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Hydrogen peroxide in the acclimation of colored-fiber cotton genotypes to salt stress

Peróxido de hidrogênio na aclimatação de genótipos de algodão colorido ao estresse salino

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ABSTRACT - The excess of salts in irrigation water restricts agricultural exploitation in arid and semi-arid regions. Thus, searching for strategies of cultivation under salt stress conditions is important for the expansion of irrigated agriculture in these regions. Thus, the objective of this study was to evaluate the gas exchange and growth rates of naturally colored-fiber cotton genotypes irrigated with saline water and under exogenous foliar application of hydrogen peroxide concentrations. The experiment was carried out under greenhouse conditions, in Campina Grande - PB, using the randomized block experimental design and 4 × 3 × 2 factorial arrangement, with four concentrations of hydrogen peroxide - H_2O_2 (0, 25, 50, and 75 µM), three colored-fiber cotton genotypes - CG (BRS Rubi; BRS Topázio; BRS Verde) and two levels of electrical conductivity of water - ECw (0.8 and 5.3 dS m^{-1}), with three replicates. Irrigation using water with electrical conductivity of 5.3 dS m^{-1} associated with foliar application of 50 µM of hydrogen peroxide favors gas exchange and growth rates of BRS Rubi cotton, at 60 days after sowing. Salinity of 5.3 dS m^{-1} associated with foliar applications of 50 µM of hydrogen peroxide increased the percentage of cell damage and the internal CO_2 concentration, but reduced the stomatal conductance, transpiration, CO_2 assimilation rate, and growth rates of BRS Topázio cotton.

Keywords: *Gossypium hirsutum* L. Salinity. H_2O_2.

RESUMO - O excesso de sais na água de irrigação restringe a exploração agrícola em regiões áridas e semiáridas. Assim, a busca por estratégias de cultivo sob condições de estresse salino é importante para expansão da agricultura irrigada nestas regiões. Assim, objetivou-se com a pesquisa, avaliar as trocas gasosas e as taxas de crescimento de genótipos de algodoeiro de fibra naturalmente colorida irrigados com águas salinas e sob aplicação exógena foliar de concentrações de peróxido de hidrogênio. A pesquisa foi desenvolvida em condições de casa de vegetação, em Campina Grande – PB, utilizando o delineamento experimental de blocos casualizados e arranjo fatorial 4 × 3 × 2, sendo quatro concentrações de peróxido de hidrogênio – H_2O_2 (0; 25; 50 e 75 µM), três genótipos de algodoeiro de fibra colorida - GA (BRS Rubi; BRS Topázio e BRS Verde) e dois níveis de condutividade elétrica da água - CEa (0,8 e 5,3 dS m^{-1}) e com três repetições. A irrigação com água de condutividade elétrica de 5,3 dS m^{-1} associada a aplicação foliar de 50 µM de peróxido de hidrogênio favorece as trocas gasosas e as taxas de crescimento do algodoeiro BRS Rubi, aos 60 dias após a semeadura. A salinidade de 5,3 dS m^{-1} associado a aplicações foliares de 50 µM de peróxido de hidrogênio aumenta a porcentagem dano celular e a concentração interna de CO_2, no entanto reduz a condutância estomática, transpiração, taxa de assimilação de CO_2 e as taxas de crescimento do algodoeiro BRS Topázio.

Palavras-chave: *Gossypium hirsutum* L. Salinidade. H_2O_2.

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INTRODUCTION

The edaphoclimatic conditions of the arid and semi-arid regions can cause water scarcity and an increase in the salt content of water. Thus, aiming at the continuity of sustainable exploitation in these areas, most agricultural producers use saline groundwater/surface water for irrigating crops (LIMA et al., 2015; SANTOS et al., 2016; SALES et al., 2021).

Commonly, plants irrigated with saline water tend to have their growth and production reduced, due to osmotic and ionic stresses, which reduce water absorption by plants and trigger a range of other adversities such as oxidative stress, nutritional imbalance, ionic toxicity, reduction of cell division and expansion, and interruption of major metabolic processes (NOBRE et al., 2012; WANI et al., 2020).

In this context, the use of saline water in irrigation is conditioned on the management of cultural practices and the use of tolerant species, aiming to reduce the damage caused by salinity to crops (SOUZA et al., 2016; BEZERRA et al., 2018). Thus, some studies aiming to identify the mitigating capacity of the exogenous application of hydrogen peroxide in plants under salt stress were

carried out with several crops, such as soursop (SILVA et al., 2019a; SILVA et al., 2021), wheat (LIU et al., 2020), red pitaya (SANTOS et al., 2020) and sunflower (SILVA et al., 2019b). However, studies on foliar applications of hydrogen peroxide (H_2O_2) in naturally colored cotton genotypes under semi-arid conditions in northeastern Brazil are scarce in the literature.

