



*Research article*

## **Photochemical efficiency and growth of soursop rootstocks subjected to salt stress and hydrogen peroxide**

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**Abstract:** Hydrogen peroxide has been used in agriculture as a way to minimize the negative effects caused by biotic and abiotic stresses on plants. Thus, the objective of this study was to evaluate the mitigating effect of hydrogen peroxide on chlorophyll *a* fluorescence and growth of soursop subjected to salt stress in the rootstock production phase. The study was conducted in plastic bags under greenhouse conditions. The treatments were distributed in randomized blocks, in a 5 × 2 factorial arrangement, corresponding to five levels of irrigation water electrical conductivity—ECw (0.6; 1.2; 1.8; 2.4 and 3.0 dS m<sup>-1</sup>) and two concentrations of hydrogen peroxide—H<sub>2</sub>O<sub>2</sub> (0 and 20 μM), with four replicates and two plants per plot. Irrigation water salinity hamper the quantum efficiency of photosystem II in soursop plants, at 120 days after sowing and it inhibits the growth of rootstocks in the period from 80 to 140 days after sowing. Hydrogen peroxide applications at concentration of 20 μM minimized the negative effects of salinity on the soursop initial fluorescence and favored the variable fluorescence and quantum efficiency of PSII.

**Keywords:** *Annona muricata* L.; salinity; chlorophyll fluorescence; acclimatation; antioxidant enzyme

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**Abbreviations:** DAS: Days after sowing; Fo: initial fluorescence; Fm: maximum fluorescence; Fv: variable fluorescence; Fv/Fm: maximum quantum efficiency of photosystem II; RGRph: relative growth rates of plant height; AGRph: absolute growth rates of plant height; RGRsd: relative growth rates of stem diameter; AGRsd: absolute growth rates of stem diameter; RGRla: relative growth rates of leaf area; AGRla: absolute growth rates of leaf area

## 1. Introduction

Soursop (*Annona muricata* L.) is a crop belonging to the Annonaceae family, considered as the second most significant in this family, in terms of production and cultivated area in the Brazilian territory. The expansion of the cultivated area has grown considerably in the last years, especially in Southern Bahia, due to the favorable edaphoclimatic conditions and due to the increase in the demand for fresh fruit and mainly by the agribusiness, making the crop one of the main alternatives of investment in the fruit sector in Northeastern Brazil [1]. In addition, the interest in the fruit by the pharmaceutical industry has been growing, since soursop fruit powder and leaf/stem powder showed *in-vitro* and *in-vivo* anti-cancer efficacy against breast and pancreas cancer cells [2].

Although it is a fruit crop with prospects of production and commercialization in the Northeastern semiarid region, the cationic composition of the water used in irrigated plantations in this region is the greatest limitation for establishment. Under salt stress conditions, plants undergo reduction of shoot or root growth [3] and changes in physiology due to the decrease in the osmotic potential of the soil solution, which may also cause ionic toxicity, nutritional imbalances or both simultaneously, as a consequence of the excessive accumulation of chloride and sodium in cell protoplasm [4,5]. Additionally salinity-induced ROS formation causes oxidative damage to lipids, proteins and other cellular components [6].

In this context, it is necessary to search for techniques capable of reducing the harmful effects of salts on plants. Thus, studies have been carried out with application of hydrogen peroxide in the pre-treatment of seeds and also through the exogenous application on leaves, in order to verify its capacity to attenuate effects of salinity in some crops, such as maize [7], barley [8] and cowpea [9].

Hydrogen peroxide is one of the reactive oxygen species (ROS) that are present in the plant cell as a normal byproduct of aerobic metabolism and photo-oxidative processes. When it is found at low levels, the plant is able to adapt and live with the ROS because of the enzymatic mechanisms involved in its detoxification, especially superoxide dismutases (SODs), catalases (CATs), ascorbate peroxidases (APXs) and phenol peroxidases (POXs) [8,10].

Thus, the process of acclimation to stress conditions constitutes an alternative to increase the survival capacity of plants under adverse conditions. Acclimation is a process in which an individual is previously exposed to a particular type of stress, which causes metabolic changes that are responsible for increasing its tolerance to a new exposure to the stress [1].

Given the above, the present study aimed to evaluate the photochemical efficiency and growth of soursop rootstocks subjected to salt stress and exogenous applications of hydrogen peroxide under semiarid conditions.

## 2. Materials and methods

### 2.1. Location, treatments, plant material and experimental procedure

The experiment was carried out between June and November 2018 in a protected environment (greenhouse) at the Center for Technology and Natural Resources (CTRN) of the Federal University of Campina Grande (UFCG), located in the municipality of Campina Grande-PB, Brazil (7°15'18" S; 35°52'28" W; 550 m).

