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Original Article

Gas exchange and growth of soursop under salt stress and H_2O_2 application methods

Trocas gasosas e crescimento de graviola sob estresse salino e métodos de aplicação de H_2O_2

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Abstract

The production of soursop seedlings in the Northeast region of Brazil has faced limitations due to the high concentrations of salts in the water, so it is necessary to use techniques that enable its cultivation, and the application of hydrogen peroxide to minimize the deleterious effects of salt stress stands out. In this context, the objective was to evaluate forms of application of hydrogen peroxide as an attenuator of salt stress on the gas exchange and growth of soursop seedlings cv. Morada Nova. The experiment was conducted under greenhouse conditions, in an Regolithic Neosol of sandy loam texture, from the municipality of Lagoa Seca - PB. A completely randomized design was used in a 5 × 4 factorial arrangement, whose treatments resulted from the combination of five levels of electrical conductivity of irrigation water - ECw (0.6 – control, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}) and four forms of hydrogen peroxide application (M1 - without H_2O_2 application, M2 - application by seed soaking, M3 - application by foliar spraying, and M4 - application by seed soaking + foliar spraying), with four replicates and two plants per experimental unit, totaling 160 plants. The concentration of H_2O_2 used in the different forms of application was 20 µM. Irrigation water salinity from 0.6 dS m^{-1} reduced the gas exchange and growth of soursop. The method of H_2O_2 application by foliar spraying minimized the effects of salt stress on gas exchange. The method of H_2O_2 application by seed soaking reduced the effect of salt stress on the growth of soursop at 85 days after sowing.

Keywords: *Annona muricata* L, acclimatization, hydrogen peroxide, saline water.

Resumo

A produção de mudas de gravioleira na região semiárida do Brasil tem enfrentado limitações devido às elevadas concentrações de sais nas águas, sendo necessário o uso de técnicas que viabilizem seu cultivo, destacando-se a aplicação do peróxido de hidrogênio. Neste contexto, objetivou-se avaliar as formas de aplicação do peróxido de hidrogênio como atenuante do estresse salino nas trocas gasosas e no crescimento de mudas de gravioleira cv. Morada Nova. O estudo foi conduzido sob condições de casa de vegetação, em um Neossolo Regolítico de textura franco-arenosa, utilizando-se o delineamento inteiramente casualizado, no arranjo fatorial 5 × 4, cujos tratamentos resultaram da combinação de cinco níveis de condutividade elétrica da água de irrigação - CEa (0,6 - testemunha; 1,2; 1,8; 2,4 e 3,0 dS m^{-1}) e quatro formas de aplicação de peróxido de hidrogênio (M1- sem aplicação de H_2O_2, M2 - aplicação via embebição das sementes, M3 -aplicação por pulverização foliar e M4 - aplicação via embebição das sementes + pulverização foliar), com quatro repetições e duas plantas por unidade experimental, perfazendo o total de 160 plantas. A concentração de H_2O_2 utilizada nas diferentes formas de aplicação foi de 20 µM. A salinidade da água de irrigação a partir de 0,6 dS m^{-1} diminuiu as trocas gasosas e o crescimento das plantas de gravioleira. O método de aplicação do H_2O_2 via pulverização foliar minimizou o efeito do estresse salino sobre as trocas gasosas. O método de aplicação do H_2O_2 via embebição das sementes diminuiu o estresse salino no crescimento de gravioleira aos 85 dias após o semeio.

Palavras-chave: *Annona muricata* L., aclimatação, peróxido de hidrogênio, águas salinas.

1. Introduction

Belonging to the Annonaceae family, soursop (*Annona muricata* L.) has been gaining prominence in the national market, due to the growing demand for its fruits for fresh consumption and in the agroindustry, in addition to the possibility of use in the pharmaceutical and cosmetics industry (Freitas et al., 2013). In Brazil, it is one of the

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best-known fruits in the market in the North and Northeast regions and the State of Bahia is the largest producer with 4,509.000 tons of fruits according to Censo Agro (IBGE, 2017), standing out for its commercialization potential in the domestic market, with relevant economic importance and prospects for export, due to the high acceptance of its fruit and pulp (Cavalcante et al., 2016).

In the semi-arid region of northeastern Brazil, soursop cultivation is limited, because the water available for irrigation commonly contains high concentrations of salts resulting from high evaporation and low precipitation, which reduces the quality of this resource for use in agriculture (Veloso et al., 2018; Sá et al., 2018).

Salt stress negatively affects leaf gas exchange and reduces photosynthesis, transpiration, and stomatal conductance of plants (Pan et al., 2021). The excess of salts also reduces the osmotic potential of the soil solution, limits the availability of water for plants, causing a reduction in turgidity and expansion of the leaf area, harming the growth and development of crops (Sá et al., 2021; Silva et al., 2022).

Excessive accumulation of ions in plant tissues, especially Na^+ and Cl^-, can affect other processes, such as cell division and differentiation, enzyme activity, and absorption and distribution of nutrients (Voigt et al., 2009). However, the degree to which salt stress influences germination and the initial growth of seedlings is dependent on many factors such as plant species, irrigation and fertilization management, and climatic conditions (Dutra et al., 2017).