Hydrogen peroxide is a by-product of photosynthesis, normally produced by the plant and eliminated by the enzymatic and non-enzymatic antioxidant defense system. At low concentrations, H_2O_2 can act as a stress-signaling molecule, playing a role in the activation of the plant defense system. Thus, the pretreatment of plants with adequate concentrations of H_2O_2 can contribute to their acclimation, through the production of soluble proteins and carbohydrates, which can aid in osmotic adjustment (CARVALHO et al., 2011; VELOSO et al., 2021).

In addition to hydrogen peroxide, the cultivation of genotypes with lower sensitivity to salt stress may be an alternative to make the use of saline water more promising. Cotton is a crop characterized as tolerant to salinity, with a threshold level of 5.1 dS m^{-1} in irrigation water (OLIVEIRA et al., 2013). However, the tolerance of naturally colored cotton can vary according to genotype, phenological stage and time of exposure to stress (SOARES et al., 2018; PINHEIRO et al., 2022).

In this context, the objective of this study was to evaluate the gas exchange and growth rates of naturally colored-fiber cotton genotypes to salt stress under foliar applications of hydrogen peroxide concentrations, through gas exchange and growth.

MATERIAL AND METHODS

The experiment was carried out between November 2020 and March 2021 in a protected environment (greenhouse), belonging to the Academic Unit of Agricultural Engineering (UAEA) at the Federal University of Campina Grande (UFCG), located in Campina Grande, Paraíba, Brazil, whose local geographical coordinates are 07° 15' 18" S, 35° 52' 28" W and average altitude of 550 m. During the experimental period, the meteorological variables were monitored inside the greenhouse and expressed as a climograph (Figure 1).

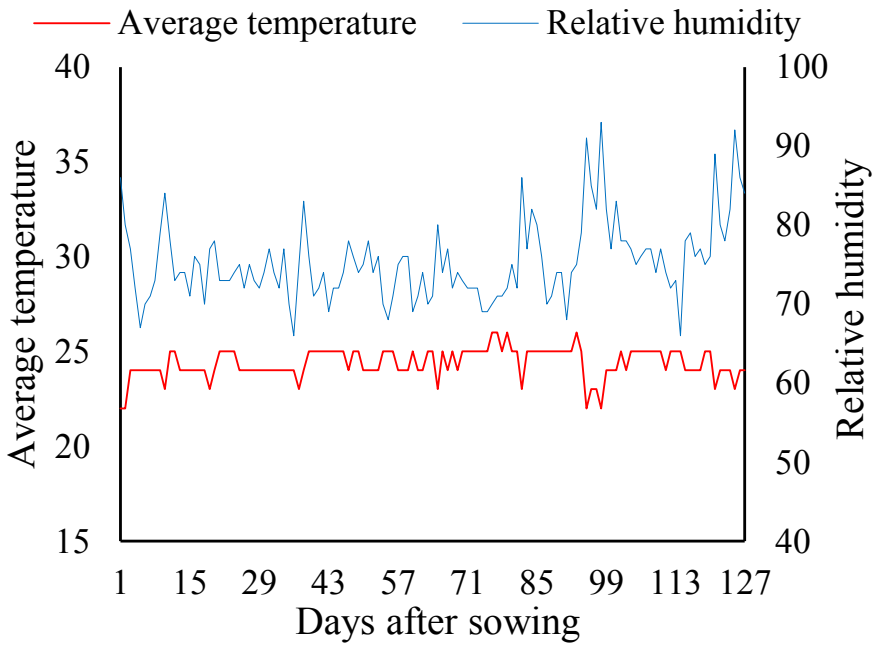


Figure 1. Climograph with the meteorological variables recorded during the experimental period.

The experimental design adopted was randomized blocks, arranged in a 4 × 3 × 2 factorial scheme, corresponding to four concentrations of hydrogen peroxide - H_2O_2 (0; 25; 50 and 75 µM), three colored-fiber cotton genotypes - CG (BRS Rubi; BRS Topázio and BRS Verde) and two levels of electrical conductivity of water - ECw (0.8 and 5.3 dS m^{-1}), resulting in 24 treatments, with three replicates and one plant per plot.

The plants were grown in plastic pots adapted as drainage lysimeters with capacity of 20 L (35 cm height, 31 cm upper diameter, 20 cm lower diameter). Each lysimeter was filled with 24 kg of soil with sandy loam texture, whose physical-chemical attributes (Table 1) were determined according to Teixeira et al. (2017) by the soil laboratory of UFCG – Campus of Campina Grande.

Table 1. Physical-chemical attributes of the soil used in the experiment, before applying the treatments.

Chemical characteristics									
pH (H_2O) (1:2.5)	OM dag kg^{-1}	P (mg kg^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	$Al^{3+} + H^+$	ESP (%)	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-hydraulic characteristics									
Particle-size fraction (g kg^{-1})			Class xtural	Moisture (kPa)		AW	Porosity total %	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 mol L^{-1} KCl at pH 7.0; Na^+ and K^+ extracted with 1 mol L^{-1} NH_4OAc at pH 7.0; Al^{3+} and H^+ extracted with 1 mol 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.