The experimental design was completely randomized blocks, in a 5 × 2 factorial scheme, corresponding to five levels of irrigation water electrical conductivity—EC<sub>w</sub> (0.6; 1.2; 1.8; 2.4 and 3.0 dS m<sup>-1</sup>) and two concentrations of hydrogen peroxide—H<sub>2</sub>O<sub>2</sub> (0 and 20 μM) applied by immersion and leaf spraying. The experiment was composed of four replications and two plants per plot. Hydrogen peroxide concentrations were based on a study conducted by [5].

The electrical conductivity levels of 1.2, 1.8, 2.4 and 3.0 dS m<sup>-1</sup> were prepared by dissolving the salt NaCl, CaCl<sub>2</sub> · 2H<sub>2</sub>O and MgCl<sub>2</sub> · 6H<sub>2</sub>O, in equivalent proportion of 7:2:1, respectively, in water from the local supply (EC<sub>w</sub> = 1.10 dS m<sup>-1</sup>). This proportion is commonly found in sources of water used for irrigation in small properties of the Northeast region [11], based on the relationship between EC<sub>w</sub> and the concentration of salts (mmol<sub>c</sub> L<sup>-1</sup> = 10 \* EC<sub>w</sub> dS m<sup>-1</sup>). The level of 0.6 dS m<sup>-1</sup> was obtained by diluting water from the local supply in rainwater (EC<sub>w</sub> = 0.02 dS m<sup>-1</sup>). The concentration of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), previously established, was obtained by diluting H<sub>2</sub>O<sub>2</sub> in distilled water.

The experiment used rootstocks of soursop cultivar ‘Morada Nova’, because it is the genetic material preferred by farmers in the Northeast region, besides composing most of the commercial orchards in Brazil [12].

### 2.2. Experimental setup and analyses

The rootstocks were grown in plastic bags with a capacity of 2.6 kg, filled with substrate composed of 85% soil, 14% sand and 1% humus. The soil used to fill the bags was classified as *Neossolo Regolítico Eutrófico* (Entisol) with sandy loam texture (0–30 cm layer), collected in the municipality of Esperança-PB, Brazil, and its physical and chemical attributes (Table 1) were determined according to the methodologies proposed by [13].

The seeds used to produce the rootstocks were obtained from fruits harvested in a commercial orchard (Boi Bravo Farm) located in the municipality of Sousa—PB (6°45'33" S; 38°13'41" W; 220 m). Seeds were manually extracted, air-dried at temperature of 25.7 °C (mean temperature in the month of June in the municipality of Sousa—PB) and its dormancy was broken by means of a distal cut to the embryo.

Before sowing, a pre-treatment with hydrogen peroxide was applied to the seeds, by soaking them in solution with the concentrations of the respective treatments for a period of 36 h, and seeds of the control treatment (0 μM) were soaked in distilled water for the same period of time. Next, sowing was performed by equidistantly planting three seeds of soursop cv. ‘Morada Nova’ at 3 cm depth. At 20 days after germination, thinning was performed leaving only one plant per bag, the one with highest vigor.

**Table 1.** Chemical and physical characteristics of the eutrophic Entisol used in the experiment.

Chemical characteristics							
pH 1:2.5	EC <sub>se</sub> dS m <sup>-1</sup>	P mg kg <sup>-1</sup>	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	H + Al
5.90	1.36	6.80	.....cmol <sub>c</sub> kg <sup>-3</sup> .....				
			2.22	1.60	26.00	36.60	19.30
Physical characteristics							
	Sand	Silt	Clay	AD	DP	Total Porosity %	Textural Class
	.....dag kg <sup>-1</sup> .....			.....kg dm <sup>3</sup> .....		47,74	Sandy loam
	73.29	14,21	12.50	1.39	2.66		

Note: pH = hydrogen potential; EC<sub>se</sub> = electrical conductivity of the saturation extract; Ca<sup>2+</sup> e Mg<sup>2+</sup> extra flos com KCl 1 M pH 7.0; Na<sup>+</sup> e K<sup>+</sup> extra flos utilizando-se NH<sub>4</sub>OAc 1 M pH 7.0; Al<sup>3+</sup> e H<sup>+</sup> extra flos utilizando-se CaOAc 0.5 M pH 7.0; AD = Apparent density; PD = Particle density; dag kg<sup>-1</sup> = decagrama por kilograma; cmol<sub>c</sub> kg<sup>-3</sup> = centimol de carga por quilograma.

