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Tolerance to salt stress in soursop seedlings under different methods of H_2O_2 application[1]

Tolerância ao estresse salino de mudas de gravioleira sob diferentes métodos de aplicação de H_2O_2

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ABSTRACT - The aim of this study was to evaluate the effect of different methods of hydrogen peroxide application on biomass production and quality in soursop seedlings under salt stress. The study was conducted in a greenhouse, in a Psammitic Regolithic Neosol of a sandy-loam texture. The treatments were distributed in a completely randomised design, in a 5 x 4 factorial scheme, with five levels of electrical conductivity for the irrigation water - ECw (0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}) and four methods of hydrogen peroxide application (Control, application by soaking the seeds, application by spraying, and application by soaking and spraying), with two plants per plot and four replications. From 0.6 dS m^{-1}, the salinity of the irrigation water inhibited dry matter production and altered the leaf morpho-physiological index of the soursop seedlings 145 days after sowing. The method of hydrogen peroxide application by soaking the seeds minimised the effect of salt stress on root dry matter production and the quality of the soursop seedlings, and increased leaf succulence, standing out as a tolerance mechanism to salt stress. The application of hydrogen peroxide by soaking the seeds favoured an increase in the root to shoot ratio in soursop exposed to salinity of the irrigation water.

Key words*: Annona Muricata* L. Saline water. Hydrogen peroxide.

RESUMO - Objetivou-se com o presente trabalho, avaliar o efeito de diferentes métodos de aplicação de peróxido de hidrogênio sobre a produção de biomassa e qualidade de mudas de gravioleira sob estresse salino. O estudo foi conduzido em casa de vegetação, utilizando-se de um Neossolo Regolítico Psamitico de textura franco-arenosa. Os tratamentos foram distribuídos em delineamento inteiramente casualizados, em arranjo fatorial 5 x 4, sendo cinco níveis de condutividade elétrica da água de irrigação – CEa (0,6; 1,2; 1,8; 2,4 e 3,0 dS m^{-1}) e quatro métodos de aplicação de peróxido de hidrogênio – (Testemunha; aplicação por embebição das sementes; aplicação por pulverização e aplicação por embebição e pulverização), com duas plantas por parcela e quatro repetições. A salinidade da água de irrigação a partir de 0,6 dS m^{-1} inibiu a produção de biomassa seca e altera os índices morfofisiológicos foliares de mudas de gravioleira, aos 145 dias após o semeio; O método de aplicação de peróxido de hidrogênio por embebição das sementes minimizou o efeito do estresse salino sobre a produção de biomassa seca radicular e na qualidade das mudas de gravioleira e aumentou da suculência foliar, destacando-se como mecanismo de tolerância ao estresse salino; A aplicação de peróxido de hidrogênio por embebição das sementes favoreceu o aumento da relação raiz/parte aérea da gravioleira quando exposta a salinidade da água de irrigação.

Palavras-chave: *Annona Muricata* L. Águas salinas. Peróxido de hidrogênio.

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INTRODUCTION

The salinity of the water and/or the soil is one of the main factors that limit agricultural production worldwide, especially in semi-arid regions (SCUDEIRO; SKAGGS; CORWIN, 2016). According to Lhissou, El Harti and Chokmani (2014), salinity negatively affects one billion hectares worldwide, and this is expected to increase at a rate of two million hectares per year, mainly due to natural factors and anthropogenic action.

In the semi-arid region of north-eastern Brazil, spatial and temporal irregularity of the rainfall is common, together with high evaporation that favours an increase in the levels of salinity of the water sources available for irrigation. An excess of salts in the water hampers the absorption of water and nutrients by plants due to a reduction in the osmotic potential of the soil, thereby affecting the growth and development of the crops (SILVA *et al*., 2019a).

According to Zhu *et al*. (2019), salinity causes significant reductions in morphological and physiological parameters, stomatal density and water conductance, which are the result of the reduced osmotic potential of the soil, the toxicity caused by excessive absorption of Na^+ and Cl^- ions, nutritional imbalance and oxidative stress, especially in plants sensitive to salt stress.

Faced with the problem of salinity in the semi-arid region of north-eastern Brazil, studies that make it possible to use saline water in agriculture have become a necessity to guarantee crop sustainability. In this context, the use of hydrogen peroxide in acclimatising plants has emerged as an alternative way of minimising the harmful effects caused by the high salt content of the plants (HASAN *et al*., 2016).

