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ORIGINAL ARTICLE

Priming seeds with hydrogen peroxide attenuates damage caused by salt stress in sorghum[1]

Condicionamento de sementes com peróxido de hidrogênio atenua os danos causados pelo estresse salino em sorgo

Marcelo A. R. Limão[2], Joicy L. Barbosa[3], Aldair de S. Medeiros[4*], Sebastião de O. Maia Júnior[5], Ivomberg D. Magalhães[3], Thiago A. Pimenta[3], Giordano B. M. Gonzaga[3], Valéria F. de O. Sousa[6], Glécio M. Siqueira[4], Jordânio I. Marques[7], Washington da S. Sousa[7] & Patrício G. Leite[7]

[1] Research developed at Universidade Federal de Campina Grande, Centro de Ciências e Tecnologia Agroalimentar, Pombal, PB, Brazil
[2] Universidade Federal de Viçosa/Departamento de Agronomia, Viçosa, MG, Brazil
[3] Universidade Federal de Alagoas/Centro de Ciências Agrárias, Rio Largo, AL, Brazil
[4] Universidade Federal do Maranhão/Programa de Pós-Graduação em Biodiversidade e Biotecnologia, São Luís, MA, Brazil
[5] Universidade Estadual do Maranhão/Campus Paulo VI, São Luís, MA, Brazil
[6] Universidade Federal de Campina Grande/Unidade Acadêmica de Ciências Agrárias, Pombal, PB, Brazil
[7] Universidade Federal do Maranhão/Centro de Ciências de Chapadinha, Chapadinha, MA, Brazil

HIGHLIGHTS:

Soaking seeds with H_2O_2 increases salt stress acclimatization in sorghum plants, improving photosynthesis.
Hydrogen peroxide in alleviating salt stress in sorghum plants depends on the dose used.
Dose of 8.2 μM of H_2O_2 attenuates the negative effects of salt stress on sorghum plants.

ABSTRACT: Salinity affects physiological processes, such as photosynthesis, in various agricultural crops, such as sorghum, around the world. Thus, mitigating techniques such as priming seeds with hydrogen peroxide (H_2O_2) can increase plant tolerance to salt stress. Thus, the objective of present study was to evaluate the priming of seeds with hydrogen peroxide on gas exchange and shoot phytomass of sorghum grown under salt stress. The treatments were distributed in a randomized block design, in a 4 × 4 factorial arrangement, with four levels of electrical conductivity of irrigation water - (ECw- 0.3, 1.5, 3.5, and 5.5 dS m^{-1}) and four concentrations of H_2O_2 (0, 6, 12, and 18 μM L^{-1}), with three replications. The salinity of the water reduced gas exchange, shoot fresh and dry mass, in addition to shoot moisture content in sorghum plants. However, priming the seeds with H_2O_2 improved gas exchange and the accumulation of plant dry mass. Seed priming with H_2O_2 at dose of 8.2 μM increases the acclimatization of sorghum plants under salt stress.

Key words: *Sorghum bicolor* (L.) Moench, salinity, acclimatization, photosynthesis

RESUMO: A salinidade afeta processos fisiológicos, como a fotossíntese, em diversas culturas agrícolas, como o sorgo, em todo o mundo. Dessa forma, técnicas mitigadoras como o condicionamento de sementes com peróxido de hidrogênio (H_2O_2) podem aumentar a tolerância das plantas ao estresse salino. Assim, o objetivo do presente estudo foi avaliar o condicionamento de sementes de sorgo com peróxido de hidrogênio nas trocas gasosas e na fitomassa da planta sob estresse salino. Os tratamentos foram distribuídos em delineamento de blocos casualizados, em arranjo fatorial 4 × 4, com quatro níveis de condutividade elétrica da água de irrigação (CEa- 0,3, 1,5, 3,5 e 5,5 dS m^{-1}) e quatro concentrações de H_2O_2 (0, 6, 12 e 18 μM L^{-1}), com três repetições. A salinidade da água reduziu as trocas gasosas, a massa fresca e seca da parte aérea, além do teor de água nas plantas de sorgo. Entretanto, o condicionamento das sementes com H_2O_2 melhorou as trocas gasosas e o acúmulo de massa seca das plantas. O condicionamento de sementes com dose de H_2O_2 de 8,2 μM aumenta a aclimatação de plantas de sorgo sob estresse salino.

Palavras-chave: *Sorghum bicolor* (L.) Moench, salinidade, aclimatação, fotossíntese

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* Corresponding author - E-mail: aldair.medeiros@ufma.br
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Introduction

The population increase, associated with conditions of high temperatures, high evapotranspiration rates, water deficit, and water salinity, requires the agricultural sector to use techniques to increase food production and ensure food security (Castro & Santos, 2020). Sorghum (*Sorgum bicolor* L. Moench - Poaceae) is a forage species that stands out for its high biomass production and tolerance to environmental adversities such as salinity and mainly for its adaptability to semi-arid conditions, with predominance of higher salinity waters (Guimarães et al., 2019; Calone et al., 2020). However, sorghum tolerance to salinity is approximately 4.5 dS m^{-1} of electrical conductivity, above which there is a reduction in yield of approximately 16% for each unit increase, with significant reduction in its production with higher salinity (Guimarães et al., 2022), because the salt stress affects different parts of plants.