Prior to sowing, soil moisture content was raised to the maximum retention capacity using the solutions according to each treatment. After sowing, irrigation was performed daily by applying in each plastic bag a volume of water to maintain the soil moisture close to field capacity. The volume applied was determined according to the water requirement of the plants, estimated through water balance, by subtracting the volume drained from the volume applied in the previous irrigation, plus a leaching fraction of 0.10 applied every fifteen days, in order to avoid excessive accumulation of salts in the soil.

As the emergence of soursop seedlings established only at 40 DAS, foliar applications of H<sub>2</sub>O<sub>2</sub> began at 75 DAS, carried out manually at 17 h, at the appropriate concentrations, every 15 days, by spraying the solution of hydrogen peroxide, in such a way to fully wet the leaves (spraying their abaxial and adaxial sides), using a sprayer.

Fertilization with nitrogen, potassium and phosphorus was based on the recommendations described by [14]. 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<sup>-1</sup> of the substrate of N, K<sub>2</sub>O and P<sub>2</sub>O<sub>5</sub>, respectively, were applied as top-dressing in four equal portions through fertigation, at 15-day intervals, with the first application performed at 15 DAS. In order to meet micronutrient deficiencies, 2.5 g L<sup>-1</sup> of Ubyfol [(N (15%); P<sub>2</sub>O<sub>5</sub> (15%); K<sub>2</sub>O (15%); Ca (1%); Mg (1.4%); S (2.7%); Zn (0.5%); B (0.05%); Fe (0.5%); Mn (0.05%); Cu (0.5%); Mo (0.02%)] was applied through spraying at 60, 75, 90, 105, 120 and 135 DAS.

### 2.3. Variables analyzed

The initial fluorescence (F<sub>o</sub>), maximum fluorescence (F<sub>m</sub>), variable fluorescence (F<sub>v</sub> = F<sub>m</sub>-F<sub>o</sub>) and maximum quantum efficiency of photosystem II (F<sub>v</sub>/F<sub>m</sub>) were evaluated at 110 DAS, using a portable pulse-modulated fluorometer (OS5p model—Opti Science). Evaluations were performed after maintaining leaf under dark conditions for 30 minutes, using the dark-adaptation clip of the device, to ensure that all primary receptors were oxidized, that is, the reaction centers were open.

Treatment effects were also measured by determining the relative and absolute growth rates of plant height (AGR<sub>ph</sub> and RGR<sub>ph</sub>), stem diameter (AGR<sub>sd</sub> and RGR<sub>sd</sub>) and leaf area (AGR<sub>la</sub> and RGR<sub>la</sub>) in the interval from 90 to 140 days after sowing.

The absolute growth rate (AGR) was obtained using the methodology proposed by [15] as described in the equation:  $AGR = [(A_2 - A_1)/(t_2 - t_1)]$ , where: AGR = absolute growth rate,  $A_2$  = growth in height, stem diameter or leaf area at time  $t_2$ ,  $A_1$  = growth in plant height, stem diameter or leaf area at time  $t_1$ , and  $t_2 - t_1$  = time difference between samplings.

The relative growth rates were obtained by the equation:  $RGR = [(\ln A_2 - \ln A_1)/(t_2 - t_1)]$ , in which plant growth is measured based on the pre-existing matter height, diameter or leaf area, according to [15], where: RGR = relative growth rate,  $A_2$  = growth in height, stem diameter or leaf area at time  $t_2$ ,  $A_1$  = growth in height, stem diameter or leaf area at time  $t_1$ ,  $t_2 - t_1$  = time difference between samplings, and  $\ln$  = natural logarithm.

#### 2.4. Statistical analysis

The collected data were subjected to analysis of variance by F test at 0.05 probability level and, when significant, linear and quadratic regression analysis was carried out for the salinity factor. For the  $H_2O_2$  concentrations, means were compared by Tukey test at 0.05 probability level using the statistical program SISVAR - ESAL [16].

### 3. Results and discussion

According to the summary of the F test (Table 2), there was significant effect ( $p < 0.01$ ) of the interaction between salinity levels and  $H_2O_2$  concentrations on the initial fluorescence (Fo) and variable fluorescence (Fv). The isolated effect of salinity levels was also significant for the quantum efficiency of photosystem II (Fv/Fm), but the  $H_2O_2$  concentrations had significant influence on maximum fluorescence (Fm) and quantum efficiency of photosystem II (Fv/Fm).

