7^{ns}	38.61^{ns}	0.425^{ns}	0.091^{ns}	0.0093^{ns}	0.0013*	0.0531^{ns}
Hydrogen peroxide (H_2O_2)	4	76.89**	5.75^{ns}	15.37**	1.859^{ns}	0.163^{ns}	0.0167^{ns}	0.0046**	0.0030*
Linear regression	1	4.845^{ns}	0.247^{ns}	13.89^{ns}	0.890^{ns}	0.007^{ns}	0.0424^{ns}	0.0081*	0.0002^{ns}
Quadratic regression	1	213.7**	3.050^{ns}	28.90**	1.477^{ns}	0.285^{ns}	0.0006^{ns}	0.00003^{ns}	0.0083**
Interaction (SL × H_2O_2)	16	50.78^{ns}	21.23**	105.2**	0.111^{ns}	0.540^{ns}	0.0279^{ns}	0.0108**	0.0298**
Blocks	3	7.855^{ns}	9.151^{ns}	24.97^{ns}	0.022^{ns}	0.257^{ns}	0.0107^{ns}	0.0010^{ns}	0.0033^{ns}
Residual	30	5.808	2.876	3.783	0.0594	0.224	0.033	0.00077	0.0008
CV (%)		5.33	2.08	14.32	14.92	17.59	17.32	5.03	12.18

ns, *, and ** - not significant and significant at p≤0.05 and p≤0.01, respectively. DF - degrees of freedom. CV - coefficient of variation.

The maximum and minimum values of leaf nitrogen (N) in soursop cv. Morada Nova were estimated at 45.45 and 42.15 g kg^{-1}, respectively, in plants subjected to H_2O_2 application of 15 and 30 µM (Figure 2A). These foliar N levels fall within the sufficiency range recommended by Batista et al. (2003) and Viégas and Frazão (2004), who indicated adequate levels above 14.30 g kg^{-1} and deficient levels around 8.5 g kg^{-1}. Adequate concentrations of N can support enzyme activity, protein synthesis, amino acid production, and osmotic adjustment in plants grown under salt stress conditions (S. S. Silva et al., 2019b). According to T. A. Shalaby et al. (2021), H_2O_2 acts as a signaling molecule, triggering mechanisms that significantly increase the levels of enzymatic and non-enzymatic antioxidants, thereby effectively combating oxidative stress.