Among the effects, salinity affects the photosynthetic process of plants, reducing stomatal conductance with direct implications for carbon assimilation and transpiration rate, which causes irreversible damage to shoot growth and plant biomass production (Veloso et al., 2022).

Therefore, in regions where there is a history of salinity in irrigation water, management techniques have been adopted to minimize the effects of salinity on plants, such as the use of hydrogen peroxide (H_2O_2). Its use in plants under salinity improves water use efficiency, i.e., preserving water balance in leaf tissue, chlorophyll content, and accumulating more compatible solutes (Bagheri et al., 2021; Chattha et al., 2022), in addition to minimizing the reductions in stomatal conductance (Carvalho et al., 2011; Iqbal et al., 2018; Araújo et al., 2021), photosynthetic rate, and dry mass accumulation (Silva et al., 2016; Silva et al., 2019a).

In this context, the use of hydrogen peroxide can be an alternative for acclimation to salt stress in sorghum plants, functioning as an important intracellular signal, as observed in several studies (Carvalho et al., 2011; Silva et al., 2016; Iqbal et al., 2018; Araújo et al., 2021; Chattha et al., 2022). Thus, the objective of present study was to evaluate the priming of seeds with hydrogen peroxide on gas exchange and shoot phytomass of sorghum grown under salt stress.

Material and Methods

Experiment location

The experiment was carried out under drainage lysimeter conditions, with plants grown in a greenhouse at the Center for Science and Agri-Food Technology at the Universidade Federal de Campina Grande (CCTA/UFCG), in Pombal, Paraíba, Brazil (6° 48' 16" S, 37° 49' 15" W, 144 m), located in the Brazilian semi-arid region. Climatic conditions were monitored inside the greenhouse throughout the experimental period, using the thermohygrometer model HT-208 (ICEL-Manaus). The maximum and minimum air temperatures recorded were 40.32 °C and 32.76 °C, respectively, while the respective maximum and minimum relative air humidity were 78.89 and 56.65%.

Treatments and experimental design

The treatments were composed of four electrical conductivities of irrigation water (ECw- 0.3 - control, 1.5, 3.5, and 5.5 dS m^{-1}) and four concentrations of H_2O_2 (0 - control; 6, 12, and 18 μM L^{-1}). The experimental design used was randomized blocks in a 4 × 4 factorial scheme, with three replications and one plant per plot (lysimeters), totaling 48 experimental units.

Salinity levels

The salinity levels (electrical conductivities of the water) applied as treatments were obtained according to Blanco et al. (2008), preparing the solutions to contemplate the equivalent ratio of 7:2:1 for Na:Ca:Mg, respectively, dissolving the salts NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ in public-supply water (0.3 dS m^{-1}), considering the relationship between electrical conductivity of irrigation water and salt concentration (Richards, 1954), following Eq.1. In the Brazilian semi-arid region, this proportion of salts is predominant in water sources used for irrigation on small properties (Silva et al., 2019b).

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

where:

Q - quantity of salts to be added ($mmol_c$ L^{-1}); and,
ECw - electrical conductivity of the water (dS m^{-1}).

For the present study, due to the lack of research evaluating the application of H_2O_2 in sorghum to mitigate salt stress, the concentrations of H_2O_2 used were adapted from the study with the corn crop (Silva et al., 2016), in which concentrations of up to 8 μM promoted greater growth and above 15 μM accentuated the negative effects caused by salinity. The concentrations of H_2O_2 used in this study were prepared by diluting pure hydrogen peroxide (CINÉTICA - 99%) in deionized water. To apply the concentrations of H_2O_2, according to the treatments, before sowing, the sorghum seeds were soaked in a solution with the respective concentrations for 24 hours in the dark (Veloso et al., 2022).

Growing conditions

Next, the seeds were planted in lysimeters with capacity of 10 dm^3, with the lower part covered with geotextile, a 5-cm-thick layer of crushed stone (No. 1) and connected to a drain to collect the drained water. The lysimeters were filled with soil material classified as *Neossolo Flúvico* (EMBRAPA, 2018), Fluvents (USDA, 2022), collected in the municipality of São Domingos, Paraíba. The physical and chemical attributes of the soil are presented in Barbosa et al. (2023).

The sorghum cultivar used in the experiment was BRS Ponta Negra, with 96% germination and good health, being the most suitable for silage production (Gois et al., 2019). Before sowing, soil moisture in the lysimeters was raised to the level of field capacity with water from the control treatment (0.30 dS m^{-1}) according to Veloso et al. (2022), to promote acclimatization to the lysimeter conditions, with irrigation kept in this treatment until 15 days after sowing (DAS). Sowing was carried out by five seeds per lysimeter, distributed equidistantly.

Germination started at three and stabilized at seven DAS. Plant thinning was performed 15 days after emergence (DAE), leaving only the most vigorous plant per lysimeter, cultivated until the end of the experimental period.

Applications of saline treatments were manually carried out every day from 16 days after emergence (DAE) onwards, with the volume of water equivalent to that obtained by the water balance of the previous irrigation being applied to each lysimeter (Ramos et al., 2022), as proposed in 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), respectively; and,

LF - leaching fraction of 0.2, applied fortnightly, to reduce the excessive accumulation of salts in the root zone of the plants.

