

## Use of hydrogen peroxide for acclimation of sorghum plants to salt stress

### Uso de peróxido de hidrogênio na aclimação do sorgo ao estresse salino

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**ABSTRACT** - The use of chemical conditioners, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), is important for mitigating deleterious effects caused by salt stress on plants. This practice can increase the production of agricultural crops, including sorghum, in the Semiarid region of Brazil. In this sense, the objective of this study was to evaluate effects of different electrical conductivities of the irrigation water and H<sub>2</sub>O<sub>2</sub> concentrations on plant growth and biomass accumulation of sorghum plants grown in the Semiarid region of Brazil. The experiment was conducted from November 2020 to January 2021 in a greenhouse at the Center for Agri-Food Sciences and Technologies of the Federal University of Campina Grande, in Pombal, Paraíba, Brazil. A randomized block experimental design was used, in a 4×4 factorial arrangement, consisted of four electrical conductivities of the irrigation water [0.30 (control), 1.50, 3.50, and 5.50 dS m<sup>-1</sup>] and four H<sub>2</sub>O<sub>2</sub> concentrations [0 (control), 6, 12, and 18 μM], with three replications and one plant per plot, totaling 48 experimental units. Plant height, stem diameter, flag leaf length, and fresh and dry weights of leaves and stems were evaluated. The results showed that applying irrigation water with electrical conductivities higher than 1.50 dS m<sup>-1</sup> decreases plant growth and biomass accumulation in sorghum plants. Treating sorghum seeds with H<sub>2</sub>O<sub>2</sub> concentrations of up to 12 μM mitigates adverse effects caused by salt stress on sorghum plants subjected to the salinity levels evaluated in the present study.

**Keywords:** *Sorghum bicolor* (L.) Moench. H<sub>2</sub>O<sub>2</sub>. Brackish water. Semiarid region.

**RESUMO** - A utilização de condicionadores químicos, como o peróxido de hidrogênio (H<sub>2</sub>O<sub>2</sub>), é extremamente importante para atenuação dos efeitos deletérios causados pelo estresse salino nas plantas. Essa técnica pode inclusive, aumentar a produção agrícola como de sorgo no semiárido brasileiro. Neste sentido, objetivou-se com este estudo avaliar os efeitos de diferentes condutividades elétricas da água de irrigação e concentrações de H<sub>2</sub>O<sub>2</sub> no crescimento e acúmulo de fitomassa do sorgo no semiárido brasileiro. O experimento foi conduzido entre os meses de novembro de 2020 e janeiro de 2021, em casa de vegetação no Centro de Ciências e Tecnologias Agroalimentar da Universidade Federal de Campina Grande, no município de Pombal, Paraíba, Brasil. O delineamento experimental adotado foi o de blocos ao acaso, em esquema fatorial 4 x 4, referentes a quatro condutividades elétricas da água de irrigação: 0,30 (controle); 1,50; 3,50 e 5,50 dS m<sup>-1</sup> e quatro concentrações de H<sub>2</sub>O<sub>2</sub>: 0 (controle); 6; 12 e 18 μM, com três repetições e uma planta por parcela, totalizando 48 unidades experimentais. Foi avaliada a altura de planta, diâmetro de colmo, comprimento da folha bandeira, fitomassa fresca e seca de folhas e colmos. Os resultados denotam que a água de irrigação com condutividade elétrica superior a 1,50 dS m<sup>-1</sup> reduz o crescimento e acúmulo de fitomassa em plantas de sorgo. Tratamento das sementes de sorgo com concentrações de peróxido de hidrogênio até 12 μM, reduz os efeitos adversos causados pelo estresse salino nas plantas, em todos os níveis de salinidade.

**Palavras-chave:** *Sorghum bicolor* (L.) Moench. H<sub>2</sub>O<sub>2</sub>. Água salina. Região semiárida.

**Conflict of interest:** The authors declare no conflict of interest related to the publication of this manuscript.



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## INTRODUCTION

Climate change has caused production losses for the main crops grown in tropical and temperate regions, with a worsening trend due to increases in greenhouse gas emissions (IPCC, 2022). Droughts, among climate-related adversities, accounts for 80% of global food losses, increasing food insecurity (FAO, 2021).

Climate change tends to intensify water scarcity in arid and Semiarid regions worldwide (IPCC, 2022). In this sense, future scenarios have indicated that this phenomenon could reduce rainfall in the Semiarid region of Brazil by 10% to 20% by 2100 (MEDEIROS et al., 2021), directly impacting agricultural production in this region.

The Semiarid region of Brazil is strongly affected by long periods (7 to 8 months) of water deficit (BRASIL, 2020) due to low and irregular annual rainfall depths (usually lower than 800 mm) combined with high mean air temperatures that result in a mean evapotranspiration of 2,000 mm year<sup>-1</sup> (GOIS et al., 2019; MEDEIROS et al., 2020). These unfavorable climate conditions in the Semiarid of Brazil result in insufficient water sources for irrigated crops (VELOSO et al., 2022). Additionally, most groundwater sources are characterized by high salt concentrations (GOIS et al., 2019). Thus, the quantity and quality of the available water in the Semiarid region of Brazil restricts the expansion of irrigated

agriculture in this region.

The use of brackish water is an alternative for growing crops in semiarid regions; however, it requires some management measures, such as the use of plants that are more tolerant to salt stress (GOIS et al., 2019; GUIMARÃES et al., 2022; LACERDA et al., 2022) and application of elicitors, such as hydrogen peroxide ( $H_2O_2$ ), for plant acclimation to salt stress (ANDRADE et al., 2022; PEREIRA et al., 2023).