Despite the negative effects of salts, irrigation with saline water can be made possible through the use of hydrogen peroxide (H_2O_2) (Andrade et al., 2019). Seed pre-treatment or plant exposure to H_2O_2 is an important mechanism to modulate cross-tolerance through action on various physiological processes, such as photosynthesis and stomatal regulation, and on different stress-responsive pathways, such as detoxification of reactive species of oxygen (ROS) (Veloso et al., 2022).

In this context, several studies have evaluated the effects of hydrogen peroxide as an attenuator of salt stress, such as that of Silva et al. (2016), who found that the application of H_2O_2 in concentrations of 7 to 8 µM at sowing and by foliar spraying promoted acclimatization of maize plants to irrigation water salinity of 2.0 dS m^{-1} in the initial growth stage. Similarly, Silva et al. (2018), working with soursop plants cv. 'Morada Nova' subjected to irrigation water salinity ranging from 0.7 to 3.5 dS m^{-1}, found that pre-treatment with seed soaking and foliar spraying mitigated the deleterious effects of irrigation water salinity on the emergence and growth of soursop at 85 and 145 days after sowing.

Although there are studies evaluating the effects of hydrogen peroxide on soursop, they are limited only to the application of different concentrations. Thus, studies are needed to evaluate the best way to apply hydrogen peroxide during the formation of soursop seedlings, considering that the beneficial effect of H_2O_2 does not depend only on the concentration but also on the way it is applied. Given the above, the objective of this study was to evaluate the effects of hydrogen peroxide application methods as attenuators of salt stress on the gas exchange and growth of soursop seedlings cv. 'Morada Nova'.

2. Material and Methods

2.1. Location and characterization of the area

The experiment was conducted between April and September 2019, under greenhouse conditions at the Academic Unit of Agricultural Engineering of the Federal University of Campina Grande (UAEA/UFCG), in Campina Grande, PB, Brazil, located at the geographic coordinates of 7º 15' 18" S, 35º 52' 28" W and altitude of 550 m. The data of temperature (maximum and minimum) and average relative humidity of air observed during the experimental period at the experimental site are shown in Figure 1.

2.2. Treatments

The treatments consisted of the combination of five levels of electrical conductivity of irrigation water - ECw (0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}) and four methods of ap plication of hydrogen peroxide - H_2O_2 (M1 - without application of H_2O_2, M2 - application by seed soaking, M3 -

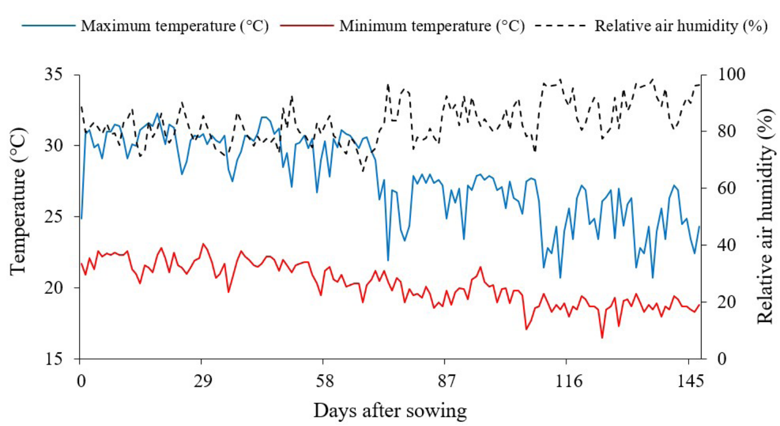

Figure 1. Air temperature (maximum and minimum) and average relative air humidity inside the greenhouse during the experimental period.

application by foliar spraying and M4 - application by seed soaking + foliar spraying), in a 5 × 4 factorial arrangement, distributed in a completely randomized design, with four replicates and two plants per experimental unit, totaling 160 plants. The salinity levels used here were based on studies conducted by Silva et al. (2019a).

2.3. Preparation of water and doses of hydrogen peroxide

The irrigation waters with different electrical conductivity 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 water from the municipal supply (ECw = 0.38 dS m^{-1}). Irrigation waters were prepared considering the relationship between ECw and the sum of cations (Richards, 1954). The irrigation events with the saline waters were performed manually and daily, applying the volume corresponding to that obtained by the water balance, and the volume of water to be applied to the plants was determined by Equation 1:

$$\mathrm{VI} = \frac{(\mathrm{Va} - \mathrm{Vd})}{(1 - \mathrm{LF})} \tag{1}$$

where: VI = Volume of water to be applied in the next irrigation event (mL); Va and Vd = volume applied and drained in the previous irrigation event (mL); and LF = leaching fraction of 0.10, applied every 15 days.

The seeds of the treatments Soaking and Soaking + Foliar spraying underwent a pre-treatment with H_2O_2, in which they were soaked in a solution at a concentration of 20 µM for a period of 36 h in the dark. The H_2O_2 concentration and soaking time were established according to Silva et al. (2019b), who observed that the gas exchange and the growth variables of soursop seedlings cv. 'Morada Nova' showed deleterious effects caused by salinity of irrigation water, mitigated by exogenous application of H_2O_2 at the concentration of 20 µM. The concentration was obtained by dilution of H_2O_2 in distilled water, which was stored in a dark environment soon after preparation.

