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MULTIVARIATE ANALYSIS OF SOURSOP UNDER SALT STRESS AND EXOGENOUS APPLICATION OF HYDROGEN PEROXIDE[1]

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ABSTRACT - The objective of this study was to evaluate, through multivariate data analysis, the effect of exogenous application of hydrogen peroxide on the photosynthetic pigments, gas exchange and growth of soursop seedlings under salt stress. The study was conducted in a greenhouse, at Federal University of Campina Grande - Paraíba. The assay was carried out from May to October 2018. The treatments were distributed in a randomized block design, in a 5 × 5 factorial arrangement, corresponding to five levels of irrigation water electrical conductivity – ECw (0.6-control, 1.2, 1.8, 2.4, and 3.0 dS m^{-1}) and five concentrations of hydrogen peroxide – H_2O_2 (0, 10, 20, 30, and 40 µM), with two plants per plot and four replicates. Irrigation water salinity from 1.2 dS m^{-1} negatively affected the biosynthesis of photosynthetic pigments, gas exchange and growth of soursop. Application of hydrogen peroxide at the concentration of 20 µM resulted in attenuation of salt stress effects on the biosynthesis of photosynthetic pigments, gas exchange and growth of soursop. Hydrogen peroxide concentrations above 30 µM intensified the deleterious effect of irrigation water salinity on the photosynthetic pigments, gas exchange and growth of soursop.

Keywords: *Annona muricata* L.. Saline water. Mitigation.

ANÁLISE MULTIVARIADA DE GRAVIOLEIRA SOB ESTRESSE SALINO E APLICAÇÃO EXÓGENA DE PERÓXIDO DE HIDROGÊNIO

RESUMO - Objetivou-se com o presente trabalho, avaliar, por meio da análise multivariada de dados, o efeito da aplicação exógena de peróxido de hidrogênio sobre os pigmentos fotossintéticos, as trocas gasosas e o crescimento de mudas de gravioleira sob estresse salino. O estudo foi conduzido em casa de vegetação, na Universidade Federal de Campina Grande - Paraíba. A pesquisa foi conduzida durante o período de maio a outubro de 2018. Os tratamentos foram distribuídos em delineamento de blocos casualizados, em arranjo fatorial 5 × 5, sendo cinco níveis de condutividade elétrica da água de irrigação – CEa (0,6 - testemunha, 1,2, 1,8, 2,4 e 3,0 dS m^{-1}) e cinco concentrações de peróxido de hidrogênio – H_2O_2 (0, 10, 20, 30 e 40 µM), com duas plantas por parcela e quatro repetições. A salinidade da água de irrigação a partir de 1.2 dS m^{-1} afetou negativamente a biossíntese de pigmentos fotossintéticos, as trocas gasosas e o crescimento da gravioleira. Aplicação de peróxido de hidrogênio na concentração de 20 µM resultou em atenuação do estresse salino sobre a biossíntese de pigmentos fotossintéticos, as trocas gasosas e o crescimento da gravioleira. Concentrações de peróxido de hidrogênio acima de 30 µM intensificaram o efeito deletério da salinidade da água de irrigação sobre os pigmentos fotossintéticos, trocas gasosas e o crescimento da gravioleira.

Palavras-chave: *Annona muricata* L.. Águas salinas. Mitigação.

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INTRODUCTION

Soil salinity is an abiotic restriction that hampers crop yield and already affects 20% of all irrigated areas worldwide (SABAGH et al., 2021). Within this percentage, a portion corresponds to the semiarid region of northeastern Brazil, an area of marked water scarcity, due to rainfall irregularity and high evaporation rate, which makes groundwater a promising alternative for irrigation, but most of these sources have excess dissolved salts (NOBRE et al., 2012; SÁ et al., 2019).

Water salinity has limited the production of some crops of significant economic potential and that are adapted to the edaphoclimatic conditions of northeastern Brazil, such as soursop (*Annona muricata* L.). This fruit crop is considered moderately tolerant to salinity and with significant capacity for export, and its fruit is widely used in the preparation of juices, ice cream, nectars, yogurts, among other industrialized products, and can also be consumed fresh. In addition, soursop has recently aroused the interest of the medical and pharmaceutical sector, because its leaves and seeds have been used to treat diseases such as malaria, gastrointestinal diseases, infections, cancer, respiratory diseases, among others (CORIA-TÉLLEZ et al., 2018; VELOSO et al., 2020).