In this context, sorghum plants (*Sorghum bicolor* (L.) Moench) exhibit a high growth potential in Semiarid regions due to their high energy value and soluble carbohydrate contents, which are important for animal feed; in addition, they are tolerant to several abiotic stresses, such as drought, salt, and high temperatures, which are limiting factors for producing most forage species (GOIS et al., 2019; CALONE et al., 2020; GUIMARÃES et al., 2022). Studies have shown that sorghum plants can tolerate salinity levels from 4.19 (GUIMARÃES et al., 2022) to 4.50  $dS\ m^{-1}$  (CALONE et al., 2020), whereas higher salinity levels can cause significant production losses for these plants.

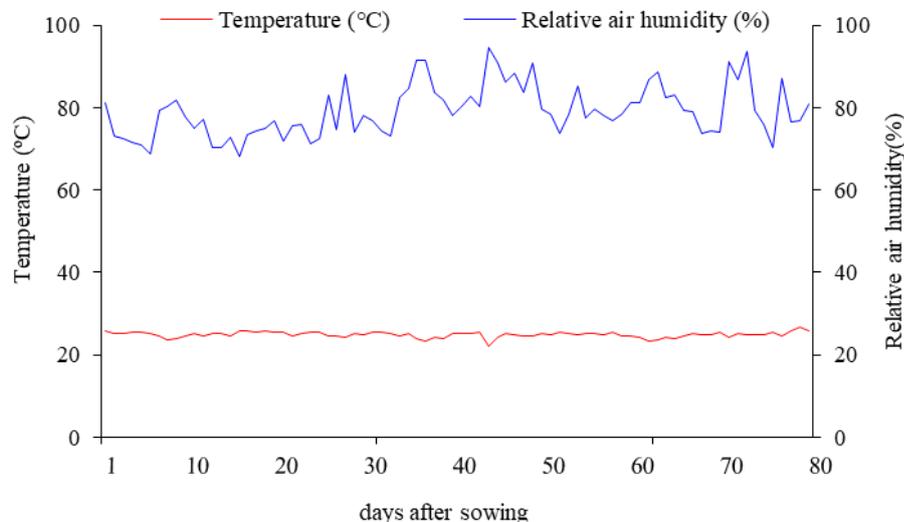
Regarding the application of elicitors for plant acclimation,  $H_2O_2$  is a reactive oxygen species that, at small concentrations, acts as an intracellular signal for activating stress responses and plant defenses (VELOSO et al., 2023). Promising results have indicated that the application of small

amounts of  $H_2O_2$  induces acclimation to salt stress in different crop species (DANTAS et al., 2022; VELOSO et al., 2022, 2023; PEREIRA et al., 2023). However, the ideal  $H_2O_2$  concentration to trigger this physiological process in sorghum plants is still unclear.

Therefore, the hypothesis of this study is that the acclimation technique using  $H_2O_2$  enables the production of sorghum plants irrigated with brackish water in Semiarid regions. Thus, the objective of this study was to evaluate the effects of different electrical conductivities of the irrigation water and  $H_2O_2$  concentrations on plant growth and biomass accumulation of sorghum plants grown in the Semiarid region of Brazil.

## MATERIAL AND METHODS

The experiment was conducted from November 2020 to January 2021, using drainage lysimeters under greenhouse conditions at the Center for Agri-Food Sciences and Technologies of the Federal University of Campina Grande, Pombal, Paraíba, Brazil ( $6^{\circ}48'16''S$ ,  $37^{\circ}49'15''W$ , and altitude of 144 m). Data of maximum and minimum temperatures and relative air humidity during the experimental period are shown in Figure 1.



**Figure 1.** Mean temperature and relative air humidity inside the greenhouse during the experimental period.

A randomized block experimental design was used, in a  $4 \times 4$  factorial arrangement consisted of four electrical conductivities of the irrigation water ( $EC_w$ ) [ $0.30$  (control),  $1.50$ ,  $3.50$ , and  $5.50\ dS\ m^{-1}$ ] and four hydrogen peroxide ( $H_2O_2$ ) concentrations [ $0$  (control),  $6$ ,  $12$ , and  $18\ \mu M$ ], with three replications and one plant per lysimeter, totaling 48 experimental units.

The  $EC_w$  levels were determined according to Blanco et al. (2008). The solutions were prepared to correspond to a respective equivalent ratio of 7:2:1 for Na:Ca:Mg by dissolving the salts  $NaCl$ ,  $CaCl_2 \cdot 2H_2O$ , and  $MgCl_2 \cdot 6H_2O$  in the water available for irrigation ( $0.30\ dS\ m^{-1}$ ) in the study

region, considering the correlation between  $EC_w$  and salt concentration proposed by Richards (1954), as shown in Equation 1. This salt proportion is predominant in most water sources used for irrigation in small rural properties in the Semiarid region of Brazil (ANDRADE et al., 2022).

$$Q = 10 \times EC_w \quad (1)$$

where,  $Q$  = quantity of salts to be added ( $mmol\ L^{-1}$ ) and  $EC_w$  = electrical conductivity of the irrigation water ( $dS\ m^{-1}$ ).

Considering the absence of studies on the application

of  $H_2O_2$  to sorghum plants under salt stress, the  $H_2O_2$  concentrations used in this experiment were adapted from a study with maize plants conducted by Silva et al. (2016), who found that  $H_2O_2$  concentrations up to 8  $\mu M$  promoted higher plant growth, whereas concentrations higher than 15  $\mu M$  increased the damage caused by salt stress. The solutions with the different  $H_2O_2$  concentrations used in the present study were prepared by diluting pure peroxide (99%) in deionized water and then applied through seed imbibition. The sorghum seeds were soaked in solutions with different  $H_2O_2$  concentrations (according to each treatment) for 16 hours

before sowing.