At 70, 85, 100, 115 and 130 days after sowing (DAS), plants of the treatment M3 (foliar spraying) and M4 (seed soaking + foliar spraying) were subjected to applications of H_2O_2 (20 µM). The applications were performed manually at 17:00 h, by spraying the abaxial and adaxial sides of the leaves, in order to completely wet the foliage, using a sprayer.

2.4. Acquisition of soil and seeds

Plastic bags of 2 dm^3 were filled with 2.6 kg of an air-dried substrate composed (v/v) of soil (84%), sand (15%) and humus (1%). The soil used in the experiment was an Neossolo Regolítico of sandy loam texture, collected in the 0-20 cm layer, from the rural area of the municipality of Lagoa Seca, PB, properly pounded to break up clods and sieved. Its physical-hydraulic and chemical characteristics (Table 1) were determined according to the methodologies of Teixeira et al. (2017).

The soursop cultivar 'Morada Nova' was evaluated in this study because it is the genetic material preferred by farmers in the Northeast region, composing the majority of commercial orchards in Brazil, as it has higher number of favorable characters, such as high yield, better fruit quality, and lower susceptibility to fruit borer (*Cerconota anonella* Sepp.) (Costa et al., 2016).

Then, three soursop seeds were sown at 3 cm depth and distributed equidistantly; at 40 days after sowing, thinning was carried out, in order to leave only one plant per bag, preserving the one with best vigor.

Prior to sowing, the moisture content of the substrate was increased until reaching the maximum water retention capacity in the container, using water according to each treatment. Irrigation was performed daily by applying in each plastic bag a volume of water sufficient to keep the substrate moisture content close to the maximum capacity of the container. The volume applied was determined according to the water requirement of the plants, estimated by the water balance by subtracting the volume drained from the volume applied in the previous irrigation, plus a leaching fraction of 0.10 every 20 days (Ayers and Westcot, 1999).

2.5. Fertilization

Fertilization with nitrogen, potassium, and phosphorus was carried out as top-dressing, based on the recommendation

Table 1. Chemical and physical-hydraulic attributes of the soil used in the experiment, before the application of the treatments.

Chemical characteristics									
pH (H_2O) (1:2.5)	**OM %**	**P (mg kg^{-1})**	**K^+**	**Na^+**	**Ca^{2+}**	**Mg^{2+}**	**$Al^{3+} + H^+$**	**ESP (%)**	**ECse (dS m^{-1})**
			..........(cmol$_c$ kg^{-1})..........						
5.90	1.36	6.80	0.22	0.16	2.60	3.66	1.93	1.87	1.0

Physical-hydraulic characteristics									
Size fraction (dag kg^{-1})			**Textural class**	**Water content (kPa)**		**AW**	**Total porosity %**	**AD**	**PD**
Sand	**Silt**	**Clay**		**33.42**	**1519.5 dag kg^{-1}**			**(kg dm^{-3})**	
732.9	142.1	125.0	FA	11.98	4.32	7.66	47.74	1.39	2.66

OM = Organic matter: Walkley-Black Wet Digestion; Ca^{2+} and Mg^{2+} extracted with 1 M L^{-1} KCl at pH 7.0; Na^+ and K^+ extracted with 1 M L^{-1} NH_4Oac at pH 7.0; Al^{3+} and H^+ extracted with 0.5 M L^{-1} calcium acetate at pH 7.0; ESP = Exchangeable sodium percentage; ECse = Electrical conductivity of saturation extract; SL = Sandy loam; AW = Available water; AD = Apparent density; PD = Particle density proposed by Novais et al. (1991), applying 0.58 g of urea, 0.65 g of potassium chloride and 1.56 g of monoammonium phosphate, equivalent to 100, 150 and 300 mg kg^{-1} of N, K_2O and P_2O_5, respectively, in four equal portions via fertigation, at intervals of 15 days, with the first application performed at 15 DAS. In order to meet the need for micronutrients, the leaves were sprayed at 60, 75, and 90, 105, 120 and 135 DAS, with 2.5 g L^{-1} of a solution with the following composition: 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%).

2.6. Analyzed variables

Gas exchange was evaluated at 145 DAS through stomatal conductance - *gs* (mol H_2O m^{-2} s^{-1}), transpiration - *E* (mmol H_2O m^{-2} s^{-1}), CO_2 assimilation rate - *A* (μmol CO_2 m^{-2} s^{-1}) and internal CO_2 concentration - *Ci* (μmol CO_2 m^{-2} s^{-1}), measured on the third leaf, counted from the apex, using the portable photosynthesis meter "LCPro+" from ADC BioScientific Ltda. These data were then used to quantify the instantaneous water use efficiency (*WUEi*) (*A/E*) [(μmol m^{-2} s^{-1})(mol $H_2O m^{-2}$ s^{-1})$^{-1}$] and the instantaneous carboxylation efficiency (*CEi*) (*A/Ci*) [(μmol m^{-2} s^{-1})(μmol mol^{-1})$^{-1}$]. At 85 and 145 DAS, the following growth variables were evaluated: plant height (PH), stem diameter (SD), number of leaves (NL), leaf area (LA).

Plant height (cm) was measured using as reference the distance from the collar to the insertion of the apical meristem, stem diameter (mm) was measured at 2 cm from the collar, and the number of leaves was obtained by counting fully expanded leaves with a minimum length of 3 cm in each plant.

