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# $H_2O_2$ alleviates salt stress effects on photochemical efficiency and photosynthetic pigments of cotton genotypes[1]

## $H_2O_2$ alivia o estresse salino na eficiência fotoquímica e pigmentos fotossintéticos de genótipos de algodoeiros

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*HIGHLIGHTS:*

*Increase in the electrical conductivity of water affects the photochemical efficiency of the cotton plant.*
*Application of 50 µM of $H_2O_2$ increases the photosynthetic pigments and the fluorescence of the cotton plant.*
*Salinity and application of $H_2O_2$ had a distinct influence on the physiology of each cotton genotype.*

**ABSTRACT:** The objective of this study was to evaluate the quantum yield and concentrations of photosynthetic pigments of naturally colored cotton genotypes under irrigation with waters of different electrical conductivities and foliar applications of hydrogen peroxide. The design used was randomized blocks in a 4 × 3 × 2 factorial arrangement, corresponding to four concentrations of hydrogen peroxide - $H_2O_2$ (0, 25, 50 and 75 µM), three genotypes of colored fiber cotton ('BRS Rubi', 'BRS Topázio' and 'BRS Verde') and two values of electrical conductivity of water (0.8 and 5.3 dS $m^{-1}$), with three replicates. Irrigation with water of 5.3 dS $m^{-1}$ reduces the chlorophyll a, chlorophyll b and total chlorophyll concentrations of 'BRS Rubi' cotton. The concentrations of photosynthetic pigments, maximum fluorescence, variable fluorescence and quantum efficiency of photosystem II of 'BRS Rubi' cotton increased under irrigation with 0.8 dS $m^{-1}$ water and foliar application of 50 µM of hydrogen peroxide. Water of 5.3 dS $m^{-1}$ and foliar applications of 75 µM of hydrogen peroxide reduce the concentrations of photosynthetic pigments, but did not cause damage to the efficiency of photosystem II of the colored cotton genotypes.

**Key words:** *Gossypium hirsutum* L., salinity, acclimatization, hydrogen peroxide

**RESUMO:** O objetivo deste trabalho foi avaliar o rendimento quântico e os teores de pigmentos fotossintéticos de genótipos de algodão naturalmente coloridos sob irrigação com águas de diferentes níveis de salinidade e aplicações foliares de peróxido de hidrogênio. O delineamento utilizado foi em blocos casualizados em esquema fatorial 4 × 3 × 2, correspondendo a quatro concentrações de peróxido de hidrogênio - $H_2O_2$ (0, 25, 50 e 75 µM), três genótipos de algodão de fibra colorida ('BRS Rubi', 'BRS Topázio' e 'BRS Verde') e dois valores de condutividade elétrica da água (0,8 e 5,3 dS $m^{-1}$), com três repetições. A irrigação com água de 5,3 dS $m^{-1}$ reduz os teores de clorofila a, clorofila b e clorofila total do algodão 'BRS Rubi'. Os teores de pigmentos fotossintéticos, fluorescência máxima, fluorescência variável e eficiência quântica do fotossistema II do algodão 'BRS Rubi' aumentaram sob irrigação com 0,8 dS $m^{-1}$ de água e aplicação foliar de 50 µM de peróxido de hidrogênio. O uso da água de 5,3 dS $m^{-1}$ e aplicações foliares de 75 µM de peróxido de hidrogênio reduzem os teores de pigmentos fotossintéticos, mas não prejudicaram a eficiência do fotossistema II dos genótipos de algodão colorido.

**Palavras-chave:** *Gossypium hirsutum* L., salinidade, aclimatação, peróxido de hidrogênio

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## Introduction

The agricultural areas of the Brazilian semi-arid region are characterized by the occurrence of water sources with high concentrations of salts. Thus, the use of saline water can be an alternative for the expansion of irrigated cultivation in this region (Nobre et al., 2012).

Therefore, an alternative to minimize the effects of salinity on plants is the application of elicitors, such as hydrogen peroxide ($H_2O_2$). When subjected to pre-treatment with adequate concentrations of $H_2O_2$, plants undergo metabolic alterations, through the activation of the enzymatic and non-enzymatic antioxidant defense system, which gives them greater tolerance to abiotic stresses such as salinity (Silva et al., 2019; Veloso et al., 2022).

$H_2O_2$ performs the function of hormonal signaling, controlled by its production and elimination, and acts in the regulation of biological processes, such as growth, elevation of Ca concentration in plants and osmotic adjustment through the increase of proline. However, the biological effects of $H_2O_2$ depend on its concentration, as well as on the stage of plant development and previous exposure to other types of stress (Liu et al., 2020).

Another way to enable the use of saline water in irrigation is to grow crops that have lower sensitivity to salinity. Cotton (*Gossypium hirsutum* L.) is a crop that has a salinity threshold of 5.1 dS $m^{-1}$ in irrigation water, but this tolerance may vary with genotype, development stage, time of exposure to stress and cultivation strategy (Soares et al., 2021).

Thus, the objective of this study was to evaluate the quantum yield and concentrations of photosynthetic pigments of naturally colored cotton genotypes under irrigation with waters of different electrical conductivity values and foliar applications of hydrogen peroxide.

