

Growth and quality of soursop (*Annona muricata*, L.) seedlings under saline stress and hydrogen peroxide (H₂O₂)

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Abstract

This study aimed to evaluate growth and quality of soursop seedlings cv. Morada Nova, as a function of saline water irrigation and exogenous applications of hydrogen peroxide (H₂O₂), under greenhouse conditions. The experimental design used was randomized blocks in 5 x 5 factorial scheme, corresponding to the combination of five levels of irrigation water electrical conductivity – ECw (0.7, 1.4, 2.1, 2.8 and 3.5 dS m⁻¹) and five concentrations of hydrogen peroxide (0, 25, 50, 75 and 100 µM). The results showed that Hydrogen peroxide concentrations were applied by soaking the seed for 24 h and by spraying on all leaves of soursop seedlings. Irrigation water above electrical conductivity of 0.7 dS m⁻¹ negatively affected growth and quality of soursop seedlings cv. Morada Nova. The dry phytomass of root was the most sensitive variable to saline stress. Hydrogen peroxide concentrations of 31 and 100 µM led to highest relative growth rate in leaf area and dry phytomass of leaves and stem, respectively. The quality of soursop seedlings cv. Morada Nova was not compromised by using water with electrical conductivity of 3.5 dS m⁻¹ in irrigation.

Key words: *Annona muricata* L., salinity, acclimation

Abbreviations: DAS_ days after sowing; ECw_ irrigation water electrical conductivity; RGR_{PH}_ relative growth rate in plant height; RGR_{SD}_ relative growth rate in stem diameter; RGR_{LA}_ relative growth rate in leaf area; LDP_ dry phytomass of leaves; SDP_ dry phytomass of stem; RDP_ dry phytomass of roots; TDP_ dry phytomass total; DQI_ Dickson quality index.

Introduction

Belonging to the Annonaceae family, soursop (*Annona muricata*, L.) occupies a promising position in Brazilian fruticulture, especially in the Northeast region. Its consumption, either fresh or industrially processed, has increased due to its nutritional importance and forms of use in human diet, besides the medicinal properties of its leaves, fruits, seeds and roots (Freitas et al., 2013).

Although the Northeast region has favorable soil and climatic conditions to produce soursop, that is not enough to promote a great potential of exploitation of this crop because such potential has been limited by irregular rainfall regimes (Souza et al., 2018), causing water deficit for plants because evaporation rates exceed rainfall during most of the year, leading to elevation of salinity in the water sources (Lima et al., 2018).

Consequently, irrigation using waters with high concentration of soluble salts, especially sodium, became a common practice. In addition, using these waters for long periods may cause negative effects on soils and crops in these areas. This compromises their growth and

development due to the toxic, osmotic and nutritional imbalance effects, leading to morphological and physiological alterations, consequently reducing the production (Silva et al., 2017; Shrivastava and Kumar, 2015). Thus, soursop seedling production in this region can be optimized by using techniques that allow for the management of waters with excess salts, such as the acclimation process, which consists of previous exposure of seeds to a certain type of stress, causing metabolic changes, responsible for the increase of their tolerance to a new stress (Savvides et al., 2016).

Using exogenous hydrogen peroxide (H₂O₂) at low concentrations in plants emerged as a promising alternative of acclimation to saline stress. In general, this molecule enhances the capacity of the plant antioxidant system, which rapidly acts on reactive oxygen species – ROS produced by the stress, neutralizing their action or preventing their generation, resulting in lower ROS concentration and consequently fewer cell damages (Íseri et al., 2013).

Seed pre-treatment with hydrogen peroxide can also act to increase stomatal conductance, photosynthesis, chlorophyll contents and protection of chloroplast membranes; consequently, plants in general exhibit higher growth and dry matter accumulation (Ahmad et al., 2013). However, the information of seed pre-treatment and foliar application of H₂O₂ in the soursop crop and their effects on the tolerance to saline stress is incipient. Thus, studies that allow for the utilization of hydrogen peroxide in soursop acclimation to saline stress become important for its full development in the semi-arid region of Northeast Brazil.

