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Effects of saline water and exogenous application of hydrogen peroxide (H_2O_2) on Soursop (*Annona muricata* L.) at vegetative stage

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Abstract

Soursop is a fruit of great socioeconomic importance for the northeastern region of Brazil. However, the quantitative and qualitative limitation of the water resources of this region has reduced its production. The objective of this study was to evaluate the growth of 'Morada Nova' soursop plants irrigated with saline water and subjected to exogenous application of hydrogen peroxide through seed immersion and foliar spray. The study was conducted in plastic pots adapted as lysimeters, using a eutrophic Regolithic Neosol with sandy loam texture under greenhouse conditions. Treatments were distributed in randomized blocks, in a 4 x 4 factorial arrangement, corresponding to four levels of irrigation water electrical conductivity – ECw (0.7; 1.7; 2.7 and 3.7 dS m^{-1}) and four concentrations of hydrogen peroxide – H_2O_2 (0, 25, 50 and 75 µM), with three replicates and one plant per plot. Foliar applications of H_2O_2 began 15 days after transplanting (DAT) and were carried out every 15 days at 17:00 h, after the sunset, by manually spraying the H_2O_2 solutions with a sprayer in such a way to completely wet the leaves (spraying the abaxial and adaxial faces). Treatment effects were evaluated based on plant height (PH), stem diameter (SD), number of leaves (NL) and leaf area (LA) at 60 and 90 DAT, and dry phytomass of leaves (LDP), stem (SDP), roots (RDP) and total (TDP) and leaf succulence (LS) at 90 DAT. Irrigation using water with estimated ECw of 1.55 dS m^{-1} caused acceptable mean reduction of 10% in soursop growth. Increasing concentrations of hydrogen peroxide did not attenuate the effects of salinity on plant height and leaf area during the vegetative stage. Hydrogen peroxide concentration of 20 µM led to higher leaf succulence in soursop plants.

Key words: *Annona Muricata* L., salinity and hydrogen peroxide.
Abbreviations: DAT_days after transplanting; ECw_irrigation water electrical conductivity; PH_plant height; SD_stem diameter; NL_number of leaves; LA_leaf area; LDP_leaf dry phytomass; SDP_stem dry phytomass; RDP_root dry phytomass; TDP_total dry phytomass of plant; LS_leaf succulence.

Introduction

The increase in the economic potential of soursop in the national and international markets, in recent years, is justified by its characteristics of pleasant aroma and taste, besides nutritional and medicinal value. Among the soursops exploited in Brazil, the cultivar 'Morada Nova' has stood out due to its easy adaptation to the most diverse types of soil and for being able to produce more than 40 kg of pulp per year. Currently, the Northeast region in Brazil stands out as the largest producer of this fruit crop, especially the states of Bahia, Alagoas, Ceará, Paraíba and Pernambuco (São José et al., 2014; Almeida et al., 2015).

This region has scarce rainfall and high evaporation rates, naturally causing a water deficit which limits the growth and production of crops under natural conditions, i.e., rational agricultural exploitation is only possible using irrigation; however, the waters available for irrigation in these regions are not always of good quality, causing the use of saline waters to become a reality, characterizing a problem that affects the economy of the region, the society and the environment (Medeiros et al., 2012; Nascimento et al., 2017).

Using water with high contents of salts usually causes toxicity to plant metabolism due to the excessive accumulation of Na^+ and Cl^- ions and to the reduction in the osmotic potential of the soil, decreasing water availability to plants and consequently causing stomatal closure, limiting conductance and transpiration, which reduces the photosynthesis rate (Silva et al., 2010). This situation severely affects seed emergence speed (Nobre et al., 2003) and growth (Veloso et al., 2018), thus compromising crop development.