## Material and Methods

The experiment was carried out in a protected environment (greenhouse) of the Academic Unit of Agricultural Engineering (UAEA) at the Federal University of Campina Grande (UFCG), located in Campina Grande, Paraíba, Brazil, whose geographic coordinates are 07° 15' 18" S, 35° 52' 28" W and average altitude of 550 m. The data of average temperature and relative humidity obtained during the experimental period are presented in Figure 1.

The experiment was set up in a randomized block design with treatments arranged in a 4 × 3 × 2 factorial scheme, referring to four concentrations of hydrogen peroxide - $H_2O_2$ (0, 25, 50 and 75 µM), three cotton genotypes - CG ('BRS Rubi', 'BRS Topázio' and 'BRS Verde') and two values of water electrical conductivity - ECw (0.8 and 5.3 dS $m^{-1}$) with three replicates and one plant per plot.

The values of water electrical conductivity chosen were based on a study conducted by Sousa et al. (2018), and the solutions were prepared in such a way as to have an equivalent ratio of 7:2:1 for Na:Ca:Mg, respectively, from the dissolution 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 concentration of salts (Richards, 1954), according to Eq. 1.

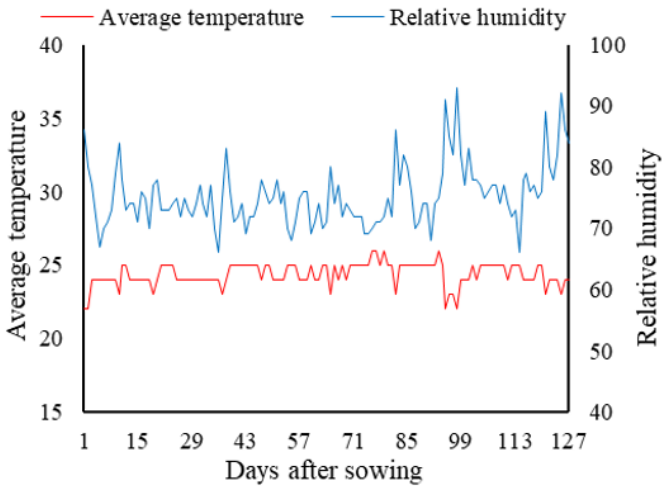



**Figure 1.** Average air temperature and relative air humidity recorded within the greenhouse during the experimental period

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

where:

C - concentration of salts to be added (mmol$_c$ $L^{-1}$); and,

ECw - electrical conductivity of water (dS $m^{-1}$).

Hydrogen peroxide ($H_2O_2$) concentrations were established according to a study conducted by Silva et al. (2019). The concentrations were prepared in each application event by diluting $H_2O_2$ in distilled water, while the treatment of 0 µM was obtained using only distilled water.

At 15 days after sowing (DAS), hydrogen peroxide began to be applied by foliar spray, fortnightly, until the appearance of flowers (100 DAS), totaling five applications. A Jacto XP-12 backpack sprayer, a pump with a working pressure (maximum) of six bar, a JD-12 nozzle and a flow rate of approximately 770 mL $min^{-1}$ were used. Initially, an average volume of 125 mL of the solution was applied to the three plants present in each lysimeter, and through thinning, this volume continued to be applied to the remaining plant until the end of the experiment.

The plants were grown in pots adapted as drainage lysimeters with capacity of 20 L, arranged in single rows with spacing of 0.6 × 0.3 m. The bottom of each lysimeter was covered with geotextile (Bidim®), a 500-g layer of crushed stone and connected to a drain for collection of drained water.

The lysimeters were filled with 24 kg of an Entisol with sandy loam texture, collected from the 0-20 cm layer in Lagoa Seca, PB, Brazil, with physical and chemical attributes obtained according to Teixeira (2017): $Ca^{2+}$, $Mg^{2+}$, $Na^+$, $K^+$, $Al^{3+}$ + $H^+$ = 2.60, 3.66, 0.16, 0.22 and 1.93 cmol$_c$ $kg^{-1}$, respectively; pH (water, 1:2.5) = 5.9; ECse = 1.0 dS $m^{-1}$; organic matter = 1.36 dag $kg^{-1}$; sand, silt and clay = 73.29, 14.21 and 12.50 dag $kg^{-1}$, respectively; bulk density (kg $dm^{-3}$) = 1.39; moisture content at 33.42 and 1519.5 kPa = 11.98 and 4.32 dag $kg^{-1}$, respectively.

Prior to sowing, soil moisture was increased to the level corresponding to field capacity with public-supply water (0.28 dS $m^{-1}$) and irrigation with low-salinity water continued until 17 DAS. Sowing was performed using five seeds per lysimeter

planted at 3 cm depth and distributed equidistantly. At 25 days after the emergence, 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, which was grown until the end of the experiment.

Irrigation with saline waters started at 18 DAS, in order to maintain soil moisture at a level corresponding to the maximum water retention capacity in all experimental units. Irrigation was applied manually and daily, using a volume of water equivalent to that obtained by the water balance, determined by Eq. 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); and,

LF - leaching fraction of 0.2, applied fortnightly.