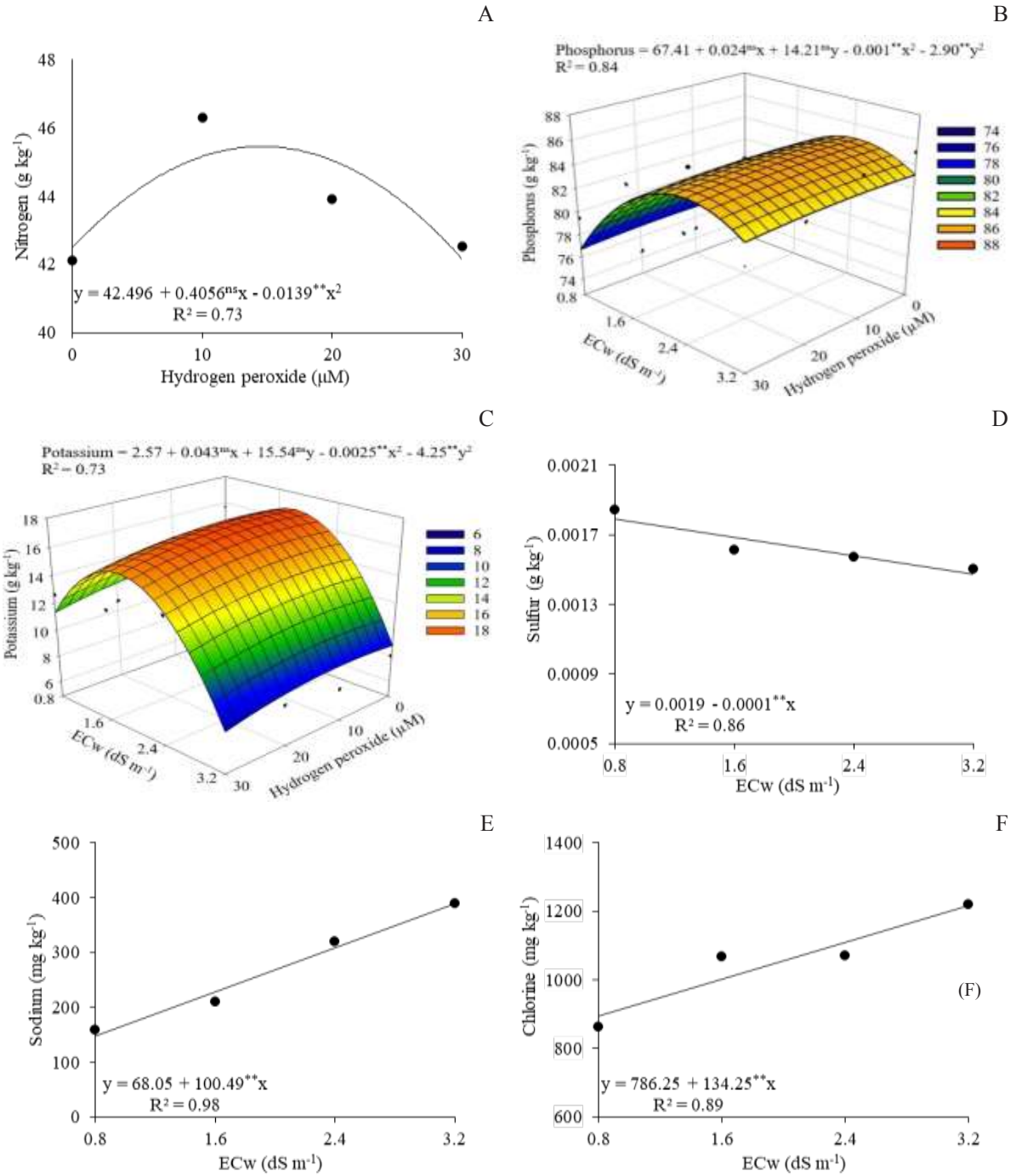

Figure 2. Leaf nitrogen - N (A) levels in soursop cv. Morada Nova plants as a function of H_2O_2 concentration; phosphorus - P (B) and potassium - K (C) levels as a function of irrigation water salinity (ECw) and H_2O_2 concentration; and sulfur - S (D), sodium - Na (E), and chlorine - Cl (F) contents as a function of ECw levels, 780 days post-transplantating.
ns, ** not significant and significant at p ≤ 0.01 by F test, respectively. X and Y correspond to the concentrations of hydrogen peroxide and ECw, respectively.

The interaction between water salinity levels and hydrogen peroxide concentrations significantly influenced leaf P contents in soursop cv. Morada Nova plants (Figure 2B). The highest estimated value (84.91 g kg-¹) was obtained in plants irrigated with ECw of 2.5 dS m-¹ and a foliar application of H_2O_2 of 12 µM. Plants under irrigation with water of 0.8 dS m-¹ and foliar application of 30 µM of H_2O_2 achieved the lowest estimated P content of 76.73 g kg-¹. Viégas and Frazão (2004) suggest leaf P levels between 0.8 and 1.0 g kg-¹ as adequate, while H. Silva & Silva (1986) propose levels between 1.4 and 1.5 g kg-¹ for soursop. However, regardless of the hydrogen peroxide concentration applied, the P contents observed in this study exceeded the considered adequate range. Phosphorus stimulates the synthesis of organic solutes, increases energy availability, and enhances the selectivity of the plant membrane in the absorption of beneficial ions, aiding in the exclusion of toxic ions and contributing to ionic homeostasis (Carneiro et al., 2017).

For K content (Figure 2C), the highest values were observed in plants irrigated with water having an ECw of 1.8 dS m-¹ and a H_2O_2 concentration of 9 µM (16.96 mg kg-¹). Conversely, plants subjected to a foliar application of 30 µM of H_2O_2 and irrigation with water having an ECw of 3.2 dS m-¹ exhibited the lowest K content (7.83 mg kg-¹). Avilán (1975) and S. A. de Oliveira (2004) consider leaf K content below 12.6 g kg-¹ as deficient for soursop. Thus, a hydrogen peroxide concentration of up to 20 µM in ECw of 2.8 dS m-¹ (12.63 g kg-¹) could mitigate the deleterious effects of salinity on leaf K content, possibly through biochemical mechanisms and signaling pathways, contributing to plant acclimation to abiotic stress and the maintenance of ionic homeostasis (Javed et al., 2018). The increase in potassium content in soursop leaves may serve as a tolerance mechanism to salt stress, as potassium ions act as osmoregulators, maintaining turgor pressure and relative water content in plant cells (Geng et al., 2016).