To meet the nutritional needs of the plants, fertilization with N, P and K was performed as recommended by Novais, Neves and Barros (1991), by applying 100 mg of N kg^{-1}, 300 mg of N kg^{-1}, 300 mg P_2O_5 kg^{-1} and 150 mg K_2O kg^{-1} of soil, in the forms of urea, monoammonium phosphate and potassium chloride, respectively. Phosphorus was applied as basal, while N and K were applied as top-dressing, via fertigation, at 30 and 60 days after sowing (DAS). The pots were arranged in single rows with spacing of 0.6 m × 0.3 m. Foliar application of micronutrients was performed monthly, with a concentration of 1.0 g L^{-1} of the commercial product Dripsol® micro, containing: Mg (1.1%), Zn (4.2%), B (0.85%), Fe (3.4%), Mn (3.2%), Cu (0.5%) and Mo (0.05%), applied to the adaxial and abaxial sides.

The colored cotton seeds came from Embrapa Cotton. Five seeds were planted in each pot, placed at 1.5 cm deep and distributed equidistantly. Twenty-five days after germination, the first thinning was carried out, leaving the three most vigorous plants per pot. At 50 DAS, the second thinning was carried out, leaving only one plant per pot, which was grown until the end of the experiment.

The salinized waters were prepared so as to have an equivalent proportion of 7:2:1, of Na:Ca:Mg, respectively, from the dilution of NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ salts in local-supply water (0.28 dS m^{-1}), considering the relationship between ECw and salt concentration according to Richards (1954), Equation 1.

$$C \approx 10 \times ECw \quad (1)$$

Where:
C - Concentration of salts to be added ($mmol_c$ L^{-1});
ECw - electrical conductivity of water (dS m^{-1}).

Hydrogen peroxide (H_2O_2) concentrations were established according to a study previously conducted by Silva et al. (2019a). The concentrations were prepared by diluting H_2O_2 in distilled water. H_2O_2 applications were carried out before the beginning of irrigation with saline water, via foliar sprays, at 15 DAS, and were repeated with intervals of 15 days until the bolls opened (100 DAS), totaling approximately 5 applications.

The applications were carried out with a Jacto XP-12 backpack sprayer, using a pump with working pressure (maximum) of 6 bar, with JD-12 nozzle and flow rate of approximately 770 mL min^{-1}. Approximately 125 mL of the solution was applied, initially in the 3 plants per lysimeter, and after thinning this volume was maintained during the vegetative growth of the remaining plant.

Irrigations with saline waters according to treatments started at 18 DAS, in order to maintain soil moisture at a level proportional to maximum retention capacity in all experimental units, using water according to each treatment. Irrigations were performed manually and daily, applying the volume corresponding to that obtained by the water balance, and the volume of water applied to the plants was determined by Equation 2:

$$VI = \frac{(Va - Vd)}{(1 - LF)} \quad (2)$$

Where: VI - Volume of water to be applied in the next irrigation event (mL);
Va and Vd - Volume applied and drained in the previous irrigation event (mL);
LF - Leaching fraction of 0.2.

During the experimental period, cultural practices of manual weeding, scarification of soil surface and staking to vertically train the plant stems were performed. For phytosanitary control, insecticides of the Neonicotinoid chemical group, fungicide of the Triazole chemical group and acaricide of the Abamectin chemical group were used preventively.

The absolute growth rates for plant height (AGRph) and stem diameter (AGRsd) and relative growth rates for plant height (RGRph) and stem diameter (RGRsd) were obtained at 30 and 60 days after sowing (DAS) according to Benincasa (2003), using Equations 3 and 4:

$$AGR = \frac{(A2 - A1)}{t2 - t1} \quad (3)$$

Where: AGR - Absolute growth rate of PH (cm day^{-1}) and SD (mm day^{-1});
A_1 - Plant growth at time t_1;
A_2 - Plant growth at time t_2;
$t_2 - t_1$ - Time difference between samplings.

$$RGR = \frac{lnA2 - lnA1}{t_2 - t_1} \quad (4)$$

Where: RGR - Relative growth rate of PH (cm cm^{-1} day^{-1}) and SD (mm mm^{-1} day^{-1});
A_2 - Plant growth at time t_2,
A_1 - Plant growth at time t_1;
$t_2 - t_1$ - Time difference between samplings;
ln - Natural logarithm.