Fertilization with nitrogen, phosphorus and potassium was performed according to Novais et al. (1991), applying the equivalent to 100 mg N, 300 mg $P_2O_5$ and 150 mg $K_2O$ $kg^{-1}$ soil, in the forms of urea, monoammonium phosphate and potassium chloride, respectively. Potassium was applied as basal, while N and K were applied as top-dressing, via fertigation, at 30 and 60 DAS, through manual irrigation. A micronutrient solution at 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%), was applied monthly through the leaves, on the adaxial and abaxial sides, using a backpack sprayer.

During the experiment, cultural practices of manual weeding, surface scarification of the soil and staking of plants to grow them vertically were carried out. Phytosanitary control was performed with applications of insecticide of the Neonicotinoid chemical group, fungicide of the Triazole chemical group and acaricide of the Abamectin chemical group.

At 60 DAS, during the vegetative stage of the plants, because in the productive stage there is a natural degradation of the cotton leaves, chlorophyll a fluorescence was determined using a pulse-modulated fluorometer, OS5p model from Opti Science. The Fv/Fm protocol was used to determine the fluorescence induction variables: initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv) and the quantum efficiency of photosystem II (Fv/Fm). The protocol was performed after adaptation of the leaves to the dark for a period of 30 min and at 7 a.m., using a clip of the device, in order to ensure that all primary acceptors were fully oxidized, that is, the reaction centers were open.

Also using the pulse-modulated fluorometer, the evaluations were performed under light conditions, using the 'Yield' protocol, applying an actinic lighting source with multi-flash saturating pulse, coupled to a photosynthetically active radiation determination clip (PAR-Clip) in order to determine the following variables: initial fluorescence before the saturation pulse (F'), maximum fluorescence after adaptation to saturating light (Fm'), electron transport rate (ETR), quantum efficiency of photosystem II (YII). These results were then used to determine the following variables: minimum fluorescence of illuminated plant tissue (Fo'), photochemical quenching coefficient by the "lake" model (qL), quantum yield of regulated photochemical quenching ($Y_{NPQ}$) and quantum yield of non-regulated photochemical quenching ($Y_{NO}$), through Eqs. 3, 4, 5 and 6, respectively.

$$Fo' = \frac{(Fm - Fo)}{\frac{(Fm + Fo)}{Fm'}} \quad (3)$$

$$qL = \frac{(Fm' - F')}{(FM' - Fo')} \times \left(\frac{FO'}{F'}\right) \quad (4)$$

$$YNPQ = \frac{(F')}{(Fm')} - \left(\frac{F'}{Fm}\right) \quad (5)$$

$$YNO = \frac{F'}{Fm} \quad (6)$$

The concentrations of photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids) were quantified according to the methodology of Arnon (1949), expressed in mg $g^{-1}$ of fresh matter (FM). From the plant extracts corresponding to each treatment, the concentration of chloroplast pigments were determined using the spectrophotometer at absorbance wavelength (ABS) (470, 647 and 663 nm) through Eqs. 7, 8, 9 and 10:

$$Chl\ a = (12.25 \times ABS_{663}) - (2.79 \times ABS_{647}) \quad (7)$$

$$Chl\ b = (21.5 \times ABS_{647}) - (5.10 \times ABS_{663}) \quad (8)$$

$$Chl\ t = (7.15 \times ABS_{663}) + (18.71 \times ABS_{647}) \quad (9)$$

$$Car = \frac{\left[(1000 \times ABS_{470}) - (1.82 \times Chl\ a) - (85.02 \times Chl\ b)\right]}{198} \quad (10)$$

The data collected were standardized as zero mean (M = 0.0) and unit variance ($\sigma^2$ = 1.0). The multivariate structure of the data was evaluated by principal components analysis (PCA), compiling the amount of relevant information contained in the original data set in a smaller number of variables, resulting from linear combinations of the original variables generated from the highest eigenvalues ($\lambda > 1.0$) in the correlation matrix, explaining a percentage greater than 10% of $\sigma^2$ (Govaerts et al., 2007).

From the reduction of dimensions, the original data of the variables of each component were subjected to multivariate analysis of variance by the Hotelling test at 0.05 probability level for the factors: $H_2O_2$ concentrations, electrical conductivity levels and cotton genotypes, as well as for their interactions.

Only variables with correlation coefficient greater than 0.60 and with eigenvalues greater than one (λ > 1.00) were maintained in the composition of each principal component (PC) (Hair et al., 2009; Kaiser, 1960). The analyses were processed using Statistica software v. 7.0.

## Results and Discussion

The multidimensional space of the original variables was reduced to two dimensions represented by the first two principal components (PC1 and PC2). The eigenvalues and percentage of variance explained for each component can be observed in Table 1.

Together, the PCs explained 66.29% of the total variance. PC1 explained 41.0% of the total variance, formed mainly by the concentrations of photosynthetic pigments, quantum efficiency of photosystem II (Fv/Fm), minimum fluorescence of illuminated plant tissue (Fo') and quantum yield of non-regulated photochemical quenching ($Y_{NO}$). PC2 represented 25.29% of the remaining variance, being formed by the initial fluorescence before the saturation pulse (F'), maximum fluorescence after adaptation to saturating light (Fm') and photochemical quenching coefficient (qL).