Nutritional supplementation was carried out through the application of 140, 300, and 180 mg dm^{-3} of soil of N, P and K, respectively, in the form of urea, super phosphate and potassium chloride (Novais et al., 1991), divided into 4 applications, the first as basal and the others via fertigation (except P_2O_5) at 20, 30 and 40 DAE. Micronutrient supplementation was performed through the monthly application of a solution at a concentration of 1.0 g L^{-1} (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%), applying about 10 mL plant^{-1} through the leaves (adaxial and abaxial sides) using a knapsack sprayer.

The cultural treatments carried out throughout the experimental period consisted of surface scarification of the soil in lysimeters, manual weeding and staking of plants to prevent lodging and breakage. For phytosanitary control, insecticides from the Neonicotinoids chemical group, fungicide from the Triazol chemical group and acaricide from the Abamectin chemical group were applied whenever necessary.

Variables analyzed

The gas exchange analyses of sorghum leaves were carried out in the pre-flowering stage, which refers to the period of highest photosynthetic rate (Coelho et al., 2018); in the present study, it corresponded to the period after 55 days under salt stress, when net CO_2 assimilation (A - μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs - mol H_2O m^{-2} s^{-1}), transpiration rate (E - mmol H_2O m^{-2} s^{-1}), instantaneous water use efficiency (WUE = A/E - [μmol CO_2 m^{-2} s^{-1})(mmol H_2O m^{-2} s^{-1})$^{-1}$]), were evaluated in the youngest fully expanded leaf of each plant, using an infrared gas analyzer LCpro+ (Analytical Development, Kings Lynn, UK) with a constant light source of 1200 μmol m^{-2} s^{-1} photons, under standard temperature of 27 °C. These analyses were carried out between 8:00 and 11:00 a.m.

At the end of the experimental period (80 DAE), 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 reaching constant weight to determine shoot phytomass (g). Shoot moisture content was calculated as the difference between the shoot fresh mass and shoot dry mass, and expressed in g plant^{-1}.

Statistical analyses

For statistical analyses, initially, the data obtained were subjected to the normality test (Kolmogorov-Smirnov). The analyses were conducted using the R 3.6.3 platform with the ExpDes.pt package (Ferreira et al., 2018), and the response surface equations were generated by the rsm package (Lenth, 2009) and GA (Scrucca, 2013); figures were created in SigmaPlot software version 11.0 (Systat Software, San Jose, CA, USA); a graphical description of hydrogen peroxide doses within salt stress levels was demonstrated for variables with significant interactions between factors. Principal component analysis (PCA) was performed in order to assess the interrelation between variables and factors with FactoMineR computer package (Factor Analysis and Data Mining with R) (Lê et al., 2008).

Results and Discussion

Water salinity, individually, had an effect on stomatal conductance and shoot dry mass, in addition to shoot moisture content, while hydrogen peroxide influenced all variables evaluated (Table 1). The interaction between the factors did not affect only shoot fresh mass and shoot moisture content.

The net photosynthesis rate decreased linearly by 10.7% per unit increase in water salinity, reaching 55.8% between 0.3 and 5.5 dS m^{-1} in the absence of hydrogen peroxide (Figure 1A). On the other hand, with doses of 6 and 12 μM L^{-1} of H_2O_2, photosynthesis increased linearly by 17.2 and 3.8%, respectively, per unit increase in water salinity, reaching 89.2

Table 1. Summary of analysis of variance for photosynthetic rate (A), stomatal conductance (gs), transpiration (E), instantaneous water use efficiency (WUE), 55 days under stress and shoot fresh mass (SFM), shoot dry mass (SDM), and shoot moisture content (SMC) of sorghum, 80 days under salt stress, as a function of seed priming with hydrogen peroxide and electrical conductivity of irrigation water

Source of variation	DF	Mean squares						
		A	gs	E	WUE	SFM	SDM	SMC
Blocks	2	1.126^{ns}	0.0001^{ns}	0.053^{ns}	2.53^{ns}	950.92^{ns}	25.46^{ns}	1168.84^{ns}
Salinity (S)	3	3.608^{ns}	0.0004*	0.079^{ns}	5.18^{ns}	40238.51**	3545.55**	19905.97**
Hydrogen peroxide (H_2O_2)	3	272.61**	0.0019**	1.33**	47.45**	1500.44**	4227.13**	758.75^{ns}
Interaction (S × H_2O_2)	9	51.87**	0.0028**	0.258**	10.81**	158.56^{ns}	90.44*	138.67^{ns}
Residual	30	4.66	0.0001	0.079	2.46	358.88	32.40	353.11
CV (%)		14.00	10.71	15.52	18.40	5.86	3.39	12.07

ns, *, **: not significant, significant at p ≤ 0.05 and at p ≤ 0.01 by F test; CV: coefficient of variation; DF- degree of freedom