In this context, this study aimed to evaluate the effects of exogenous application of different concentrations of hydrogen peroxide on the growth and quality of soursop seedlings cv. Morada Nova irrigated using solutions with different saline levels.

Results and discussion

Effect of saline stress and exogenous application of hydrogen peroxide for soursop relative growth rates

According to the summary of the analysis of variance (Table 1), the relative growth rate of height (RGR_{PH}), diameter (RGR_{SD}) and leaf area (RGR_{LA}) of soursop seedlings cv. Morada Nova were not influenced ($p > 0.05$) and interaction between saline levels and hydrogen peroxide (SL x H₂O₂). Saline levels in the irrigation water had significant ($p < 0.01$) influence on relative growth rate of diameter and leaf area. H₂O₂ concentrations caused significant effect just on the relative growth rate in leaf area (RGR_{LA}).

Based on the regression equation (Figure 1) of relative growth rate in stem diameter, there was a reduction of 7.57% per unit increase in irrigation water salinity. Plants irrigated with 3.5 dS m⁻¹ water showed a reduction of 21.21% in RGR_{SD} compared with those subjected to 0.7 dS m⁻¹, i.e., the diameter of plants under the lowest saline level grew 0.003 mm mm⁻¹ per day more than that of plants cultivated at the highest saline level, from 85 to 145 DAS.

Irrigation water salinity negatively affected plant growth, possibly due to the osmotic and specific effects of the ions, retarding cell expansion and division, causing negative consequences on the photosynthetic rate and compromising plant physiological and biochemical processes (Bezerra et al., 2018). Sena et al. (2017), studied the guava crop under saline stress conditions (EC_w from 0.3 to 3.5 dS m⁻¹). They also found reduction in RGR_{SD}, equal to 5.31% per unit increase in EC_w.

The relative growth rate in leaf area (RGR_{LA}) showed a decreasing linear response as irrigation water salinity increased, according to the regression equation (Figure 2A), EC_w of 0.7 dS m⁻¹ led to RGR_{LA} of 0.022 cm² cm⁻² day⁻¹. However, plants under EC_w of 3.5 dS m⁻¹ showed RGR_{LA} of 0.013 cm² cm⁻² day⁻¹, i.e., a reduction of 0.009 cm² cm⁻² day⁻¹ (35.72%) in plants irrigated at the highest saline level, compared with those at the lowest saline level. Comparatively, the magnitude of the deleterious effects of irrigation water salinity on the morphological behavior of soursop seedlings was higher for RGR_{LA} than for RGR_{SD}, which demonstrates higher sensitivity of leaves to salinity. It also shows that different plant organs may respond differently to the action of salts.

In addition to salinity, such reduction may be related to the imbalanced absorption of nutrients by plants grown under saline stress conditions, which causes damages in the leaf tissues, leading to greater inhibition in leaf elongation, decreasing the transpiring surface and resulting in reduction of water absorption. At these conditions, reduction in transpiration is desirable since it culminates reduction of Na⁺ and Cl⁻ movement in the xylem and in water conservation in plant tissues (Munns and Tester, 2008).

The regression equation (Figure 2B) allows to estimate the effect of hydrogen peroxide concentration on RGR_{LA} from 85 to 145 DAS. A quadratic response was observed for RGR_{LA} with positive effect of H₂O₂ application up to the concentration of 31 μM (0.0189 cm² cm⁻² day⁻¹). From this point on, RGR_{LA} decreased until the estimated value of 0.0151 cm² cm⁻² day⁻¹ in plants under 100 μM of H₂O₂. Based on the results, it can be inferred that adequate H₂O₂ applications can cause higher plant growth because H₂O₂ can stimulate the accumulation of proteins and soluble carbohydrates. This will act as organic solutes, performing osmotic adjustment in plants under saline stress conditions, culminating in higher absorption of water and nutrients. In addition, it can minimize the effects of salinity on stomatal conductance, resulting in adequate physiological functioning of the plant (Liao et al., 2012).