Considering the correlation coefficient (r) between original variables and PCs, all variables were important (r > 0.6) to explain the influence of electrical conductivity values, $H_2O_2$ concentrations and cotton genotypes on concentrations of photosynthetic pigments and photosynthetic efficiency. In order of importance, the variables were classified in the following sequence: qL > F' > Chl t > Chl b > Chl a > Car > $Y_{NO}$ > Fm' > Fm > Fv > Fo' > Fo > Fv/Fm (Table 1).

The results of the MANOVA are presented in Table 1. The significant effect of the interaction between the electrical conductivity of water (ECw), hydrogen peroxide concentrations and cotton genotypes was observed for the two PCs. There were also significant effects of the factors alone,

**Table 1.** Eigenvalues, variance, correlation coefficients between principal components ($PC_1$) and ($PC_2$) and means of treatments for relative water content, water saturation deficit, concentration of photosynthetic pigments and chlorophyll a fluorescence of colored cotton genotypes

| | Principal components | |
|---|---|---|
| | PC1 | PC2 |
| Eigenvalues (λ) | 5.33 | 3.28 |
| Percentage of total variance ($S^2$%) | 41.0 | 25.29 |
| Hotelling test ($T^2$) for electrical conductivity (ECw) | < 0.001 | 0.02 |
| Test of Hotelling ($T^2$) for $H_2O_2$ concentrations ($H_2O_2$) | < 0.001 | < 0.001 |
| Hotelling test (T2) for cotton genotypes (CG) | < 0.001 | < 0.001 |
| Hotelling test (T2) for interaction (ECw × $H_2O_2$) | < 0.001 | < 0.001 |
| Hotelling test (T2) for interaction (ECw × CG) | < 0.001 | < 0.001 |
| Hotelling test (T2) for interaction ($H_2O_2$ × CG) | < 0.001 | < 0.001 |
| Hotelling test (T2) for interaction (ECw × $H_2O_2$ × CG) | < 0.001 | < 0.001 |