The increase in irrigation water electrical conductivity induced a linear decreasing effect on S content (Figure 2D), which declined by 6.99% per unit increase in ECw. Plants irrigated with water of higher salinity (3.2 dS m-¹) exhibited a reduction of 17.7% (0.34 g kg-¹) in S content compared to those irrigated with water having an ECw of 0.8 dS m-¹. This value falls below the range considered suitable for soursop, which, according to Viégas and Frazão (2004), varies between 3.88 and 5.96 g kg-¹, with values below 1.99 g kg-¹ indicating deficiency. Sulfur plays a crucial role in plant metabolism, being part of several compounds such as amino acids, proteins, chloroplast molecules, coenzymes, sulfolipids, flavonoids, lipids, glucosinolates, polysaccharides, unsaturated compounds, reduced compounds, among other metabolic functions. Additionally, it participates in physiological processes, hormonal control, photosynthesis, and defense mechanisms, contributing to protection against pests and diseases (Stipp & Casarin, 2010; R. J. Oliveira et al., 2020).

There was an increase in foliar Na (Figure 2E) and Cl (Figure 2F) contents of soursop cv. Morada Nova plants in response to irrigation with saline water. Data indicated

a linear increasing behavior, with an increase of 147.67% (241.17 mg kg-¹) for Na and 17.08% (322.32 mg kg-¹) for Cl with each unit increment of ECw. Comparing the foliar Na and Cl contents of plants irrigated with water of 0.8 dS m-¹ with those grown under water salinity of 3.2 dS m-¹, an increase of 162.47% and 36.06%, respectively, was observed.

Similarly, Sousa et al. (2019), in their study on early dwarf cashew (*Anacardium occidentale* L.) rootstock, observed an increase in Na and Cl contents in the leaves regardless of the rootstock used. This increase represented linear accumulation with the rise in ECw, with proportions of 13.6% and 18.3%, respectively. Alvarenga et al. (2019) reported that elevated concentrations of Cl^- and Na^+ can induce ionic toxicity in plants, resulting in the degradation of chlorophylls, the pigments responsible for photosynthesis, and potentially hindering plant growth. Moreover, high salt concentrations in plants may manifest symptoms of nutrient deficiency due to imbalances in nutrient uptake, resulting in decreased K content.

Foliar application of hydrogen peroxide and irrigation water salinity significantly affected the N/P ratio in soursop plants (Figure 3A). The highest N/P ratio observed was 0.6070 in plants irrigated with water having an ECw of 1.2 dS m^{-1} and treated with 14 µM H_2O_2. Conversely, the lowest N/P ratio was recorded in plants subjected to the highest salinity level (3.2 dS m^{-1}) and treated with 30 µM H_2O_2. Nitrogen and phosphorus are components that integrate proteins that build cellular structures and plant tissues, thus playing a significant role in plant growth and development (Ashraf et al., 2018). The N/P ratio is a critical indicator of nutrient limitation in terrestrial ecosystems, emphasizing the need for a balanced ratio for optimal nutrient absorption, which can be adversely affected by the salinity of irrigation water and soil (Huang et al., 2018).

For plants treated with 30 µM hydrogen peroxide and irrigated with water salinity level of 3.2 dS m^{-1}, the Na/K ratio was 0.5546 (Figure 3B). It was observed that the Na/K ratio increased with the salinity of the irrigation water, regardless of the H_2O_2 concentration. This increase in the Na/K ratio in soursop leaves, with higher irrigation water salinity, indicates a preference for sodium absorption over potassium, suggesting a salt stress tolerance mechanism. Azevedo et al. (2004) noted that the ionic imbalance, reflected in the Na/K ratio, affects potassium ion selectivity in the roots due to compromised membrane integrity under saline conditions.

A significant interaction between the salinity levels of irrigation water and H_2O_2 concentrations was observed on the chlorophyll *total* and carotenoid contents in soursop plants (Table 3). The salinity of irrigation water significantly impacted all measured variables, except for maximum fluorescence and carotenoid content. Hydrogen peroxide concentrations significantly affected maximum fluorescence, variable fluorescence, and carotenoid levels.

