Growth and gas exchange of soursop under salt stress and hydrogen peroxide application

Crescimento e trocas gasosas de gravioleira sob estresse salino e aplicações de peróxido de hidrogênio


ABSTRACT: The cultivation of irrigated soursop in semiarid Northeastern Brazil highlights the need for information regarding its responses to the salinity of irrigation water and the use of techniques that allow its exploration, such as the use of hydrogen peroxide. Thus, the study aimed to evaluate the effect of soaking of seeds and foliar application of hydrogen peroxide on soursop plant growth and physiology under conditions of salt stress. The study was conducted in lysimeters in a greenhouse, and the treatments were distributed in a randomized block design and 4 × 4 factorial scheme, with four values of electrical conductivity of the irrigation water - ECw (0.7, 1.7, 2.7, and 3.7 dS m⁻¹) and four concentrations of H₂O₂ (0, 25, 50, and 75 μM), with three replicates and one plant per plot. H₂O₂ concentrations were applied via seed imbibition and foliar spray. Irrigation with water from 0.7 dS m⁻¹ impairs gas exchange and absolute growth rates of plant height and stem diameter and relative growth rate in height of soursop plants. Concentrations of 35, 33 and 23 μM of hydrogen peroxide favored the relative and absolute growth rates of plant height and transpiration, respectively. Compared to the aerial part, the root of soursop plants is more affected when irrigated with water from 1.6 dS m⁻¹.

Key words: Annona muricata L., salinity, acclimatization

RESUMO: O cultivo da graviola irrigada no semiárido do Nordeste brasileiro evidencia a necessidade de informações sobre suas respostas à salinidade da água de irrigação e o uso de técnicas que permitam sua exploração, como a utilização de peróxido de hidrogênio. Assim, objetivou-se com este estudo avaliar efeitos de embebição e aplicações foliares de peróxido de hidrogênio sobre o crescimento e a fisiologia de graviolerias submetidas à estresse salino. O estudo foi conduzido em lisímetros em casa de vegetação, e os tratamentos foram distribuídos em delineamento de blocos casualizados em esquema fatorial 4 × 4, com quatro valores de condutividade elétrica da água de irrigação - CEa (0.7; 1.7; 2.7 e 3.7 dS m⁻¹) e quatro concentrações de H₂O₂ (0; 25, 50 e 75 μM), com três repetições e uma planta por parcela. As concentrações de H₂O₂ foram aplicadas via embebição das sementes e pulverização foliar. A irrigação com água à partir de 0.7 dS m⁻¹ prejudicou as trocas gasosas e as taxas de crescimento absoluto da altura de planta e diâmetro de caule e crescimento relativo da altura da graviola. A aplicação foliar com concentrações estimadas de 35, 33 e 23 μM de peróxido de hidrogênio favoreceram as taxas de crescimento absoluto e relativo da altura da células de transpiração, respectivamente. Em comparação com a parte aérea, a raiz de graviola foi mais afetada quando irrigadas com água à partir de 1.6 dS m⁻¹.

Palavras-chave: Annona muricata L., salinidade, aclimatização

HIGHLIGHTS:
The decrease in stomatal conductance restricts soursop leaf transpiration under salt stress.
Exogenous application of hydrogen peroxide at low concentration favors soursop growth and transpiration.
Application of high concentrations of hydrogen peroxide intensifies salt stress in soursop.

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**INTRODUCTION**

Soursop (*Annona muricata* L.) is a tropical fruit crop that has a significant potential of commercialization in the internal market, with relevant economic importance and prospects of exportation, due to its use by the food and cosmetics industry, in addition to its medicinal characteristics (Freitas et al., 2013). Despite being considered a moderately tolerant crop to irrigation water salinity and with considerable production in the states of Northeast Brazil, soursop yield may be limited by the high salt content present in the irrigation water in the region (Cavalcante et al., 2001). Usually, the excess salts can reduce the osmotic potential of the soil solution, which limits water availability, resulting in reduced photosynthetic activity and plant growth (Najar et al., 2019).

Under stressing environmental conditions, such as water deficit, salinity, and heat, there is an imbalance between the production and removal of reactive oxygen species (ROS). Consequently, there is an increase in ROS levels triggering oxidative stress. However, the acclimation to stress with the application of H$_2$O$_2$ can cause metabolic changes in plants which will be responsible for increasing their tolerance to a new exposure to the stress, making it possible to use saline water for irrigation (Gohari et al., 2020).