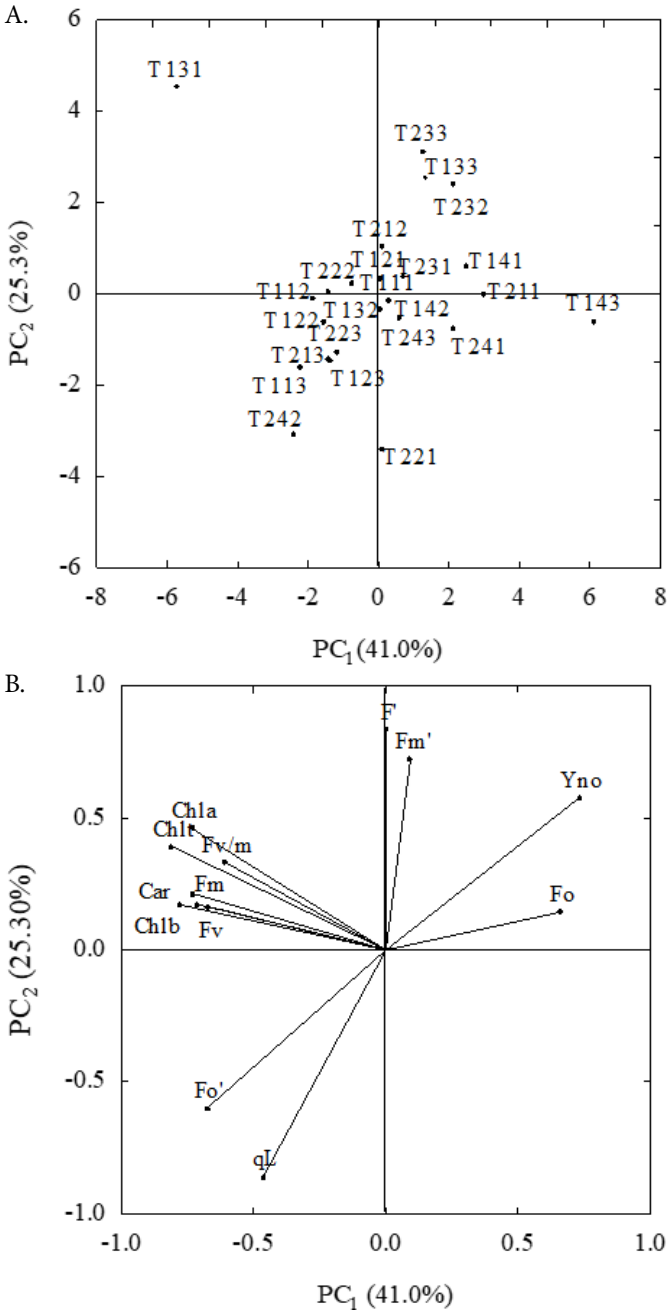
| | Chl a | Chl b | Chl t | Car | Fo | Fm | Fv | Fv/Fm | F' | Fm' | Fo' | qL | $Y_{NO}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PC1 | -0.73 | -0.77 | -0.80 | -0.73 | 0.65 | -0.71 | -0.67 | -0.60 | 0.00 | -0.09 | -0.67 | -0.46 | 0.72 |
| PC2 | 0.46 | 0.17 | 0.39 | 0.21 | -0.14 | 0.17 | 0.01 | -0.33 | 0.83 | -0.72 | -0.60 | -0.85 | -0.57 |
| Means | | | | | | | | | | | | | |
| T111 | 951.5 | 804.36 | 1756.0 | 258.0 | 416.0 | 1972.0 | 1575.0 | 0.79 | 99.0 | 312.0 | 21 x $10^{-4}$ | 1.47 x $10^{-5}$ | 0.05 |
| T121 | 925.5 | 811.33 | 1739.3 | 250.6 | 384.0 | 1939.0 | 1587.0 | 0.81 | 95.0 | 352.0 | 20 x $10^{-4}$ | 1.47 x $10^{5}$ | 0.05 |
| T131 | 1797.2 | 925.32 | 2676.4 | 309.1 | 356.0 | 2120.0 | 1647.0 | 0.89 | 93.0 | 331.0 | 22 x $10^{-4}$ | 1.28 x $10^{-5}$ | 0.05 |
| T141 | 866.3 | 664.18 | 1518.1 | 216.4 | 406.0 | 1936.0 | 1565.0 | 0.77 | 99.0 | 333.0 | 20 x $10^{-4}$ | 1.30 x $10^{-5}$ | 0.05 |
| T211 | 747.2 | 627.01 | 1374.2 | 163.2 | 402.0 | 1968.0 | 1571.0 | 0.80 | 101.0 | 327.0 | 20 x $10^{-4}$ | 1.34 x $10^{-5}$ | 0.05 |
| T221 | 872.7 | 672.11 | 1553.6 | 221.1 | 388.0 | 1983.0 | 1595.0 | 0.80 | 89.0 | 282.0 | 22 x $10^{-4}$ | 1.75 x $10^{-5}$ | 0.04 |
| T231 | 1017.4 | 799.52 | 1804.8 | 227.6 | 420.0 | 1972.0 | 1552.0 | 0.80 | 99.0 | 320.0 | 20 x $10^{-4}$ | 1.41 x $10^{-5}$ | 0.05 |
| T241 | 830.3 | 729.36 | 1559.7 | 224.3 | 415.0 | 1865.0 | 1509.0 | 0.80 | 96.0 | 309.0 | 21 x $10^{-4}$ | 1.47 x $10^{-5}$ | 0.05 |
| T112 | 1289.7 | 819.10 | 2103.9 | 264.2 | 359.5 | 1932.5 | 1573.0 | 0.81 | 95.0 | 314.0 | 22 x $10^{-4}$ | 1.53 x $10^{-5}$ | 0.05 |
| T122 | 1140.8 | 879.85 | 2024.3 | 278.2 | 340.0 | 1893.0 | 1551.0 | 0.79 | 92.0 | 329.0 | 21 x $10^{-4}$ | 1.64 x $10^{-5}$ | 0.05 |
| T132 | 1112.1 | 806.15 | 1918.2 | 255.3 | 378.0 | 2010.0 | 1632.0 | 0.81 | 100.0 | 305.0 | 22 x $10^{-4}$ | 1.42 x $10^{-5}$ | 0.05 |
| T142 | 1012.4 | 733.20 | 1715.4 | 240.7 | 320.0 | 1854.5 | 1533.5 | 0.81 | 96.0 | 335.0 | 21 x $10^{-4}$ | 1.57 x $10^{-5}$ | 0.05 |
| T212 | 985.7 | 781.22 | 1767.0 | 231.3 | 378.5 | 1992.0 | 1617.0 | 0.76 | 100.0 | 338.0 | 20 x $10^{-4}$ | 1.38 x $10^{-5}$ | 0.05 |
| T222 | 1124.5 | 833.8 | 1952.5 | 227.7 | 374.0 | 1948.0 | 1606.0 | 0.81 | 93.0 | 336.0 | 19 x $10^{-4}$ | 1.54 x $10^{-5}$ | 0.05 |
| T232 | 1038.6 | 783.6 | 1836.7 | 258.2 | 385.0 | 1988.0 | 1600.0 | 0.81 | 94.0 | 334.0 | 17 x $10^{-4}$ | 1.06 x $10^{-5}$ | 0.05 |
| T242 | 1022.1 | 829.9 | 1874.2 | 286.2 | 363.0 | 1962.0 | 1585.0 | 0.81 | 87.0 | 296.0 | 23 x $10^{-4}$ | 1.89 x $10^{-5}$ | 0.06 |
| T113 | 1043.7 | 816.2 | 1858.9 | 264.4 | 344.0 | 2034.0 | 1611.0 | 0.80 | 92.0 | 322.0 | 23 x $10^{-4}$ | 1.75 x $10^{-5}$ | 0.05 |
| T123 | 953.9 | 633.7 | 1577.0 | 216.2 | 348.0 | 2095.0 | 1691.5 | 0.85 | 92.0 | 322.0 | 22 x $10^{-4}$ | 1.68 x $10^{-5}$ | 0.05 |
| T133 | 1085.8 | 641.5 | 1713.3 | 251.5 | 407.0 | 1932.0 | 1592.0 | 0.80 | 99.0 | 351.0 | 18 x $10^{-4}$ | 1.18 x $10^{-5}$ | 0.06 |
| T143 | 870.1 | 573.5 | 1469.1 | 182.3 | 423.0 | 1547.0 | 1287.5 | 0.80 | 93.0 | 306.0 | 18 x $10^{-4}$ | 1.30 x $10^{-5}$ | 0.06 |
| T213 | 1188.5 | 763.5 | 1957.8 | 229.3 | 391.0 | 2006.0 | 1615.0 | 0.75 | 92.0 | 305.0 | 22 x $10^{-4}$ | 1.68 x $10^{-5}$ | 0.05 |
| T223 | 1043.8 | 784.6 | 1828.1 | 275.8 | 370.0 | 1978.0 | 1569.0 | 0.81 | 109.0 | 358.0 | 21 x $10^{-4}$ | 1.68 x $10^{-5}$ | 0.05 |
| T233 | 1003.3 | 727.9 | 1730.1 | 243.8 | 397.0 | 1992.0 | 1624.0 | 0.81 | 91.0 | 342.0 | 1 9x $10^{-4}$ | 1.17 x $10^{-5}$ | 0.06 |
| T243 | 1135.3 | 712.8 | 1837.4 | 181.4 | 390.0 | 1932.0 | 1571.0 | 0.81 | 95.0 | 323.0 | 21 x $10^{-4}$ | 1.56 x $10^{-5}$ | 0.05 |