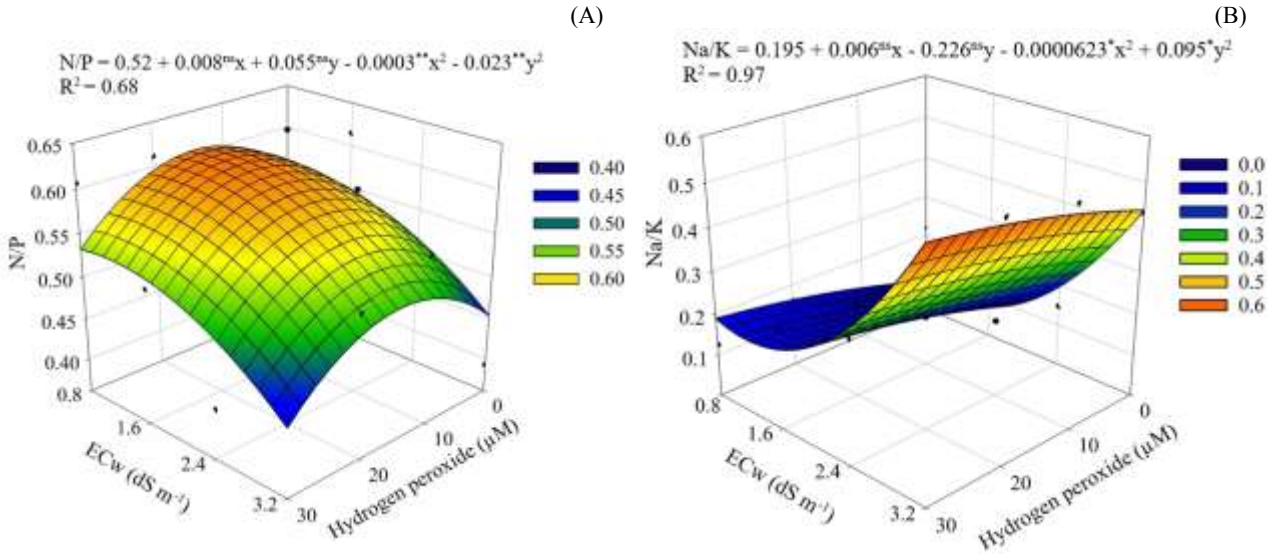

Figure 3. N/P (A) and Na/K (B) ratios in the leaves of soursop cv. Morada Nova plants as a function of ECw levels and hydrogen peroxide (H_2O_2) concentrations, 780 days post-transplantating. ns, *, not significant and significant at p≤0.05 by the F test, respectively. X and Y correspond to the concentrations of hydrogen peroxide and ECw, respectively.

Table 3
Summary of analysis of variance of photosynthetic variables in soursop cv. Morada Nova plants irrigated with saline water and subjected to foliar application of hydrogen peroxide, 370 days post-transplanting

Source of variation	DF	Mean square							
		F_0	Fm	Fv	Fv/Fm	Chl *a*	Chl *b*	Car	Chl *t*
Salt level (SL)	4	981.83**	26536.07^{ns}	41892.1**	0.00133*	0.937*	0.405*	0.0079^{ns}	8.066**
Linear regression	1	2926.01**	3816.03^{ns}	61248.15^{ns}	0.00345^{ns}	2.711^{ns}	1.025**	0.0006^{ns}	6.520^{ns}
Quadratic regression	1	14.08^{ns}	567.18^{ns}	47628.0**	0.00043**	0.099^{**s}	0.001^{ns}	0.0003^{ns}	13.27**
Hydrogen peroxide (H_2O_2)	4	846.94^{ns}	7365.74**	11471.25**	0.00036^{ns}	0.447^{ns}	0.047^{ns}	0.3551*	0.191^{ns}
Linear regression	1	0.066^{ns}	19747.2**	18096.06^{ns}	0.00037^{ns}	0.056^{ns}	0.090^{ns}	1.040**	0.105^{ns}
Quadratic regression	1	18.75^{ns}	123.52^{ns}	15624.08**	0.00014^{ns}	1.270^{ns}	0.008^{ns}	0.0012^{ns}	0.410^{ns}
Interaction (SL × H_2O_2)	16	499.96^{ns}	15198.39^{ns}	19360.56^{ns}	0.00020^{ns}	1.686^{ns}	0.508^{ns}	0.8036*	3.275*
Blocks	3	13.56^{ns}	126.06^{ns}	645.02^{ns}	0.000008^{ns}	0.1212^{ns}	0.725^{ns}	0.0060^{ns}	0.279^{ns}
Residual	30	49.49	2276.44	283.75	0.000289	0.3122	0.103	0.077	0.401
CV (%)		1.18	1.99	0.93	2.27	10.33	6.40	14.36	6.10

ns, *, and ** - not significant and significant at p≤0.05 and p≤0.01, respectively. F_0 - initial fluorescence; Fm - maximum fluorescence; Fv - variable fluorescence; Fv/Fm - quantum efficiency of photosystem II; Chl *a* - chlorophyll a; Chl *b* - chlorophyll b; Cl *t* - chlorophyll *total*; Car - carotenoids; DF - degrees of freedom. CV - coefficient of variation.