The feasibility of the use of low-quality waters in agricultural production, besides being associated with the identification of salt-tolerant genetic materials, is also conditioned on the development of strategies that allow the management of saline water and soil, in a way not to compromise crop growth and the environment (Sá et al., 2015).
Acclimation using the pre-treatment of plants with low concentrations of hydrogen peroxide is a practice that can make viable the use of saline water in irrigation. This process consists in previous exposure of an individual to a certain type of stress which causes metabolic changes responsible for increasing its tolerance to a new exposure to the stress. In this context, hydrogen peroxide (H_2O_2) is a signaling molecule of biotic and abiotic stress which plays important role in plant development and physiological processes, besides being one of the promising alternatives in delaying the effects of saline stress on plants (Gondim et al., 2011; Silva et al., 2016).
In this context, this study aimed to evaluate the growth of 'Morada Nova' soursop plants irrigated with saline water and subjected to exogenous application of hydrogen peroxide through seed immersion and foliar sprays during the initial growth.

Results and discussion

Effect of saline stress and exogenous application of hydrogen peroxide on soursop growth

According to the results of the analysis of variance (Table 1), irrigation water salinity had significant effect ($p <0.01$) on all variables studied. On the other hand, hydrogen peroxide and its interaction with irrigation water salinity caused significant effect ($p <0.01$) only on plant height (PH) and leaf area (LA) at 90 DAT.
The saline stress caused by the increase in irrigation water electrical conductivity linearly reduced the height of soursop plants (Fig 1A), by 6.20% per unit increase in ECw, i.e., when plants were subjected to irrigation using 3.7 dS m^{-1} water, PH decreased by 13.71 cm in comparison to plants irrigated with 0.7 dS m^{-1} water. Osmotic and specific effects of the ions, which are characteristic of the saline stress, were possibly the causes for the reduction in soursop height. These effects delay cell expansion and division, leading to negative consequences for the photosynthetic rate, compromising physiological and biochemical processes of the plants (Bezerra et al., 2018).
Increasing levels of water salinity in interaction with hydrogen peroxide concentrations at 90 DAT inhibited the growth in height of soursop plants (Fig 1B). According to the regression equations, plants subjected to the concentrations of 0, 25, 50 and 75 µM showed reductions of 6.74, 7.68, 8.58 and 7.48%, respectively, per unit increase in ECw, i.e., decreases of 17.42, 20.67, 23.85 and 18 cm, respectively, in plants subjected to the highest (3.7 dS m^{-1}) level of salinity compared to those under the lowest level (0.7 dS m^{-1}). Thus, the increase in H_2O_2 concentration under saline stress was not able to alleviate the effects of increasing water salinity, because of the relative reduction in PH as H_2O_2 concentrations increased in interaction with water salinity. Such reduction is possibly related to the intensity of the time of application; probably, due to the frequency of applications, H_2O_2 accumulated in the tissues and became toxic, resulting in cell death (Forman et al., 2010).
Stem diameter of soursop plants was negatively affected by the increase in water salinity (Fig 2A). The reduction was equal to 5.87% at 60 DAT and to 6.33% at 90 DAT, per unit increase in irrigation water salinity. This decrease in SD can be associated with the decline in the photosynthetic rate and with the diversion of energy, previously used for growth, to the activate and maintain the metabolic activity, associated with acclimation to salinity as a way to maintain membrane integrity, synthesis of organic solutes for osmoregulation and/or protection of macromolecules and regulation of the transport and distribution of ions in various organs inside the cells (Souza et al., 2016).
The study conducted by Nobre et al. (2003) corroborates the results found here. These authors found that the increase in irrigation water salinity up to a level of 5.5 dS m^{-1} linearly reduced stem diameter by 9.8% per unit increase in water electrical conductivity.
According to the regression equation (Fig 2B), the increase in water salinity resulted in linear reductions of 5.46 and 12.70% in the number of leaves per unit increase in ECw at 60 and 90 DAT, respectively. Similarly to what occurred with stem diameter (Fig 2A), the longer duration of exposure to saline stress intensified the harmful effects caused by the high concentrations of salts, directly affecting plant growth. The action of the osmotic component hampers the entry of water in plant cells, leading to alterations in the photosynthetic capacity (CO_2 assimilation rate, leaf transpiration and stomatal conductance), inhibiting plant growth (Lima et al., 2016). In addition, the reduction in NL is a form of adaptation of plants to saline stress, and this mechanism is adopted by the plant to reduce water losses through transpiration and exclude the salts accumulated in the leaves (Willadino and Câmara, 2010).
Irrigation water salinity also inhibited soursop leaf area (Fig 3A) and, according to the regression equation, the data fitted to a linear model, with reduction of 25.26% as the ECw increased from 0.7 to 3.7 dS m^{-1}, which is equivalent to a decrease of 404.49 cm². Reduction in transpiring surface leads to the decrease in the balanced absorption of water and nutrients by plants grown under saline stress conditions, which causes damages to the leaf tissues, resulting in more accentuated inhibition of leaf expansion, because, under these conditions, it is interesting for plants to have a reduction in transpiration and, consequently, decrease in the transport of Na^+ and Cl^- in the xylem, and water conservation in plant tissues (Lima et al., 2015).
According to the analysis of the effect of irrigation water salinity at each hydrogen peroxide concentration (Fig 3B), leaf area data fitted to a linear model, with reductions of 10.25, 9.25, 23.61 and 19.14% per unit increase in ECw, respectively at concentrations of 0, 25, 50 and 75 µM of H_2O_2. Higher leaf area (1082.094 cm²) was observed in plants in the treatment with H_2O_2 concentration of 25 µM, when subjected to salinity of 3.7 dS m^{-1}. Despite the reductions in soursop leaf area at all H_2O_2 concentrations as salinity increased, the concentration of 25 µM led to maximum LA under the highest salinity level. This situation demonstrates the efficiency of application of the product on plant acclimation to saline stress. Possibly, the application of 25 µM of hydrogen peroxide stimulated the accumulation of proteins and soluble carbohydrates, which act as organic