Chl a - Chlorophyll a; Chl b - Chlorophyll b; Chl t - Total chlorophyll; Car - Carotenoids; Fo - Initial fluorescence; Fm - Maximum fluorescence; Fv - Variable fluorescence; Fv/Fm - Quantum yield of PSII; F' - Initial fluorescence before saturation pulse; Fm'- Maximum fluorescence after adaptation to saturating light; Fo' - Minimum fluorescence of illuminated plant tissue; qL - Photochemical quenching coefficient; and, $Y_{NO}$ - Quantum yield of non-regulated photochemical quenching; T123: T – Treatment; 1 - Corresponds to ECw (ranging from 1 to 2: 1 = 0.8 dS $m^{-1}$ and 2 = 5.3 dS $m^{-1}$); 2 - Corresponds to $H_2O_2$ concentrations (ranging from 1 to 4: 1= 0 µM, 2 = 25 µM, 3 = 50 µM and 4 = 75 µM); 3 - Corresponds to cotton genotypes (ranging from 1 to 3: 1= 'BRS Rubi'; 2 = 'BRS Topázio' and 3 = 'BRS Verde')

except for the electrical conductivity of water, which exhibited significant effect ($p < 0.01$) only for $PC_1$.

The biplot projections for the effects of treatments and variables in the first and second principal components (PC1 and PC2) are shown in Figures 2A and B. The two principal components constructed from the original characteristics described the differences between electrical conductivity values, hydrogen peroxide concentrations and cotton genotypes.

In principal component 1 (PC1), the highest relative values for Chl a (1797.2 mg $g^{-1}$ of FM), Chl b (925.32 mg $g^{-1}$ of FM), Chl t (2676.4 mg $g^{-1}$ of FM), Car (309.1 mg $g^{-1}$ of FM), Fm (2120.0), Fv (1647.0) and Fv/Fm (0.89) were found in the




T123: T – Treatment; 1 - Corresponds to ECw (ranging from 1 to 2: 1 = 0.8 dS $m^{-1}$ and 2 = 5.3 dS $m^{-1}$); 2 - Corresponds to $H_2O_2$ concentrations (ranging from 1 to 4: 1= 0 µM, 2 = 25 µM, 3 = 50 µM and 4 = 75 µM); 3 - Corresponds to cotton genotypes (ranging from 1 to 3: 1= 'BRS Rubi'; 2 = 'BRS Topázio' and 3 = 'BRS Verde')

**Figure 2.** Two-dimensional projection of treatments (A) and analyzed variables (B) in the two firsts principal components ($PC_1$) and ($PC_2$)

treatment T131, which corresponds to the irrigation of the 'BRS Rubi' cotton genotype with 0.8 dS $m^{-1}$ water and foliar application of 50 µM of hydrogen peroxide; for Fo (423.0) and $Y_{NO}$ (0.06), the highest values were obtained in the treatment T143; for Fo' (23 x $10^{-4}$), the highest value was verified in the treatment T242 (Table 1 and Figures 2A and B).

When comparing the results obtained in plants of the T131 treatment with the results obtained in plants of T111 (Control for the genotype 'BRS Rubi'), there were increments of 47.05% (Chl a), 13.07% (Chl b), 34.38% (Chl t), 16.5% (Car), 6.98% (Fm), 4.37% (Fv) and 11.23% (Fv/Fm). Therefore, it can be inferred that the foliar application of 50 µM of hydrogen peroxide was able to favor the physiology of 'BRS Rubi' without salt stress (Table 1 and Figures 2A and B).

Also in the principal component 1, there were increments of 17.7 and 12.9% in Fo and $Y_{NO}$ in plants of the treatment T143, compared to plants of the treatment T113 (Control for 'BRS Verde' genotype). For Fo', there was an increase of 13.04% in the treatment T242 compared to plants of 'BRS Topázio' that were irrigated with saline water and that did not receive foliar applications of $H_2O_2$ (T212).