Leaf area (cm^2) was determined as recommended by Almeida et al. (2006), considering Equation 2:

$$LA = 5.71 + 0.647 LW \tag{2}$$

where: LA - Leaf area (cm^2); L - Leaf length (cm); W- Leaf width (cm).

2.7. Statistical analysis

The data were analyzed for normality (Shapiro-Wilk test) and subsequently the data were subjected to analysis of variance by the F test at 0.05 and 0.01 probability levels and, when significant, linear and quadratic polynomial regression analysis was performed for the salinity levels while for the methods of H_2O_2 application Tukey test ($p \leq 0.05$) was applied, using the statistical program SISVAR-ESAL (Ferreira, 2019).

3. Results and Discussion

There was a significant effect ($p \leq 0.01$) of the interaction between methods of H_2O_2 application and irrigation water salinity on stomatal conductance (*gs*), transpiration (*E*), and CO_2 assimilation rate (*A*) (Table 2). The irrigation water salinity, as single factor, influenced ($p \leq 0.01$) all variables analyzed, except the internal CO_2 concentration (*Ci*). On the other hand, the methods of H_2O_2 application promoted significant effects ($p \leq 0.01$) on *gs*, *E*, *A*, and *Ci*.

The increase in irrigation water salinity from 1.2 dS m^{-1} negatively affected the stomatal conductance (Figure 2A) and transpiration (Figure 2B) of soursop plants, causing reductions of 36.37% (0.024 mol H_2O m^{-2} s^{-1}) in *gs* and 37.31% (0.5 mmol H_2O m^{-2} s^{-1}) in *E* in plants that did not receive H_2O_2 and irrigated with the water of highest salinity (3.0 dS m^{-1}). The reductions in *gs* and *E* occurred due to osmotic stress, which causes physiological changes in plants, such as stomatal closure, with a consequent decrease in transpiration and reduction of photosynthetic rate (Neves et al., 2009).

However, despite the reductions in *gs* and *E* with increasing salinity, plants that had their leaves sprayed with H_2O_2 reached higher *gs* (0.077, 0.0725, 0.070 and 0.0625 mol H_2O m^{-2} s^{-1}), respectively at 0.6, 1.2, 1.8 and 2.4 dS m^{-1} and the highest values of *E* (1.62, 1.55, 1.40, 1.25 and 1.11 mmol H_2O m^{-2} s^{-1}) at levels of salinity of 0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}, respectively when compared with the other application methods. For Forman et al. (2010), pre-exposure of plants to either signaling metabolites such as H_2O_2 or moderate stresses may result in metabolic signaling of the cell and hence better physiological performance when these plants are exposed to severe stress conditions, which may have occurred in this study with plants subjected to the application method by foliar spraying with H_2O_2.

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' irrigated with saline waters and subjected to different methods of hydrogen peroxide application, at 145 days after sowing.

Source of variation	DF	Mean Squares					
		gs	***E***	***A***	***Ci***	***CEi***	***WUEi***
Salinity levels (SL)	4	0.00075**	0.2056**	5.061**	1623.80^{ns}	0.000173**	0.1202**
Linear regression	1	0.00066**	0.0695**	1.637**	-	0.000061**	0.367**
Quadratic regression	1	0.00031^{ns}	0.0796^{ns}	1.525^{ns}	-	0.000153^{ns}	0.0001^{ns}
Application methods (AM)	3	0.00080**	0.3654**	8.0284**	4180.24**	0.000084^{ns}	0.0385^{ns}
Interaction (SL × AM)	12	0.00051**	0.1466**	5.013**	1604.05^{ns}	0.000191^{ns}	1.130^{ns}
Residue	3	0.00004	0.1734	0.2555	498.91	0.000031	0.176
CV (%)		14.63	17.18	8.87	12.87	10.26	6.39

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

As with *gs* and *E* (Figure 2A and 2B), the CO_2 assimilation rate of soursop plants was also influenced by the interaction between the factors (salinity levels and methods of H_2O_2 application) and, according to the means comparison test (Figure 3A), there was a reduction in *A* with the increase in ECw. The decrease in CO_2 assimilation rate may be a result of the low availability of water to the plants due to the increase in ECw, causing stomatal closure, which in turn restricts the entry of CO_2 into leaf mesophyll cells (Munns and Tester, 2008; Silva et al., 2010).

Despite the reduction in *A*, it can be noted that soursop plants that received H_2O_2 through foliar spraying obtained the highest values of *A* (8.35, 7.94, 7.32, 6.39 and 5.17 μmol CO_2 m^{-2} s^{-1}), respectively for 0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}. For plants that did not receive H_2O_2 application and were irrigated with water of 3.0 dS m^{-1}, there was a reduction of 24.87% (1.51 μmol CO_2 m^{-2} s^{-1}) compared to those under the lowest salinity level (0.6 dS m^{-1}). Silva et al. (2019c), studying the effects of salt stress (ECw ranging from 0.7 to 3.5 dS m^{-1}) and exogenous application of H_2O_2 on the photosynthetic variables of soursop, verified that the pre-treatment of soursop plants with hydrogen oxide by seed soaking (25 and 50 μM) attenuated the deleterious effects of salt stress on stomatal conductance and CO_2 assimilation rate, which disagrees with the results observed in the present study.