The degree of cellular integrity was estimated based on electrolyte leakage according to Silva et al. (2011). A hole punch with known area (10 mm) was used to collect 5 five discs of leaf blades, which were immersed in 50 mL of distilled water contained in Erlenmeyer® flask for 90 min. Then, the initial electrical conductivity of the medium (ECi) was measured using a bench-top conductivity meter. Subsequently, the Erlenmeyer® flasks were closed with aluminum foil and subjected to a temperature of 80 °C for 90 minutes in a drying oven and, after cooling the contents, the final conductivity (ECf) was measured. The percentage of cell damage was estimated by Equation 5.

$$\%\ CD = \frac{(ECi)}{(ECf)} \times 100 \quad (5)$$

The relative water content (RWC) was determined with the collection of 6 discs (10 mm) from the leaf blade. Immediately after collection, the discs were weighed on a precision scale (0.0001) to obtain the fresh mass (FM) of the disc. Then, the samples were immersed in 40 mL of distilled water for 90 min., and after this period the discs were weighed again to determine the turgid mass (TM). Dry mass (DM) was obtained after drying the discs at 80 ºC, for 48 h in a forced air circulation oven. RWC was estimated by Equation 6.

$$RWC\ (\%) = \frac{(FM - DM)}{(TM - DM)} \times 100 \quad (6)$$

Where:
RWC - Relative water content (%)
FM - Fresh mass of the discs (g)
DM - Dry mass of the discs (g)
TM - Turgid mass of the discs (g).

Leaf gas exchange was analyzed at 60 DAS in the vegetative stage, by determining stomatal conductance (*gs* - mol H_2O m^{-2} s^{-1}), CO_2 assimilation rate (*A* - µmol m^{-2} s^{-1}), transpiration (*E* - mmol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration (*Ci* - µmol CO_2 mol^{-1}), with a portable infrared gas analyzer (LCPro+ - Portable Photosynthesis System®), with irradiation of 1200 pmol photons m^{-2} s^{-1} and airflow of 200 mL min^{-1}. These data were then used to calculate the instantaneous water use efficiency - WUEi (*A/E*) [(µmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$] and the instantaneous carboxylation efficiency - CEi (*A/Ci*) [(µmol m^{-2} s^{-1}) (µmol mol^{-1})$^{-1}$].

The collected data were subjected to multivariate analysis, being normalized to zero mean (M = 0.0) and unit variance (σ^2 = 1.0). The multivariate composition of the results was evaluated by exploratory Principal Component Analysis (PCA), condensing the amount of relevant variables contained in the original data set into a smaller number, resulting from linear combinations of the original variables generated from the highest eigenvalues (λ > 1.0) in the correlation matrix, explaining a percentage greater than 10% of σ^2 (GOVAERTS et al., 2007).

The variables of each PC were subjected to multivariate analysis of variance (MANOVA) by the Hotelling test at 0.05 probability level for the factors H_2O_2 concentrations, cotton genotypes and salinity levels, as well as for their interactions.

Only variables with correlation coefficient greater than 0.5 were maintained in the composition of each Principal Component (PC) (HAIR et al., 2009). Variables not associated with the PCs (r < 0.5) were removed from the database and subjected to a new analysis. The analyses were processed in Statistica software v. 7.0.

RESULTS AND DISCUSSION

The sum of the first two principal components (PCs) was responsible for 67.73% of the total variance. The first principal component (PC1) contributed with 51.12% of the total variance and was composed of the linear combination between the percentage of cell damage (%CD), relative water content (RWC), stomatal conductance (*gs*), transpiration (*E*), CO_2 assimilation rate (*A*), instantaneous water use efficiency (WUEi), instantaneous carboxylation efficiency (CEi), relative (RGRph) and absolute (AGRph) growth rates of plant height and relative (RGRsd) and absolute (AGRsd) growth rates of stem diameter. The second principal component (PC2) accounted for 16.61% of the remaining variance and consisted of the internal CO_2 concentration (*Ci*).

Given the highest correlation coefficient (r) between original variables and PCs, it was found that all variables were important (r >0.6) to explain the influence of salinity levels, H_2O_2 concentrations and colored cotton genotypes on their physiology and growth. In order of importance, the variables were classified in the following sequence: *A* > RGRsd > *gs* > CEi > AGRsd > RGRph > %CD > *E* > AGRph >WUEI > RWC> *Ci* (Table 2).

The results of the multivariate analysis of variance (MANOVA) are presented in Table 2, where it is possible to observe a significant effect of salinity levels (SL), hydrogen peroxide concentrations (H_2O_2) and colored-fiber cotton genotypes individually and their interactions for the two PCs, by the Hotelling correlation test.

Table 2. Eigenvalues, percentage of total variance explained, multivariate analysis of variance, correlation coefficient, means of the original variables and the principal components.