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

According to the summary of F test (Table 1), there were no significant interactions between the salinity and hydrogen peroxide for the dry phytomass of different plant parts of soursop cv. Morada Nova. Saline levels of irrigation water had significant influence on all the dry phytomass of different plant parts. However, H₂O₂ concentrations caused significant effect on dry phytomass of leaves (LDP) and the stem (SDP).

According to the regression equations obtained for stem (SDP) and leaf dry phytomass (LDP) of soursop cv. Morada Nova (Figure 3A), there was a decreasing linear effect, with reductions of 14.04% and 14.24% in SDP and LDP per unit increase in EC_w, respectively. This is equivalent to reductions of 0.48 and 0.74 g in plants subjected to the highest level of irrigation water salinity (3.5 dS m⁻¹), compared to those under the lowest EC_w level, 0.7 dS m⁻¹. Reduction in dry phytomass is closely related to the effects of the accumulation of soluble salts and is a limiting factor for the development of most crops. Such behavior can be understood as a possible mechanism of adjustment by plants to reduce the effects of salinity.

The increased salinity may reduce turgescence pressure and result in reduced cell expansion, causing negative effects on metabolism, growth, and establishment of plants (Campos Junior et al., 2018). Besides, the modifications in the ionic balance, water potential, mineral nutrition, stomatal closure, photosynthetic efficiency and carbon allocation. Sá et al. (2015), studied the initial growth of custard apple under saline water irrigation and found reduction in plant dry matter with the increment in water salinity. They attributed it to osmotic and ionic effects caused by salinity.

Regarding the effects of H₂O₂ concentrations on the phytomass production of soursop cv. Morada Nova (Fig 3B).

Table 1. Summary of F test for relative growth rate in plant height (RGR_{PH}), stem diameter (RGR_{SD}) and leaf area (RGR_{LA}) during the period 85 to 145 days after sowing (DAS), dry phytomass of leaves (LDP), stem (SDP), roots (RDP) and total (TDP), and Dickson quality index (DQI) at 145 DAS of soursop seedlings cv. Morada Nova under saline water irrigation and exogenous application of hydrogen peroxide.

Source of variation	F-test							
	RGR _{PH}	RGR _{SD}	RGR _{LA}	LDP	SDP	RDP	TDP	DQI
Saline levels (SL)	ns	**	**	**	**	**	**	**
Linear regression	ns	**	ns	**	**	**	**	**
Quadratic regression	ns	ns	**	ns	ns	ns	ns	ns
Hydrogen peroxide (H ₂ O ₂)	ns	ns	**	*	**	ns	ns	ns
Linear regression	ns	ns	**	**	**	**	**	ns
Quadratic regression	ns	ns	**	ns	ns	ns	ns	**
Interaction (SL x H ₂ O ₂)	ns	ns	ns	ns	ns	ns	ns	ns
Blocks	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	12.06	15.78	15.89	23.02	21.52	28.67	22.09	24.52

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

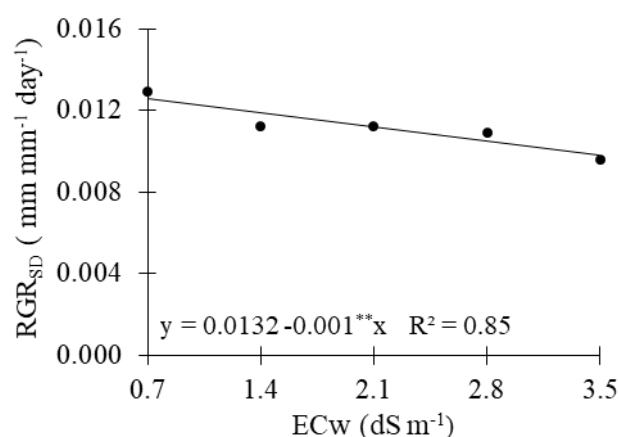


Fig 1. Relative growth rate in stem diameter (RGR_{SD}) of soursop cv. Morada Nova as a function of irrigation water salinity during the period 85 to 145 days after sowing.