Generally, when salt-sensitive plants are exposed to a highly saline environment, there may be significant reductions in their morphological and/or physiological parameters, stomatal density and water conductance, which are consequences of the reduction in soil osmotic potential, toxicity caused by excessive absorption of Na^+ and/or Cl^- ions, nutritional imbalance and oxidative stress (SOUZA et al, 2016; ZHU et al., 2019). Thus, the search for management strategies capable of minimizing the negative effects caused by irrigation with saline water on soursop plants is extremely important and, among the alternatives, the use of hydrogen peroxide in the acclimation of plants to salt stress stands out (SILVA et al., 2019a).

Hydrogen peroxide is a reactive oxygen species (ROS), a byproduct of photosynthesis that, under adequate environmental conditions, i.e., with absence of biotic and abiotic stresses, is produced by plants in quantities that are naturally controlled by an antioxidant system that includes a large number of hydrophilic antioxidant compounds, such as ascorbic acid (ASA) and glutathione (GSH), and also antioxidant enzymes, the most important of which are superoxide dismutases (SOD), catalases (CAT) and ascorbate peroxidases (APX) (AMOR et al., 2019).

However, under stress conditions hydrogen peroxide acts as a stress-signaling molecule and, moreover, experimental evidence shows that a transitory pre-exposure of plants to this molecule can induce tolerance to a subsequent stress. Thus, pretreatment with hydrogen peroxide for acclimation has been studied in several crops, such as pistachio – *Pistacia vera* (BAGHERI; GHOLAMI; BANINASAB, 2019), basil – *Ocimum basilicum* (SILVA et al., 2019b), and rice – *Oryza sativa* (ROY et al., 2016).

Thus, the objective of this study was to evaluate, through multivariate data analysis, the effect of exogenous application of hydrogen peroxide on the acclimation of soursop seedlings under salt stress.

MATERIAL AND METHODS

The experiment was carried out from May to October 2018 in a greenhouse of the Center of Technology and Natural Resources - CTRN of the Federal University of Campina Grande - UFCG, located in the municipality of Campina Grande, Paraíba State, in the northeast region of Brazil, at the geographic coordinates 07° 15' 18" S latitude, 35° 52' 28" W longitude and average altitude of 550 m. The data of temperature (maximum and minimum) and mean relative humidity of air observed during the experimental period at the experimental site are shown in Figure 1.

The experimental design adopted was randomized blocks in a 5 x 5 factorial arrangement, which corresponded to five levels of irrigation water electrical conductivity – ECw (0.6 - control, 1.2, 1.8, 2.4, and 3.0 dS m^{-1}) and five concentrations of hydrogen peroxide – H_2O_2 (0, 10, 20, 30, and 40 μM), applied during seed imbibition and through foliar spray, with two plants per plot and four replicates, totaling two hundred experimental units (Table 1).

The soursop cultivar used in the experiment was 'Morada Nova', chosen because it is the most appreciated by producers, composing most commercial plantations in Brazil, besides having larger fruits, which can weigh up to 15 kg, and having higher yield compared to other cultivars (VELOSO et al., 2020).

The seeds used in the experiment were obtained from fruits harvested in a commercial plantation located in the municipality of Aparecida, PB, Brazil. The seeds were separated manually and dried outdoors in the shade. After drying, dormancy was interrupted by cutting the distal end of the seed.