Subsequently, the seeds were sown in 10  $dm^{-3}$  lysimeters, with bottoms covered with geotextile and a 5 cm layer of crushed stones (9.5 to 19 mm), connected to a drain for collecting drained water. The soil used to fill the lysimeters was classified Neossolo Fluvico (SANTOS et al., 2018) or Fluvisol, according to the classification of IUSS (2015); it was collected in Sao Domingos, Paraiba, and its physical and chemical attributes (Table 1) were determined according to Teixeira et al. (2017).

**Table 1.** Physical and chemical characteristics of the soil (0.0-0.3 m layer) used in the experiment.

Chemical attributes									
pH	P ( $mg\ dm^{-3}$ )	$K^+$	$Na^+$	$H^+ + Al^{+3}$ ..... $cmol_c\ dm^{-3}$ .....	$Ca^{+2}$	$Mg^{+2}$	SB	CEC	OM $g\ kg^{-1}$
6.5	6.14	0.37	0.07	0.81	1.20	0.71	2.28	3.09	4.79
Physical attributes									
Particles ( $g\ kg^{-1}$ )			Density ( $g\ cm^{-3}$ )		Porosity (%)		Texture		
Sand	Silt	Clay	Soil	Particle			Sandy-loam		
851	99	50	1.26	2.71	53.50				

pH = hydrogen potential; P = available phosphorus extracted with Mehlich<sup>-1</sup>;  $K^+$  = exchangeable potassium;  $Na^+$  = exchangeable sodium;  $H^+ + Al^{+3}$  = hydrogen + aluminum;  $Ca^{+2}$  = exchangeable calcium;  $Mg^{+2}$  = exchangeable magnesium; SB = sum of bases; OM = organic matter; CEC = cation exchange capacity in pH 7.0.

The sorghum seeds used (cultivar BRS Ponta Negra) present a 96% germination rate and good health. The choice of this cultivar for the experiment was based on its suitability for silage production (GOIS et al., 2019). Before sowing, the soil moisture in the lysimeters was increased to the field capacity using a similar water to that of the control treatment (0.30  $dS\ m^{-1}$ ) to promote good acclimation to the lysimeter conditions; irrigations with this treatment continued until 15 days after sowing (DAS). Five seeds were sown to a depth of 3 cm in each lysimeter, uniformly distributed. Germination started at 3 DAS and stabilized at 7 DAS. Thinning was performed at 15 days after emergence (DAE), leaving only the most vigorous seedling in each lysimeter until the end of the experimental period.

The application of ECw in the treatments started at 16 DAE to maintain soil moisture close to field capacity in all experimental plots. Manual irrigation was performed daily, based on the recommendations of Ramos et al. (2022), by applying a volume of water equivalent to the water balance obtained from the previous irrigation in each lysimeter, according to 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 = volumes of water applied and drained in the previous irrigation event (mL), respectively; and LF = a leaching fraction of 0.2 applied every 15 days to decrease excessive salt accumulation in the plant root zone.

Nutritional management was carried out as recommended by Novais, Neves, and Barros (1991), with application of N, P, and K (140, 300, and 180  $mg\ dm^{-3}$ , respectively) using urea,  $P_2O_5$ , and  $K_2O$ , divided into four applications, except for  $P_2O_5$ : the first at planting and the others at 20, 30, and 40 DAE, through fertigation during the manual irrigations. A micronutrient fertilizer solution (Dripsol<sup>®</sup> micro) at concentration of 1.0  $g\ L^{-1}$  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 to the leaves (adaxial and abaxial surfaces), using a backpack sprayer.

Cultural practices during the experimental period included manual weeding, surface soil scarification in the lysimeters, and staking of plants to prevent lodging and breakage. Plant health protection was carried as needed through application of insecticides, fungicides, and acaricides from the chemical groups Neonicotinoids, Triazoles, and Abamectin, respectively.

Plant growth and biomass accumulation of sorghum plants were evaluated at 80 DAE by determining plant height (cm), measured from the ground to the base of the flag leaf ligule; stem diameter (mm), measured at 5 cm from the ground level using a digital caliper; and flag leaf length (cm),

measured from the base to the apex of the leaf using a ruler. Subsequently, the plants were cut at the ground level and separated into leaves and stems to determine their fresh weights (g) by weighing on a precision digital balance. These materials were then dried in an oven at 65 °C until constant weight to determine the leaf and stem dry weights (g).

The obtained data were subjected to normality test (Kolmogorov-Smirnoff), followed by analysis of variance at a probability level of 0.05, and significant means were subjected to polynomial regression analysis ( $p \leq 0.05$ ), using the statistical software SISVAR (FERREIRA, 2019).

**Table 2.** Analyses of variance for plant height (PH), stem diameter (SD), and flag leaf length (FLL) of sorghum plants (*Sorghum bicolor* (L.) Moench) subjected to different electrical conductivities of the irrigation water (ECw) and hydrogen peroxide concentrations ( $H_2O_2$ ).

Source of variation	DF	Mean squares		
		PH	SD	FLL
ECw	3	6213.50**	71.49**	155.81 <sup>ns</sup>
Linear regression	1	17673.08**	103.43**	345.60 <sup>ns</sup>
Quadratic regression	1	862.75 <sup>ns</sup>	89.65 <sup>ns</sup>	67.68 <sup>ns</sup>
$H_2O_2$	3	2837.47**	31.55**	874.34**
Linear regression	1	4828.55**	0.04 <sup>ns</sup>	300.38 <sup>ns</sup>
Quadratic regression	1	84.00 <sup>ns</sup>	81.90**	2146.68**
Interaction (ECw × $H_2O_2$ )	9	1107.39 <sup>ns</sup>	3.55 <sup>ns</sup>	62.96 <sup>ns</sup>
Blocks	2	308.31	5.35	143.34
CV (%)	-	11.60	12.09	13.08

CV = coefficient of variation; DF = degrees of freedom; <sup>ns</sup> and \*\* = not significant and significant at  $p \leq 0.01$  by the F test, respectively.