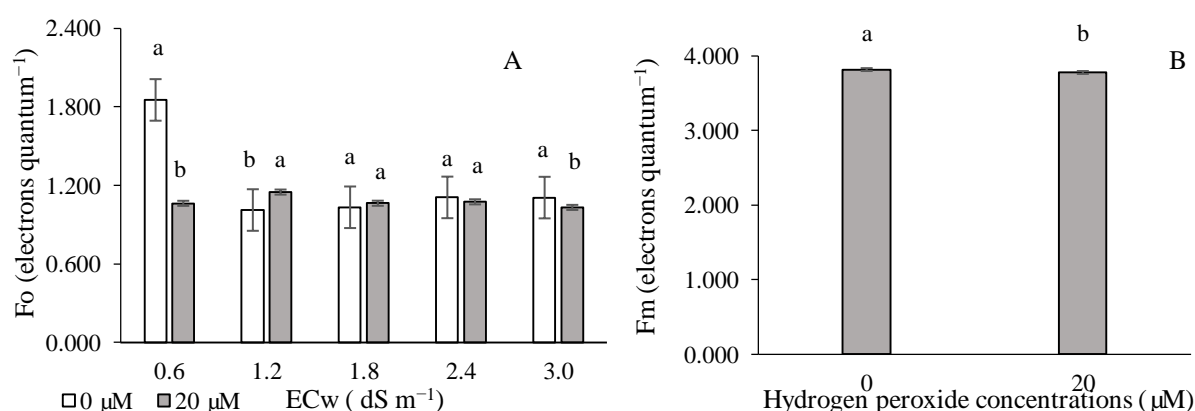
**Table 2.** Summary of F test for initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv) and efficiency of photosystem II (Fv/Fm) of soursop rootstock irrigated with saline water and subjected to exogenous application of hydrogen peroxide, at 110 days after the sowing.

Source of variation	F test			
	Fo	Fm	Fv	Fv/Fm
Saline levels (SL)	**	ns	**	**
Linear regression	**	ns	**	**
Quadratic regression	**	ns	**	**
Hydrogen peroxide ( $H_2O_2$ )	**	*	**	**
Interaction (SL $\times$ $H_2O_2$ )	**	ns	**	ns
Blocks	ns	ns	ns	ns
CV%	4.60	1.46	0.92	1.45

Note: \*, \*\*, ns, significant at 0.05, 0.01 probability levels and not significant, respectively.

By analyzing the interaction between the distinct  $H_2O_2$  concentrations and the irrigation water electrical conductivity, it is possible to note that the applications of 0 and 20  $\mu\text{m}$ , respectively, did not significantly influence the initial fluorescence of soursop rootstocks when irrigated using water with salinity of 1.8 and 2.4  $\text{dS m}^{-1}$  (Figure 1A). However, there was a significant difference in Fo at

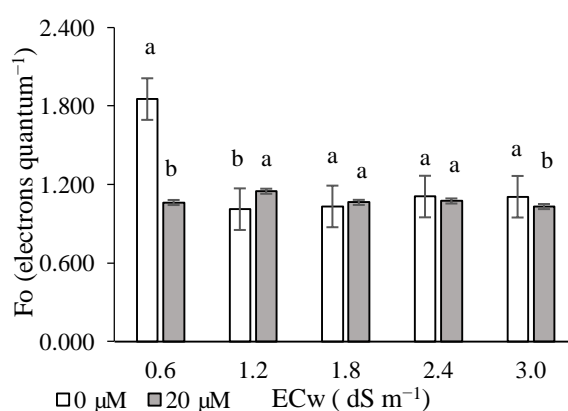
the levels of 0.6, 1.2, and 3.0  $\text{dS m}^{-1}$  and there was an expressive reduction in the initial fluorescence of plants sprayed with  $\text{H}_2\text{O}_2$  concentration of 20  $\mu\text{M}$ . In addition, at the highest salinity level (3.0  $\text{dS m}^{-1}$ ), spraying with 20  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$  caused the reduction of initial fluorescence by 0.075 electrons quantum $^{-1}$ , compared to the control treatment (0  $\mu\text{M}$ ). The initial fluorescence explains the fluorescence emission when the quinone of the primary electron (QA) of the photosystem II (PSII) is fully oxidized and the reaction center ( $\text{P}_{680}$ ) is open, indicating the activation of photochemical reactions. In general, plants subjected to stress tend to have an increase in  $F_o$ , which stands out as an indicative of occurrence of damage to the PSII reaction center, because the stress effect can cause damage to the reaction center and inhibit the repair cycle of PSII, in particular the D1 protein. In addition, the increase in  $F_o$  can be related to the increase in leaf thickness due to lipid peroxidation by reactive oxygen species [17,18]. This contributes to demonstrate that, in the present study, there was no damage to PSII because the initial fluorescence decreased with the increase in water salinity and  $\text{H}_2\text{O}_2$  application of 20  $\mu\text{M}$ . Hydrogen peroxide had once been considered a toxic substance to plant metabolism and its reaction with  $\text{O}_2$  a possible responsible for the dissociation of the pigment-protein complex from the antenna of the central nucleus of the light-harvesting system of PSII, within the photosynthetic apparatus, causing inactivation of enzymes, discoloration of pigments, lipid peroxidation and proteolysis, consequently reducing plant growth and development [8]. This disagrees with the results of the present study, which shows that adequate concentrations of  $\text{H}_2\text{O}_2$  may have positive effects on the plant, especially on the initial fluorescence of PSII.