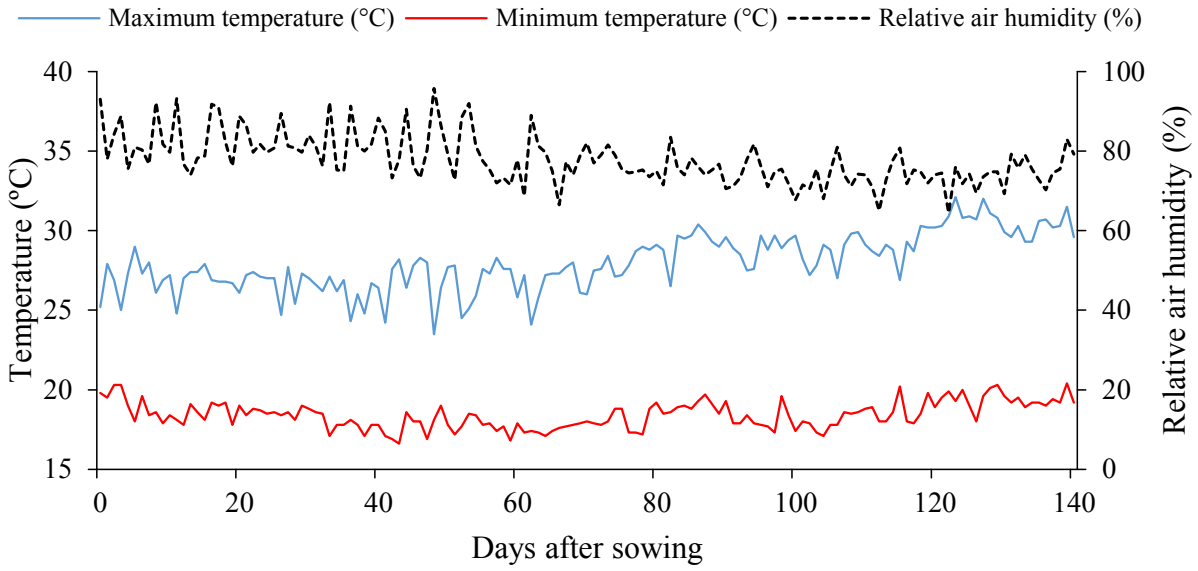

Figure 1. Air temperature (maximum and minimum) and mean relative air humidity inside the greenhouse during the experimental period.

Table 1. Description of the analyzed treatments.

Salinity levels dS m^{-1}	Concentrations of hydrogen peroxide (µM)				
	0	10	20	30	40
0.6	S1H1	S1H2	S1H3	S1H4	S1H5
1.2	S2H1	S2H2	S2H3	S2H4	S2H5
1.8	S3H1	S3H2	S3H3	S3H4	S3H5
2.4	S4H1	S4H2	S4H3	S4H4	S4H5
3.0	S5H1	S5H2	S5H3	S5H4	S5H5

Solutions with different salinity levels were prepared by adding the salts sodium chloride (NaCl), calcium chloride ($CaCl_2.2H_2O$) and magnesium chloride ($MgCl_2.6H_2O$) in an equivalent proportion of 7:2:1, and the quantities were determined considering the relationship between ECw and salt concentration ($mmol_c\ L^{-1} \approx 10 \times ECw\ dS\ m^{-1}$) recommended by Richards (1954). After preparation, the electrical conductivity of the solutions was checked and adjusted.

The hydrogen peroxide (H_2O_2) concentrations used were based on a study conducted by Panngom et al. (2018), obtained by diluting H_2O_2 in deionized water. Prior to sowing, the seeds were immersed in H_2O_2 solutions according to the treatments for 36 hours in the dark; immediately after this period, the seeds were sown. Foliar applications of H_2O_2 concentrations began at 85 days after sowing (DAS), with sprays on the abaxial and adaxial sides of the leaves, at 15-day intervals, performed with a manual sprayer between 17:00 and 17:30 h.

For seedling production, three seeds were sown in plastic bags with capacity for 2 dm^3 of soil, perforated on the sides and bottom to allow free drainage. The bags were arranged on wooden benches at 0.80 m height and filled with substrate composed (w/w) of soil (84%) + sand (15%) + humus (1%).

The soil used in the experiment was of sandy loam texture, classified as *Neossolo Regolítico* (*Entisol* - UNITED STATES, 2014), collected in the 0-20 cm layer, from 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 Teixeira et al. (2017).

Along the experiment, the soil was kept close to field capacity by daily irrigations and each bag received the salinity levels according to the treatments. The volume applied was estimated by the water balance: water volume applied minus water volume drained in the previous irrigation, plus a leaching fraction of 0.20 (AYERS; WESTCOT, 1999), in order to avoid excessive accumulation of salts in the root zone. The leaching fraction was applied every 20 days.

Table 2. Chemical and physical-hydraulic attributes of the soil used in the experiment, before applying the treatments.

Chemical characteristics									
pH (H_2O) (1:2.5)	OM %	P (mg kg^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	$Al^{3+} + H^+$	ESP (%)	ECse (dS m^{-1})
				.....(cmolc kg^{-1})					
5.90	1.36	6.80	0.22	0.16	2.60	3.66	1.93	1.87	1.0
Physical characteristics									
Particle-size fraction (g kg^{-1})			Textural class	Water content (kPa)		AW	Total porosity %	BD	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

OM - Organic matter: Walkley-Black Wet Digestion; Ca^{2+} and Mg^{2+} extracted with 1 M L^{-1} KCl at pH 7.0; Na^+ and K^+ extracted using 1 M L^{-1} NH_4OAc at pH 7.0; Al^{3+} and H^+ extracted with 0.5 M L^{-1} calcium acetate at pH 7.0; ESP - Exchangeable sodium percentage; ECse - Electrical conductivity of saturation extract; SL – Sandy Loam; AW - Available water; BD - Bulk density; PD - Particle density.