Table 2. Chemical and physical characteristics of the eutrophic Regolithic Neosol used in the experiment.

Chemical characteristics									
pH (H ₂ O) (1:2.5)	O.M. (%)	P (mg kg ⁻¹)	K ⁺	Na ⁺	Ca ²⁺	Mg ²⁺	Al ³⁺ + H ⁺	ESP (%)	EC _{se} (dS m ⁻¹)
5.90	1.36	6.80	2.22	1.60	26.00	36.60	19.30	1.87	1.0
Physical characteristics									
Size fraction (g kg ⁻¹)			Textural class	Water content (kPa)			Total porosity (%)	AD (kg dm ⁻³)	PD
Sand	Silt	Clay		33.42	1519.5	AW			
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; Ca²⁺ and Mg²⁺ extracted with 1 M KCl at pH 7.0; Na⁺ and K⁺ extracted using 1 M NH₄OAc at pH 7.0; Al³⁺+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.

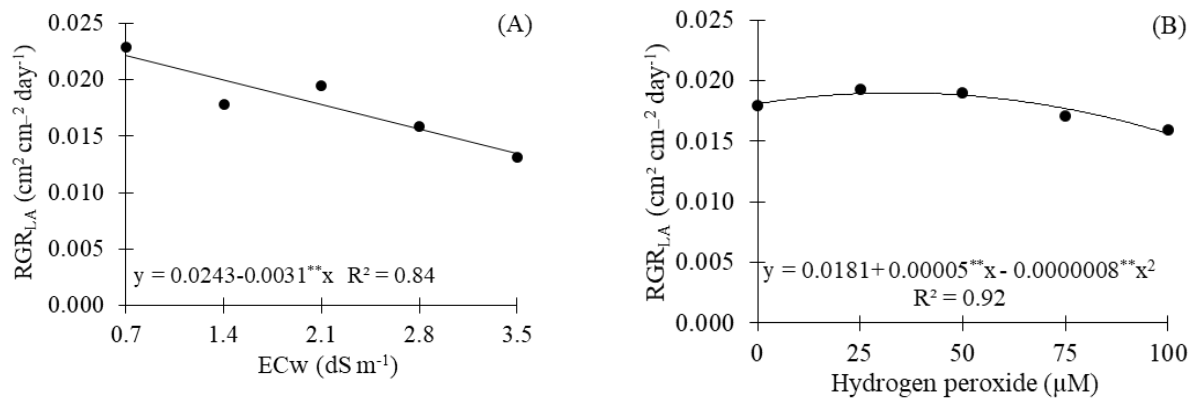


Fig 2. Relative growth rate in leaf area (RGR_{LA}) of soursop cv. Morada Nova as a function of irrigation water salinity – ECw (A) and hydrogen peroxide – H₂O₂ (B) during the period 85 to 145 days after sowing.