Table 1. Summary of F test for plant height (PH), stem diameter (SD), number of leaves (NL) and leaf area (LA) at 60 and 90 days after transplanting (DAT) of 'Morada Nova' soursop seedlings irrigated with saline water and subjected to exogenous application of hydrogen peroxide.

Source of variation	F test							
	PH		SD		NL		LA	
	days after transplanting							
	60	90	60	90	60	90	60	90
Saline levels (SL)	**	**	**	**	**	**	**	**
Linear regression	**	**	**	**	**	**	**	**
Quadratic regression	ns	ns	ns	ns	ns	ns	ns	ns
Hydrogen peroxide (H_2O_2)	ns	**	ns	ns	ns	ns	ns	**
Linear regression	ns	*	ns	ns	ns	ns	ns	ns
Quadratic regression	ns	**	ns	ns	ns	ns	ns	**
Interaction (SL × H_2O_2)	ns	**	ns	ns	ns	ns	ns	**
Blocks	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	12.33	4.30	8.56	9.05	11.09	13.47	9.58	18.19

*; **; ns, significant at 0.05, 0.01 probability levels and not significant, respectively.

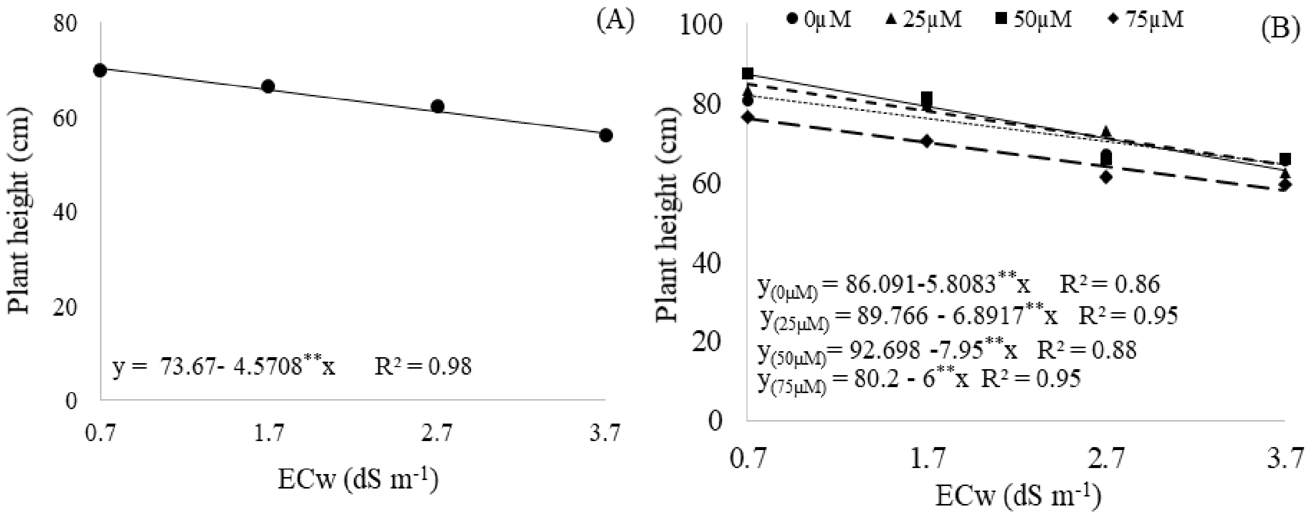

Fig 1. Plant height (PH) of 'Morada Nova' soursop as a function of water salinity - ECw (A) at 60 days after transplanting - DAT and interaction between water salinity and hydrogen peroxide (B) at 90 DAT.