Plant height (PH) decreased linearly as the ECw was increased, regardless of the  $H_2O_2$  concentration (Figure 2A), presenting excellent predictive ability ( $R^2 = 0.98^{**}$ ). A decrease of 4.59% was found for each unit increase in ECw. Plants irrigated with the highest ECw ( $5.5 \text{ dS m}^{-1}$ ) presented a decrease of 23.86% (0.50 m) in PH compared to those in the control (ECw of  $0.30 \text{ dS m}^{-1}$ ). Lacerda et al. (2022) evaluated maize crops and found decreases of 9.43% in PH for an ECw of  $2.0 \text{ dS m}^{-1}$  compared to the initial ECw of  $0.30 \text{ dS m}^{-1}$ . These different results may be due to the highest ECw ( $2.0 \text{ dS m}^{-1}$ ) used by them, which was lower than that evaluated in the present study ( $5.50 \text{ dS m}^{-1}$ ).

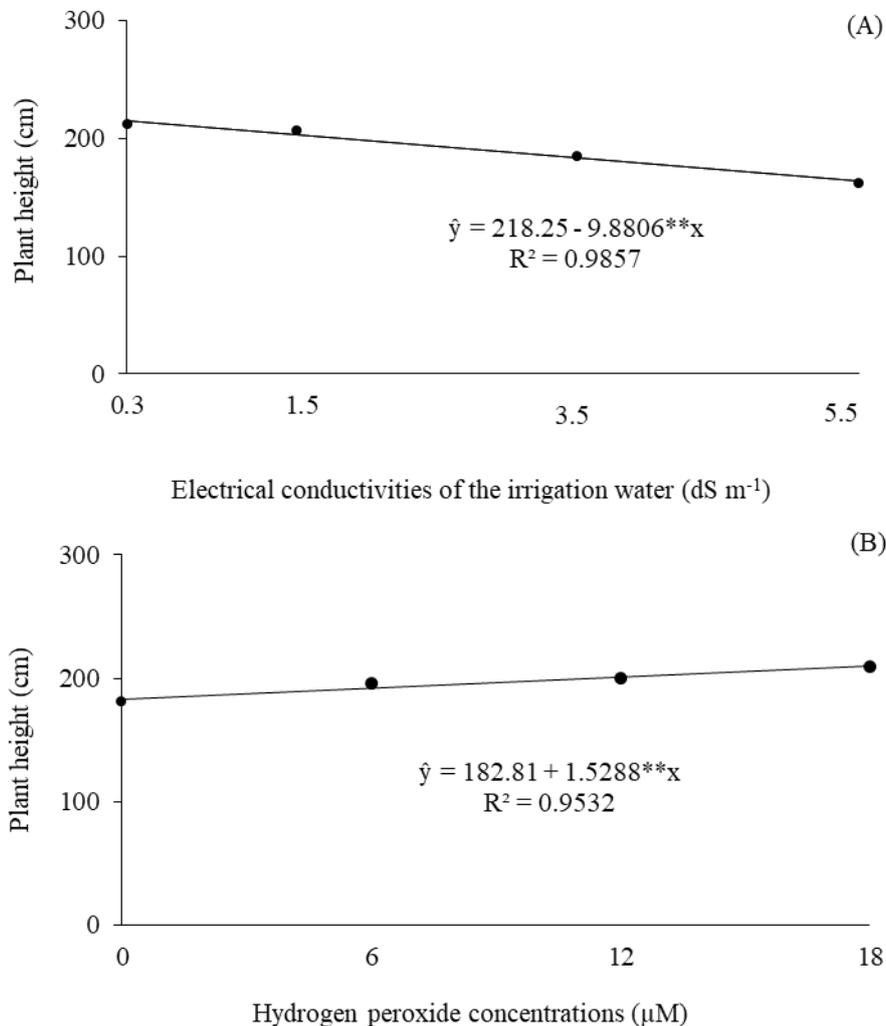
Salt stress causes several physiological and biochemical disturbances in plants, affecting the photosynthesis process and, consequently, decreasing plant growth (SILVA et al., 2019). According to Veloso et al. (2022), the most common effect caused by salt stress in plants is a decreased plant growth due to increases in osmotic potential as the concentration of soluble salts in the soil solution increases, thus restricting water uptake by plants. Additionally, this effect also can be connected to a nutritional imbalance in the soil solution, when the absorption of nutrients by plants is affected by the competition of some specific ions, such as competition of  $Na^+$  with other essential nutrients to plants (PEREIRA et al., 2023).

## RESULTS AND DISCUSSION

According to the analysis of variance (Table 2), the interaction between electrical conductivity of the irrigation water and hydrogen peroxide concentration (ECw ×  $H_2O_2$ ) was not significant for the evaluated variables related to sorghum plant growth. However, the factors (ECw and  $H_2O_2$ ) had significant effect ( $p \leq 0.01$ ) on plant height and stem diameter, whereas flag leaf length was significantly affected ( $p \leq 0.01$ ) only by  $H_2O_2$ .