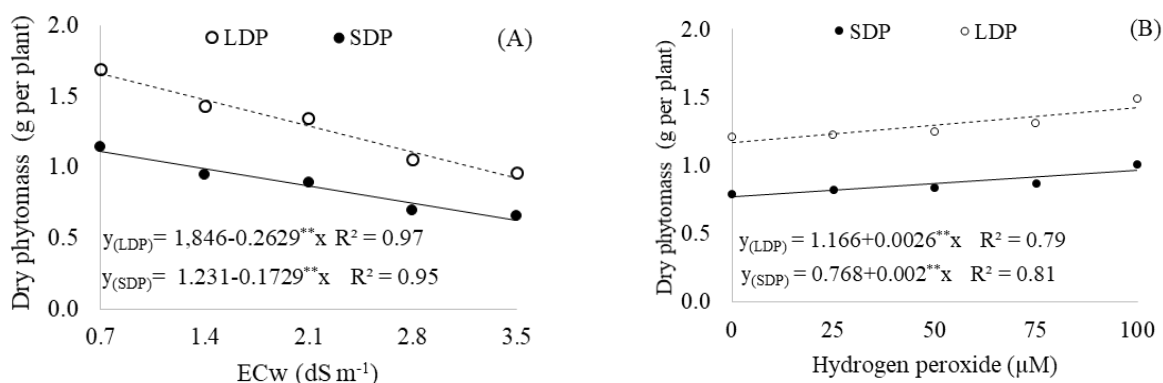


Fig 3. Leaf dry phytomass – LDP and stem dry phytomass – SDP of soursop cv. Morada Nova, as a function of irrigation water salinity – ECw (A) and hydrogen peroxide – H₂O₂ (B), at 145 days after sowing.

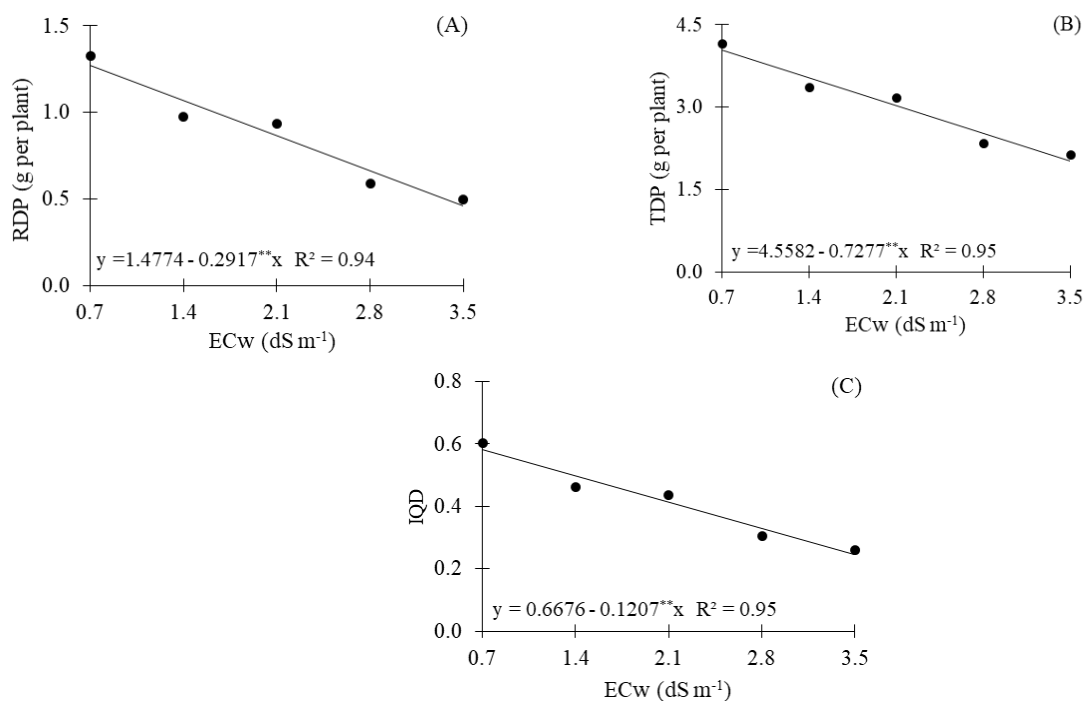


Fig 4. Root dry phytomass – RDP (A), total dry phytomass – TDP (B) and Dickson quality index – DQI (C) of soursop cv. Morada Nova, as a function of irrigation water salinity at 145 days after sowing.