The exogenous application of hydrogen peroxide in low concentrations promotes a condition of moderate stress, which results in the accumulation of latent signals in different parts of the plant. When exposed to a condition of more severe stress, the stored signals lead to molecular adjustments that result in various mechanisms of acclimatisation (SAVVIDES *et al*., 2016).

The soursop (*Annona muricata* L.) is a crop belonging to family *Annonaceae*, and has great economic value, especially for north-eastern Brazil, where the state of Bahia is the largest domestic producer (LEMOS *et al*., 2014). Studies involving the soursop have been on the increase in recent years, which can be explained in part by the crop having food and nutritional properties, and pharmaceutical characteristics that are used in the treatment of various diseases (BENTO *et al*., 2016).

Some research has been carried out on the use of hydrogen peroxide in soursop seedlings under salt stress (SILVA *et al*., 2019b; VELOSO *et al*., 2020). However, there is no information on identifying a method of H_2O_2 application. The aim of this study, therefore, was to evaluate the effect of different methods of hydrogen peroxide application on biomass production and quality in soursop seedlings under salt stress.

MATERIAL AND METHODS

The experiment was conducted between April and September 2019, using plastic bags with a capacity of 2 dm^3 under greenhouse conditions at the Centre for Technology and Natural Resources of the Federal University of Campina Grande (CTRN/UFCG), in the district of Campina Grande in the state of Paraíba (PB), located at 7º15'18" S, 35º52'28" W, at a mean altitude of 550 m.

The treatments resulted from a combination of five levels of electrical conductivity for the irrigation water - ECw (0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}) and four methods of hydrogen peroxide application - H_2O_2 (M1 = Control – with no H_2O_2 application, M2 = soaking, M3 = spraying, and M4 = soaking and spraying), in a 5 x 4 factorial scheme, in a completely randomised design with four replications and two plants per plot, giving a total of one hundred and sixty plants.

Sodium chloride (NaCl) was used to prepare the irrigation water, adjusting the concentration of the water supply (0.4 dS m^{-1}) in the district of Campina Grande, PB, based on the ratio between the ECw and the salt concentration ($mmol_c$ L^{-1} = 10*ECw dS m^{-1}) recommended by Rhoades, Kandiah and Mashali (2000). After preparing and calibrating the ECw, the saline water was stored in plastic vessels with a capacity of 120 L, properly protected to avoid evaporation.

The plastic bags were filled with 2.6 kg of an air-dried substrate composed of soil (84%), sand (15%) and humus (1%). The soil used in the experiment was a Psammitic Regolithic Neosol of a sandy-loam texture collected from the 0-20 cm layer in the rural area of the district of Lagoa Seca, PB, properly broken up and sieved. The physical and chemical characteristics (Table 1) were determined as per the method proposed by Teixeira *et al*. (2017).

The seeds used in the experiment were obtained from fruit harvested in a commercial orchard located in the district of Sousa, PB. The seeds were extracted manually; they were then air-dried, and the dormancy broken by distal cut to the embryo, following the method proposed by Mendonça *et al*. (2007).

The seeds for the soaking treatment, and the soaking and spraying treatment were pre-treated with hydrogen peroxide prior to sowing, by soaking at a concentration of 20 µM for 36 hours in the dark. The seeds for the control and spraying treatments were not pre-treated with hydrogen peroxide. The concentration of hydrogen peroxide (20 µM) and the soaking time

Table 1 – Chemical, physical and hydraulic attributes of the soil used in the experiment

Chemical characteristics									
pH (H_2O) (1:2.5)	OM %	P (mg kg^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	$Al^{3+} + H^+$	PES (%)	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 and hydraulic characteristics									
Particle size fraction (dag kg^{-1})			Textural class	Moisture (kPa)		AW	Total porosity %	Ds	PD
Sand	Silt	Clay		33.42	1519.5 dag kg^{-1}			(g cm$^{-3)}$	
73.29	14.21	12.50	SL	11.98	4.32	7.66	47.74	1.39	2.66

OM – Organic Matter; PES- Percentage exchangeable sodium; ECse – Electrical conductivity of the saturation extract; SL – Sandy Loam; AW – Available water; Ds- Bulk desnsity; PD- Particle density

were determined as per a study developed by Veloso *et al*. (2020), the concentrations were achieved by diluting the H_2O_2 in deionised water.