	Principal components	
	PC1	PC_2
Eigenvalues (λ)	6.13	8.12
Percentage of total variance (σ^2 %)	51.12	16.61
Hotelling test (T^2) for salinity levels (SL)	<0.001	<0.001
Hotelling test (T^2) for H_2O_2 concentrations (H_2O_2)	<0.001	<0.001
Hotelling test (T^2) for cotton genotypes (CG)	<0.001	<0.001
Hotelling test (T^2) for interaction (SL × H_2O_2)	<0.001	<0.001
Hotelling test (T^2) for interaction (SL × CG)	<0.001	<0.001
Hotelling test (T^2) for interaction (H_2O_2 × CG)	<0.001	<0.001
Hotelling test (T^2) for interaction (SL × H_2O_2 × CG)	<0.001	<0.001

Correlation coefficient (r)												
	%CD	RWC	*gs*	*E*	*Ci*	*A*	WUEi	CEi	RGRph	AGRph	RGRsd	AGRsd
PC1	0.68	-0.62	0.80	-0.68	0.23	-0.88	-0.67	0.77	-0.72	-0.68	-0.81	-0.77
PC2	-0.16	0.06	-0.07	-0.11	-0.61	0.38	-0.44	0.57	-0.50	-0.58	-0.38	-0.36
Means												
T111	20.58	76.10	0.39	3.65	230	18.33	4.64	0.08	1.12	1.00	1.17	0.12
T121	19.66	76.36	0.47	4.32	230	23.42	5.42	0.10	1.05	0.98	1.17	0.14
T131	18.59	75.27	0.38	3.35	234	20.43	5.25	0.09	0.98	0.90	1.18	0.13
T141	17.71	75.56	0.46	3.92	230	22.50	5.74	0.10	1.16	0.87	1.12	0.15
T211	17.27	74.84	0.44	4.26	233	20.91	5.09	0.09	1.10	1.03	1.16	0.14
T221	17.31	76.54	0.51	4.56	231	24.08	5.28	0.10	1.00	1.00	1.24	0.14
T231	17.78	80.99	0.54	5.31	234	24.08	5.42	0.10	1.65	1.63	1.19	0.15
T241	16.15	75.70	0.53	5.09	231	25.45	6.01	0.11	1.71	1.92	1.40	0.17
T112	17.66	79.06	0.53	4.36	241	25.27	5.58	0.10	1.06	0.87	1.19	0.14
T122	15.65	80.29	0.43	4.10	240	22.08	5.37	0.09	1.12	0.90	1.26	0.14
T132	17.73	79.37	0.47	4.35	237	24.14	5.29	0.10	1.09	0.88	1.33	0.16
T142	18.22	76.79	0.46	4.01	251	21.03	5.26	0.08	0.89	0.73	1.08	0.13
T212	18.41	77.70	0.46	4.12	244	21.46	5.32	0.09	1.06	0.88	1.09	0.13
T222	18.10	77.91	0.46	4.00	232	21.08	5.44	0.09	1.37	1.17	1.21	0.13
T232	23.64	73.00	0.38	3.81	254	17.09	4.27	0.07	0.65	0.57	0.98	0.11
T242	18.43	77.44	0.46	4.21	252	22.12	5.53	0.09	1.40	1.65	1.31	0.17
T113	22.41	73.71	0.48	3.70	241	20.71	5.45	0.09	1.00	0.83	1.13	0.14
T123	23.20	75.28	0.49	4.20	241	22.65	5.40	0.09	0.83	0.82	1.10	0.13
T133	21.62	76.10	0.48	4.55	255	20.21	5.25	0.08	1.24	1.20	1.19	0.15
T143	23.21	78.00	0.47	4.00	238	20.52	5.13	0.09	0.75	0.70	1.06	0.14
T213	20.64	76.39	0.41	3.95	228	21.11	5.31	0.09	0.65	0.57	0.99	0.11
T223	19.83	73.09	0.39	3.85	238	21.29	5.32	0.09	0.92	0.81	1.00	0.12
T233	20.25	75.02	0.4	3.95	237	20.62	5.27	0.09	0.95	1.00	1.10	0.14
T243	21.05	74.07	0.41	4.19	242	20.62	4.92	0.09	0.92	1.03	1.12	0.14