Fertilization with nitrogen (N), potassium (K), and phosphorus (P) was performed based on the recommendations of Novais, Neves, and Barros (1991): 0.58 g urea, 0.65 g potassium chloride, and 1.56 g 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, applied as top-dressing in four equal portions, via fertigation, at 15-day intervals, with the first application at 15 days after sowing (DAS).

Treatment effects were evaluated at 120 DAS by determining growth variables: plant height (PH), stem diameter (SD), number of leaves (NL), and leaf area (LA); photosynthetic pigments: chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl t), and carotenoids (Car); and gas exchange variables: internal CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance (*gs*), CO_2 assimilation rate (*A*), and instantaneous water use efficiency (*WUEi*).

Gas exchange variables, *Ci* (µmol CO_2 m^{-2} s^{-1}), *E* (mmol H_2O m^{-2} s^{-1}), *gs* (mmol H_2O m^{-2} s^{-1}), *A* (µmol CO_2 m^{-2} s^{-1}) and *WUEi* [(µmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1}) $^{-1}$], were evaluated on the third leaf, counted from the apex, with photon irradiation of 1200 µmol m^{-2} s^{-1} and air flow of 200 mL min^{-1}, using the portable photosynthesis meter "LCPro+" from ADC BioScientific Ltda.

Plant height was obtained by taking as reference the distance from the collar to the insertion of the apical meristem, SD (mm) was measured 2 cm above the collar, and the number of leaves was obtained by counting fully expanded leaves with minimum length of 3 cm in each plant. Leaf area was obtained by measuring the length and width of all leaves of the plants according to the methodology described by Almeida et al. (2006), considering the following equation:

$$LA = 5.71 + 0.647X$$

Where:
LA - leaf area (cm^2); and,
X - product of leaf length (cm) and width (cm).
Photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids) were quantified by following the laboratory method developed by Arnon (1949), by preparing plant extracts from samples of discs from the blade of the third fully expanded leaf from the apex. From these extracts, chlorophyll and carotenoid concentrations (µg mL^{-1}) were determined in the solutions with a spectrophotometer at the absorbance wavelength (ABS) (470, 646, and 663 nm), using the following equations:

$$\text{Chlorophyll } a \text{ (Chl } a) = (12.21 \times ABS_{663}) - (2.81 \times ABS_{646})$$

$$\text{Chlorophyll } b \text{ (Chl } b) = (20.13 \times ABS_{646}) - (5.03 \times ABS_{663})$$

$$\text{Chlorophyll total (Chl t)} = 17.3 \times ABS_{646} + 7.18A \times BS_{663}$$

$$\text{Carotenoids (Car)} = ((1000 \times ABS_{470}) - (1.82 \times \text{Chl } a) - (85.02 \times \text{Chl } b)) / 198$$

The data were standardized to zero mean ($\bar{x} = 0.0$) and unit variance (S^2=1.0). The multivariate structure of the results was evaluated by exploratory principal component analysis (PCA), summarizing the amount of relevant information contained in the original dataset in a smaller number of dimensions, resulting from linear combinations of the original variables generated from the eigenvalues ($\lambda > 1.0$) in the covariance matrix, explaining a percentage greater than 10% of the total variance (GOVAERTS et al., 2007).

Based on the reduction of the dimensions, the

original data of the variables of each component were subjected to multivariate analysis of variance (MANOVA) by the test of Hotelling (1947) at 0.05 level of probability for the salinity levels and hydrogen peroxide concentrations, as well as for the interaction between the factors studied.

Only variables with correlation coefficient above 0.5 were maintained in the composition of each Principal Component (PC) (HAIR et al., 2009). Variables not associated with the PCs ($r < 0.6$) were removed from the standardized database and a new analysis was performed. The analyses were performed using Statistica v. 7.0 software (STATSOFT, 2004).