Three of the soursop seeds were then planted, spaced equally apart, at a depth of three centimetres. Forty days after sowing (DAS), the plants were thinned out to leave one plant showing physiological vigour per bag.

Before sowing, the soil moisture content was increased until field capacity was reached. After sowing, irrigation was carried out daily by applying a volume of water to maintain the substrate moisture close to field capacity. The volume to be applied was determined from the water balance, by subtracting the drained volume from the volume applied during the previous irrigation, plus a leaching fraction of 0.10 every 20 days (AYERS; WESTCOT, 1999) in order to avoid the excessive accumulation of salts in the root zone.

A top dressing of nitrogen, potassium and phosphorus was applied based on the recommendation contained in Novais, Neves and Barros (1991), including 0.58 g urea, 0.65 g potassium chloride and 1.56 g monoammonium phosphate, equivalent to 100, 150 and 300 mg kg^{-1} of N, K_2O and P_2O_5 respectively. Application was in four equal doses via fertigation at 15-day intervals, with the first application 15 days after sowing (DAS). In order to meet the micronutrient requirement, 1.0 g L^{-1} of a solution of Mg (1.1%), Zn (4.2%), B (0.85%), Fe (3.4%), Mn (3.2%), Cu (0.5%), and Mo (0.05%) was applied via the leaves at 60, 75, 90, 105, 120 and 135 DAS.

At 80, 95, 110 and 125 DAS the plants from treatment M3 (spraying) and treatment M4 (soaking and spraying) were submitted to foliar applications of H_2O_2 (20 μM), carried out manually at 17:00, with the abaxial and adaxial surfaces being sprayed to wet the leaves completely.

The effects of the treatments were evaluated at 145 DAS and the following determined: leaf succulence (SUC), specific leaf area (SLA), leaf area ratio (LAR), the Dickson quality index (DQI), leaf dry matter (LDM), stem dry matter (STDM), shoot dry matter (SDM), root dry matter (RDM) and total dry matter (TDM), in addition to the root dry matter to shoot dry matter ratio (R/S).

Leaf succulence (SUC) was determined according to the methodology proposed by Mantovani (1999), expressed by Equation 1.

$$SUC = \frac{(LFM - LDM)}{LA} \quad (1)$$

where:

SUC – Leaf succulence (g H_2O cm^2);

LFM – Leaf fresh matter (g); and

LDM – Leaf dry matter.

The leaf area (cm^2) was determined as recommended by Almeida *et al*. (2006). The methodology proposed by Benincasa (2003) was used to determine the specific leaf area (SLA, cm^2 g^{-1}) and leaf area ratio (LAR, cm^2 g^{-1}).

To obtain the dry matter, the stem of each plant was cut close to the ground and the different parts (stem, leaf and roots) were then separated and packed in paper bags and left to dry in a forced-air ventilation oven at 65 °C to constant weight. The material was then weighed, and the leaf, stem, root and total dry matter were determined.

Seedling quality was estimated by means of the Dickson quality index (DQI) for seedlings, using the formula by Dickson, Leaf and Hosner (1960), described by Equation 4.

$$DQI = \frac{TDM}{(PH / SD) + (SDM / RDM)} \quad (4)$$

where:

DQI – Dickson quality index;

PH – plant height (cm);

SD – stem diameter (mm);

TDM – total plant dry matter (g);

SDM – plant shoot dry matter (g); e,

RDM – plant root dry matter (g).

The collected data were submitted to analysis of variance by F-test at a level of 0.05 probability and, when significant, linear and quadratic polynomial regression analysis was carried out for the levels of salinity, while the methods of application were submitted to Tukey's test ($p<0.05$) using the SISVAR statistical software (FERREIRA, 2014).

RESULTS AND DISCUSSION

From the summary of the analysis of variance (Table 2), a significant effect ($p <0.01$) can be seen from the interaction between the salinity of the irrigation water and the methods of hydrogen peroxide application on the shoot and root dry matter. The salinity of the irrigation water analysed separately had a significant effect ($p<0.01$) on all the variables under study. The methods of hydrogen peroxide application influenced the SDM and RDM only.