Based on the regression equations, there was a linear increase in SDP and LDP in plants receiving maximum H₂O₂ concentration (100 µM) with increments of 26.04 and 22.29%, respectively, compared to the control (0 µM). Thus, H₂O₂ application to attenuate saline stress was satisfactory because plant exposure to moderate stresses or to signal metabolites such as H₂O₂ may lead to metabolic signaling in the cell (increase in metabolites and/or antioxidative enzymes) and, consequently, to better physiological and metabolic performance when the plant is exposed to more severe stress conditions (Forman et al., 2010). This results to higher tolerance to stress.

Root dry phytomass (RDP) and total dry phytomass (TDP) in soursop seedlings cv. Morada Nova were negatively affected by the increase in irrigation water salinity. According to the regression equations (Figures 4A and B), the data fitted to a linear model and the estimated reductions were 19.74 and 15.96%, respectively, per unit increase in ECw, i.e., reductions of approximately 0.82 g in RDP and 2.04 g in TDP in plants irrigated with the highest ECw level (3.5 dS m⁻¹), compared with those under ECw of 0.7 dS m⁻¹.

Reduction in phytomass formation due to saline water stress may be a strategy of tolerance by plants to reduce the absorption of toxic ions, allowing for ionic homeostasis in its metabolism (Sá et al., 2013). In addition, plants under saline stress tend to perform osmotic adjustment. But this activity requires a large amount of energy, which will be used for the accumulation of sugars, organic acids and ions in the vacuole, energy that under normal conditions would be converted into phytomass production (Wenji et al., 2018).

Effect of saline stress and nitrogen doses on quality of soursop seedlings

On the variable related to the quality of soursop seedlings (DQI), there was significant effect ($p < 0.01$) of the levels of irrigation water salinity. According to Fig 4C, the quality of soursop seedlings cv. Morada Nova (DQI) was negatively affected by the increase in irrigation water salinity. Also according to the regression equation, there was a decreasing linear effect with reduction of about 18.08% in DQI with per unit increase in ECw at 145 DAS. Comparing plants at the highest saline level (3.5 dS m⁻¹) with those at ECw of 0.7 dS m⁻¹, there was a reduction of 50.62% in the DQI. According to Oliveira et al. (2013), DQI is an important morphological parameter used to express quality and rusticity of seedlings, assessing their capacity for growth and survival. In the present study, even plants irrigated with the highest saline level (3.5 dS m⁻¹) showed acceptable DQI, since seedlings with DQI higher than 0.2 are considered as of good quality. In addition, the higher the DQI, the better the seedling quality, because it expresses robustness and balance in biomass distribution.

Materials and methods

Localization and treatments

The experiment was carried out from May to October 2017 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, PB,

located at the geographic coordinates 7° 15'18" S and 35° 52' 28" W, at altitude of 550 m.

The experimental design consisted of completely randomized blocks in a 5 x 5 factorial scheme, with four replicates, corresponding to five levels of irrigation water salinity, expressed by values of electrical conductivity – ECw (0.7; 1.4; 2.1; 2.8 and 3.5 dS m⁻¹), and five concentrations of hydrogen peroxide (0; 25; 50; 75 and 100 µM).

Plant material

The soursop cultivar Morada Nova was used in the experiment because it is the most appreciated by the producers, composing most commercial plantations in Brazil. Besides, it has larger fruits, which may weigh up to 15 kg, and higher production in comparison to the others (Veloso et al., 2018).

Establishment and management of the experiment

The seeds used in the experiment were obtained from fruits harvested from a commercial plantation located in the municipality of Macaparana, PE. Seeds were manually removed and dried outdoors in the shade. After drying, dormancy was broken by cutting the distal end of the seed. Solutions with different saline levels were prepared by adding sodium chloride (NaCl), calcium chloride (CaCl₂.2H₂O) and magnesium chloride (MgCl₂.6H₂O) salts to obtain an equivalent proportion of 7:2:1. The amounts were determined considering the relationship between ECw and salt concentration ($10 \text{ mmol}_e \text{ L}^{-1} = \text{ECw} - \text{dS m}^{-1}$).