Seed pretreatment or plant exposure to H$_2$O$_2$ is an important mechanism to modulate cross-tolerance through action on various physiological processes, such as photosynthesis, and on various stress-responsive pathways, such as ROS and methylglyoxal detoxification. In addition, it can increase antioxidant metabolism and reduce lipid peroxidation in leaves and roots (Hossain et al., 2015).

Some authors in biochemical and genetic studies defend the role of molecular signaling of H$_2$O$_2$ under conditions of salt stress in soursop (Veloso et al., 2020a), wheat (Liu et al., 2020a), broad bean (Latef et al., 2021). Thus, this study aimed to evaluate the effects of soaking and foliar applications of hydrogen peroxide on the growth and physiology of soursop subjected to salt stress.

**Material and Methods**

The experiment was conducted from October 2017 to February 2018 under greenhouse conditions at the Center of 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 and altitude 550 m asl). Data of temperature (maximum and minimum) and mean relative air humidity were recorded inside the greenhouse during the experimental period shown in Figure 1.

The concentrations of H$_2$O$_2$, according to the treatments, were applied to the soursop plant via seed imbibition and foliar sprays. So, before sowing, the seeds were soaked in a solution with different concentrations of hydrogen peroxide (H$_2$O$_2$) for 24 hours in the dark and planted immediately after this period in polyethylene bags filled with substrate. This immersion period was based on preliminary studies carried out with soursop seeds.

Eight months after sowing, the plants were transplanted to 23 dm$^3$ lysimeters, whose bottom was covered with a geotextile (Bidim) and a 5 cm layer of crushed stone and connected to a drain to collect the drained water. The soil used to fill the lysimeters was classified as Entisol with clay loam texture (0-30 cm layer), collected in the municipality of Esperança-PB, and its physical and chemical attributes were determined according to the methodologies proposed by Teixeira et al. (2017). The soil had the following characteristics: pH (soil:water 1:2.5) = 5.9; EC$_{s}$ (dS m$^{-1}$) = 1.0; P (mg kg$^{-1}$) = 6.80; exchangeable Ca, Mg, Na, K (cmolc kg$^{-1}$) = 2.6, 3.66, 0.16, and 0.22, respectively. Organic matter - OM (dag kg$^{-1}$) = 1.36; bulk density - BD (kg dm$^{-3}$) = 1.39; and total porosity, Pt (%) = 47.74.

Hydrogen peroxide (H$_2$O$_2$) concentrations were established according to a study conducted by Panngom et al. (2018), obtained by diluting H$_2$O$_2$ in deionized water. Foliar applications with H$_2$O$_2$ concentrations began at 30 days after transplanting (DAT), with sprays on the abaxial and adaxial sides of the leaves, at 15-day intervals, totaling eight applications, performed with a manual sprayer between 17:00 and 17:30 hour. The sprayer used was a Jacto XP model with a capacity of 12 L and the average volume applied per plant was 125 mL.

The values of irrigation water electrical conductivity (0.7, 1.7, 2.7, and 3.7 dS m$^{-1}$) were based on a study carried out by Silva et al. (2019) and were prepared by dissolving the salts NaCl, CaCl$_2$,2H$_2$O, and MgCl$_2$,6H$_2$O, in the equivalent proportion of 7:2:1, respectively, in water from the local supply system (EC$_w$ = 1.1 dS m$^{-1}$). The water salinity treatments were applied from the beginning of the experiment, raising the soil moisture until reaching free drainage through manual irrigation, and thereafter using in each lysimeter the volume of water corresponding to each treatment. The applied volume was estimated by water balance: water volume applied minus water volume drained in the previous irrigation, plus a leaching fraction of 0.15 to avoid excessive accumulation of salts in the soil, applied at an interval of 30 days.
Fertilization with N, K, and P was carried out as recommended by Novais et al. (1991) for pot experiments, applying 5.75 g of potassium chloride and 13.8 g of monoammonium phosphate, equivalent to 100, 150, and 300 mg kg⁻¹ of the substrate of N, K, and P₂O₅, respectively. These nutrients were applied as top-dressing, in four equal applications through irrigation water, at 15 days intervals, with the first application being performed at 10 days after transplanting (DAT). Cultural practices regarding control of pests, diseases, and weeds were performed as and when necessary according to recommendations of Silva et al. (2019).