The increase in irrigation water electrical conductivity linearly decreased the initial fluorescence (F_0) in soursop cv. Morada Nova (Figure 4A). Relative comparisons showed that plants irrigated with higher salinity water (3.2 dS m^{-1}) exhibited a 3.63% decrease in F_0 compared to those irrigated with water at an ECw of 0.8 dS m^{-1}. This reduction in F0 might indicate a reduction in photochemical efficiency under salt stress, potentially impairing chlorophyll a activity and, consequently, ATP and NADPH synthesis. Dias et al. (2018) reported similar findings in a study on West Indian cherry (*Malpighia emarginata*) plants irrigated with saline water (ECw ranging from 0.8 to 3.8 dS m^{-1}), where water salinity decreased F0 by 21.49% as ECw increased from 0.8 to 3.8 dS m^{-1}.

The salinity of irrigation water had a quadratic effect on the variable fluorescence (Fv) in soursop cv. Morada Nova (Figure 4B), with the peak estimated Fv value of 1860.80 observed in plants irrigated with an ECw of 1.1 dS m^{-1} and the lowest value of 1732.90 in plants grown under an ECw of 3.2 dS m^{-1}. Comparatively, plants irrigated with the highest salinity level (3.2 dS m^{-1}) exhibited a 6.76% reduction in Fv, amounting to 125.68, relative to those grown under the lowest water salinity level (0.8 dS m^{-1}). Fv represents the capacity of a plant to transfer energy from electrons ejected from pigment molecules for the synthesis of NADPH, ATP, and reduced ferredoxin (Freire et al., 2014). Thus, a decrease in electron transport and the production of these compounds may inhibit photosynthesis, as they are crucial for CO_2 fixation in the Calvin cycle (Andrade et al., 2022). Studies by Dias et al. (2018) on West Indian cherry cv. BRS 366 Jaburu (ECw of 0.8 and 3.8 dS m^{-1}) and by Fernandes et al. (2022) on custard apple (ECw of 1.3 and 4.0 dS m^{-1}) under salt stress conditions showed reductions in Fv of 39.57% and 62.91%, respectively, as water salinity increased.

The impact of hydrogen peroxide on Fv in soursop plants (Figure 4C) indicated that a foliar application of 19 µM H_2O_2 resulted in the highest Fv value of 1839.53. Conversely, plants exposed to a 0 µM H_2O_2 concentration had the lowest Fv value of 1790.40. Hydrogen peroxide serves as a signaling molecule in hormonal regulation, balancing its synthesis and degradation, and is involved in biological processes such as osmotic adjustment through proline accumulation. Nonetheless, the beneficial effects of H_2O_2 depend on its concentration, the plant's developmental stage, and prior exposure to different types of stress (Liu et al., 2020).