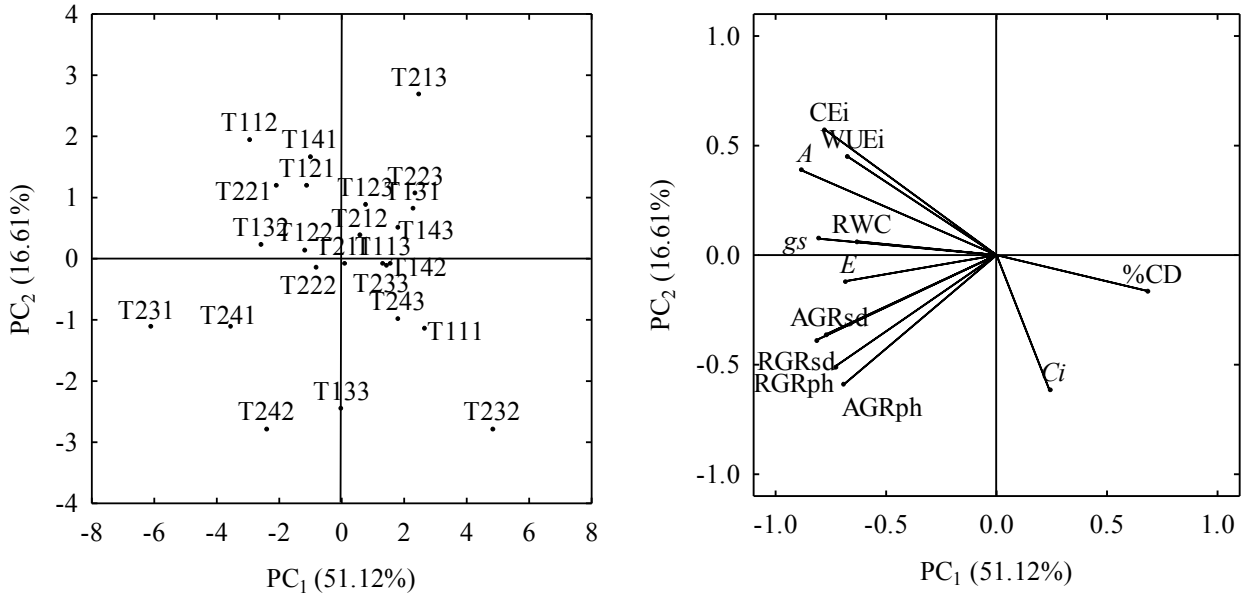
Percentage of cell damage (%CD), relative water content (RWC), stomatal conductance (*gs*), transpiration (*E*), internal CO_2 concentration (*Ci*), CO_2 assimilation rate (*A*), instantaneous water use efficiency (WUEi), instantaneous carboxylation efficiency (CEi), relative growth rate (RGRph) and absolute growth rate (AGRph) of plant height and relative growth rate (RGRsd) and absolute growth rate (AGRsd) of stem diameter. T- Treatment; first number corresponds to ECw (ranging from 1 to 2, where 1 = 0.8 dS m^{-1} and 2 = 5.3 dS m^{-1}); second number corresponds to the H_2O_2 concentrations (ranging from 1 to 4, where 1 = 0 µM, 2 = 25 µM, 3 = 50 µM and 4 = 75 µM); third number corresponds to the cotton genotypes (ranging from 1 to 3, where 1 = BRS Rubi; 2 = BRS Topázio and 3 = BRS Verde).

The two-dimensional projections of the effects of treatments and variables on the first and second principal components (PC1 and PC2) are shown in Figures 2A and 2B. It is observed that the two principal components constructed from the original information portrayed the treatments and characteristics responsible for the differences between these systems.

In the principal component 1 (PC1), the most significant relative value for %CD (24.21%) was verified in plants subjected to treatment T232, which corresponds to irrigation with water of 5.2 dS m^{-1} with application of 50 µM of hydrogen peroxide in the BRS Topázio genotype. The highest values for RWC (80.99%), *gs* (0.53 mol H_2O m^{-2} s^{-1}), *E* (5.05 mmol H_2O m^{-2} s^{-1}), *A* (25.45 µmol CO_2 m^{-2} s^{-1}), WUEi (6.01 [(µmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$]), CEi (0.11 [(µmol m^{-2} s^{-1}) (µmol mol^{-1})$^{-1}$]), RGRph (1.71 cm cm day^{-1}), AGRph (1.92 cm day^{-1}), RGRsd (1.40 mm mm day^{-1}), AGRsd (0.17 mm day^{-1}) were obtained in BRS Rubi irrigated with water of 5.3 dS m^{-1} and foliar spraying of 50 µM of hydrogen peroxide (T231) (Figures 2A and 2B).

When comparing the percentage of cell damage observed in plants of the T232 treatment, with the results obtained in BRS Topázio plants irrigated with water of 5.3 dS m^{-1} without the application of H_2O_2 (T212), there was an increase of 21.82% in cell damage. Thus, it is evident that, under conditions of high salinity, the foliar application of 50 µM of H_2O_2 intensified the cell damage of BRS Topázio cotton. However, there were increments of 1.14% (RWC), 16.98% (*gs*), 16.30% (*E*), 13.16% (*A*), 6.07% (WUEi), 2.17% (CEi), 32.99% (RGRph), 36.93% (AGRph), 2.35% (RGRsd) and 4.76% (AGRsd) when irrigating BRS Rubi cotton with water of 5.3 dS m^{-1} and under 50 µM of H_2O_2 (T231) compared to plants of the T211 treatment. Thus, it can be inferred that the application of 50 µM of H_2O_2 favored the physiology and growth of BRS Rubi cotton genotype under conditions of salt stress, a situation that was not observed for BRS Topázio (Table 2 and Figures 2A and 2B).