The internal CO_2 concentration of soursop plants differed significantly among the methods of H_2O_2 application. As shown in Figure 3B, the application of H_2O_2 by seed soaking resulted in the highest value of *Ci* (208 μmol CO_2 m^{-2} s^{-1}), corresponding to an increase of 16.82% (35 μmol CO_2 m^{-2} s^{-1}) compared to plants that were not subjected to H_2O_2 application.

The method of H_2O_2 application via seed soaking led to a higher value in *Ci,* possibly because the seeds had a previous contact before exposure to salt stress. Similarly, Silva et al. (2019d) conducted a study with passion fruit plants under salt stress (0.7, 1.4, 2.1 and 2.8 dS m^{-1}) and observed that H_2O_2 application at 0.25, 50 and 75 μM by seed soaking mitigated the deleterious effects caused by irrigation water salinity on the internal CO_2 concentration.

The salinity of irrigation water inhibited the instantaneous carboxylation efficiency (*CEi*) (Figure 4A) and

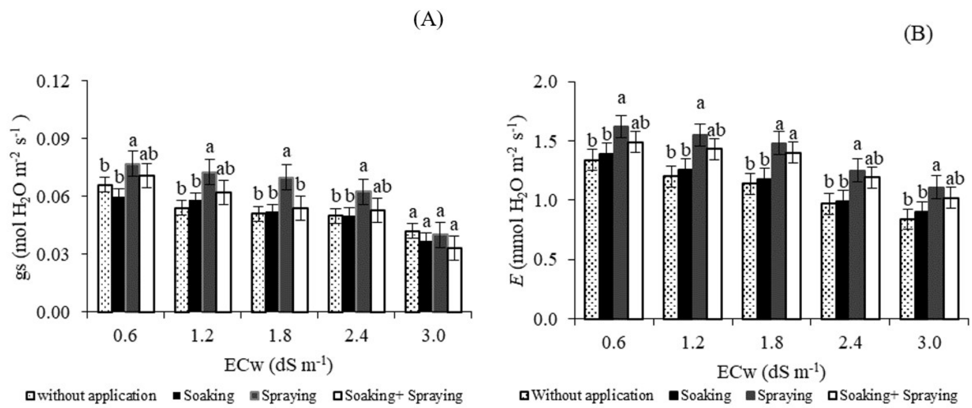

Figure 2. Stomatal conductance - *gs* (A) and transpiration - (*E*) (B) of soursop plants cv. Morada Nova as a function of the interaction between irrigation water salinity - ECw and methods of hydrogen peroxide application, at 145 days after sowing. Means followed by the same letter do not differ statistically from each other by the Tukey test (p≤0.05). Vertical bars represent the standard error of the mean (n=4).

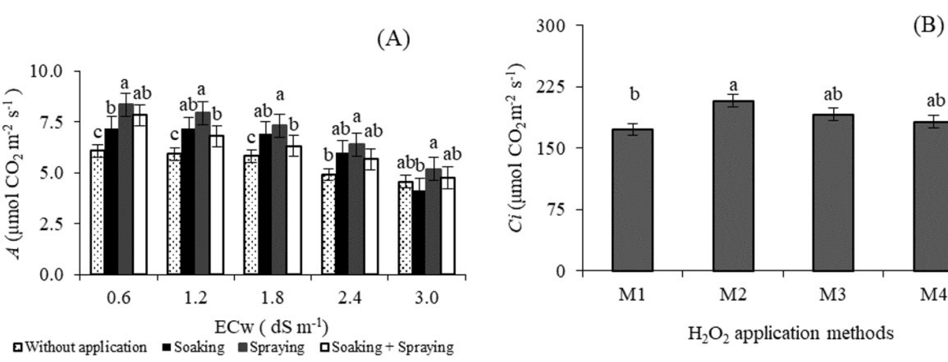

Figure 3. CO_2 assimilation rate - *A* (A) of soursop cv. Morada Nova, as a function of the interaction between irrigation water salinity - ECw and methods of hydrogen peroxide application, and internal CO_2 concentration - *Ci* (B) as a function of the methods of hydrogen peroxide application, at 145 days after sowing. M1= without H_2O_2 application, M2 = application via seed soaking, M3 = application via foliar spraying, and M4 = application via seed soaking and foliar spraying; Means followed by the same letter do not differ statistically from each other by Tukey test (p<0.05).

instantaneous water use efficiency (*WUEi*) (Figure 4B) of soursop at 145 DAS; according to the regression equations, there were reductions of 8.50% in CEi and 4.97% in *WUEi* with per unit increment of ECw. In relative terms, a comparison of the results obtained in plants irrigated with water of highest salinity (3.0 dS m^{-1}) to those of plants irrigated with the lowest salinity level (0.6 dS m^{-1}) pointed to reductions of 24.32% [0.009 (μmol m^{-2} s^{-1})(μmol mol^{-1})$^{-1}$] in *CEi* and 13.54% [0.75 (μmol m^{-2} s^{-1})(mol H_2O m^{-2} s^{-1})$^{-1}$] in *WUEi*.