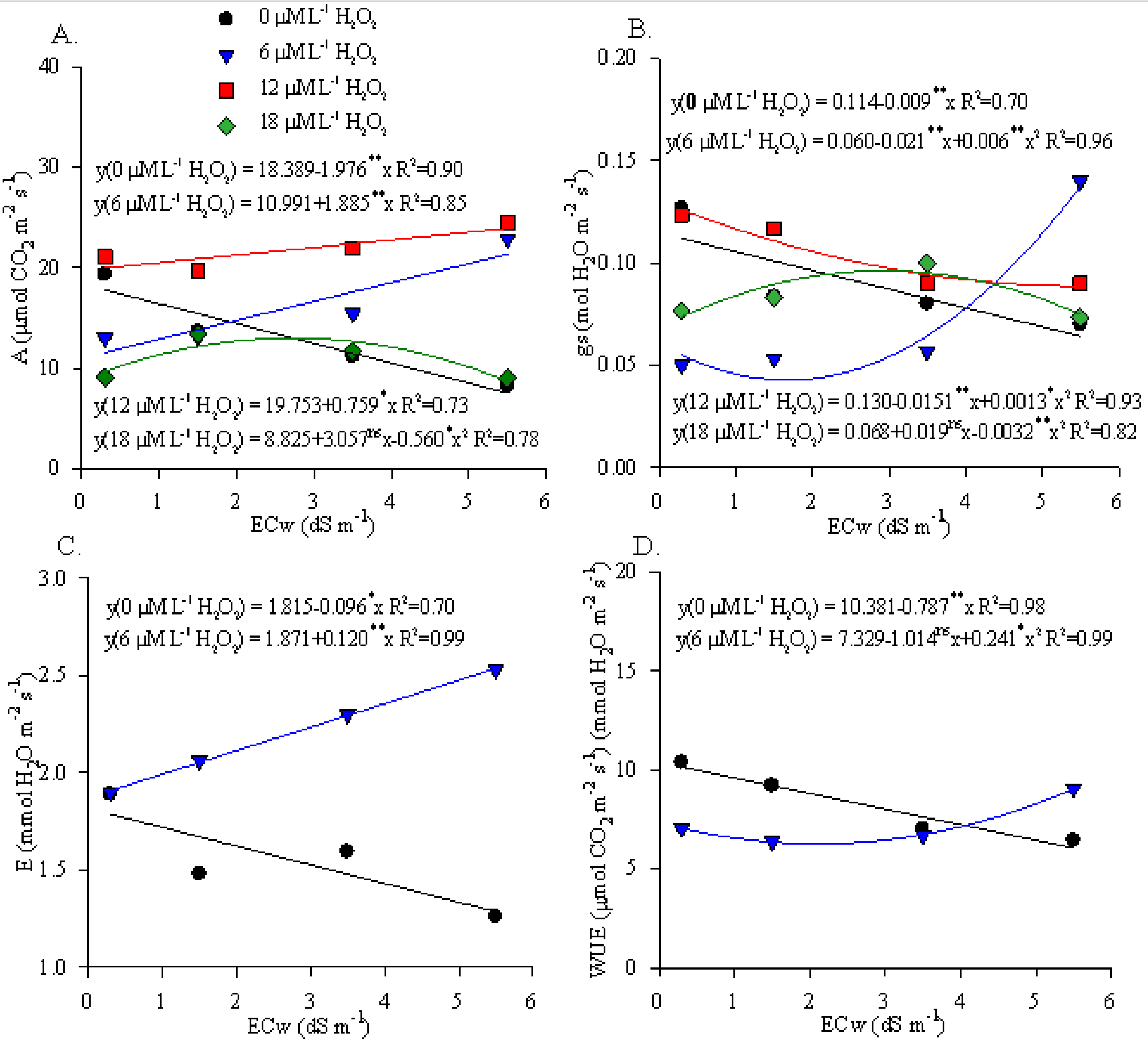


Figure 1. Effect of salt stress and seed priming with hydrogen peroxide on photosynthetic rate (A- A), stomatal conductance (gs- B), transpiration rate (E- C), and instantaneous water use efficiency (WUE- D) of sorghum after 55 days growth under salt stress

and 19.9% between 0.3 and 5.5 dS m^{-1}. The dose of 18 μM L^{-1} of H_2O_2 led to the maximum estimated values of 12.98 μmol CO_2 m^{-2} s^{-1}, when plants were irrigated with electrical conductivity of irrigation water of 2.72 dS m^{-1}, decreasing afterwards.

The reduction in photosynthetic rate with increasing salinity has also been reported in sorghum (Coelho et al., 2018), corn (Gondim et al., 2013), and rice (Carvalho et al., 2011) plants. However, salinity attenuation was observed on the photosynthesis of sorghum plants that received seed treatment mainly with 6 μM L^{-1} of H_2O_2, indicating that this concentration alleviated the intensity of salt stress in the present study. Similar results have been observed in corn (Gondim et al., 2013), passion fruit (Silva et al., 2019a), and sunflower (Silva et al., 2022) plants, in which pretreatment with H_2O_2 attenuated the effects of salinity on photosynthetic rate.

This may have occurred due to the activation of antioxidant enzymes that reduced oxidative stress (Carvalho et al., 2011; Iqbal et al., 2018), reducing excess energy induced by salt stress and preserving thylakoid stacking, resulting in improved photosynthetic process (Araújo et al., 2021; Silva et al., 2022). Furthermore, it minimizes damage to the chlorophyll content, which also reflects on the photosynthetic rate (Gondim et al., 2013; Bagheri et al., 2021; Silva et al., 2023).