Contrastingly,  $H_2O_2$  concentrations positively affected ( $p \leq 0.01$ ) the PH of the evaluated sorghum plants (Figure 2B), with an increase of 0.84% for each unit increase in  $H_2O_2$ , i.e., when the plants were subjected to the highest  $H_2O_2$  concentration ( $18 \mu\text{M}$ ), PH increased by 15.05% (0.29 m) compared to that found in plants grown under absence of  $H_2O_2$  (control). These results denoted that the increases in the  $H_2O_2$  concentration contributed to plant growth, regardless of the ECw applied. Similarly, Lacerda et al. (2022) found linear increases in PH of maize crops subjected to salt stress due to the use of  $H_2O_2$  concentrations. Furthermore, Silva et al. (2016) evaluated the effects of different  $H_2O_2$  concentrations on initial growth of maize plants under salt stress and found positive effects on PH up to an  $H_2O_2$  concentration  $8 \mu\text{M}$ . Therefore, these results confirm the effectiveness of applying  $H_2O_2$  for plant acclimation under salt stress (FAROUK; AMIRA, 2018; VELOSO et al., 2023).

Hydrogen peroxide acts as a signaling molecule when plants are subjected to biotic and abiotic stresses (VELOSO et al., 2022); when applied at low concentrations, it induces the defense system of antioxidant enzymes, which reduces deleterious effects caused by stress (GONDIM et al., 2013). This probably occurred in the sorghum plants evaluated in the present study.



**Figure 2.** Plant height of sorghum plants under effects of electrical conductivities of the irrigation water (A) and hydrogen peroxide concentrations (B).

The results showed that linear decreases in stem diameter (SD) due to increasing EC<sub>w</sub> are independent from the applied H<sub>2</sub>O<sub>2</sub> concentrations (Figure 3A), with a good predictive ability ( $R^2 = 0.98^{**}$ ). A decrease of approximately 2.98% was found for each unit increase in EC<sub>w</sub>, resulting in a total decrease of 15.48% in SD of plants subjected to the EC<sub>w</sub> of 5.50 dS m<sup>-1</sup> compared to that found for plants in the control treatment (EC<sub>w</sub> of 0.30 dS m<sup>-1</sup>). These results are important for sorghum crops, as plants with small SD present less resistance to breakage and lodging, decreasing yield and generating harvesting difficulties (GOIS et al., 2019).

Similar results were reported by Souza et al. (2014), who evaluated maize crops irrigated with EC<sub>w</sub> ranging from 0.50 to 4.50 dS m<sup>-1</sup> and found decreases of 15.70% in SD of plants in the treatment with the highest EC<sub>w</sub>. Several studies have evaluated irrigation with brackish water in different crops and reported deleterious effects on plant growth and development (BLANCO et al., 2008; SAFDAR et al., 2019; LACERDA et al., 2022; PEREIRA et al., 2023). Excess salts in the soil solution triggers several physiological and

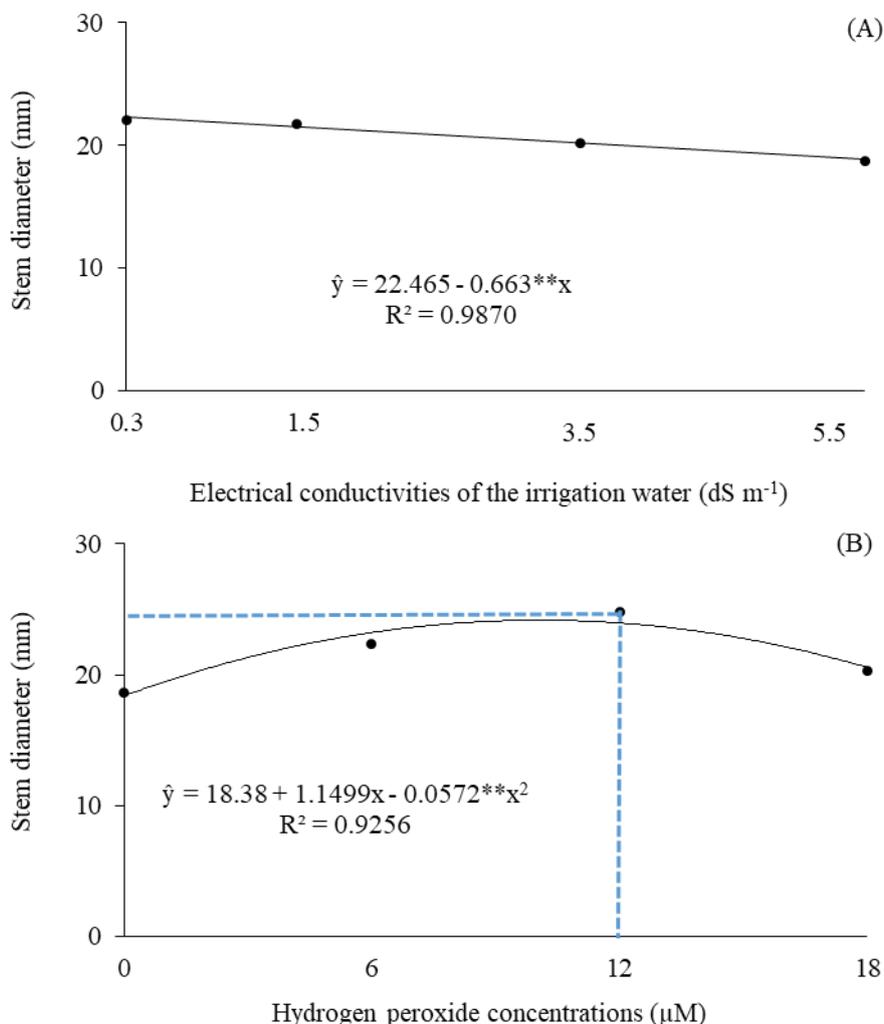
biochemical processes in plants that can cause toxicity and water stress, directly and indirectly affecting plant growth, development, and production (SHANKAR; EVELIN, 2019).