Table 2. Dry phytomass of leaves (LDP), stem (SDP), roots (RDP) and total (TDP) and leaf succulence (LS) of 'Morada Nova' soursop seedlings irrigated with saline water and subjected to exogenous application of hydrogen peroxide, at 90 days after transplanting (DAT).

Source of variation	F test				
	LDP	SDP	RDP	TDP	LS
Saline levels (SL)	**	**	**	**	**
Linear regression	**	**	**	**	**
Quadratic regression	ns	ns	ns	ns	**
Hydrogen peroxide (H_2O_2)	ns	ns	ns	ns	**
Linear regression	ns	ns	**	**	**
Quadratic regression	ns	ns	ns	ns	**
Interaction (SL x H_2O_2)	ns	ns	ns	ns	ns
Blocks	ns	ns	ns	ns	ns
CV (%)	18.51	17.37	12.27	22.67	18.26

*; **; ns, significant at 0.05, 0.01 probability levels and not significant, respectively.

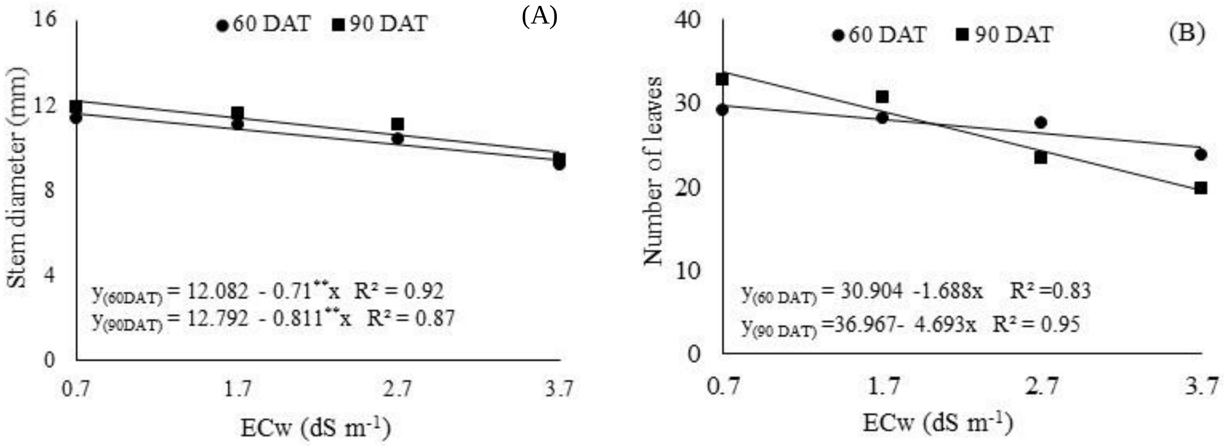

Fig 2. Stem diameter (A) and number of leaves (B) of 'Morada Nova' soursop as a function of water salinity – ECw, at 60 and 90 days after transplanting.

Table 3. Chemical and physical characteristics of the eutrophic Regolithic Neosol used in the experiment.