The stomatal conductance of sorghum plants decreased linearly by 7.9% per unit increase in water salinity, reaching 41.1% between 0.3 and 5.5 dS m^{-1} when hydrogen peroxide was not used to treat the seeds (0 μM L^{-1}, Figure 1B). With a dose of 6 μM L^{-1} of H_2O_2, stomatal conductance reached a minimum of 0.041 mol H_2O m^{-2} s^{-1} with a salinity of 1.75 dS m^{-1}, followed by an increase of approximately 207% up to 5.5 dS m^{-1}, in which it reached 0.126 mol H_2O m^{-2} s^{-1}. On the other hand, with doses of 12 and 18 μM L^{-1} of H_2O_2 it reached estimated maximum values of 0.087 and 0.153 mol H_2O m^{-2} s^{-1}, respectively, when the plants were irrigated with electrical conductivities of irrigation water of 5.0 and 3.16 dS m^{-1}, respectively, and decreasing with increasing salinity. Thus, by using appropriate concentrations of hydrogen peroxide in plants subjected to salt stress, it is possible to stimulate their defense system, triggering metabolic changes that contribute to increasing plant tolerance in the face of subsequent exposure to stress (Veloso et al., 2023).

The reduction in stomatal conductance is a response commonly observed in plants under salt stress due to the two dysfunctions caused by salt, osmotic and ionic stresses (Silva et al., 2019a; Bagheri et al., 2021). These dysfunctions, either together or individually, lead to the inhibition of stomatal opening, resulting in a direct effect of decrease in CO_2 availability due to limitations on diffusion through the stomata (Silva et al., 2010). Such results occurred in sorghum plants with an increase in conductivities of irrigation water without acclimating the seeds with H_2O_2.

In contrast, with the application of 6 μM L^{-1} of H_2O_2, gs was partially improved with the increase in conductivities of irrigation water, possibly due to the effect of H_2O_2 on signaling enzymatic activation (Gondim et al., 2010; Chattha et al., 2022), resulting in improvements in the K^+/Na^+ ratio, increasing water relations and, consequently, the opening of stomata (Iqbal et al., 2018).

Similar to what occurred with stomatal conductance when hydrogen peroxide was not used to soak the seeds (0 μM L^{-1}), the transpiration rate decreased linearly by 5.3% per unit increase in water salinity, reaching 27.5% between 0.3 and 5.5 dS m^{-1} (Figure 1C). With a dose of 6 μM L^{-1} of H_2O_2, transpiration increased linearly by 6.4% per unit increase in water salinity, reaching 33.3% between 0.3 and 5.5 dS m^{-1}. On the other hand, H_2O_2 doses of 12 and 18 μM L^{-1} had no significant effect on transpiration rate with the increase in salinity from 0.3 to 5.5 dS m^{-1} and, therefore, were not described by any polynomial equation.

As the transpiration rate generally follows the behavior of stomatal conductance, increasing salinity reduced transpiration of sorghum plants particularly in those not treated with H_2O_2, as reported in sorghum plants (Coelho et al., 2018) and other agricultural crops (Araújo et al., 2021; Veloso et al., 2022). However, in the present study, priming sorghum seeds with 6 μM L^{-1} of H_2O_2 attenuated the effects of salinity on plants, as reported in soursop (Veloso et al., 2022) and corn (Gondim et al., 2013). These authors observed that plants treated with H_2O_2 and subjected to water salinity had transpiration less affected by stress. This is probably due to the fact that treating seeds with hydrogen peroxide favors the water status of plants under salinity (Silva et al., 2023).

Instantaneous water use efficiency (WUE) without the use of H_2O_2 decreased linearly by 7.6% per unit increase in water salinity, reaching 39.4% between 0.3 and 5.5 dS m^{-1} (Figure 1D), while with the dose of 6 μM L^{-1} of H_2O_2 it reached the estimated minimum value of 6.26 [(μmol CO_2 m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})], when irrigated with electrical conductivity of irrigation water of 2.10 dS m^{-1}, followed by an increase of approximately 38.2% up to 5.5 dS m^{-1}. Whereas, as occurred with transpiration, hydrogen peroxide doses of 12 and 18 μM L^{-1} had no significant effect on WUE.

The improvements in water use efficiency observed in sorghum plants after priming the seeds with 6 μM L^{-1} H_2O_2 are similar to those reported for corn plants treated with 10 μM H_2O_2 (Araújo et al., 2021). These responses occur as a consequence of improvements in photosynthesis, and even plants with little water absorption under saline conditions are able to maintain higher photosynthetic rates than those not treated with hydrogen peroxide (Gondim et al., 2013).

Thus, this characteristic is quite important to be considered in plants under salt stress, as it generally reduces with the intensity of stress (Silva et al., 2019b; Veloso et al., 2022) and, therefore, when attenuated by treatment with H_2O_2, seems to be a good alternative to improve plant tolerance to salinity, including through seed priming (Veloso et al., 2022).

Shoot fresh mass had significant isolated effects of water salinity levels and hydrogen peroxide concentrations. The fresh mass increased with the increase in salinity, reaching the maximum estimated value of 374.35 g with the maximum salinity of 2.25 dS m^{-1} (Figure 2A), while with the maximum concentration of 8.40 μM L^{-1} of hydrogen peroxide it reached 333.59 g $plant^{-1}$ (Figure 2B).

Shoot fresh mass did not reduce linearly with increasing salinity, as observed in bean (Silva et al., 2010) and passion fruit (Silva et al., 2019a). This is due to the fact that sorghum is a plant that is more tolerant to salinity, including the Ponta Negra variety (Guimarães et al., 2019) and, therefore, can withstand moderate water salinity with no effect on biomass production, as found in this study, in which plants produced maximum fresh biomass at 2.25 dS m^{-1}. Similar results were reported by Guimarães et al. (2019) when they found the salinity threshold of approximately 4.5 dS m^{-1}.