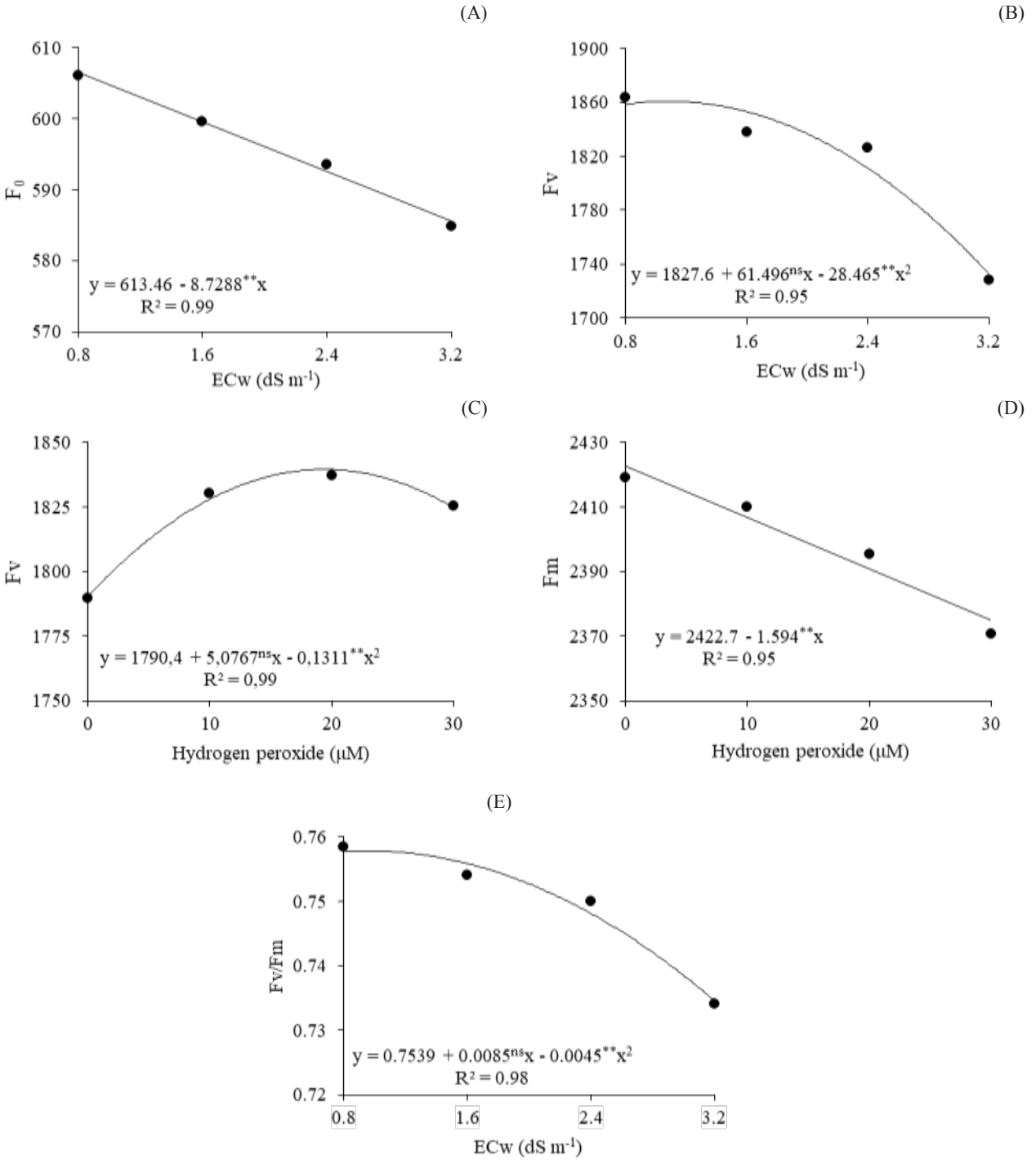

Figure 4. Initial fluorescence - F0 (A), variable fluorescence - Fv (B) and quantum efficiency of photosystem II - Fv/Fm (E) in soursop cv. Morada Nova plants as a function of irrigation water salinity (ECw); and variable fluorescence - Fv (C) and maximum fluorescence - Fm (D) as a function of hydrogen peroxide (H2O2) concentrations, 370 days post-transplantation. ns, *, ** not significant and significant at p≤0.05 and at p ≤ 0.01 by the F test, respectively.

Maximum fluorescence (Fm) was notably influenced by hydrogen peroxide foliar application (Figure 4D), with the estimated maximum value of 2422.70 observed in plants treated with 0 µM H_2O_2. In relative terms, there was a 2.01% decrease in Fm, equivalent to 47.82, as H_2O_2 concentration increased from 0 to 30 µM. Fm signals when the PSII reaction centers are closed and reach their maximum; hence, a reduction in this parameter with rising H_2O_2 levels may indicate constraints in PSII's capacity to reduce plastoquinone A (Akhter et al., 2021). At elevated concentrations, H_2O_2 can cause damage to different cellular biomolecules through mechanisms like protein oxidation, lipid peroxidation, enzyme deactivation, chlorophyll breakdown, and nucleic acid degradation under stress conditions (Hasanuzzaman et al., 2021).

The quantum efficiency of photosystem II in soursop was found to decline as the electrical conductivity of irrigation water increased (Figure 4E). Plants irrigated with water at an ECw of 3.2 dS m^{-1} exhibited a 13.76% (0.092) decrease in efficiency compared to those irrigated with 0.8 dS m^{-1} water. It was further noted that photoinhibition occurred in the FSII reaction centers or they became photochemically inactive from an ECw of 1.4 dS m^{-1} onwards, as evidenced by the alteration of Fv/Fm values, which typically range between 0.75 and 0.85 when the photosynthetic apparatus is intact (Reis & Campostrini, 2011). Carvalho et al. (2011) stated that salt stress-induced alterations in the photosynthetic process lead to an overproduction of reactive oxygen species (ROS), and in the absence of effective protective mechanisms (whether enzymatic or otherwise), oxidative damage can ensue due to metabolic disruptions.