Chemical characteristics									
pH (H_2O) (1:2.5)	O.M. %	P (mg kg^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	$Al^{3+} + H^+$	ESP (%)	EC_{se} (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 characteristics									
Size fraction (g 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	SL	11.98	4.32	7.66	47.74	1.39	2.66

pH – hydrogen potential, O.M – Organic matter: Walkley-Black Wet Digestion; Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted using 1 M NH_4OAc at pH 7.0; Al^{3+}+H^+ extracted using 0.5 M CaOAc pH 7.0; EC_{se} – electrical conductivity of the saturation extract; ESP – Exchangeable sodium percentage; SL – Sandy loam; AW – Available water; AD - Apparent density; PD- Particle density.

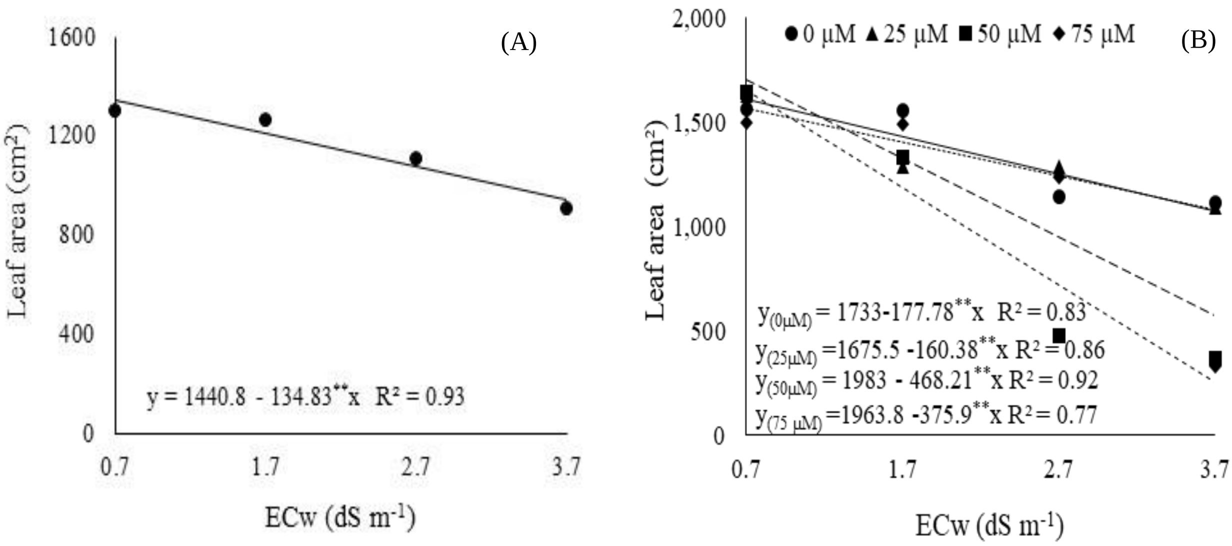

Fig 3. Leaf area (LA) of soursop as a function of water salinity – ECw (A), at 60 days after transplanting – DAT and its interaction with hydrogen peroxide (B), at 90 DAT.