According to Oliveira et al. (2017), with the increase in salt concentration in the soil, the plant has greater difficulty in absorbing water and nutrients. Consequently, to avoid excessive water loss, they close their stomata, restricting the entry of CO_2 into the sub-stomatal chamber, compromising carboxylation efficiency and water use efficiency, in addition to other variables.

According to the summary of the analysis of variance (Table 3), there were significant effects ($p \le 0.01$) of the interaction between irrigation water salinity and methods of H_2O_2 application on plant height (PH), stem diameter (SD), leaf area (LA) and the number of leaves (NL) of soursop plants, at 85 DAS, and on leaf area (LA) at 145 DAS. The irrigation water salinity as a single factor significantly affected ($p \le 0.01$) all variables analyzed at 85 and 145 DAS. On the other hand, the methods of H_2O_2 application caused significant effects on PH, SD, LA, and NL at 85 DAS and PH at 145 DAS.

By analyzing the interaction between the salinity levels of irrigation water and the methods of H_2O_2 application for plant height (Figure 5A), it is possible to observe that the height of soursop plants cv. 'Morada Nova' was reduced at 85 DAS due to increased salinity. It was observed that the method of H_2O_2 application via seed soaking led to the highest PH (23.5, 21.75, 18.23, 16.6 and 15 cm) for the ECw levels of 0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}, respectively. Gondim et al. (2010), analyzing the pre-treatment with H_2O_2 in maize seeds and its effects on germination and acclimatization of seedlings to salt stress, concluded that pre-treatment via seed soaking decreases the deleterious effects of salinity on maize growth. This result may have occurred due to the role of H_2O_2 as a signaling molecule under biotic and abiotic stresses. In addition, H_2O_2 acts by inducing the antioxidant enzyme defense system when applied at low concentrations, attenuating the deleterious effects of salinity (Carvalho et al., 2011; Petrov and van Breusegem, 2012).

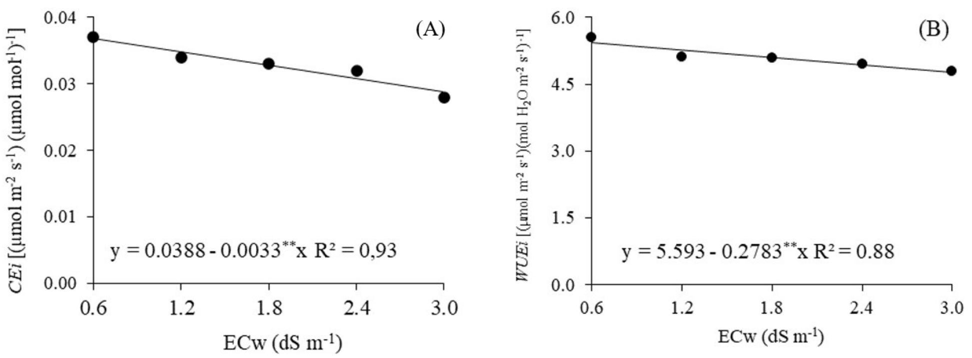

Figure 4. Instantaneous carboxylation efficiency - *CEi* (A) of soursop plants cv. Morada Nova and instantaneous water use efficiency - *WUEi* (B) as a function of the levels of electrical conductivity of irrigation water (ECw), at 145 days after sowing. **Significant at $p \le 0.01$ by F test.

Table 3. Summary of the analysis of variance for plant height (PH), stem diameter (SD), leaf area (LA), and number of leaves (NL) of soursop plants cv. 'Morada Nova' irrigated with saline waters and subjected to different methods of hydrogen peroxide application, at 85 and 145 days after sowing (DAS).

Source of variation	DF	Mean Squares							
		PH		SD		LA		NL	
		Days after sowing							
		85	145	85	145	85	145	85	145
Salinity levels (SL)	4	213.19**	763.97**	8.56**	33.09**	100882.3**	7400070.8**	78.68**	109.50**
Linear regression	1	788.68**	3043.2**	31.60**	131.18**	393131.7**	1558713.8**	391.93**	431.22**
Quadratic regression	1	60.14ns	1.43ns	2.55ns	0.453ns	6536.16ns	32524.8ns	1.08ns	1.264ns
Application methods (AM)	3	21.70**	11.42ns	1.51**	0.833ns	12340.4**	53953.95**	8.60**	7.37ns
Interaction (SL × AM)	12	6.83**	33.79ns	0.29**	1.94ns	2344.54ns	54587.0**	6.57**	16.0ns
Residue	3	1.49	2.21	0.263	0.202	692.62	5967.1	0.492	7.90
CV (%)		6.41	9.09	9.29	11.99	12.73	17.64	10.87	15.27

DF = degrees of freedom; CV = coefficient of variation; ns, **, * respectively not significant, significant at $p \le 0.01$ and at $p \le 0.0$.

The increase in irrigation water salinity caused a linear reduction in the height of soursop plants at 145 DAS and, according to the regression equation (Figure 5B), there were decreases of 18.73% per unit increment in ECw, equivalent to a reduction of 50.63% (17.44 cm) in plants irrigated with ECw of 3.0 dS m^{-1}, compared to those that were subjected to water salinity of 0.6 dS m^{-1}. The reduction in PH with the increase in water salinity may be related to water deficit induced by the osmotic effect, causing stomatal closure and a reduction of gas exchange, consequently reducing the absorption of water and nutrients by plants, which results in lower growth (Lima et al., 2015).