However, SD of sorghum plants increased quadratically with increasing H<sub>2</sub>O<sub>2</sub> concentration up to 10.05 μM, presenting a 23.92% increase compared to that found under absence of H<sub>2</sub>O<sub>2</sub>; however, significant decreases were found for higher concentrations (Figure 3B). These results denoted that the low H<sub>2</sub>O<sub>2</sub> concentrations induced tolerance to salt stress in the sorghum plants, whereas high concentrations (> 10.05 μM) combined with high EC<sub>w</sub> resulted in taller plants, but with smaller stem diameters; similar results were found by Lacerda et al. (2022) for maize plants.

These results reinforce the mitigating effect of H<sub>2</sub>O<sub>2</sub> on salt stress in cultivated plants (SILVA et al., 2016; DANTAS et al., 2022; ANDRADE et al., 2022). The application of appropriate H<sub>2</sub>O<sub>2</sub> concentrations induces the defense system of antioxidant enzymes, which minimizes deleterious effects caused by salt stress (GONDIM et al., 2013). However, the

application of higher  $H_2O_2$  concentrations results in excess reactive oxygen species (ROS), which cause oxidative damages to nucleic acids, lipids, and proteins, resulting in secondary oxidative stress (VELOSO et al., 2022; PEREIRA et al., 2023). The plant height and stem strength are important

characteristics of sorghum plants, as shorter plants with resistant stems are less susceptible to lodging and breakage, which are essential for decreasing yield losses (GUIMARÃES et al., 2022).



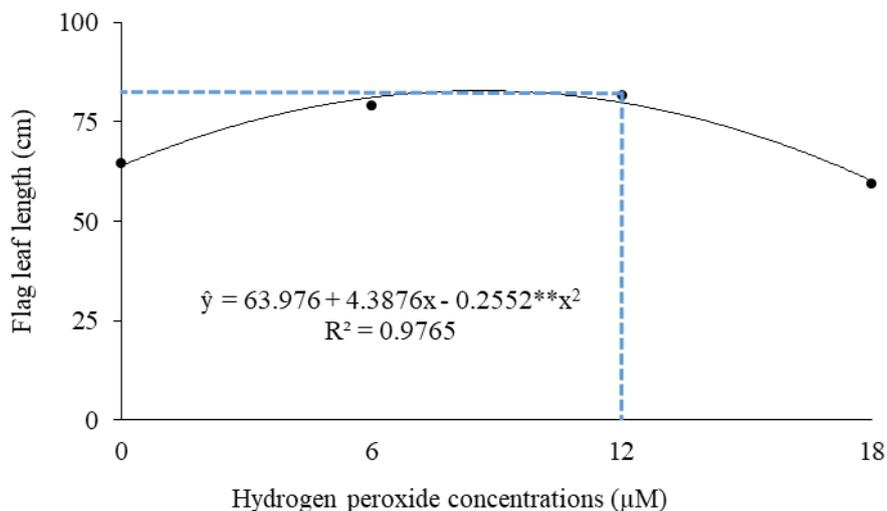
**Figure 3.** Stem diameter of sorghum plants as a function of electrical conductivities of the irrigation water (A) and hydrogen peroxide concentrations (B).

The regression analysis (Figure 4) showed a quadratic effect ( $p \leq 0.01$ ) of  $H_2O_2$  concentrations on flag leaf length (FLL), with a coefficient of determination ( $R^2$ ) of  $0.97^{**}$ . The maximum increase in FLL (22.76%; 18.86 cm) was found for the optimal  $H_2O_2$  concentration of  $8.60\ \mu M$ , compared to that found in plants grown under absence of  $H_2O_2$ . However, the smallest FLL (60.26 cm) was found for the highest  $H_2O_2$  concentration tested.

$H_2O_2$  acts in plants as one of the most stable ROS, which is involved in adaptation processes to different environments, mainly in plants under stress conditions, and is formed during processes involving electron transport, such as photosynthesis and mitochondrial respiration (RAMOS et al.,

2022). However,  $H_2O_2$  concentrations higher than the optimal level combined with stress conditions can trigger formation of ROS, which can exceed the cell's metabolism capacity, causing oxidative stress (SILVA et al., 2016). This may explain the effects found in the present study when using the highest  $H_2O_2$  concentrations.

The analysis of variance (Table 3) showed a significant effect of ECw ( $p \leq 0.05$ ) on leaf dry weight (LDW) and stem fresh weight (SFw) and a significant effect of  $H_2O_2$  concentrations ( $p \leq 0.01$ ) on all evaluated biomass-related variables. Moreover, a significant interaction between the factors ( $ECw \times H_2O_2$ ) ( $p \leq 0.05$ ) was found for SFw.



**Figure 4.** Flag leaf length of sorghum plants as a function of hydrogen peroxide concentrations.

**Table 3.** Analysis of variance for leaf fresh and dry weights (LFW and LDW, respectively) and stem fresh and dry weights (SFW and SDW, respectively) of sorghum plants (*Sorghum bicolor* L. Moench) under different electrical conductivities of the irrigation water (ECw) and hydrogen peroxide concentrations (H<sub>2</sub>O<sub>2</sub>).