RESULTS AND DISCUSSION

The multidimensional space of the original variables was reduced to two dimensions represented by the first two principal components (PC_1 and PC_2) with eigenvalues greater than $\lambda > 1.0$, according to Kaiser (1960). Based on these results, the respective eigenvalues and percentages of variation explained for each component can be observed in Table 3. Together, these components explained 85.63% of the total variation. PC_1 explained 74.77% of the total variance, formed by most of the variables analyzed. PC_2 represented 10.86% of the remaining variance, consisting only of the variables internal CO_2 concentration and instantaneous water use efficiency.

The results of multivariate analysis of variance (MANOVA) are presented in Table 3, where it is possible to observe significant effects ($p \leq 0.01$) of the irrigation water salinity levels (SL) and hydrogen peroxide concentrations (H_2O_2), as well as the interaction of SL × H_2O_2 in both PCs.

The two-dimensional projections of treatment effects and variables in the first and second principal components (PC_1 and PC_2) are shown in Figures 2A and B. In the first principal component (PC_1), a process was identified possibly characterized by the effect of the interaction between irrigation water salinity and peroxide concentrations, since the coefficients of correlation between PH, SD, NL, LA, Chl *a*, Chl *b*, Chl t, Car, *E*, *gs*, and *A* were higher than 0.85.

In principal component 1, it is possible to observe the beneficial effect of hydrogen peroxide at the concentration 20 µM (T13 and T23), especially in soursop plants irrigated with waters of 0.6 and 1.2 dS m^{-1}, considering that these treatments had the highest values (Table 3) of PH (47 cm), SD (6.52 mm), NL (18.3), LA (750.6 cm^2), Chl *a* (2192 µg mL^{-1}), Chl *b* (952.2 µg mL^{-1}), Chl t (3028.4 µg mL^{-1}), Car (483.7 µg mL^{-1}), *E* (1.28 mmol H_2O m^{-2} s^{-1}), *gs* (0.09 mmol H_2O m^{-2} s^{-1}) and *A* (7.31 µmol CO_2 m^{-2} s^{-1}).

A comparison in relative terms of the results obtained in the plants from the treatment S2H3 (1.2 dS m^{-1} and 20 µM) with those of plants from the treatment S2H1 (1.2 dS m^{-1} and 0 µM) showed increments of 15.4% (PH), 6.4% (SD), 24.1% (NL), 17.4% (LA), 19.4% (Chl *a*), 31.9% (Chl *b*), 22.5% (Chl t), 40.7% (Car), 23.3% (*E*), 60% (*gs*) and 43.1% (*A*), thus demonstrating the beneficial effect of exogenous application of hydrogen peroxide at the concentration of 20 µM on soursop plants.

Also in the principal component 1, the lowest values of PH (20.5 cm), SD (4.16 mm), NL (8.8), LA (324.5 cm^2), Chl *a* (1137.8 µg mL^{-1}), Chl *b* (401.4 µg mL^{-1}), Chl t (1539.2 µg mL^{-1}), Car (206.9 mg g^{-1} FM), *E* (0.62 mmol H_2O m^{-2} s^{-1}), *gs* (0.03 mmol H_2O m^{-2} s^{-1}) and *A* (2.97 µmol CO_2 m^{-2} s^{-1}) were found in the treatment S5H5, i.e. in plants irrigated using water with electrical conductivity of 3.0 dS m^{-1} and subjected to hydrogen peroxide at the concentration of 40 µM, thus evidencing the deleterious effects caused by salt stress and high concentrations of hydrogen peroxide on soursop plants.

For principal component 2 (PC_2), it is observed that the internal CO_2 concentration and instantaneous water use efficiency are the most important variables for the second principal component, due to the highest values of correlation found (Table 3). Plants under 0 µM of H_2O_2 and irrigated with 3.0 dS m^{-1} water obtained the highest value of *Ci* (280 µmol CO_2 m^{-2} s^{-1}), hence confirming the stress of plants due to irrigation water salinity, since the increase of *Ci* associated with the reduction in the CO_2 assimilation rate is an indication of inhibition of enzymatic activity (SILVA et al., 2019a).

With respect to the instantaneous water use efficiency, Table 3 shows that the highest value (5.87 (µmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$) was obtained in plants irrigated with 1.2 dS m^{-1} water and subjected to 20 µM of hydrogen peroxide (S2H3); when comparing in relative terms the results obtained in plants from treatment S2H3 (1.2 dS m^{-1} and 20 µM) to those of plants from treatment S2H1 (1.2 dS m^{-1} and 0 µM), there was an increase of 17.6% [0.88 (µmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$] in instantaneous water use efficiency, thus highlighting the beneficial effect of H_2O_2 (20 µM) in mitigating the deleterious effect of irrigation water salinity.