The absolute and relative growth rates of plant height (AGRph and RGRph) and stem diameter (AGRsd and RGRsd) were determined for the interval from 30 to 90 DAT, and root/shoot (R/S - g g⁻¹) ratio at 90 DAT. Absolute (AGR) and relative (RGR) growth rates in terms of plant height and stem diameter were determined using the methodology proposed by Benincasa (2003), according to the equations:

\[
AGR = \frac{(A_{t_2} - A_{t_1})}{(t_{2} - t_{1})}, \quad (1)
\]

\[
RGR = \frac{\ln(A_{t_2} - A_{t_1})}{(t_{2} - t_{1})}, \quad (2)
\]

where:
- \(A_{t_2}\) - plant height or stem diameter at time \(t_2\);
- \(A_{t_1}\) - plant height or stem diameter at time \(t_1\);
- \(t_{2} - t_{1}\) - time difference between measurements; and,
-ln - natural logarithm.

At 45 DAT, in the vegetative stage, the gas exchange of the soursop leaf was measured by determining stomatal conductance (mol H₂O m⁻² s⁻¹), transpiration (mmol H₂O m⁻² s⁻¹), CO₂ assimilation rate (µmol m⁻² s⁻¹), and internal CO₂ concentration (µmol mol⁻¹). The analysis was performed between 7:00 to 10:00 a.m., using the portable photosynthesis meter LCPRO⁺ from ADC BioScientific Ltda. These data were used to quantify the instantaneous water use efficiency - WUEi (A/E) [(µmol m⁻² s⁻¹) (mol H₂O m⁻² s⁻¹)] and the instantaneous carboxylation efficiency – CEi (A/Ci) [(µmol m⁻² s⁻¹) (µmol mol⁻¹)].

The data were subjected to analysis of variance by F test at \(p \leq 0.05\), and when significant, linear and quadratic regression analysis was carried out using the statistical program SISVAR. Due to the heterogeneity of the data, verified through the normality and homogeneity of variances tests, they were transformed into √x before the analysis of variance.

### Results and Discussion

According to the summary of the analysis of variance (Table 1), the electrical conductivity of irrigation water had a significant effect on the absolute and relative growth rates of plant height (AGRph and RGRph), absolute growth rate of stem diameter (AGRsd), and on root/shoot (R/S) ratio, stomatal conductance (gs), transpiration (E), CO₂ assimilation rate (A) and instantaneous carboxylation efficiency (CEi). Furthermore, hydrogen peroxide concentrations caused a significant effect on AGRph, RGRph, and E. The interaction between ECw × H₂O₂ did not significantly affect any of the studied variables, indicating that the factors acted independently.

The increase in ECw from 0.7 to 3.7 dS m⁻¹ negatively affected the absolute growth rate of plant height. Based on the regression analysis (Figure 2A), in plants irrigated with 3.7 dS m⁻¹ water, the absolute growth rate decreased by 0.20 cm per day (29.04%) compared to those subjected to the lowest values of ECw (0.7 dS m⁻¹). Reduction of AGRph of soursop plants may be related to the reduction of the osmotic potential, limiting water absorption by the plant, affecting cell division and elongation, impairing plant growth (Wang et al., 2019). Adverse effects of water salinity on soursop plant growth have also been reported by Silva et al. (2020).

The absolute growth rate of plant height (AGRph) was significantly affected by the hydrogen peroxide concentrations (Figure 2B), and its data were described by a quadratic equation, with the highest value (0.671 cm per day) in plants subjected to the estimated H₂O₂ concentration of 33 µM. Above this concentration, there was a reduction and, at the H₂O₂ concentration of 75 µM, the AGRph was equal to 0.527 cm per day. Hydrogen peroxide is a reactive oxygen species (ROS), considered as a byproduct of the aerobic and photosynthetic metabolism and, at concentrations compatible with the cellular redox homeostasis, is a component of various species (ROS), considered as a byproduct of the aerobic and photosynthetic metabolism.
signaling pathways. On the other hand, the excess of ROS causes oxidative damage in proteins, lipids, and nucleic acids, characterizing the secondary oxidative stress (Maia et al., 2012).