Source of variation	DF	Mean squares			
		LFW	LDW	SFW	SDW
ECw	3	81.83 <sup>ns</sup>	76.53*	21006.59**	10690.60 <sup>ns</sup>
Linear regression	1	166.66 <sup>ns</sup>	191.42*	51627.84**	25240.83 <sup>ns</sup>
Quadratic regression	1	16.10 <sup>ns</sup>	25.40 <sup>ns</sup>	11327.07*	2024.10 <sup>ns</sup>
H <sub>2</sub> O <sub>2</sub>	3	1725.13**	173.72**	13002.25**	4319.09**
Linear regression	1	3360.46 <sup>ns</sup>	172.41 <sup>ns</sup>	21767.29**	8466.63 <sup>ns</sup>
Quadratic regression	1	1277.40**	337.39**	13950.31**	1533.41**
Interaction (ECw × H <sub>2</sub> O <sub>2</sub> )	9	180.78 <sup>ns</sup>	30.08 <sup>ns</sup>	3888.89*	4620.10 <sup>ns</sup>
Blocks	2	4.04	14.12	916.70	369.71
CV (%)	-	13.62	15.08	15.15	15.64

CV = coefficient of variation; DF = degrees of freedom; <sup>ns</sup>, \*\*, and \* = not significant and significant at  $p \leq 0.01$  and  $p \leq 0.05$  by the F test, respectively.

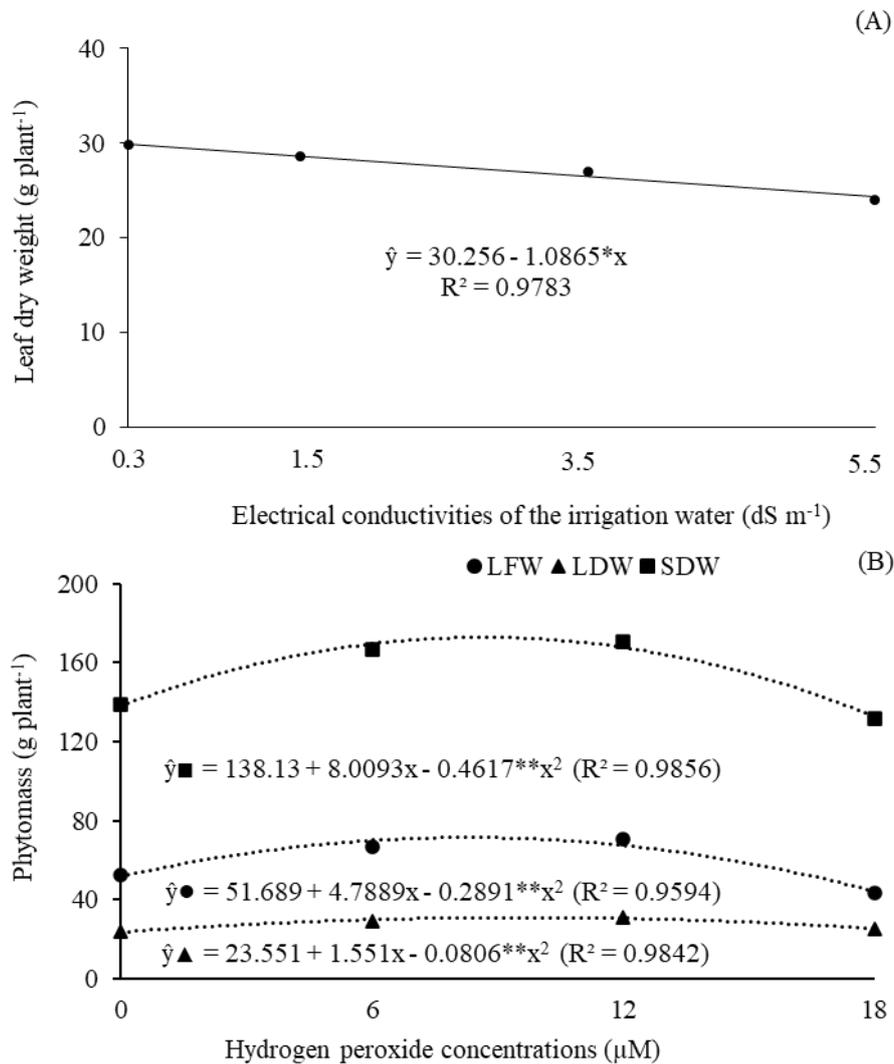
LDW in the evaluated sorghum plants presented a linear decrease of 3.63% for each unit increase in ECw (Figure 5A), thus, plants irrigated with the highest ECw (5.50 dS m<sup>-1</sup>) presented a 18.88% decrease (6.47 g per plant) in LDW compared to those in the control (ECw of 0.30 dS m<sup>-1</sup>). Similar results of biomass accumulation were found by Silva et al. (2019) and Lacerda et al. (2022) in maize plants subjected to ECw ranging from 0.30 to 2.0 dS m<sup>-1</sup>. These results confirm the deleterious effects caused by salt stress on plant physiology, resulting in decreases in biomass accumulation over time.

The decreases found for sorghum biomass accumulation as a function of increasing ECw were expected, as this factor negatively affected ( $p \leq 0.01$ ) plant height (Figure 2A) and stem diameter (Figure 3A). Salt stress in plants inhibits their growth due to osmotic stress resulting from a low soil water potential, consequently decreasing plant turgor (ANDRADE et al., 2022; DANTAS et al., 2022). Additionally, it restricts photosynthesis through leaf

abscission and reduction in leaf area caused by an early senescence due to the toxic action of excess salts in the irrigation water (TAIZ et al., 2017; DANTAS et al., 2022).