In principal component 2 (PC2), the highest relative values for F' (109.0) and Fm' (358.0) were observed in the treatment T223, and for qL (1.75 x $10^{-5}$) in T221. In turn, the lowest values for F' (89.0) were found in the treatment T242 and the lowest values referring to Fm' (282.0) and qL (1.06 x $10^{-5}$) were identified in plants of the treatment T221 and T232, respectively (Table 1 and Figures 2A and B).

Salt stress negatively affected physiological variables. These changes may be related to osmotic and ionic effects, especially of $Na^+$ and $Cl^-$, which interfere in normal metabolic processes, causing membrane damage, nutritional imbalance, changes in levels of growth regulators, reduction of photosynthesizing pigment concentration, enzymatic inhibition and metabolic dysfunction, including photosynthesis (Shobha et al., 2021).

However, application of $H_2O_2$ at adequate concentrations can mitigate the effects of salinity, promoting the maintenance of photosynthetic pigments and the functioning of the photosynthetic apparatus, responsible for the development and production of plants, or even assist in the increase of these factors in plants under normal environmental conditions, that is, with absence of stressful factors, as observed in the treatment T131 (Figures 2A and B).

In this study, the foliar application of 50 µM of $H_2O_2$ increased the concentration of photosynthetic pigments of 'BRS Rubi' cotton irrigated with water of 0.8 dS $m^{-1}$ (T131), possibly due to the multiple physiological functions performed by $H_2O_2$ in the plant, such as the capacity to increase chlorophyll concentration, since the use of signaling agents in the plant, such as $H_2O_2$, can promote metabolic alteration in the cell and activation of antioxidant enzymes such as superoxide dismutase, catalase, guaiacol peroxidase and ascorbate peroxidase, resulting in decreased oxidative stress in plants (Nazir et al., 2020).

Silva et al. (2019), in their study evaluating the concentration of photosynthetic pigments of 'Morada Nova' soursop seedlings irrigated with saline water and subjected to $H_2O_2$ application by seed imbibition and foliar spray, found that the application

of 50 µM of $H_2O_2$ increased chlorophyll c concentration in soursop plants irrigated with water up to 3.5 dS $m^{-1}$.

With regard to the reduction in chlorophyll a, chlorophyll b and total chlorophyll concentrations observed (Table 1) in the 'BRS Rubi' cotton genotype irrigated with water of 5.3 dS $m^{-1}$ without application of $H_2O_2$ (T211), it is inferred that this decrease may be related to water salinity, since excess salts may alter the structure of the organelles, the concentration of pigments and metabolites, in addition to the enzymatic activities involved in the photosynthetic process (Shahverdi et al., 2019). In addition, the negative correlation of chlorophyll concentration with salinity may be associated with the action of chlorophyll degradation enzyme, chlorophyllase, which is more active under salt stress, and the low absorption of some ions, such as $Mg^{+2}$ and $Fe^{+2}$, which are involved in chlorophyll formation and whose absorption is limited by competition with other ions (Soares et al., 2021).

Zhang et al. (2014) observed a significant reduction in the chlorophyll a and b concentrations of the CCRI-79 and Simian 3 genotypes with the increase of NaCl from 0 to 240 mM. According to these authors, this reduction may have been caused by the elimination of specific enzymes associated with chlorophyll synthesis, hence considering chlorophyll content a good indicator for the selection of salinity-tolerant varieties. Ibrahim et al. (2019) observed that chlorophyll a, chlorophyll b and carotenoid concentrations of Zhongmian 23 and Zhongmian 41 cotton genotypes were significantly inhibited by the increase in salt stress.

The reduction in carotenoid concentration (Table 1) in cotton plants of the T211 treatment can also compromise their yield, due to the decline in photosynthetic activity, because carotenoids, besides being considered important antioxidant agents, are key pigments in the photosynthetic apparatus as they are involved in the capture of light energy during the photosynthesis process. In addition, in plants exposed to high salinity oxidative stress can be triggered due to excess ROS, which is the main cause of the degradation of photosynthesizing pigments (Sharif et al., 2019).

Regarding the quantum efficiency of photosystem II, it is possible to note a beneficial effect of the application of 50 µM of $H_2O_2$, which caused reduction in initial fluorescence (Fo) and increase in maximum fluorescence (Fm), variable fluorescence (Fv) and quantum efficiency of photosystem II (Fv/Fm) in plants subjected to the T131 treatment.

In addition, the highest Fo value was verified in plants subjected to the T143 treatment, that is, when exposed to the application of 75 µM of $H_2O_2$ and irrigation with water of 0.8 dS $m^{-1}$, the 'BRS Verde' cotton plants probably suffered damage in the reaction center of photosystem II, or reduction in the transfer of excitation energy from the light-harvesting system to the reaction center (Veloso et al., 2020). In a way, this result may be related to the high concentration of $H_2O_2$ used, since hydrogen peroxide is the most stable reactive oxygen species in cells and, at high concentrations, can spread rapidly through the subcellular membrane, resulting in oxidative damage to the cell membrane (Farouk & Amira, 2018).