Table 3. Eigenvalues, percentage of total variance explained, in the multivariate analysis of variance (MANOVA) and correlations (r) between original variables and principal components.

	Principal Components - PCs	
	PC_2	PC_2
Eigenvalues (λ)	9.72	1.41
Percentage of total variance (S^2%)	74.77	10.86
Hotelling test (T^2) for salinity levels (SL)	0.01	0.01
Hotelling test (T^2) for hydrogen peroxide (H_2O_2)	0.01	0.01
Hotelling test (T^2) for interaction (SL × H_2O_2)	0.01	0.01

PCs	Correlation coefficient												
	PH	SD	NL	LA	Chl *a*	Chl *b*	Chl t	Car	*Ci*	*E*	*gs*	*A*	*WUEi*
PC_1	-0.91	-0.91	-0.94	-0.95	-0.92	-0.95	-0.96	-0.94	0.03	-0.95	-0.85	-0.93	-0.49
PC_2	-0.02	-0.02	0.10	-0.06	-0.08	0.14	-0.00	0.06	-0.92	0.11	0.21	-0.11	-0.67

	Medium values												
	PH	SD	NL	LA	Chl *a*	Chl *b*	Chl t	Car	*Ci*	*E*	*gs*	*A*	*WUEi*
S1H1	43.3	5.98	14.8	669.6	1931.4	763.6	2694.9	351.1	250	1.07	0.06	5.41	5.06
S1H2	45.0	6.03	15.3	686.6	1978.3	809.2	2787.4	364.0	245	1.23	0.08	6.78	5.51
S1H3	47.0	6.52	18.3	737.4	2076.2	952.2	3028.5	483.7	220	1.28	0.09	6.94	5.42
S1H4	42.8	6.18	16.0	621.1	1848.3	832.6	2680.9	452.4	233	1.11	0.06	5.31	4.81
S1H5	36.5	5.43	14.8	601.9	1784.8	647.0	2431.8	366.0	234	1.01	0.05	4.91	4.86
S2H1	39.0	5.96	13.5	639.2	1835.7	617.4	2453.0	324.0	251	1.03	0.05	5.11	4.99
S2H2	40.5	6.28	14.8	632.4	1960.6	734.0	2694.6	357.2	256	1.18	0.07	6.82	5.78
S2H3	45.0	6.48	16.8	750.6	2192.0	824.2	3006.1	455.9	232	1.27	0.08	7.31	5.87
S2H4	42.6	5.93	14.5	648.3	1735.6	802.3	2538.0	366.5	226	1.10	0.07	5.51	5.01
S2H5	38.0	5.41	13.0	543.9	1674.7	617.7	2292.3	340.0	225	1.10	0.07	4.30	5.01
S3H1	30.8	5.77	11.3	552.5	1820.3	600.9	2421.2	314.8	220	0.97	0.06	4.80	4.43
S3H2	33.3	6.18	12.8	565.7	1920.6	706.6	2794.4	330.3	260	0.98	0.04	5.82	4.93
S3H3	34.3	6.22	15.0	612.7	2056.3	799.3	2854.9	433.8	256	1.13	0.06	6.13	5.17
S3H4	27.8	6.00	12.5	510.2	1679.1	763.3	2442.5	382.1	243	1.20	0.07	5.19	5.13
S3H5	24.0	4.94	11.0	431.0	1509.6	601.4	2111.0	306.5	226	1.04	0.06	4.14	4.98
S4H1	27.0	5.24	10.5	474.3	1576.1	540.9	2116.9	292.3	269	0.75	0.04	3.69	4.92
S4H2	28.3	5.32	13.5	505.2	1659.2	654.1	2313.3	310.3	256	0.82	0.04	4.39	4.95
S4H3	30.0	6.03	14.5	538.1	1870.3	718.4	2588.7	343.8	233	0.91	0.05	3.80	4.65
S4H4	26.5	5.37	12.3	432.4	1432.7	640.8	2073.5	277.1	224	0.85	0.05	3.89	4.48
S4H5	21.5	4.46	9.5	374.8	1315.2	501.4	1816.5	231.5	220	0.71	0.04	3.34	4.72
S5H1	24.0	4.69	9.3	425.0	1376.1	428.4	1804.4	249.8	280	0.71	0.04	3.42	4.80
S5H2	27.0	4.81	10.0	454.5	1434.2	441.6	1875.8	276.6	270	0.73	0.03	4.04	4.98
S5H3	28.8	5.92	11.8	476.5	1622.8	515.9	2138.7	271.3	251	0.74	0.03	3.80	5.12
S5H4	24.8	4.68	9.8	430.6	1356.4	448.3	1804.8	226.6	239	0.67	0.02	3.18	4.75
S5H5	20.5	4.16	8.8	324.5	1137.9	401.4	1539.2	207.0	234	0.62	0.04	2.97	4.79