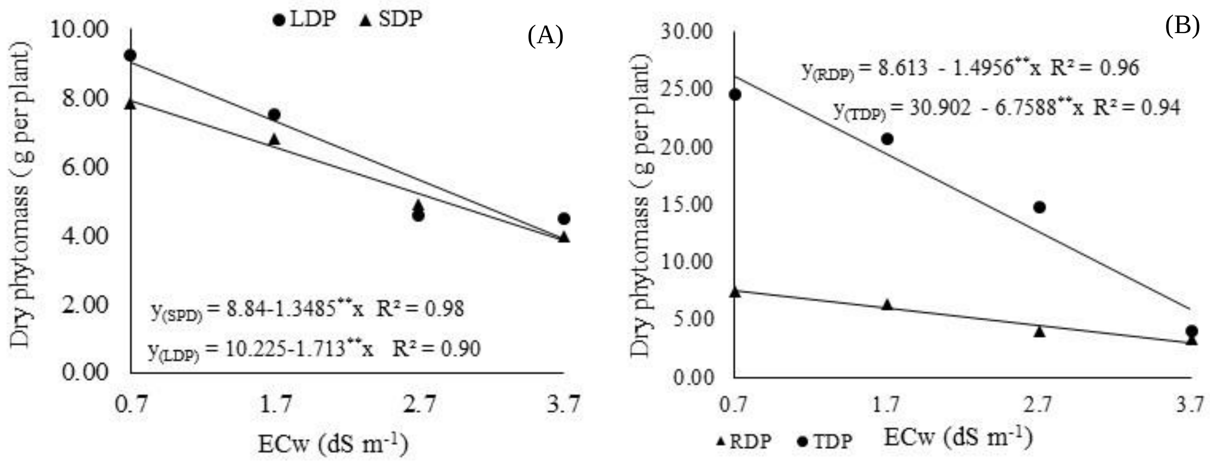

Fig 4. Leaf dry phytomass (LDP) and stem dry phytomass (SDP) (A) and root dry phytomass (RDP) and total dry phytomass (TDP) (B) of 'Morada Nova' soursop as a function of water salinity at 90 DAT.

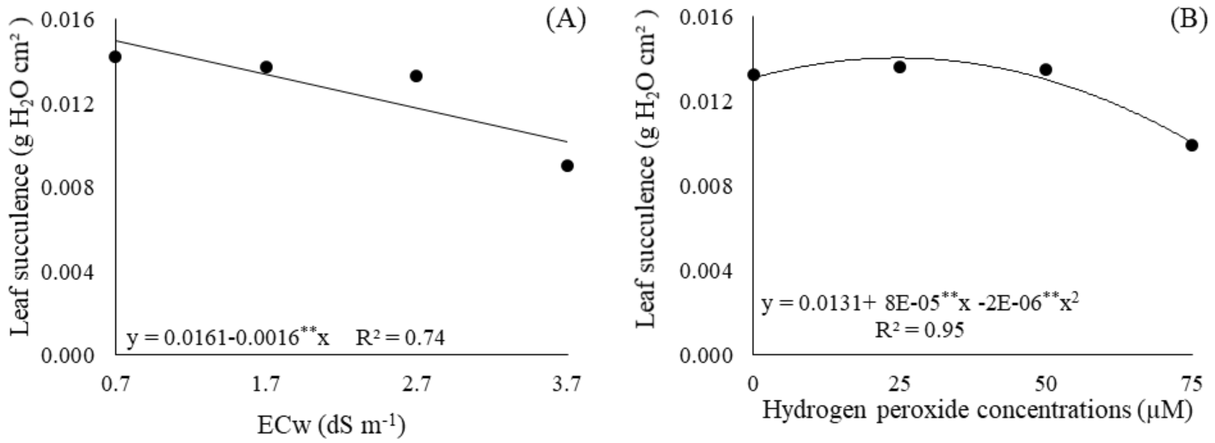

Fig 5. Leaf succulence (LS) of 'Morada Nova' soursop as a function of water salinity – ECw (A) and hydrogen peroxide concentrations (B), at 90 days after transplanting.

solutes, performing the osmotic adjustment in plants under saline stress conditions, culminating in greater absorption of water and nutrients (Carvalho et al., 2011).

Effect of saline stress and exogenous application of hydrogen peroxide on soursop dry phytomass accumulation

According to the summary of F test (Table 2), there were no significant interactions between the factors salinity and hydrogen peroxide for the variables LDP, SDP, RDP, TDP and LS. However, there were significant effects of water salinity levels on all variables and of hydrogen peroxide only on RDP, TDP and LS, at 90 DAT.

Water salinity also linearly reduced leaf dry phytomass (LDP) and stem dry phytomass (SDP). Comparing plants subjected to highest salinity (3.7 dS m^{-1}) with those at the lowest level (0.7 dS m^{-1}), there were reductions in dry matter accumulation of 45.23% in LDP and 41.19% in SDP, i.e., 5.14 and 4.04 g, respectively. It is possible to infer that the increase in irrigation water salinity directly affects the dry phytomass accumulation of soursop plants. Reduction in phytomass production can be related to both osmotic and ionic components. In addition, the low water availability resulting from the reduction in the osmotic potential, due to the high salt concentration, causes stomatal closure and, consequently, reduces CO_2 assimilation and photosynthetic rate, directly affecting phytomass production (Soares et al., 2013). In addition, the reduction of leaf biomass production can be attributed to the reduction in the number of leaves caused by the increase in water salinity observed in Figure 2B.