It should also be noted that the lowest values for RWC (73.0%) *gs* (0.38 mol H_2O m^{-2} s^{-1}), *E* (3.81 mmol H_2O m^{-2} s^{-1}), *A* (17.09 µmol CO_2 m^{-2} s^{-1}), EiUA (4.27 (µmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$]), CEi (0.07 [(µmol m^{-2} s^{-1}) (µmol mol^{-1})$^{-1}$]), RGRph (0.65 cm cm day^{-1}), AGRph (0.57 cm day^{-1}), RGRsd (0.98 mm mm day^{-1}) and AGRsd (0.11 mm day^{-1}) were found in the T232 treatment (Figures 2A and 2B), i.e., in the treatment in which the greatest cell damage was observed.



(A) (B)

T- Treatment; first number corresponds to ECw (ranging from 1 to 2, where 1 = 0.8 dS m^{-1} and 2 = 5.3 dS m^{-1}); second number corresponds to the H_2O_2 concentrations (ranging from 1 to 4, where 1 = 0 µM, 2 = 25 µM, 3 = 50 µM and 4 = 75 µM); third number corresponds to the cotton genotypes (ranging from 1 to 3, where 1 = BRS Rubi; 2 = BRS Topázio and 3 = BRS Verde).

Figure 2. Two-dimensional projection of the interaction between hydrogen peroxide concentrations, salinity levels and cotton genotypes at 60 DAS (A) and evaluated variables (B) in the first two principal components (PC1 and PC2).

In the principal component 2 (PC2), the salinity levels, H_2O_2 concentrations applied and cotton genotypes used influenced for the *Ci* to be in the lower quadrant of the biplot. It is observed that the highest relative value for *Ci* (254 μmol CO_2 mol^{-1}) was obtained in the T232 treatment, i.e., in BRS Topázio cotton plants irrigated with water of 5.2 dS m^{-1} and with applications of 50 μM of H_2O_2. On the other hand, the lowest value for *Ci* was obtained in T213 treatment, which corresponds to ECw of 5.3 dS m^{-1} and without applications of H_2O_2 in the genotype BRS Verde (Figures 2A and 2B).

Thus, it can be affirmed that irrigation with water of 5.3 dS m^{-1} and foliar applications of 50 μM of H_2O_2 in the genotype BRS Topázio (T232) contributed to an increase of 3.93% in *Ci* compared to the *Ci* of T212 plants. It is worth pointing out that the values referring to *gs*, *E*, and *A* of plants under the treatment T232 were lower than those of plants of the T212 treatment (Table 2).

Among the abiotic stresses, salt stress is the most harmful and limiting to agricultural production. Irrigation with saline water increases the contents of chlorine (Cl^-) and sodium (Na^+) ions in the soil, causing toxicity to plants, compromising the gas exchange and growth of plants cultivated under this stressful condition (SHI-YING et al., 2018).

The increase in cell damage observed in BRS Topázio cotton plants under irrigation with water of highest salinity level (5.3 dS m^{-1}) and application of 50 μM of H_2O_2 (T232) (Figures 2A and 2B) results from the nutritional imbalance caused by salinity, which is potentially harmful to plants. The increase in the concentration of salts in water can alter the nutritional balance and limit the availability of nutrients, including Ca, an element that is essential for the formation of the cell wall, thus generating an increase in the percentage of electrolyte leakage under conditions of high salinity (WANDERLEY et al., 2020).

Ferraz et al. (2015) reported that the increase in water salinity caused electrolyte leakage in castor bean leaves. These authors emphasized that the rupture of the cell membrane is associated with the effect of the phytotoxicity of salts on plant organisms and with changes in the composition of the structures of cell membranes and organelles, due to the accumulation of ions in plant tissues.

On the other hand, the lowest percentage of cell damage was observed in the T231 treatment, as well as the highest relative water content, which may be related to the capacity of hydrogen peroxide to assist the plant in osmotic adjustment and in the reduction of the peroxidation of membrane lipids through the increment of soluble sugars and proline (TERZI et al., 2014; SUN et al., 2016), a result that was not observed for the BRS Topázio genotype (T232). Yang, Lan and Gong (2009) reported in their study that the exogenous application of H_2O_2 may induce significant proline accumulation in maize seedlings, which contributed to greater plant development.

Irrigation with saline water of 5.3 dS m^{-1} and foliar application of 50 μM of H_2O_2 (T232) reduced stomatal conductance, as well as transpiration, CO_2 assimilation rate, instantaneous carboxylation efficiency and water use efficiency of BRS Topázio (Figures 2A and 2B). Physiologically, the first and rapid response to salt stress is verified in the reduction of stomatal conductance. The reduction in the *gs* of plants tends to restrict leaf transpiration and internal CO_2 concentration, culminating in the inhibition of photosynthesis (SILVA et al., 2018); however, there was an increase in the internal CO_2 concentration in this same treatment. Probably the increase in *Ci* was due to possible limitations in the metabolism of CO_2, which can be verified by the reductions in CO_2 assimilation rate and instantaneous carboxylation efficiency of BRS Topázio cotton (Figures 2A and 2B).