The shoot dry mass of sorghum plants reached the maximum estimated value of 200.95 g $plant^{-1}$, when they were irrigated with saline water of 2.24 dS m^{-1} and subjected to the maximum dose of hydrogen peroxide of 8.20 μM L^{-1} (Figure 2C). Thus, this dose of hydrogen peroxide contributed to the increase of 31.68% in shoot dry mass compared to 0 μM L^{-1}. In the absence of priming of sorghum seeds with hydrogen peroxide, the results of shoot dry mass were similar to those found by Guimarães et al. (2019), who evaluated the Ponta Negra variety and observed a value of 170 g $plant^{-1}$ at a salinity of 2.45 dS m^{-1}, with a sharp decrease up to 12 dS m^{-1}.

In contrast, the use of H_2O_2 at concentrations up to 8.20 μM L^{-1} in the treatment of sorghum seeds was able to minimize the deleterious effects of salt stress on dry mass production up to electrical conductivity of irrigation water of approximately 2.24 dS m^{-1}. This fact may have occurred because hydrogen peroxide acts as a signal, modulating the activity of enzymes such as Phosphoenolpyruvate carboxylase (PEPcase), which participates in the carbon assimilation process and, consequently, in the accumulation of photoassimilates (Araújo et al., 2021).

The attenuation of dry mass accumulation in the shoot promoted by treatment with hydrogen peroxide has also been observed in corn (Gondim et al., 2010; Gondim et al., 2013) and sunflower (Silva et al., 2022) plants, reinforcing the efficiency of H_2O_2 in mitigating the harmful effects caused by salt stress on different agricultural crops.

On the other hand, dose greater than 8.20 μM L^{-1} was not efficient in mitigating salt stress, which reduced shoot dry mass. This indicates that hydrogen peroxide is a molecule that depends on concentration to be effective in mitigating salt stress, as verified by Silva et al. (2019a), who evaluated different

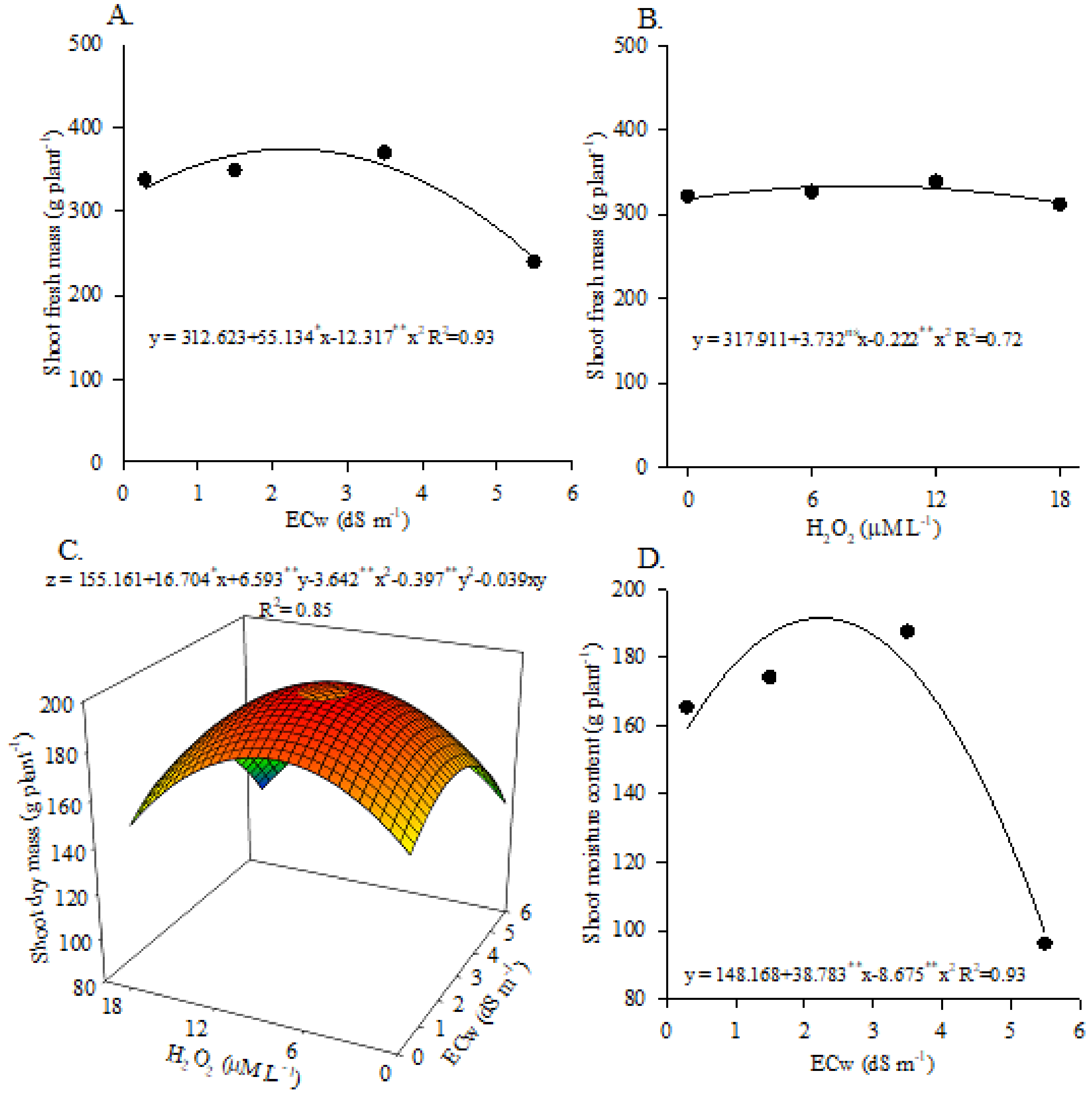
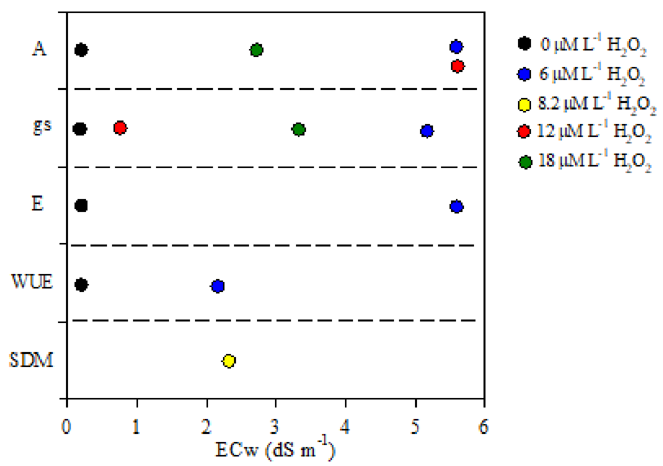