**Figure 1.** Initial fluorescence ( $F_o$ ) of the soursop rootstock as a function of interaction between water salinity—ECw and hydrogen peroxide— $\text{H}_2\text{O}_2$  (A) and maximum fluorescence as a function of the concentrations hydrogen peroxide (B). The means followed by the same letter do not differ from each other by Tukey's test. ( $p < 0.05$ ). Bars represent the standard error of the mean ( $n = 4$ ).

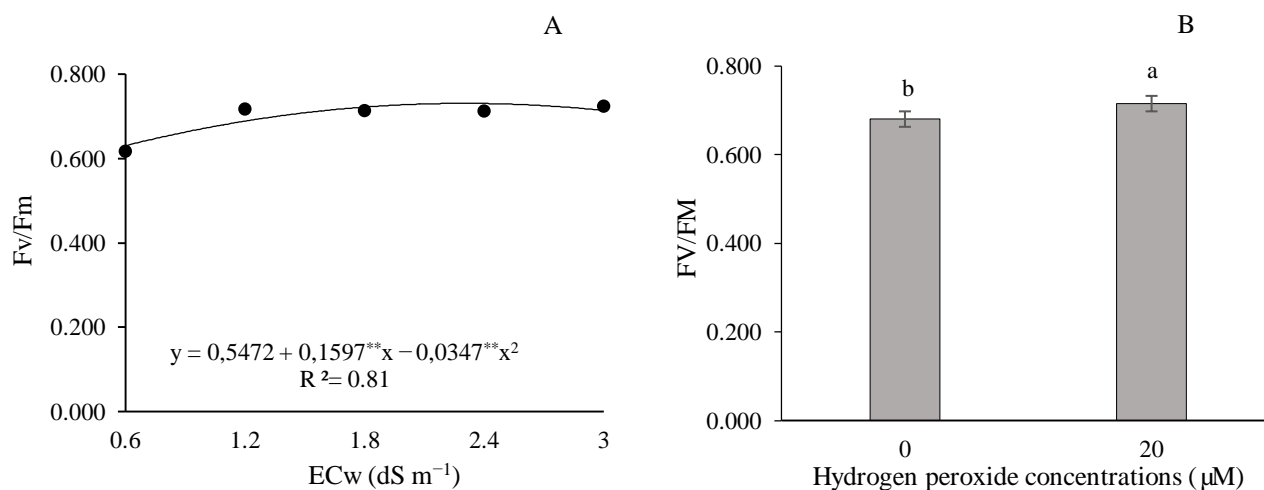
The maximum fluorescence (Figure 1B) of soursop rootstocks in the control treatment (0  $\mu\text{M}$ ) differed statistically from that of plants that received  $\text{H}_2\text{O}_2$  applications at concentration of 20  $\mu\text{M}$  (3.778), equivalent to a minimum reduction of 1.02%. Hence,  $\text{H}_2\text{O}_2$  application hampered the maximum emission of fluorescence, which represents the maximum intensity of fluorescence, demonstrating the reduced condition of all the quinone (Qa) by the electrons transferred from  $\text{P}_{680}$  [19]. In this case, the concentration of 20  $\mu\text{M}$  may have led to certain oxidative damage to the cell membrane and possibly a negative influence on the maximum fluorescence of soursop rootstocks [20].

The variable fluorescence of soursop rootstocks was influenced by the interaction between water salinity and hydrogen peroxide concentrations, with a significant difference in  $\text{H}_2\text{O}_2$  concentrations at all salinity levels studied. There was an increase in variable fluorescence with increasing salinity of irrigation water, with highest value of Fv in plants irrigated with  $1.2 \text{ dS m}^{-1}$  water ( $2.824 \text{ electrons quantum}^{-1}$ ). In addition, with  $\text{H}_2\text{O}_2$  concentration of  $20 \mu\text{M}$  and ECw of  $3.0 \text{ dS m}^{-1}$ , there was an increase of 2.17% in Fv compared to rootstocks at the same salinity level but not sprayed with  $\text{H}_2\text{O}_2$  ( $0 \mu\text{M}$ ), so the exogenous application of hydrogen peroxide favored the variable fluorescence of soursop rootstocks subjected to ECw of  $3.0 \text{ dS m}^{-1}$ . It indicates that the photochemical activity of the leaves was not compromised, since the increase in Fv represents the capacity of the rootstocks to transfer the energy from the electrons emitted by the pigment molecules for the formation of the reducing agent NADPH, ATP and reduced ferredoxin (Fdr), thus increasing the capacity to assimilate  $\text{CO}_2$  in the biochemical phase of photosynthesis [21]



**Figure 2.** Variable fluorescence (Fv) of the soursop rootstock as a function of interaction between water salinity—ECw and hydrogen peroxide— $\text{H}_2\text{O}_2$ . The means followed by the same letter do not differ from each other by Tukey's test. ( $p < 0.05$ ). Bars represent the standard error of the mean ( $n = 4$ ).