Irrigation water electrical conductivity significantly and negatively affected root dry phytomass (RDP) and total dry phytomass (TDP) in soursop plants. According to the regression equations (Fig 4B), the linear model indicates reductions of 17.36 and 21.87%, per unit increase in ECw, in RDP and TDP, resulting in decreases of about 4.48 and 20.27 g, respectively. Comparatively, the photosynthetic apparatus was more sensitive to saline stress than the root system, indicating that each plant organ/tissue responds differently to the saline stress. Furthermore, the accentuated reduction in LDP in comparison to RDP can be related to the limitations in the photosynthetic process, besides the increase in the contact surface of the root system, which helps in the absorption of specific ions (Sá et al., 2015). Veloso et al. (2018), studying phytomass accumulation in soursop seedlings under saline stress, observed reductions of 2.80 and 0.64 g in shoot dry phytomass and root dry phytomass when the seedlings were subjected to ECw of 3.5 dS m^{-1}.

Increments in leaf succulence induced by NaCl indicate the occurrence of an effective osmotic adjustment in plants grown under saline stress. However, this behavior was not observed in the present study because, according to the regression equation (Fig 5A), leaf succulence decreased linearly by 9.94% per unit increase in irrigation water salinity. Increase in succulence is important to dilute the concentration of salts in the leaf tissue, avoiding the toxic effect of salt accumulation under saline conditions, and it is a form of adaptation to the stress (Leite et al., 2017). Thus, it can be said that the plant was probably not able to acclimate to the stress through this mechanism.
The increase in hydrogen peroxide concentrations led to a quadratic response of leaf succulence. Plants subjected to H_2O_2 concentration of 20 µM had the highest leaf succulence (0.01390 g H_2O cm²), whereas the lowest value (0.00785 g H_2O cm²) was found in plants subjected to 75 µM. H_2O_2 is a reactive oxygen species which is related to the process of signaling of responses to biotic and abiotic stresses. Therefore, the pre-treatment of plants with H_2O_2 may have prepared the antioxidant system, which avoids the formation of free radicals and prevents the occurrence of damages to plant cells (Ende and El-Esawe, 2014).

Materials and methods

Location, experimental procedure, treatments

The study was conducted from October 2017 to January 2018 in a greenhouse at the Center of Technology and Natural Resources of the Federal University of Campina Grande (CTRN/UFCG), in the municipality of Campina Grande, Paraíba, Brazil, at the geographic coordinates 7º 15' 18" S and 35º 52' 28" W, at an altitude of 550 m.
The experimental design was completely randomized in a 4 x 4 factorial scheme, with three replicates. Treatments consisted of four levels of irrigation water salinity, represented by the values of electrical conductivity - ECw (0.7; 1.7; 2.7 and 3.7 dS m^{-1}), and four concentrations of hydrogen peroxide (0; 25; 50 and 75 µM).
The different concentrations of hydrogen peroxide (H_2O_2), previously established, were obtained by diluting H_2O_2 in distilled water. Prior to sowing, seeds were immersed in H_2O_2 solutions, according to the pre-established treatments, for a period of 24 h in the dark. Seeds subjected to the treatment with H_2O_2 concentration of 0 µM were immersed in distilled water. After this period, sowing was carried out.

Plant material

The seeds used in the experiment were obtained from 'Morada Nova' soursop fruits harvested in a commercial plantation located in the municipality of Macaparana- PE. The seeds were manually extracted and air-dried in the shade. After drying, dormancy was broken by cutting the distal part of the seed, as indicated by Mendonça et al. (2002).