S1 (0.6 dS m^{-1}); S2 (1.2 dS m^{-1}); S3 (1.8 dS m^{-1}); S4 (2.4 dS m^{-1}); S5 (3.0 dS m^{-1}); H1 (0 µM); H2 (10 µM); H3 (20 µM); H4 (30 µM); H4 (40 µM); PH (plant height - cm); SD (stem diameter - mm); NL (number of leaves); LA (leaf area - cm^2); Chl *a* (chlorophyll *a* - µg mL^{-1}); Chl *b* (chlorophyll *b* - µg mL^{-1}), Chl t (chlorophyll total - µg mL^{-1}); Car (carotenoids - µg mL^{-1}); Ci (internal CO_2 concentration - µmol CO_2 m^{-2} s^{-1}); *E* (transpiration - mmol H_2O m^{-2} s^{-1}); *gs* (stomatal conductance - mmol H_2O m^{-2} s^{-1}); *A* (CO_2 assimilation rate - µmol CO_2 m^{-2} s^{-1}) and *WUEi* (instantaneous water use efficiency - [(µmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$]).

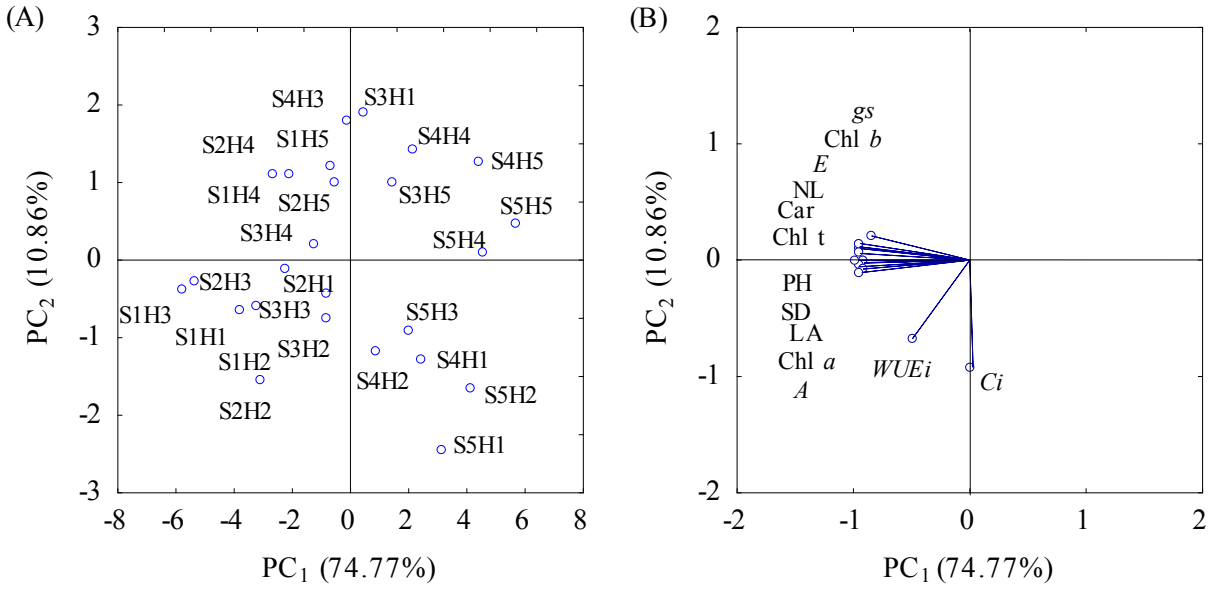

Figure 2. Two-dimensional projection of the scores of the principal components for the studied factors (SL and H_2O_2) (A) and the analyzed variables (B) in the two principal components (PC_1) and (PC_2).