The ratio between the maximum and variable fluorescence, expressed by the maximum quantum efficiency of photosystem II ( $\text{Fv}/\text{Fm}$ ), had its highest value ( $0.7309$ ) in the soursop rootstock irrigated with  $2.3 \text{ dS m}^{-1}$  water (Figure 3A). Considering that there was reduction in the initial fluorescence (Figure 1A) as a function of the highest levels of salinity, coinciding with the increase in the quantum efficiency of PSII ( $\text{Fv}/\text{Fm}$ ). Such increase was more noticeable in plants irrigated with  $2.3 \text{ dS m}^{-1}$  water, compared to the treatment of  $0.6 \text{ dS m}^{-1}$ . However, even in the face of increasing quantum efficiency of the photosystem with increasing salinity up to  $2.3 \text{ dS m}^{-1}$ , it is evident that there was damage to the photosynthetic apparatus as the maximum value obtained for the  $\text{Fv}/\text{Fm}$  ratio was below the index ( $0.75 \text{ electrons quantum}^{-1}$ ), considered as a limit to cause damage to the photosynthetic apparatus because when the photosynthetic apparatus is intact,  $\text{Fv}/\text{Fm}$  values range from  $0.75$  to  $0.85 \text{ quantum}^{-1}$  electrons. Therefore, the inhibition of FSII quantum efficiency detected in plants cultivated with saline waters indicates the occurrence of photoinhibitory damage in PSII reaction centers, which promotes the formation of reactive oxygen species [22].



**Figure 3.** Efficiency of photosystem II (Fv/Fm) of the soursop rootstock as a function of water salinity—ECw (A) and of the hydrogen peroxide concentrations—H<sub>2</sub>O<sub>2</sub> (B). The means followed by the same letter do not differ from each other by Tukey's test. ( $p < 0.05$ ). Bars represent the standard error of the mean ( $n = 4$ ).

Quantum efficiency of PSII (Fv/Fm) was also influenced by the concentrations of hydrogen peroxide. The means comparison test (Figure 3B) shows that the concentration of 20 µM caused an increase in the quantum efficiency of PSII of 0.0349 (4.88%), compared to plants that did not receive H<sub>2</sub>O<sub>2</sub> application. The beneficial effect of hydrogen peroxide may be related to its activity as a signaling molecule, acting in the regulation of several pathways [23], including responses to salt stress. Studying the influence of hydrogen peroxide on the reduction of aluminum sensitivity in canola plants, it was observed that the concentration of 0.15 M positively influenced the chlorophyll *a* fluorescence variables, highlighting that the highest concentration of hydrogen peroxide used in the soaking of canola seeds allowed the activation of an enzymatic apparatus responsible for the defense against the oxidative stress caused by the exposure to toxic aluminum [24].

**Table 3.** Summary of F test for absolute (AGRph) and relative growth rates of plant height (RGRph), absolute (AGRsd) and relative growth rates of stem diameter (RGRsd), absolute (AGRla) and relative growth rates of leaf area (RGRla).

Source of variation	Teste F					
	AGRph	RGRph	AGRsd	RGRsd	AGRla	RGRla
Saline levels (SL)	**	**	**	ns	**	ns
Linear regression	**	**	**	ns	**	ns
Quadratic regression	ns	**	*	ns	ns	ns
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	ns	ns	ns	ns	*	ns
Interaction (SL × H <sub>2</sub> O <sub>2</sub> )	ns	ns	ns	ns	*	ns
Blocks	ns	ns	ns	ns	ns	ns
CV%	15.14	14.38	19.94	14.97	21.73	13.66