Moreover, the increase in the salinity of irrigation water significantly affected the chlorophyll *a* content (Figure 5A), with the maximum estimated value recorded at 5.6131 mg g^{-1} FW in plants subjected to an ECw of 1.6 dS m^{-1}. Conversely, the minimum estimated value of 4.2515 mg g^{-1} FW was noted in plants grown under an ECw of 3.2 dS m^{-1}. The decline in Chl *a* content under salt stress suggests the disintegration of the thylakoid membrane structure (Sayyad-Amin et al., 2016) and an enhanced activity of chlorophylase, which breaks down photosynthetic pigment molecules, thereby causing a disruption and loss of function in pigment-related proteins (L. F. Cavalcante et al., 2011). Santos et al. (2023) observed similar reductions in Chl *a* content in yellow passion fruit plants exposed to salt stress, with ECw ranging from 0.6 to 3.0 dS m^{-1}.

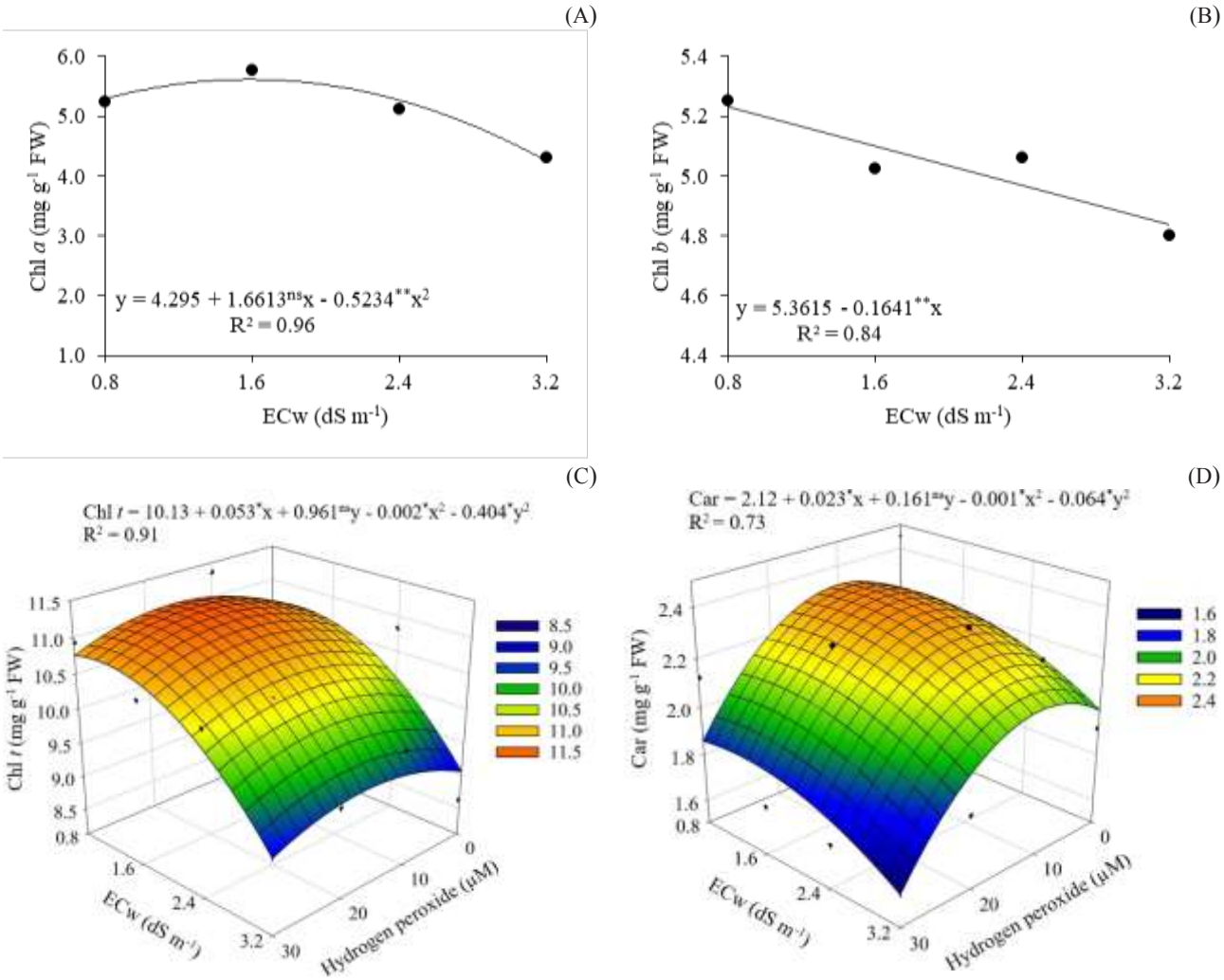

Figure 5. Chlorophyll *a* -Chl *a* (A) and chlorophyll *b* - Chl *b* (B) contents in soursop cv. Morada Nova plants as a function of irrigation water salinity (ECw); and chlorophyll total - Chl *t* (C), and carotenoid - Car (D) contents as a function of ECw levels and hydrogen peroxide (H_2O_2) concentrations, 370 days post-transplantation.
ns, *, ** not significant and significant at p≤0.05 and at p ≤ 0.01 by the F test, respectively. X and Y correspond to the concentrations of hydrogen peroxide and ECw, respectively.

The chlorophyll *b* levels in soursop exhibited a linear decrease as the salinity of the irrigation water increased (Figure 5B), with a 3.06% decline for every unit increase in ECw. A notable 8.14% decrease in Chl *b* was observed when comparing plants irrigated with the highest salinity level (3.2 dS m^{-1}) to those receiving the lowest-salinity water (0.8 dS m^{-1}). Sá et al. (2019) suggested that this reduction in chlorophyll synthesis could be due to an increase in the production of 5-aminolevulinic acid, a precursor in chlorophyll biosynthesis that also contributes to the degradation of

photosynthetic pigments. Similar reductions in Chl *b* levels under saline conditions (ECw ranging from 0.3 to 3.0 dS m^{-1}) were reported by Veloso et al. (2020) in their study on soursop physiology.