The salinity of the water inhibited the production of LDM (Figure 1A) and STDM (Figure 1B) in the soursop at 145 DAS; according to the regression equations, there is a reduction in LDM of 28.08% and in STDM of 27.86% per unit increase in ECw. When comparing in relative terms the results for plants irrigated with water at the highest level of salinity (3.0 dS m^{-1}) with those irrigated at the lowest level (0.6 dS m^{-1}), there is a reduction of 81.04% (1.93 g) in LDM and 80.3% (1.28 g) in STDM.

The reduction in dry matter (Figures 1A and B) may be related to the detrimental effects of the salt stress, since high concentrations of sodium salts negatively affect the physiological aspects of the plant, promoting ionic, osmotic, hormonal and nutritional changes, thereby causing a reduction in growth and, consequently, in biomass accumulation (SÁ *et al*., 2019). In research developed by Silva *et al*. (2019b), in soursop seedlings under salt stress (ECw ranging from 0.7 to 3.5 dS m^{-1}), the authors also found a reduction in dry matter accumulation for increases in the electrical conductivity of the irrigation water.

It was found that the methods of hydrogen peroxide application influenced shoot and root dry matter at all levels of salinity (Figures 2A and 2B). Note that the plants that were not submitted to H_2O_2 application (Control) had a reduction of 81.9% (2.16 g $plant^{-1}$) in SDM and 36.16% (0.64 g $plant^{-1}$) in RDM when irrigated at the highest level of salinity (3.0 dS m^{-1}) compared to those at the lowest level (0.6 dS m^{-1}).

However, when H_2O_2 was applied by soaking the seeds, there was a reduction in the detrimental effect of the salt stress on the SDM and RDM. It should be noted that the level of salinity of 1.2 dS m^{-1}, together with the soaking method of application, resulted in the greatest value for both SDM (4.81 g $plant^{-1}$) and RDM (2.82 g $plant^{-1}$). In research carried out by Farouk and Amira (2018) on the pea, it was found that the application of hydrogen peroxide by soaking the seeds also afforded greater plant growth.

The beneficial effect of hydrogen peroxide application by soaking the seeds, seen in SDM and RDM, can be attributed to the fact that, for this method of application, the seeds had previous contact with H_2O_2 before the exposure to salt stress; according to Forman, Maiorino and Ursini (2010), the pre-exposure of plants to moderate stress or signalling metabolites such as H_2O_2 can result in metabolic cell signalling (increased metabolites and/or antioxidative enzymes), resulting in better physiological performance when the plant is exposed to more severe stress.

Table 2 - Summary of the analysis of variance for leaf dry matter (LDM), stem dry matter (STDM), root dry matter (RDM), shoot dry matter (SDM), total dry matter (TDM), and the root/shoot ratio (R/S) in soursop seedlings irrigated with saline water and submitted to different methods of hydrogen peroxide application, 145 days after sowing

Source of variation	Mean square					
	LDM	STDM	SDM	RDM	TDM	R/S
Levels of salinity (LS)	9.53**	4.09**	2.54**	7.62**	55.65**	0.24**
Linear regression	37.25**	16.31**	9.64**	29.08**	220.29**	0.59**
Quadratic regression	0.11^{ns}	0.03^{ns}	0.22^{ns}	1.13^{ns}	0.86^{ns}	0.17^{ns}
Methods application (MA)	0.23^{ns}	0.11^{ns}	1.17**	0.34**	3.29^{ns}	0.09^{ns}
Interaction (LS x MA)	0.22^{ns}	0.12^{ns}	0.41**	0.26**	1.04^{ns}	0.21**
Residual	0.13	0.04	0.07	0.08	0.14	0.04
CV (%)	25.85	22.05	8.28	27.99	10.93	32.19

ns, **, * respectively, not significant, significant at $p<0.01$ and significant at $p<0.05$

Figure 1 – Leaf dry matter - LDM (A) and stem dry matter - STDM (B) in soursop seedlings as a function of the electrical conductivity of the water - ECw, 145 days after sowing