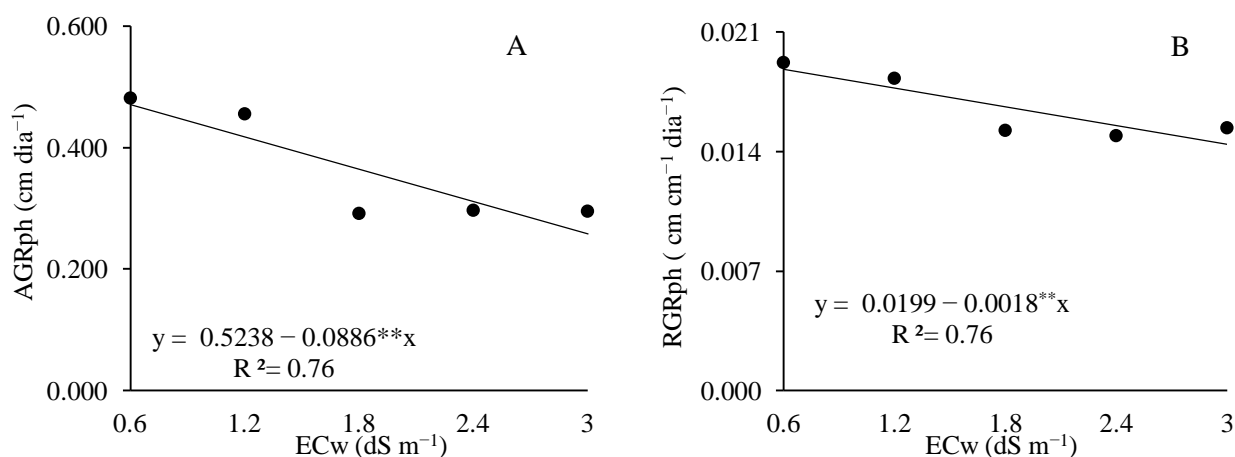
Note: \*, \*\*, ns, significant at 0.05, 0.01 probability levels and not significant, respectively.



Based on the summary of test F, the interaction between water salinity and hydrogen peroxide concentrations had a significant effect on the absolute leaf area growth rate (AGRla). Water salinity factor had a significant effect on absolute plant height (AGRph), stem diameter (AGRsd) and leaf area (AGRla) growth rates and relative plant height growth rates (RGRph). The different concentrations of hydrogen peroxide had significant effect only on AGRla.

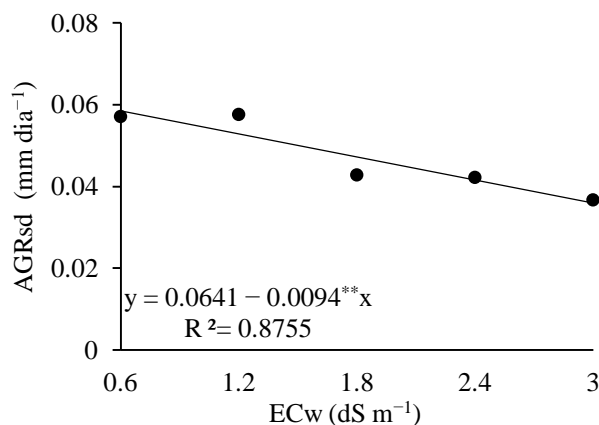
The absolute (Figure 4A) and relative (Figure 4B) growth rates of plant height of soursop decreased linearly with the increase in irrigation water salinity in the period from 80 to 140 DAS, respectively by 16.91 and 9.04% per unit increase in the electrical conductivity of irrigation water. The increase in the amount of salts in the root zone may lead to a reduction in the osmotic potential of the soil solution, inducing the plant to adjust in an attempt to minimize the osmotic stress and ionic imbalance. The osmotic adjustment in turn requires a significant expenditure of energy that would be formerly used in plant growth, but is used in the synthesis of compatible organic solutes that assist in the adjustment, thereby causing reduction in plant growth [25,26].

In agreement with the results found in the present study, [4] observed that the growth rate of plant height in passion fruit decreased when subjected to the increase in salinity levels. Also according to these authors, in plants cultivated in saline environments growth is inhibited by the water deficit caused by large amounts of salts in the root zone, which leads to a reduction of turgor, resulting in the decrease in cell expansion, reducing their growth rate.