The increase in irrigation water electrical conductivity progressively reduced the relative growth rate of height (RGRph) in soursop plants (Figure 3A). Such reduction was on the order of 7.30% per unit increase in ECw, i.e., plants under ECw of 3.7 dS m⁻¹ had a reduction of 0.0204 cm cm⁻¹ per day in the RGRph, compared to those irrigated with the lowest level of salinity (0.7 dS m⁻¹). As mentioned earlier, the inhibition of AGRph may have occurred due to the triggering of osmotic stress caused by the reduction of the soil water potential, decreasing plant turgor (Navada et al., 2020). These effects directly affect the physiological activities of the plant, reducing its growth and development. Veloso et al. (2020b) observed a reduction in the growth of soursop plants subjected to salinity and attributed it to the decrease in water availability or excessive accumulation of Na⁺ and Cl⁻ in plant tissues.

The relative growth rate of plant height was also significantly affected by the increase in hydrogen peroxide concentrations. According to the regression equation (Figure 3B), the data were described by a quadratic model and the highest value of RGRph (0.087 cm cm⁻¹ per day) was obtained when plants were subjected to the concentration of 35 µM. It can be noted from the results that adequate applications of H₂O₂ can promote greater relative plant growth, probably due to the role that hydrogen peroxide plays in normal physiological processes of plants such as photosynthesis and stomatal movement, cell cycle, growth and development, and expression of some genes in plant cells, promoting healthy plant development and production (Khandaker et al., 2012).

During the interval from 30 to 90 DAT, the AGRsd decreased linearly with an increase in electrical conductivity of irrigation water (Figure 4A). Such reduction was equal to 0.0405 mm per day in plants irrigated with 3.7 dS m⁻¹ water, compared to those subjected to 0.7 dS m⁻¹ water. This result is because the accumulation of salts causes ionic and osmotic stress, triggering a decline in plant growth, a reduction in the photosynthetic rate, and a breakdown of ionic homeostasis (Hashem et al., 2019). Souza et al. (2017), studying the guava crop, found a reduction in AGRsd under salt stress conditions (ECw ranging from 0.3 to 3.5 dS m⁻¹), with a decrease of 5.49% per unit increase in ECw.

Root/shoot ratio (R/S) was affected by the increase in ECw at 90 DAT (Figure 4B), and its maximum value (0.6758 g g⁻¹ of root dry matter per gram of shoot dry matter) was obtained at ECw of 1.6 dS m⁻¹, decreasing from this point on. This result demonstrates that the root system of soursop plants has higher sensitivity to the increase of salinity than the shoots. The damage caused to the roots may be related to physiological and biochemical changes caused by the salts, which inhibit
Growth and gas exchange of soursop under salt stress and hydrogen peroxide application

Figure 4. Absolute growth rate of stem diameter - AGRsd (A) during the period of 30 to 90 days after transplanting (DAT) and root/shoot ratio (R/S g g⁻¹) (B) at 90 DAT of soursop as a function of electrical conductivity of irrigation water (ECw) root growth (Munns & Gilliham, 2015). According to Liu et al. (2020b), roots of smaller size tend to suffer from an excess of salts due to their higher concentration in the superficial layer of the soil.

Based on the regression analysis (Figure 5A), there was a reduction in gs on the order of 0.09 mol of H₂O m⁻² s⁻¹ (38.54%) in soursop plants irrigated with water of 3.7 dS m⁻¹, compared to those irrigated with water of 0.7 dS m⁻¹. This reduction may have resulted from the osmotic effect, caused by the change in the external water potential, due to the excess of salts in the irrigation water, as the high concentration of salts in the soil compromises water absorption by roots, causing a reduction in stomatal opening to avoid losses; consequently, the decrease in gs limits the influx of CO₂ to the leaves and, as a result, there is a decrease in transpiration and CO₂ assimilation rate (Lima et al., 2018).