Establishment and management of the experiment

Seedlings were produced in plastic bags. At sowing, 3 seeds were planted in each bag. The bags had capacity for 2 dm^3 of soil and were perforated on the sides to allow free drainage. They were arranged on a wooden bench, at 0.80 m height from the soil, and filled with substrate composed of soil (84%) + medium sand (15%) + humus (1%), on volume basis.
At 145 days after sowing, which is the time required for seedlings to reach adequate growth for transplantation according to Melo et al. (2016), the seedlings were transplanted to 23-dm³ plastic pots adapted as lysimeters, which had a drain at the bottom to collect the drained water. Plants remained in the lysimeter along the entire duration of the experiment, which lasted about three months.
Foliar applications of H_2O_2 began 15 days after transplanting (DAT) and were carried out every 15 days from 17:00 h, after the sunset, by manually spraying the H_2O_2 solutions with a sprayer in such a way to completely wet the leaves (spraying the abaxial and adaxial faces).
The levels of irrigation water electrical conductivity (1.7; 2.7 and 3.7 dS m^{-1}) were prepared by dissolving the salts NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$, in equivalent proportion of 7:2:1, respectively, in water from the local supply system (ECw = 1.10 dS m^{-1}), based on the relationship between ECw and the concentration of salts ($mmol_c$ L^{-1} = 10*ECw dS m^{-1}). Such proportion is commonly found in sources of water used for irrigation in small properties of the Northeast region (Medeiros et al., 2003). The ECw level of 0.7 dS m^{-1} was obtained by diluting public-supply water in rainwater (ECw = 0.02 dS m^{-1}).
The soil used to fill the bags and the lysimeters was a eutrophic Regolithic Neosol with sandy loam texture, from the rural area of the municipality of Lagoa Seca-PB. Samples were collected in the 0-20 cm layer, properly pounded to break up clods and sieved for the determinations of chemical and physical characteristics (Table 3) at the Laboratory of Irrigation and Salinity (LIS) of the CTRN/UFCG, according to the methodologies proposed by Claessen (1997).
Soil water content was maintained at a level close to field capacity by daily irrigations, applying in each lysimeter the solutions corresponding to the treatments. The applied volume was estimated by water balance: applied water volume minus drained water volume in the previous irrigation, plus a leaching fraction of 0.15, to avoid the excessive accumulation of salts in the soil.
Nitrogen (N), potassium (K) and phosphate (P) fertilizations were carried out based on the recommendations of Novais et al. (1991) for pot experiments. 5.11 g of urea, 5.75 g of potassium chloride and 13.8 g of monoammonium phosphate, which were equivalent to 100, 150 and 300 mg kg^{-1} of the substrate of N, K_2O and P_2O_5, respectively, were applied as top-dressing in four equal portions through fertigation, at 15-day intervals, with the first application at 10 DAT.

Traits measured

Treatment effects were evaluated based on plant height (PH), stem diameter (SD), number of leaves (NL) and leaf area (LA) at 60 and 90 DAT, and dry phytomass of leaves (LDP), stem (SDP), roots (RDP) and total (TDP) and leaf succulence (LS) at 90 DAT.
Plant height (PH) was measured as the distance between the collar and the apical meristem. Stem diameter was measured 3 cm above the collar using a digital caliper.

Number of leaves was obtained by counting the fully expanded leaves with minimum length of 3 cm in each plant, and leaf area (cm^2) was determined as recommended by Almeida et al. (2006), using Equation 1:

$$\mathrm{LA} = 5.71 + 0.647X \quad (1)$$

Where:

LA - leaf area (cm^2); and,

X - product of the length by the width (cm).

To obtain dry phytomass, the stem of each plant was cut close to the soil and then the different parts were separated (stem, leaves and roots), placed in paper bags and dried in a forced-air oven at temperature of 65 °C, until constant weight. After that, the material was weighed to obtain the phytomass of leaves, stem, roots and total.

Leaf succulence (LS) was determined at 90 DAT, according to the methodology proposed by Mantovani (1999), given by Equation 2:

$$\mathrm{LS} = \frac{(\mathrm{LFP} - \mathrm{LDP})}{\mathrm{LA}} \quad (2)$$

Where:

LFP - leaf fresh phytomass (g);

LDP - leaf dry phytomass (g); and,

LA - leaf area of the leaf used (cm^2)

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 polynomial regression analyses were carried out using the statistical program SISVAR (Ferreira, 2014).

Conclusion

Growth and leaf succulence of soursop plants are reduced by the increase in irrigation water salinity, but irrigation with 1.55 dS m^{-1} water causes acceptable reductions of 10% in the growth of ‘Morada Nova’ soursop.

Increments in hydrogen peroxide concentrations did not attenuate the effects of salinity on soursop growth.

Hydrogen peroxide concentration of 20 μM led to higher leaf succulence in soursop plants.

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