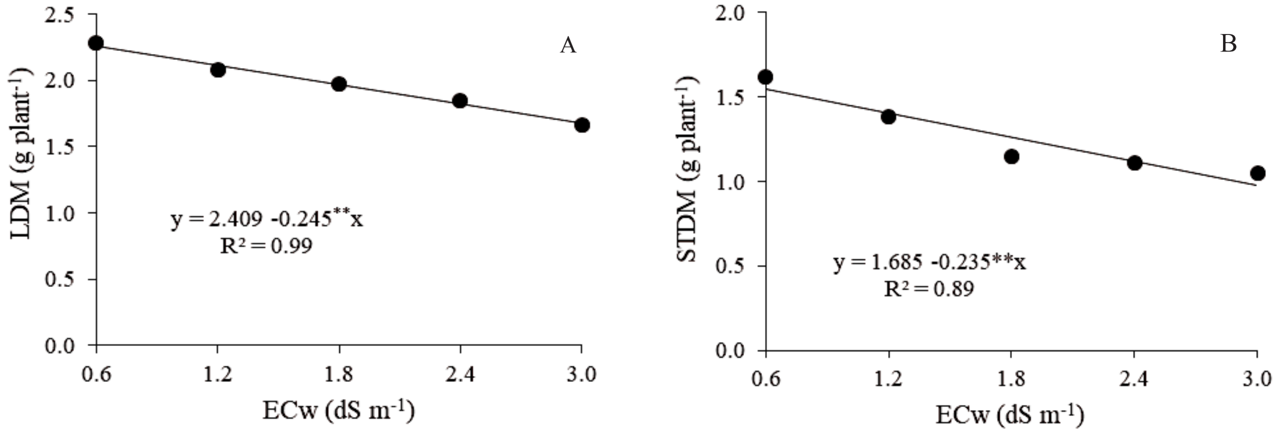
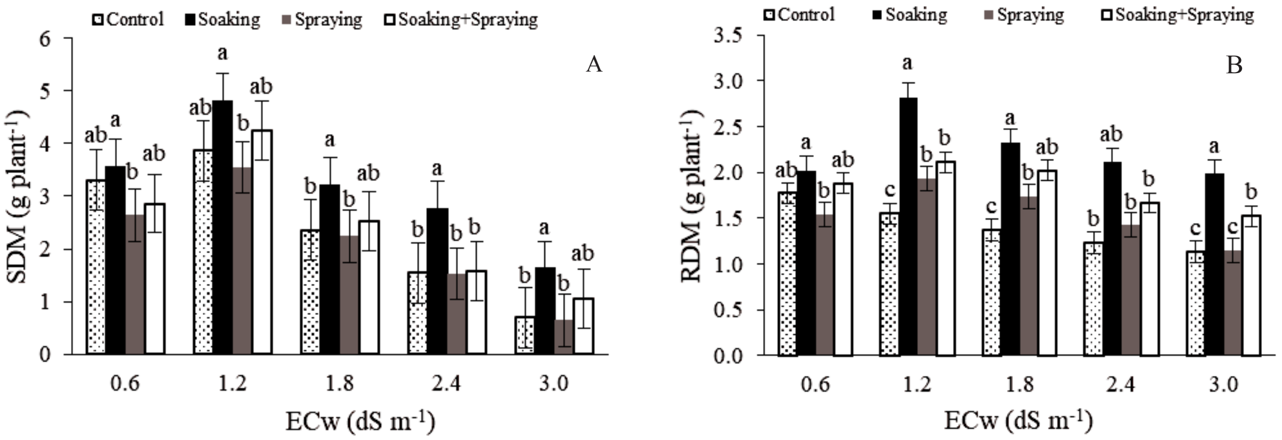


Figure 2 – Shoot dry matter - SDM (A) and root dry matter - RDM (B) in soursop seedlings irrigated with saline water – ECw and submitted to different methods of hydrogen peroxide application, at 145 days after sowing



Mean values followed by the same letter do not differ statistically by Tukey's test ($p<0.05$); vertical bars indicate the standard error of the mean (n=4)

Increases in the electrical conductivity of the irrigation water reduced the production of TDM (Figure 3A); according to the regression equation, there is a 22.93% reduction in TDM per unit increase in ECw. When comparing in relative terms the results for plants irrigated with water at the highest level of salinity (3.0 dS m^{-1}) with those irrigated at the lowest level (0.6 dS m^{-1}), there is a reduction of 63.76% (3.55g $plant^{-1}$) in TDM. Considering the ranges of relative reduction established by Richards (1954), the soursop seedlings can be classified as Sensitive (>60%).