As observed for stomatal conductance, the transpiration (E) of soursop plants was negatively affected by the increase in electrical conductivity of irrigation water (Figure 5B). It is observed that there was a decrease of 10.59% per unit increase in ECw, i.e., plants irrigated with the highest ECw (3.7 dS m⁻¹) had a reduction of 0.43 mmol of H₂O m⁻² s⁻¹ compared to those under the lowest level (0.7 dS m⁻¹). The decline in stomatal conductance (Figure 5A) certainly decreased transpiration because the stomata are an escape route that offers less resistance to gas diffusion. In addition, since stomatal opening depends on the water saturation level of stomatal cells, there may be major restriction of transpiration when water deficit in the plant is substantially high (Cerqueira et al., 2004), a fact observed in plants subjected to high levels of salinity, because the reduction in the osmotic potential of the water in the soil limits water absorption by plants, making water deficit additional stress to the salt stress.

The concentrations of hydrogen peroxide also significantly influenced the transpiration of soursop plants. The data were described by a quadratic model and, according to the regression equation (Figure 5C), its highest value was 1.1284 mmol of H₂O m⁻² s⁻¹, obtained at H₂O₂ concentration of 23 µM. The increase in transpiration of soursop plants verified up to a concentration of 23 µM of H₂O₂, can contribute to the maintenance of water absorption and ensure leaf turgor, allowing the plant to perform gas exchange with the environment (Taiz et al., 2017);
however, if the water loss through the leaves is greater than the absorption, the plant will suffer from water stress, so the reduction of transpiration can also be a form of adaptation of plants that are under salt stress to reduce water loss (Rodriguez-Zaccaro & Groover, 2019).

Thus, the beneficial effect of hydrogen peroxide at low concentrations may be associated with its role as a signaling molecule, regulating several pathways, including responses to salt stress (Baxter et al., 2014). Nonetheless, a high concentration of ROS may cause alterations in plant metabolism due to a restriction of photosynthetic processes because, under stress conditions, such as drought, salinity, and/or heat, photosynthetic processes can be affected either directly, through stomatal restriction, transpiration and, consequently, low availability of CO₂, or indirectly, through imbalance between production and removal of ROS produced during the photosynthetic process - especially H₂O₂ -, which culminate in oxidative stress (Carvalho et al., 2011).

For CO₂ assimilation rate (Figure 6A) and instantaneous carboxylation efficiency (Figure 6B), there were linear reductions of 11.46 and 11.43%, respectively, per unit increase in electrical conductivity of water. In other words, plants cultivated under ECw of 0.7 dS m⁻¹ had higher A (30.95%) and CEi (30.88%) compared to those cultivated under the highest values of electrical conductivity of water. Reduction of photosynthesis is related to the partial closure of stomata, evidenced in the present study by the reduced stomatal conductance (Figure 5A) and transpiration (Figure 5B). Silva et al. (2018) observed that soursop plants under salt stress inhibited stomatal opening, harming plant physiology, which was also observed in the present study. Therefore, it can be inferred that the saline treatments caused stress on the plants and that prolonged exposure to salts leads to alterations in their water status, inducing stomatal closure and, consequently, limitation of CO₂ entry and reduction of photosynthesis.

Instantaneous carboxylation efficiency (CEi) is a variable closely related to CO₂ assimilation rate and intracellular CO₂ concentration. Moreover, as the salt stress becomes more severe, the dehydration of mesophyll cells inhibits photosynthesis, the mesophyll metabolism is hampered, and, consequently, the carboxylation efficiency is compromised (Taiz et al., 2017). Sousa et al. (2016) also found a reduction in CEi caused by irrigation with saline water in citrus and attributed such reduction to the effects of water stress, resulting from the reduction of CEi, relating it to the loss in the activity of the enzyme RuBP, which may also have occurred with the salt stress caused by the waters with higher electrical conductivity.

**Conclusions**

1. Irrigation with water from 0.7 dS m⁻¹ electrical conductivity impairs gas exchange and absolute growth rates of plant height and stem diameter, and relative growth rate of plant height of soursop plants.

2. Concentrations of 35, 33 and 23 µM of hydrogen peroxide favor the relative and absolute growth rates of plant height and transpiration, respectively.

3. Compared to the aerial part, the root dry matter of soursop plants is more affected when irrigated with water from 1.6 dS m⁻¹.

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**Literature Cited**


Growth and gas exchange of soursop under salt stress and hydrogen peroxide application