The factor H<sub>2</sub>O<sub>2</sub> concentration had a quadratic effect on LFW, LDW, and stem dry weight (SDW), according to the regression equations (Figure 5B). The highest LFW (71.52 g), LDW (31.01 g), and SDW (172.86 g) per plant were found for the H<sub>2</sub>O<sub>2</sub> concentrations of 8.28, 9.62, and 8.67 µM, respectively, with decreases in these variables from these optimal H<sub>2</sub>O<sub>2</sub> concentrations onwards.

Similar results were found by Gondim et al. (2013), Silva et al. (2016), and Lacerda et al. (2022) for maize plants treated with H<sub>2</sub>O<sub>2</sub> concentrations ranging from 10 to 320 µmol L<sup>-1</sup> and subjected to salt stress. These effects can be attributed to the tolerance dynamics induced by optimal H<sub>2</sub>O<sub>2</sub> concentrations in plants, resulting in accumulation of proteins and soluble carbohydrates and decreases in Na<sup>+</sup> and Cl<sup>-</sup> contents in plants (RAMOS et al., 2022; PEREIRA et al., 2023).



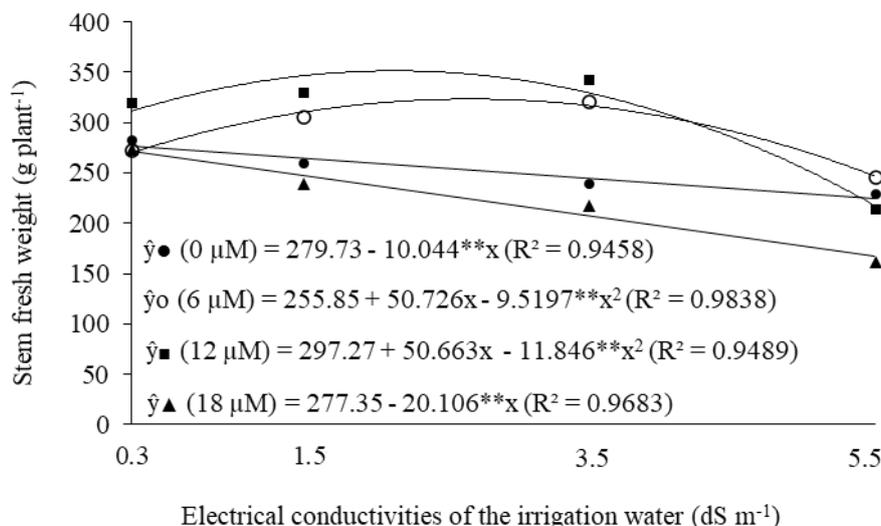
**Figure 5.** Leaf dry weight (LDW) of sorghum plants under effects of electrical conductivities of the irrigation water (A) and leaf fresh weight (LFW), LDW, and stem dry weight (SDW) as a function of hydrogen peroxide concentrations (B).

The interaction between the factors ( $EC_w \times H_2O_2$ ) had a significant effect on SFW, as shown by the F test (Table 3). According to the regression equations (Figure 6), the decreasing linear model best fitted the SFW data, with decreases of 18.87% and 38.53% from the lowest (0.0 μM) to the highest (18 μM)  $H_2O_2$  concentrations, when the plants were subjected to irrigation with  $EC_w$  of 0.30 and 5.50 dS m<sup>-1</sup>, respectively. These decreases occurred due to the high salt concentrations in the soil solution, which resulted in decreases in plant growth and biomass production; similar results were reported by Silva et al. (2016) and Lacerda et al. (2022) for maize plants.

Contrastingly, a polynomial quadratic model best fitted the SFW data of plants treated with  $H_2O_2$  concentrations of 6 and 12 μM, with increases in SFW up to the  $EC_w$  of 2.66 and 2.14 dS m<sup>-1</sup>, respectively (Figure 6). Considering the effects of the electrical conductivities of the irrigation water within each  $H_2O_2$  concentration, the SFW of sorghum plants irrigated with the  $EC_w$  of 0.30 dS m<sup>-1</sup> was significantly higher only in

plants in the treatments without  $H_2O_2$  and with the highest  $H_2O_2$  concentration. Increases in stem biomass due to seed imbibition with  $H_2O_2$  may be connected to enzyme activity in the carbon fixation processes, PSII efficiency, and protection of cellular organelles, such as chloroplasts (PEREIRA et al., 2023). Therefore, these dynamics are connected to the acclimation promoted by  $H_2O_2$  concentrations in plants under salt stress, mainly due to the activation of the oxidative enzyme system and reduction of lipid peroxidation (SILVA et al., 2016; VELOSO et al., 2023).

However, the concentrations higher than 12 μM of  $H_2O_2$  applied in the present study exceeded the tolerance of sorghum plants; therefore, the  $H_2O_2$  did not promote the expected mitigating effect of salt stress on SFW of sorghum plants. Therefore, treating sorghum seeds with up to 12 μM of  $H_2O_2$  can induce the emergence of plants that are more resistant to salt stress while maintaining metabolic processes (DANTAS et al., 2022; LACERDA et al., 2022; VELOSO et al., 2023).



**Figure 6.** Stem fresh weight (SFW) of sorghum plants as a function of H<sub>2</sub>O<sub>2</sub> concentrations for the different electrical conductivities of the irrigation water.

## CONCLUSION

Irrigation water with an electrical conductivity higher than 1.50 dS m<sup>-1</sup> decreases the growth and biomass accumulation of sorghum plants.

Treating sorghum seeds with hydrogen peroxide concentrations of up to 12 μM mitigates adverse effects caused by salt stress on plants subjected to the salinity levels evaluated in the present study.

Hydrogen peroxide concentrations higher than 12 μM is not recommended for treating sorghum seeds, as they increase deleterious effects caused by salt stress on stem diameter, flag leaf length, and biomass accumulation.

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