The concentrations of hydrogen peroxide (H₂O₂) were established according to a study developed by Panngom et al. (2018), which prepared by diluting H₂O₂ in deionized water. Before sowing, the seeds were immersed in H₂O₂ solutions according to the treatments and held in dark for 24 h. Immediately after this period, the seeds were sown. For production of seedling, three seeds were planted in plastic bags with capacity for 2 dm³ of soil, perforated on the sides to allow free drainage. The bags were arranged on wooden benches at 0.80 m height from the ground and filled with substrate composed of soil (84%) + sand (15%) + humus (1%) based on volume.

The soil used in the experiment was eutrophic Regolithic Neosol with sandy loam texture collected from 0-0.20 m layer, in the rural area of the municipality of Lagoa Seca, PB, properly pounded to break up clods and sieved. Its physical and chemical characteristics (Table 2) were determined according to the methodology proposed by Donagema et al. (2011).

Along the experiment, the soil was maintained close to field capacity by daily irrigations and each bag received solutions according to the treatments. The volume applied 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.

Nitrogen (N), potassium (K) and phosphorus (P) fertilizations were performed based on recommendations of Novais et al. (1991): 0.58 g of urea, 0.65 g of potassium chloride and 1.56 g of monoammonium phosphate, equivalent to 100, 150 and 300 mg kg⁻¹ of the substrate of N, K₂O and P₂O₅, respectively, applied as top-dressing in four equal portions, through

fertigation, at 15-day intervals, with the first application at 15 days after sowing (DAS).

Foliar application of H₂O₂ was performed manually at 17:00h with the respective concentrations, at 90, 105 and 120 DAS, by spraying on the abaxial and adaxial sides using a spray bottle in a way to fully wet all the leaves.

Traits measured

Treatment effects were evaluated based on the determination of the relative growth rate in plant height (RGR_{PH}), stem diameter (RGR_{SD}) and leaf area (RGR_{LA}) from 45 to 145 DAS. Besides, the dry phytomass of leaves (LDP), stem (SDP), roots (RDP) and total (TDP), and Dickson quality index (DQI) for seedlings were measured at 145 DAS.

Plant height, stem diameter and leaf area data were used to estimate the relative growth rate (RGR), according to the methodology of Benincasa (2003), expressed by Equation 1.

$$RGR = \frac{(\ln A_2 - \ln A_1)}{(t_2 - t_1)} \quad (1)$$

Where;

RGR - relative growth rate in cm cm⁻¹ day⁻¹ (PH), mm mm⁻¹ day⁻¹ (SD); or cm² cm⁻² day⁻¹ (LA);

A₁ - variable at time t₁ days;

A₂ - variable at time t₂ days; and,

ln - natural logarithm.

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

Seedling quality was assessed through the Dickson quality index (DQI) for seedlings, using the formula of Dickson et al. (1960), described by Equation 2.

$$DQI = \frac{TDP}{(PH/SD) + (ShDP/RDP)} \quad (2)$$

Where:

DQI - Dickson quality index;

PH - plant height (cm);

SD - stem diameter (mm);

TDP - total dry phytomass (g);

ShDP - shoot dry phytomass (g); and,

RDP - root dry phytomass (g).

Statistical analysis

The collected data were subjected to analysis of variance by F test at 0.05 and 0.01 probability levels and, when significant, linear and quadratic polynomial regressions were applied, using the statistical program SISVAR (Ferreira, 2014).

Conclusion

Irrigation water with electrical conductivity above 0.7 dS m⁻¹ negatively affected the growth of soursop cv. Morada Nova. The root dry phytomass is the most sensitive variable. Hydrogen peroxide concentrations of 31 and 100 µM led to higher relative growth rate in leaf area and dry phytomass of leaves and stem in soursop seedlings cv. Morada Nova, respectively. The quality of soursop seedlings cv. Morada Nova was not compromised by using water with electrical conductivity of 3.5 dS m⁻¹ in irrigation.

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