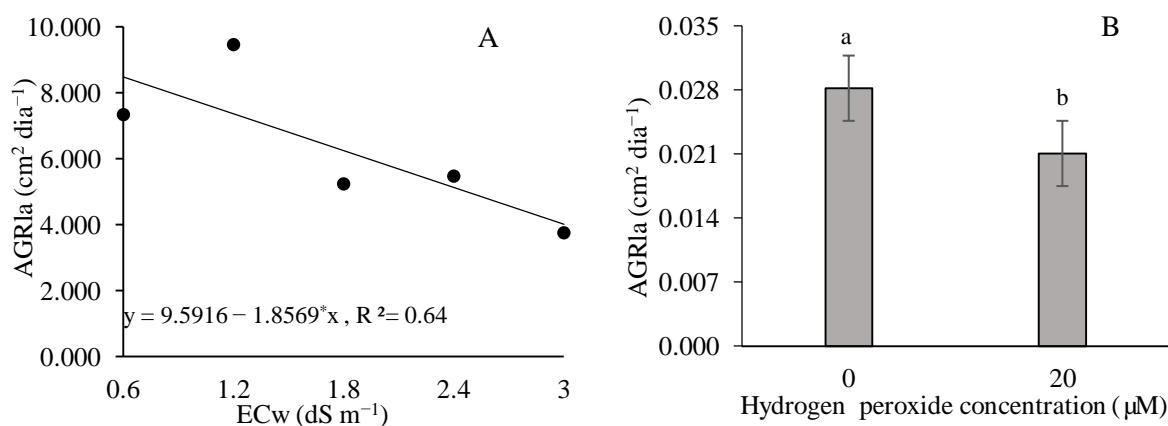


**Figure 4.** Absolute (AGRph) (A) and relative growth rates of plant height (RGRph) (B) of the soursop rootstock as a function of water salinity—ECw.

The absolute growth rate of stem diameter (Figure 5) in the period from 40 to 180 DAS was negatively influenced by the increase in irrigation water salinity, decreasing linearly by 14.66% per unit increase in ECw, i.e., plants irrigated with the highest level of salinity (3.0 dS m<sup>-1</sup>) had a reduction of 0.022 mm day<sup>-1</sup> compared to plants irrigated with 0.6 dS m<sup>-1</sup> water. Similar results were observed by [27], studying the production of guava rootstocks grown with waters of different salinity levels and nitrogen doses. These authors found that the increase in irrigation water salinity up to the level of 3.5 dS m<sup>-1</sup> reduced the growth rate and attributed such reduction to the effect of salinity on the turgor pressure in the cells, due to the decrease of water content in the tissues, resulting in a reduction in cell wall expansion, causing lower plant growth.



**Figure 5.** Absolute growth rates of stem diameter (AGRsd) of the soursop rootstock as a function of water salinity—ECw.



**Figure 6.** Absolute growth rates of leaf area (AGRla) of the soursop rootstock as a function of water salinity—ECw (A) and hydrogen peroxide concentrations—H<sub>2</sub>O<sub>2</sub> (B). The means followed by the same letter do not differ from each other by Tukey's test. ( $p < 0.05$ ). Bars represent the standard error of the mean ( $n = 4$ ).

The increase in water salinity caused a linear reduction in the absolute growth rate of leaf area in soursop rootstocks (Figure 6A). In the treatment which used low-salinity water (0.6 dS m<sup>-1</sup>), the AGRla was statistically higher compared to plants irrigated using water with highest salinity (3.0 dS m<sup>-1</sup>), whose reduction was 58.07% compared to those under ECw of 0.6 dS m<sup>-1</sup>. The absolute growth rate indicates the mean growth velocity over the observation period and, according to the results, the velocity of growth in leaf area within the time interval from 80 to 140 DAS decreased as irrigation water salinity increased. The reduction in plant growth is a reflex of salt stress in the root environment, causing, in some cases, physiological imbalance in plants due to changes in the partition of photoassimilates and to the reduction in the leaf area used in the photosynthetic process. However, the reduction in AGRla can also be seen as an important adaptive mechanism of plants grown under excess salts and water stress because, under these conditions, it is interesting to reduce

transpiration, consequently decreasing the transport of  $\text{Na}^+$  and  $\text{Cl}^-$  in the xylem and conservation of water in the tissues [28].

According to the means comparison test (Figure 6B), soursop plants that did not receive  $\text{H}_2\text{O}_2$  concentrations differed significantly from those which received 20  $\mu\text{M}$ , i.e., AGR1a decreased by  $0.0071 \text{ cm}^2 \text{ day}^{-1}$  in the treatment with 20  $\mu\text{M}$  of  $\text{H}_2\text{O}_2$ , compared to plants subjected to the control treatment (0  $\mu\text{M}$ ). Based on the results presented, the elevation in  $\text{H}_2\text{O}_2$  concentrations may have caused damage that affects metabolic functions such as photosynthesis. This effect may also be associated with oxidative stress, causing lipid peroxidation, cell membrane damage, protein degradation, DNA double strand break, and also cell death, leading to lower growth [29].

#### 4. Conclusions

Irrigation water salinity hamper the quantum efficiency of photosystem II in soursop plants, at 120 days after sowing and it inhibits the growth of rootstocks in the period from 80 to 140 days after sowing.

Applications of hydrogen peroxide at concentration of 20  $\mu\text{M}$  minimized the negative effects of salinity on the initial fluorescence and favored the variable fluorescence and quantum efficiency of PSII.

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#### Conflict of interest

All authors declare no conflict of interest in this paper.

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