Additionally, an increase in irrigation water salinity was found to decrease the chlorophyll total content in soursop (Figure 5C), which reached a minimum of 9.06 mg g^{-1} FW in plants subjected to an ECw of 3.2 dS m^{-1} and 0 µM H_2O_2. The beneficial effects of application of H_2O_2 at 16 µM are potentially due to the role of this ROS in enhancing plant tolerance to salt stress through the increased synthesis of metabolites (Nazir et al., 2020).

Regarding carotenoid content (Figure 5D), the combination of a 10 µM H_2O_2 concentration with an ECw of 2.0 dS m^{-1} resulted in the highest observed value (2.3 mg g^{-1} FW). Conversely, the lowest carotenoid content (1.67 mg g^{-1} FW) was measured in plants irrigated with water of highest saline concentration (3.2 dS m^{-1}) and treated with 30 µM of foliar H_2O_2. Carotenoids, which function between the light-harvesting complexes and the lipid phase of thylakoid membranes, reduce membrane fluidity and susceptibility to lipid peroxidation. Therefore, a decrease in carotenoid levels indicates possible degradation of ß-carotene and reduced zeaxanthin formation (Taibi et al., 2016). It is proposed that the appropriate concentration of H_2O_2 (10 µM) may activate antioxidant enzymes that help to mitigate oxidative damage and improve the physiological attributes of plants under stress conditions (Dito & Gadallah, 2019).

Conclusions

The application of hydrogen peroxide at 10 and 16 µM concentrations mitigates the impact of salt stress on carotenoid and chlorophyll *total* levels in soursop cv. Morada Nova plants up to ECw levels of 2.0 and 1.6 dS m^{-1}, respectively, 370 days post-transplanting.

Saline water irrigation starting at 0.8 dS m^{-1} adversely influences chlorophyll *b* synthesis, initial fluorescence, and the quantum efficiency of photosystem II in soursop cv. Morada Nova.

Hydrogen peroxide at 12 and 9 µM concentrations increases leaf phosphorus and potassium levels up to ECw of 2.5 and 1.8 dS m^{-1}, respectively, in soursop cv. Morada Nova plants, 780 days post-transplantation.

Sodium and chlorine levels in the leaves of soursop cv. Morada Nova increase with the salinity of irrigation water.

A 30 µM concentration of H_2O_2 intensifies salt stress effects on the foliar macronutrient content of soursop cv. Morada Nova plants, 780 days post-transplanting.

In the pre-flowering phase, nutrient accumulation in the leaves of soursop cv. Morada Nova follows a descending order of P > N > K > S > Cl > Na.

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