Figure 2. Effect of salt stress and seed priming with H_2O_2 on shoot fresh mass (A and B), shoot dry mass (C) and shoot moisture content (D) of sorghum at 80 days grown under salt stress

concentrations of H_2O_2 (0, 25, 50, and 75 μM) in attenuating salinity effects on passion fruit and observed that 25 μM was the most efficient. Possibly, at low concentrations, H_2O_2 acts as a signal, modulating the plant's tolerance to salinity, while at high concentrations it acts as a reactive oxygen species, acting in the degradation of cell membranes (Carvalho et al., 2011; Gondim et al., 2013).

Shoot moisture content was affected only by water salinity, reaching a maximum of 191.51 g plant^{-1}, with an estimated maximum salinity of 2.24 dS m^{-1} (Figure 2D). The increase in salinity above 2.24 dS m^{-1} certainly increased the ionic imbalance in sorghum plants, increasing the entry of Na^+ ions to the detriment of K^+ ions, which led to a decline in water status in the aerial part of the plants (Gondim et al., 2013; Silva et al., 2023).

A simplified graphic summary of the water salinity levels at which the highest values of the variables with significant interaction between electrical conductivities of irrigation water and H_2O_2 were recorded is presented in Figure 3. The variables stomatal conductance, transpiration, photosynthetic rate, and instantaneous water use efficiency responded in a similar way to the water salinity with the dose 0 μM L^{-1} of H_2O_2, with maximum obtained at 0.3 dS m^{-1}, while SDM was responsive at electrical conductivity of irrigation water of 2.24 dS m^{-1}. On the other hand, with a dose of 6 μM L^{-1} of H_2O_2, better responses of stomatal conductance, transpiration and photosynthetic rate were obtained with electrical conductivities of irrigation water greater than 5.0 dS m^{-1}, as well as 12 μM L^{-1}, which favored A under this saline condition. The dose of 18 μM L^{-1}



A - photosynthetic rate; gs - stomatal conductance; E - transpiration rate; WUE - instantaneous water use efficiency; and SDM - shoot dry mass

Figure 3. Electrical conductivity of irrigation water (ECw) at which the maximum values of the variables were recorded at each dose of hydrogen peroxide

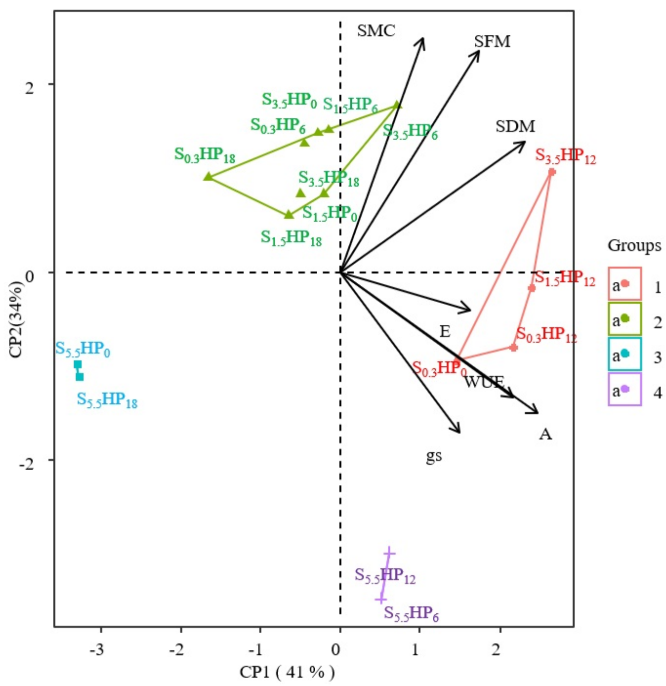
was less efficient, with higher values recorded at the electrical conductivity of irrigation water of 3.16 dS m^{-1}.