Regarding the stem diameter (SD) of soursop plants at 85 DAS (Figure 5C), the highest values (4.53, 4.06, 3.43, 3.11, and 2.72 mm) were obtained with the method of application via seed soaking at salinity levels of 0.6, 1.2, 1.8, 2.4, and 3.0 dS m^{-1}, respectively. Silva et al. (2019a) concluded that pre-treatment of seeds with H_2O_2 attenuates the deleterious effects of irrigation water salinity on the stem diameter of soursop cv. 'Morada Nova' at 110 days after sowing a result similar to that found in this study.

The salinity of irrigation water inhibited the development of stem diameter (Figure 5D) in soursop plants at 145 DAS; according to the regression equation, there was a reduction in SD of 19.38% per unit increment in ECw. In relative terms, a comparison of the results obtained in plants irrigated with water of highest salinity (3.0 dS m^{-1}) with those of plants irrigated with the lowest salinity level (0.6 dS m^{-1}) showed a reduction of 52.63% (3.62 mm) in SD. Reduction in soursop SD as a function of salt stress was also recorded by Veloso et al. (2019), studying the effects of saline water (ECw ranging from 0.3 to 3.5 dS m^{-1}) and exogenous application of H_2O_2 on soursop seedlings.

When studying the interaction between salinity levels and methods of H_2O_2 application, it was verified that the increase in the electrical conductivity of irrigation water above 1.2 dS m^{-1} negatively affected LA at 85 DAS (Figure 6A) and 145 DAS (Figure 6B). Decrease in leaf area under salt stress conditions is commonly considered a strategy of protection and/or acclimatization of plants to high salinity, reducing water losses through transpiration, and maintaining high water potential in the cell (Nascimento et al., 2019).

However, when H_2O_2 was applied by seed soaking, the harmful effect on LA, at 85 DAS caused by the increasing salinity of irrigation water was mitigated. It is also noted that the method of H_2O_2 application by foliar spraying led to the highest values of LA (797.42, 692.44, 546.7, and 516.93 cm^2) for 0.6, 1.2, 1.8, and 2.4 dS m^{-1}, respectively, at 145 DAS. Gondim et al. (2011) studied the effects of H_2O_2 on the growth and accumulation of solutes in maize plants under salt stress. Silva et al. (2016), while studying methods of application of different concentrations of H_2O_2 in maize under salt stress, verified that the pre-treatment of maize plants by spraying with H_2O_2 induced acclimatization of

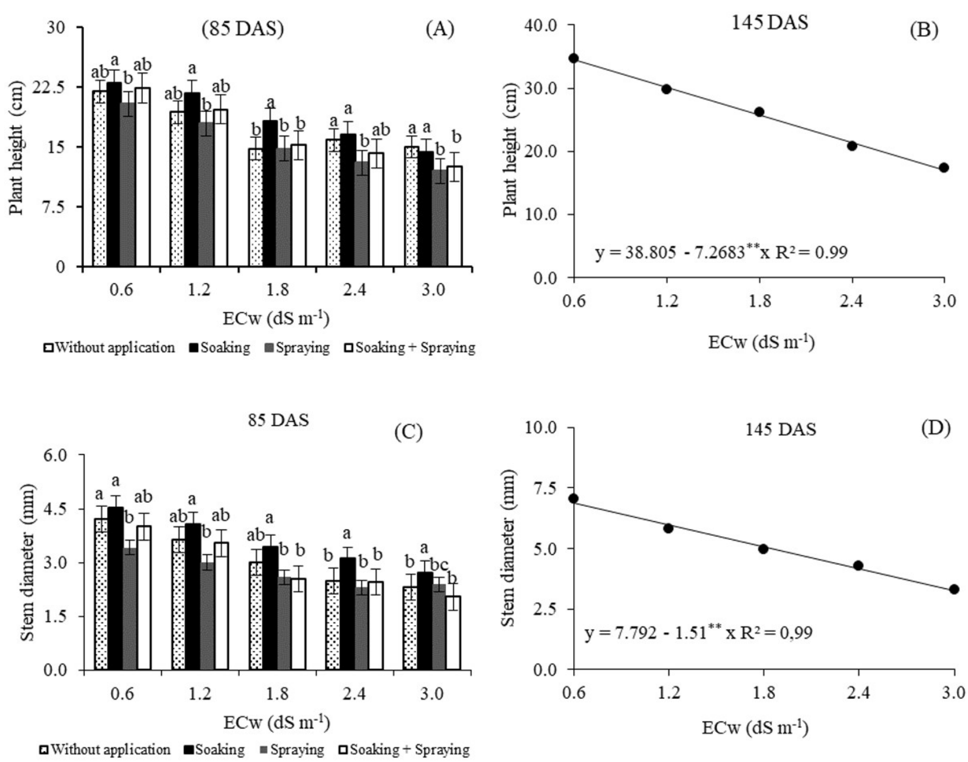

Figure 5. Plant height (A) and stem diameter (C) of soursop cv. Morada Nova at 85 days after sowing (DAS)) as a function of the interaction between irrigation water salinity - ECw and methods of hydrogen peroxide application; and plant height (B) and stem diameter (D) at 145 DAS as a function of the levels of electrical conductivity of irrigation water (ECw). **Significant at p≤0.01 by F test. Means followed by the same letter do not differ statistically from each other by Tukey test (p≤0.05).