In addition, at high concentrations, hydrogen peroxide can react with $O_2$ and become a possible responsible for dissociating the pigment-protein complex of the inner antenna of the PSII light-harvesting system, within the photosynthetic apparatus, causing enzyme inactivation, pigment discoloration, lipid peroxidation (Kilic & Ayten, 2016).

Fm is the point at which the fluorescence of the plant reaches its maximum capacity and practically the entire quinone is reduced. Thus, the treatment with hydrogen peroxide, observed in T131 treatment plants, may have helped them through metabolic alterations to achieve this maximum fluorescence. Furthermore, the absence of reduction in Fm values in plants under the treatment T131 may indicate that there was no deficiency in the photoreduction of Qa, ensuring the protection of electron flow between the photosystems and photosynthetic activity (Cintra et al., 2020).

As observed with Fm, the variable fluorescence (Fv) of 'BRS Rubi' cotton increased with the application of 50 µM of $H_2O_2$ and irrigation with water of 0.8 dS $m^{-1}$ (Table 1). Thus, it can be inferred that there was no limitation of the plant's capacity to transfer energy from electrons emitted by pigments for the formation of NADPH, ATP and reduced ferredoxin (Fdr); consequently, the plant maintained the $CO_2$ assimilation capacity in the biochemical phase of photosynthesis (Asgher et al., 2021). Probably, the $H_2O_2$ concentration of 50 µM led to the activation of the enzymatic apparatus responsible for the defense against oxidative stress, because the plant responded in different ways to the hydrogen peroxide concentrations used (Silva et al., 2019; Asgher et al., 2021; Veloso et al., 2022).

Application of 50 µM of $H_2O_2$ increased the Fv/Fm of 'BRS Rubi' cotton plant. Thus, it can be inferred that the photosynthetic apparatus integrity was not compromised, as the plants had Fv/Fm values within the range of 0.75 - 0.85. Low values indicate difficulty in fixing $CO_2$ in the leaf tissue, being an excellent indicator of plant stress (Peripolli et al., 2021). Thus, salinity can cause photoinhibitory damage to PSII reaction centers, as reported by Lima et al. (2019) in their study, in which the increase in salinity by up to 9.1 dS $m^{-1}$ reduced the quantum efficiency of the PSII of 'BRS Rubi' cotton.

The highest values for initial fluorescence before the saturation pulse (F') and maximum fluorescence after adaptation to saturating light (Fm') were identified in the treatment T233 (Table 1). Thus, with regard to F', it is inferred that the application of $H_2O_2$ associated with irrigation with 5.3 dS $m^{-1}$ water limited the use of the light energy of 'BRS Verde' cotton; however, the increase in Fm' is an indication that a greater number of electrons are passing through the photosystems, besides evidencing the importance of these elements in the protection of photosynthetic machinery (Schmidt et al., 2016).

In turn, 'BRS Rubi' cotton had the minimum fluorescence of the illuminated plant tissue (Fo') increased with the irrigation of 5.3 dS $m^{-1}$ and application of 75 µM of $H_2O_2$ (T242), which may be indicative of the occurrence of damage to the PSII reaction center (P680) or limitation in the transfer of light energy to the reaction centers, leading to PSII inactivation, caused by salt stress and high concentration of $H_2O_2$ (Taiz et al., 2017).

The photochemical quenching coefficient (qL) was increased in the T221 treatment, indicating the adequate photosynthetic efficiency of plants, since the qL is initiated as a function of the increase of electrons exported from PSII due to

the activation of enzymes involved in carbon metabolism and stomatal opening and quantifies the photochemical capacity of PSII, corresponding to the fraction of open PSII reaction centers (Azevedo Neto et al., 2011).

The increase in the quantum yield of non-regulated photochemical quenching ($Y_{NO}$) observed in the T143 treatment is a strong indication of the occurrence of photoinhibition in this treatment, since the $Y_{NO}$ values are negatively correlated with the quantum photochemical efficiency of PSII, which makes it an excellent indicator of photodamage (Colombo et al., 2018). This photodamage may be related to the $H_2O_2$ concentration used (75 µM), indicating that at this dose $H_2O_2$ became toxic to 'BRS Verde' cotton, since high concentrations of this reactive oxygen species induce oxidative stress, causing lipid peroxidation and destroying cellular integrity and pigments, leading to death (Zhang et al., 2014).

## Conclusions

1. Irrigation with water of 5.3 dS $m^{-1}$ reduces the chlorophyll a, chlorophyll b and total chlorophyll concentrations of 'BRS Rubi' cotton.

2. The concentrations of photosynthetic pigments, maximum fluorescence, variable fluorescence and quantum efficiency of photosystem II of 'BRS Rubi' cotton increased under irrigation with 0.8 dS $m^{-1}$ water and foliar application of 50 µM of hydrogen peroxide at 60 days after sowing.

3. Water electrical conductivity of 5.3 dS $m^{-1}$ and foliar applications of 75 µM of hydrogen peroxide reduce the concentrations of photosynthetic pigments, but did not cause damage to the efficiency of photosystem II of the colored cotton genotypes.

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