The root to shoot ratio of the soursop plants differed statistically when water with a ECw from 1.2 dS m^{-1} was used. It can be seen that an increase in the electrical conductivity of the irrigation water afforded an increase in the R/S ratio irrespective of the method of H_2O_2 application. However, the method of hydrogen peroxide application by soaking the seeds differed statistically from the other methods, with the highest R/S ratio (1.15 g g^{-1}) in plants irrigated with water at 3.0 dS m^{-1} and submitted to M2.

The increase in the R/S ratio with the increase in salinity was due to the higher reduction rate for shoot dry matter compared to that of the roots (Figures 2A and 2B). In addition, hydrogen peroxide applied by soaking the seeds improves the growth and development of the root system, favouring an increase in the R/S ratio (HASAN *et al*., 2016).

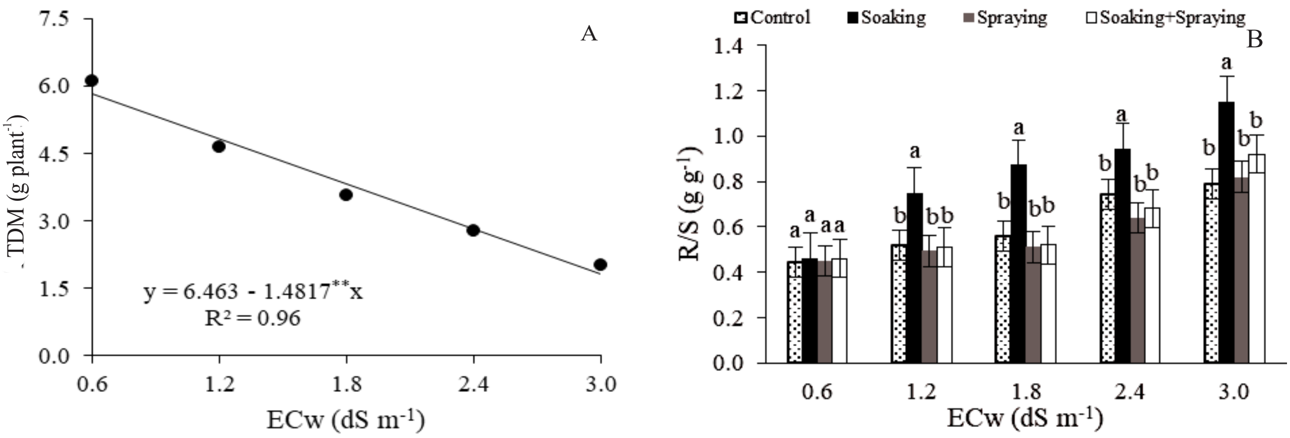
It can be seen from the summary of the analysis of variance (Table 3), that the interaction between the levels of salinity and the methods of hydrogen peroxide application significantly affected ($p<0.01$) all the variables under analysis. The levels of salinity and methods of hydrogen peroxide application had a significant effect ($p<0.01$) on leaf succulence (SUC), specific leaf area (SLA), leaf area ratio (LAR) and Dickson quality index (DQI) in the soursop seedlings at 145 DAS.

Based on Figure 4A, it can be seen that soursop plants irrigated with water at 2.4 and 3.0 dS m^{-1} and whose seeds were soaked in H_2O_2 (M2) obtained the highest mean values for SUC. It was found that M2 (application by soaking the seeds) together with a salinity level of 3.0 dS m^{-1} resulted in the highest value for leaf succulence (0.028 g H_2O cm^2). Similar results were obtained in research by Lima *et al*. (2019), using cotton (*Gossypium hirsutum* L.) under salt stress, in which they attributed the increase in SUC to a possible osmotic adjustment in the plants allowing regulation of the salt concentration in the leaf tissue.

According to GE *et al*. (2015), hydrogen peroxide acts as an osmotic regulator maintaining turgor in the protective cells and inducing the absorption of water and nutrients through the plasma membranes of the root hairs, thereby favouring the water absorption capacity and the water potential of the leaves.