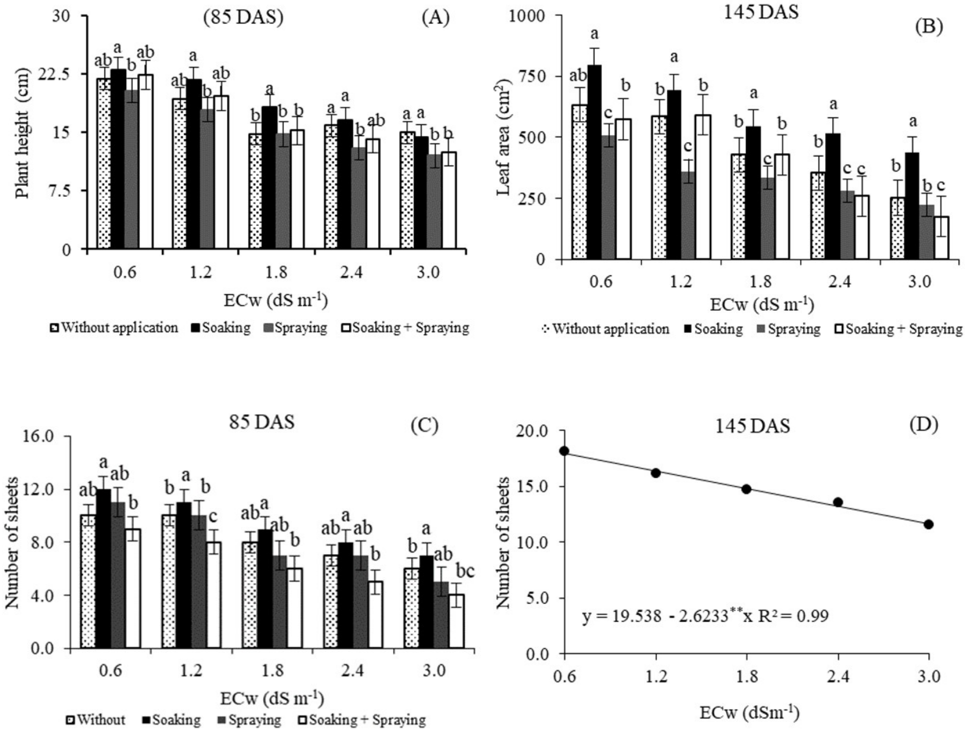

Figure 6. Leaf area at 85 (A) and 145 (B) days after sowing (DAS), and number of leaves (C) at 85 DAS of soursop plants cv. Morada Nova, as a function of the interaction between irrigation water salinity - (ECw) and methods of hydrogen peroxide application; and number of leaves (D) at 145 DAS as a function of the levels of electrical conductivity of irrigation water (ECw). **Significant at $p \leq 0.01$ by F test. Means followed by the same letter do not differ statistically from each other by the Tukey test ($p \leq 0.05$). Vertical bars represent the standard error of the mean (n = 4).

plants, a result similar to that found in this study for leaf area, at 145 DAS.

Despite the reduction in the number of leaves of soursop plants (NL) at 85 DAS, with the increase in water salinity, the means comparison test (Figure 6C) showed that the application of H_2O_2 through seed soaking promoted a higher value of NL at all salinity levels when compared to the other methods. Similarly, Santos et al. (2019) studied the influence of H_2O_2 application methods on melon plants subjected to salt stress (0.3 and 2.0 dS m^{-1}) and observed that H_2O_2 application by seed soaking mitigated the effect of salts on the number of melon leaves.

Regarding the number of leaves of soursop at 145 DAS (Figure 6D) it was observed that irrigation with waters of increasing salinity had a decreasing linear effect on NL, with reductions of 13.43% per unit increase in ECw, that is, a reduction of 35.03% in the NL of plants irrigated with the water of highest salinity (3.0 dS m^{-1}) in comparison to those subjected to the lowest salinity level (0.6 dS m^{-1}). Silva et al. (2019d) observed in a study with soursop seedlings under salt stress (ECw ranging from 0.7 to 3.5 dS m^{-1}) reductions in the number of leaves with increased electrical conductivity of irrigation water at 145 DAS.

Excess of salts in the soil hinder the entry of water into plant cells, leading to changes in photosynthetic capacity (CO_2 assimilation rate, transpiration, and stomatal conductance), inhibiting growth (Lima et al., 2016). Thus, reduced number of leaves under salt stress conditions is a stress tolerance mechanism adopted by the plant to reduce water losses by transpiration (Nobre et al., 2014).

4. Conclusions

Irrigation water salinity above 0.6 dS m^{-1} reduces the gas exchanges at 145 days after sowing and the growth of soursop plants at 85 and 145 days after sowing. The method of H_2O_2 application by foliar spraying mitigates the effect of salt stress on stomatal conductance, transpiration and CO_2 assimilation rate. The method of H_2O_2 application by seed soaking decreases the harmful effects of salt stress on the growth of soursop at 85 after sowing.

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