The application of 50 μM of H_2O_2 associated with irrigation using water of 5.3 dS m^{-1} compromised the CEi of BRS Topázio cotton and could reduce the capacity of the RuBisCO enzyme to capture carbon dioxide from the atmosphere and fix it organically, starting the Calvin cycle (CAMPOS et al., 2014). However, for BRS Rubi, there was no reduction in CEi under through irrigation with water of 5.3 dS m^{-1} and application of 50 μM of H_2O_2.

The reductions of *A*, *E*, *gs* and CEi, as well as the increase in the *Ci* of cotton under salt stress, were also observed in the study conducted by Dias et al. (2020), who stated that there was a probable decrease in the transport of cytokines from the root to the aerial part, which is involved in the restoration of RuDP (RuBisCO) during the photosynthetic process in plants under conditions of salt stress.

However, the best physiological performance was observed in BRS Rubi cotton plants under irrigation with saline water of 5.3 dS m^{-1} and foliar application of 50 μM of H_2O_2 (T231) (Figures 2A and 2B), according to the evaluated parameters (*gs*, *E* and *A*). This result may be related to the foliar application of the appropriate concentration of hydrogen peroxide and the use of the cotton genotype with lower sensitivity to salinity. This is because exposure to stress can cause changes in the photosynthetic process due to excessive production of reactive oxygen species (ROS) in the absence of enzymatic and/or non-enzymatic protection mechanisms, which lead to metabolic changes that result in oxidative damage (VASILAKOGLOU et al., 2021).

However, pretreatment of plants with hydrogen peroxide can trigger metabolic signaling in the cell, inducing the antioxidant defense system and/or the increase of a metabolite that minimizes the negative effects caused by salinity, which may benefit the physiological performance of plants that will be exposed to subsequent conditions of more severe stress (PANHWAR; KEERIO; ROBERT, 2017). This effect was observed by Silva et al. (2019a), who reported that the exogenous application of 50 μM of H_2O_2 attenuated the deleterious effects of salt stress on the physiology of soursop seedlings. Gondim et al. (2013) found that exogenous applications of 10 mM of H_2O_2 mitigated the negative effects of salinity on the stomatal conductance of maize plants irrigated with saline water.

In relation to the lower sensitivity of the BRS Rubi

genotype to salinity, Saboya, Ferreira and Cavalcanti (2017) considered from their study that the BRS Rubi genotype is moderately tolerant to salinity, while BRS Topázio was considered moderately susceptible, when they were exposed to irrigation with water of 4.0 and 8.0 dS m^{-1}. In contrast, Soares et al. (2018) stated in their study that the BRS Rubi cotton genotype was more sensitive to irrigation water salinity of 9 dS m^{-1} when compared with BRS Topázio and BRS Safira, in terms of gas exchange and production.

BRS Rubi cotton obtained higher water use efficiency when irrigated with water of 5.3 dS m^{-1} associated with the application of 50 µM of H_2O_2 (Figures 2A and 2B). Commonly, under conditions of salt stress the plant tends to suffer limitation in water absorption due to the reduction of osmotic potential; however, to ensure the absorption of water and maintenance of turgid cells, the plant tends to reduce the transpiration flow and adjusts osmotically (SILVA et al., 2019c).

In this context, the function of H_2O_2 in assisting the plant in osmotic adjustment through the synthesis of organic solutes, such as proline, stands out. By means of signal transduction, H_2O_2 can lead to proline accumulation and inhibition of the proline degradation pathway, in addition to sequential activation of glutamate and ornithine pathways, which participate in proline biosynthesis (YANG; LAN; GONG, 2009).

Regarding plant growth, there was a beneficial effect of foliar application of H_2O_2, due to the increase in absolute and relative growth rates of plant height and stem diameter observed in the treatment T231. This result can be explained by the accumulation of soluble proteins, soluble carbohydrates and NO_3^-, which ensures greater absorption of water and nutrients, as well as the reduction in the contents of toxic ions (Na^+ and Cl^-) that occurs due to the predominant action of H_2O_2 in the stress signaling pathways. According to Liu et al. (2020), hydrogen peroxide, when mediated by NADPH, is able to reduce oxidative damage caused by salt stress by regulating biosynthesis and proline degradation in wheat seedlings. Silva et al. (2019c) observed that the exogenous application of 25 µM of H_2O_2 was able to mitigate the effects of salinity on passion fruit plants, benefiting their gas exchange and growth.

CONCLUSIONS

Irrigation with waters of 5.3 dS m^{-1} associated with foliar application of 50 µM of hydrogen peroxide favored the gas exchange and growth rates of BRS Rubi cotton at 60 days after sowing. Salinity of 5.3 dS m^{-1} associated with foliar applications of 50 µM of hydrogen peroxide increased the percentage of cell damage and internal CO_2 concentration, but reduced stomatal conductance, transpiration, CO_2 assimilation rate and growth rates of BRS Topázio.

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