The increase in the electrical conductivity of the irrigation water compromised the specific leaf area (Figure 4B) and leaf area ratio (Figure 4C) in the soursop at 145 DAS, irrespective of the method of hydrogen peroxide application. When comparing the Control plants irrigated with water at the highest level of salinity (3.0 dS m^{-1}) with those irrigated at the lowest level (0.6 dS m^{-1}), a reduction of 51% (130.3 cm^2 g^{-1}) was found in SLA, and of 47.2% (56.11 cm^2 g^{-1}) in LAR.

Figure 3 - Total dry matter - TDM (A) and root/shoot ratio - R/S (B) in soursop seedlings irrigated with saline water - ECw and submitted to different methods of hydrogen peroxide application, 145 days after sowing



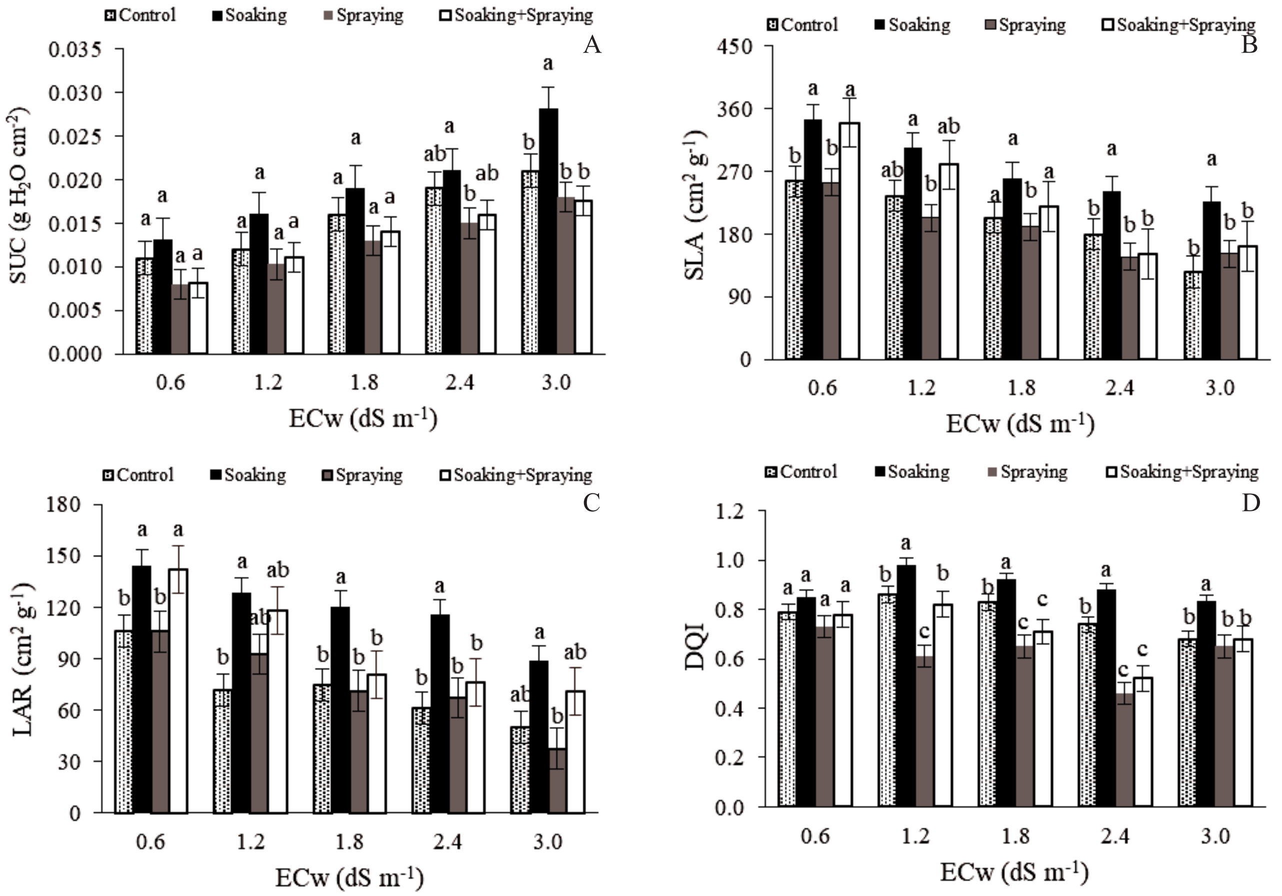
Mean values followed by the same letter do not differ statistically by Tukey's test ($p<0.05$); vertical bars indicate the standard error of the mean (n=4)