Based on principal component analysis, the first two components accounted for most of the variance, 75% (41% for PC1 and 34% for PC2). In the biplot (Figure 4), it was observed that the variables evaluated in this study were divided into four groups. The first group comprised the gas exchange variables, being related to treatments $S_{0.3}HP_0$, $S_{0.3}HP_{12}$ and $S_{1.5}HP_{12}$. The second group was composed of the other variables evaluated, more related to treatments $S_{3.5}HP_6$ and $S_{3.5}HP_{12}$. Furthermore, it was observed that treatments $S_{5.5}HP_6$ and $S_{5.5}HP_{12}$ also contributed to the distribution of gas exchange, mainly stomatal conductance and photosynthetic rate. On the other hand, the fourth group was composed of $S_{5.5}HP_0$ and $S_{5.5}HP_{18}$, which did not contribute to the distribution of any of the variables.

Thus, the interrelationships between variables and treatments confirmed the dose of 12 µM L^{-1} of H_2O_2 as the most responsive in sorghum tolerance to salinity, contributing mainly to the improvement of photosynthetic rate and shoot dry mass. Although 12 µM L^{-1} of H_2O_2 also contributes to stomatal conductance, only 6 µM L^{-1} was effective for transpiration and instantaneous water use efficiency.

The results shown in PCA confirm those presented previously (Figures 1 and 2), indicating that doses of 6 and 12 µM L^{-1} of hydrogen peroxide alleviated the negative effects of salt stress. It is noteworthy that these doses of H_2O_2 favored gas exchange in sorghum plants even under high salinity (Figure 3).

These results corroborate those of Veloso et al. (2023), who also demonstrated through PCA analysis that the application of hydrogen peroxide attenuated the harmful effects of salinity on physiological traits in cotton genotypes. The authors observed that adequate concentrations of H_2O_2 can mitigate the effects of salinity, promoting the maintenance of photosynthetic pigments and the functioning of the photosynthetic apparatus, which contributes to greater biomass accumulation. This is



Treatments: S - Water salinity; HP - Hydrogen peroxide. $S_{0.3}HP_0$ = 0.3 dS m^{-1} and 0 µM L^{-1}; $S_{0.3}HP_6$ = 0.3 dS m^{-1} and 6 µM L^{-1}; $S_{0.3}HP_{12}$ = 0.3 dS m^{-1} and 12 µM L^{-1}; $S_{0.3}HP_{18}$ = 0.3 dS m^{-1} and 18 µM L^{-1}; $S_{1.5}HP_0$ = 1.5 dS m^{-1} and 0 µM L^{-1}; $S_{1.5}HP_6$ = 1.5 dS m^{-1} and 6 µM L^{-1}; $S_{1.5}HP_{12}$ = 1.5 dS m^{-1} and 12 µM L^{-1}; $S_{1.5}HP_{18}$ = 1.5 dS m^{-1} and 18 µM L^{-1}; $S_{3.5}HP_0$ = 3.5 dS m^{-1} and 0 µM L^{-1}; $S_{3.5}HP_6$ = 3.5 dS m^{-1} and 6 µM L^{-1}; $S_{3.5}HP_{12}$ = 3.5 dS m^{-1} and 12 µM L^{-1}; $S_{3.5}HP_{18}$ = 3.5 dS m^{-1} and 18 µM L^{-1}; $S_{5.5}HP_0$ = 5.5 dS m^{-1} and 0 µM L^{-1}; $S_{5.5}HP_6$ = 5.5 dS m^{-1} and 6 µM L^{-1}; $S_{5.5}HP_{12}$ = 5.5 dS m^{-1} and 12 µM L^{-1}; $S_{5.5}HP_{18}$ = 5.5 dS m^{-1} and 18 µM L^{-1}; SFM - shoot fresh mass; SDM - shoot dry mass; SMC - shoot moisture content ; A - photosynthetic rate; gs - stomatal conductance; E - leaf transpiration; and WUE - instantaneous water use efficiency

Figure 4. Principal component analysis of gas exchange, biomass, and shoot moisture content in sorghum plants with seed priming with H_2O_2 and subjected to salt stress

possibly due to the signaling promoted by H_2O_2 priming, which favors the maintenance of the integrity of chloroplasts and photosynthetic pigments, in addition to the elimination of reactive oxygen species (Gondim et al., 2013; Araújo et al., 2021).

The elimination of reactive oxygen species in chloroplasts contributes to the maintenance of photosynthetic pigments, which are involved in the capture of light energy during the photosynthesis process, which favors the photosynthetic rate and the accumulation of dry mass (Chattha et al., 2022; Silva et al., 2022; Veloso et al., 2023).

Finally, it is worth highlighting the important findings of the present study, as it allowed us to point out appropriate management techniques, for example, soaking seeds of the sorghum cultivar BRS Ponta Negra for 24 hours before sowing at a concentration of 8.2 µM of H_2O_2, which was sufficient to mitigate the harmful effects of salt stress on gas exchange and enabled the production of sorghum biomass in a semi-arid region.

Conclusions

1. The salinity of the water reduced gas exchange, shoot fresh and dry mass, in addition to shoot moisture content in sorghum plants. However, priming the seeds with H_2O_2 improved gas exchange and the accumulation of plant dry mass.

2. Seed priming with H_2O_2 at dose of 8.2 µM increases the acclimatization of sorghum plants under salt stress.

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