The stress caused by irrigation water salinity reduces the photosynthetic pigments, gas exchange and growth of soursop plants, negatively affecting various physiological processes such as chlorophyll biosynthesis, photosynthesis, stomatal conductance and transpiration. This effect probably occurs due to the reduction in the osmotic potential caused by the concentration of soluble salts in soil, which directly affects water absorption and reduces leaf area expansion, in addition to the closing of stomata, which ultimately hampers photosynthesis and inhibits plant growth (ZHU et al., 2019; LIMA et al., 2020).

Stressful environmental conditions, such as salinity, cause imbalance between production and removal of ROS. Thus, the final balance can be an increase in ROS levels, causing oxidative damage to proteins, lipids and nucleic acids and, consequently, oxidative stress, which leads to a reduction in physiological and growth variables (CARVALHO et al., 2011).

Indeed, the results obtained here are consistent with those previously reported for different crops under salt stress, such as Dias et al. (2019), who studied soursop under salt stress conditions and found that water salinity of 3.8 dS m^{-1} induces reduction in CO_2 assimilation rate, highlighting the effects of stomatal origin as limiting factors in this process. Sá et al. (2015) in a study on sugar-apple under salt stress conditions (0.3 to 4.8 dS m^{-1}) observed reductions of growth with increasing salinity of irrigation water, which were attributed to physiological and nutritional changes related to the competition of toxic ions with nutrients that are essential to plants, such as nitrogen and potassium, preventing their absorption and leading to hormonal and osmotic imbalance.

It is worth pointing out that in this study the deleterious effects of salinity became more severe on plants subjected to hydrogen peroxide concentrations above 20 µM (Table 3), thus demonstrating that H_2O_2 at high concentrations can be harmful to plants, mainly due to its toxic effect. Hydrogen peroxide is the most stable ROS and, at high concentrations in cells, can diffuse rapidly through the subcellular membrane, thus causing oxidative damage to the cell membrane (FAROOQ et al., 2017).

However, the exogenous application of hydrogen peroxide at the concentration of 20 µM attenuated the deleterious effects of irrigation water salinity on all variables analyzed (Table 3), especially in plants irrigated with 1.2 dS m^{-1} water. This result shows the beneficial effect of hydrogen peroxide when used at adequate concentrations.

The beneficial effects of previous application of hydrogen peroxide have been observed on several crops under salt stress. Azevedo Neto et al. (2005) found that H_2O_2 induces salt tolerance in corn plants, improving the antioxidant metabolism and reducing lipid peroxidation in leaves and roots. In a study with yellow passion fruit plants, Silva et al. (2019c) observed that the exogenous application of hydrogen peroxide attenuates the deleterious effects of salinity on gas exchange and plant growth.

According to Savvides et al. (2016), exogenous application of hydrogen peroxide at low

concentrations, through sprays and/or seed soaking, promotes a moderate stress condition, which results in accumulation of latent signals in different parts of the plant and, when a condition of more severe stress occurs, the stored signals will lead to molecular adjustments, resulting in acclimation mechanisms. Moreover, the use of H_2O_2 at adequate concentrations favors greater absorption of water and nutrients, including those that are essential for plant growth and development, such as N, P, and K (FAROUK; AMIRA, 2018).

The beneficial effects of exogenous application of hydrogen peroxide on plants under salt stress may also be related to the activation of the defense system of antioxidant enzymes, such as superoxide dismutase, catalase, guaiacol peroxidase, and ascorbate peroxidase, which will act by reducing the deleterious effects of salinity (CARVALHO et al., 2011).

Thus, it is evidenced that irrigation water salinity compromised the photosynthetic pigments, gas exchange, and growth of soursop at 120 days after sowing. However, exogenous application of hydrogen peroxide at the concentration of 20 μM can be used in the induction of plant acclimation to salt stress, since the growth, chlorophyll contents, and gas exchange variables were improved with the use of H_2O_2. It is worth pointing out that hydrogen peroxide is a product of low-cost and can be applied at different stages of plant development.

CONCLUSIONS

Irrigation water salinity from 1.2 dS m^{-1} negatively affects the biosynthesis of photosynthetic pigments, gas exchange, and growth of soursop, at 120 days after sowing.

Exogenous application of hydrogen peroxide at the concentration of 20 μM can be used to mitigate salt stress in soursop seedlings.

Hydrogen peroxide concentrations above 30 μM intensify the deleterious effect of irrigation water salinity on the biosynthesis of photosynthetic pigments, gas exchange and growth of soursop.

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