Table 3 - Summary of the analysis of variance for leaf succulence (SUC), specific leaf area (SLA), leaf area ratio (LAR) and Dickson quality index (DQI) in soursop seedlings irrigated with saline water and submitted to different methods of hydrogen peroxide application, 145 days after sowing

Source of variation	Mean Square			
	SUC	SLA	LAR	DQI
Levels of salinity (LS)	91.79**	345943.66**	114410.79**	2.96**
Linear regression	352.12**	992366.55**	343572.19**	11.58**
Quadratic regression	1.47^{ns}	208000.27^{ns}	86346.87*	0.03^{ns}
Methods application (MA)	9.81**	143560.06**	41731.41**	0.26**
Interaction (LS x MA)	11.37**	112212.56**	19256.88**	0.10**
Residual	0.69	1883.41	295.60	0.01
CV (%)	16.69	10.44	10.17	11.67

ns, **, * respectively, not significant, significant at $p<0.01$ and significant at $p<0.05$

Figure 4 - Leaf succulence - SUC (A), specific leaf area - SLA (B), leaf area ratio - LAR (C) and Dickson quality index - DQI (D) in soursop seedlings irrigated with saline water - ECw and submitted to different methods of hydrogen peroxide application, 145 days after sowing



Mean values followed by the same letter do not differ statistically by Tukey's test ($p<0.05$); vertical bars indicate the standard error of the mean (n=4)

On the other hand, it can be seen that, despite the reductions found in SLA and LAR, the plants whose seeds were soaked in H_2O_2, achieved higher mean values when exposed to water salinity. Based on the mean value comparison test, the highest values for SLA (344.71 cm^2 g^{-1}) and LAR (144.06 cm^2 g^{-1}) were obtained in plants irrigated with water at 0.6 dS m^{-1}, equal to an increase of 34.9% and 35.6% in SLA and LAR respectively compared to the control plants irrigated at the same level of salinity.

The beneficial effect of H_2O_2 application by soaking the seeds is due to the use of H_2O_2 in suitable concentrations favouring a greater absorption of water and nutrients by the plants, including essential ions for plant growth and development, among them N, P and K (FAROUK; AMIRA, 2018).

The quality of the soursop seedlings, assessed by means of the Dickson quality index (DQI), was influenced by the methods of hydrogen peroxide application when irrigated with water at 1.2, 1.8, 2.4 and 3.0 dS m^{-1}. Furthermore, according to the mean value comparison test, the highest values for DQI (0.98) were obtained in plants whose seeds were soaked in hydrogen peroxide (M2), except at the lowest level of salinity, and reached maximum value in plants irrigated with water at 1.2 dS m^{-1}.

The Dickson quality index is a morphological parameter used to express the quality and rusticity of seedlings and evaluates the capacity for growth and survival (OLIVEIRA *et al*., 2013). In the present research it was found that even plants irrigated at the highest level of salinity (3.0 dS m^{-1}) showed an acceptable DQI, since seedlings with a DQI greater than 0.2 are considered to be of good quality; however, as the DQI expresses robustness and the balanced distribution of biomass, the higher the DQI, the better the quality of the seedlings.

In the present study it was found that the exogenous application of hydrogen peroxide by soaking the seeds was effective in inducing tolerance to salt stress, standing out among the other methods of application. As such, it can be concluded that in soursop seedlings it is not necessary to apply hydrogen peroxide by spraying, thereby contributing to a saving in the production of soursop seedlings, since the costs of labour and product acquisition will be reduced.

CONCLUSIONS

1. Irrigation water at a salinity of 0.6 dS m^{-1} onwards inhibits dry matter production and alters the leaf morpho-physiological index in soursop seedlings 145 days after sowing;
2. The method of hydrogen peroxide application by soaking the seeds minimises the effect of salt stress on the production of root and shoot dry biomass and on the quality of the soursop seedlings. This treatment also results in an increase in leaf succulence, and stands out as a tolerance mechanism to salt stress;
3. The application of hydrogen peroxide by soaking the seeds favours an increase in the root to shoot ratio of the soursop when exposed to salinity of the irrigation water.

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