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Okra cultivation under irrigation with saline water and foliar application of hydrogen peroxide

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ABSTRACT

Foliar application of hydrogen peroxide can induce plant defense mechanisms against salt stress, favoring plant acclimation in regions with qualitative and quantitative scarcity of water resources, such as the Brazilian semi-arid region. From this perspective, this study aimed to evaluate the effects of foliar hydrogen peroxide application on chlorophyll *a* fluorescence, growth, and production of okra under irrigation with saline water. The experiment was conducted under field conditions in Pombal, PB, using a randomized block design with a 5 × 3 factorial arrangement corresponding to five electrical conductivity levels of water – ECw (0.3, 1.3, 2.3, 3.3, and 4.3 dS m^{-1}) and three hydrogen peroxide concentrations – H_2O_2 (0, 25, and 50 µM), with five replications. Irrigation water salinity levels up to 2.2 dS m^{-1} increase the maximum fluorescence of okra plants 75 days after transplanting. Foliar application of 50 µM hydrogen peroxide proved to be beneficial for plant height, stem diameter, stem dry matter, root dry matter, and total dry matter of okra when plants were grown in low-salinity water. The hydrogen peroxide concentrations of 25 and 50 µM increased the number of leaves. However, these concentrations reduced the average weight of the okra dry fruits. Foliar application with 50 µM hydrogen peroxide had a significant effect on the dry leaf phytomass of the okra cv. *Clemson American 80* regardless of the electrical conductivity of irrigation water. Foliar hydrogen peroxide application at concentrations up to 50 µM intensifies the deleterious effects of salt stress on the total weight of dried okra fruits.

Keywords: *Abelmoschus esculentus* L., acclimation, salt stress.

Cultivo de quiabo sob irrigação com água salina e aplicação foliar de peróxido de hidrogênio

RESUMO

A aplicação foliar de peróxido de hidrogênio pode induzir mecanismos de defesa das plantas contra o estresse salino, favorecendo a aclimatação das plantas em regiões com escassez qualitativa e quantitativa de recursos hídricos, como o semiárido brasileiro. Nessa perspectiva, este estudo teve como objetivo avaliar os efeitos da aplicação foliar de peróxido de hidrogênio na fluorescência da clorofila a, no crescimento e na produção de quiabo sob irrigação com água salina. O experimento foi conduzido em condições de campo em Pombal, PB, utilizando delineamento em blocos casualizados com arranjo fatorial 5 × 3 correspondendo a cinco níveis de condutividade elétrica da água – CEa (0,3, 1,3, 2,3, 3,3 e 4,3 dS m-1) e três concentrações de peróxido de hidrogênio – H2O2 (0, 25 e 50 µM), com cinco repetições. Níveis de salinidade da água de irrigação de até 2,2 dS m-1 aumentam a fluorescência máxima das plantas de quiabo 75 dias após o transplantio. A aplicação foliar de 50 µM de peróxido de hidrogênio provou ser benéfica para a altura da planta, diâmetro do caule, matéria seca do caule, matéria seca da raiz e matéria seca total do quiabo quando as plantas foram cultivadas em água com baixa salinidade. As concentrações de peróxido de hidrogênio de 25 e 50 µM aumentaram o número de folhas. Porém, essas concentrações reduziram o peso médio dos frutos secos de quiabo. A aplicação foliar com 50 µM de peróxido de hidrogênio teve efeito significativo na fitomassa seca foliar do quiabeiro cv. *Clemson American 80* independentemente da condutividade elétrica da água de irrigação. A aplicação foliar de peróxido de hidrogênio em concentrações de até 50 µM intensifica os efeitos deletérios do estresse salino sobre o peso total dos frutos secos de quiabo.

Palavras-chave: *Abelmoschus esculentus* L, aclimatação, estresse salino.

1. INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) is a crop of great socio economic importance for northeastern Brazil, being found in virtually all tropical and subtropical areas and standing out for its early vegetative cycle, high yield, low production costs, and high food and nutritional value. It is a vegetable source of fiber, vitamin A, C, B6, calcium, iron, magnesium, phosphorus, sodium and potassium (Couto and Coqueiro, 2020; Soares *et al.*, 2020)

Although okra plants are adapted to different soil conditions of the semi-arid region of Brazil (Modesto *et al.*, 2019; Lima *et al.*, 2020), this crop faces production limitations due to the qualitative and quantitative scarcity of water resources (Goes *et al.*, 2020) as a result of long drought periods and high evapotranspiration rates (Lopes *et al.*, 2020).

Most vegetables are glycophytic, showing changes in the photosynthetic process and allocation of photoassimilates due to osmotic and ionic effects, with impacts on plant growth and production (Oliveira *et al.*, 2017; Alvarenga *et al.*, 2019). Okra plants, in turn, have a threshold salinity of 1.3 dS m^{-1} in the saturation extract (Campos, 2013; Gheyi *et al.*, 2016), but can be cultivated under higher salinity levels as long as in association with substances that mitigate the deleterious effects of salt stress in plants (Zafar *et al.*, 2021; Mendonça *et al.*, 2022a; Silva *et al.*, 2023). Although there are several strategies in the literature that aim to mitigate the effects of saline stress on okra plants, the intensity of the deleterious effects on the plants depends on the culture, cultivar, development phase, irrigation and fertilization management and climatic conditions (Pinheiro *et al.*, 2022; Mendonça *et al.*, 2022a)

In this scenario, it is essential to develop strategies that mitigate the effects of salt stress in plants. The available alternatives include foliar application of hydrogen peroxide (H_2O_2)

(Veloso *et al.*, 2023), which can act as a signaling molecule in plants under biotic and abiotic stresses and induce defense mechanisms before exposure to stress (Mendonça *et al.*, 2022b).

H_2O_2 can favor the accumulation of proteins and soluble carbohydrates that act as organic solutes and contribute to the osmotic adjustment of plants under salt stress (Ramos *et al.*, 2021; Veloso *et al.*, 2023). Furthermore, it can act in cells as a secondary messenger, increasing the flow of Ca^{2+} ions and modifying the pattern of proteins and gene expression (Bienert *et al.*, 2006). Research carried out using foliar H_2O_2 application has highlighted beneficial effects in plants under salt stress conditions, as observed in pepper (Aragão *et al.*, 2023), soursop (Silva *et al.*, 2019a), sour passion fruit (Silva *et al.*, 2019b), and colored fiber cotton (Veloso *et al.*, 2023).

The hypothesis of this study is that the foliar application of hydrogen peroxide in low concentrations works as an abiotic stress signaling molecule, playing an important role in the growth and production of okra plants under saline stress. From this perspective, this study aimed to evaluate the effects of foliar hydrogen peroxide application on chlorophyll *a* fluorescence, growth, and production of okra under irrigation with saline water.

2. MATERIAL AND METHODS

The experiment was conducted under field conditions from December 2020 to March 2021, at the Agri-food Science and Technology Center - CCTA of the Federal University of Campina Grande - UFCG, Pombal, Paraíba, located at the geographic coordinates 6º46'13" S, 37°48'06" W, and at an elevation of 193 m a.s.l.

A randomized block design with a 5×3 factorial arrangement was adopted, corresponding to five water electrical conductivity levels - ECw (0.3, 1.3, 2.3, 3.3, and 4.3 dS m^{-1}) and three hydrogen peroxide concentrations - H_2O_2 (0, 25, and 50 μM), with five replications and one plant per plot. The water salinity levels were based on research carried out by Soares *et al.* (2020). The concentrations of H_2O_2 used in this study were based on previous research carried out with summer squash (Dantas *et al.*, 2021).

Sowing was carried out in a 50-mL polyethylene tray using the cultivar Clemson Americano 80, with two seeds per cell in a substrate obtained by mixing soil, sand, and cattle manure in a ratio of 2:1:1 (on a volume basis), respectively. At this stage, the plants were irrigated daily with low-salinity water (0.3 dS m^{-1}) and subsequently thinned to one plant per cell.

Transplanting was carried out 20 days after sowing (DAS) into 20-mL pots adapted as drainage lysimeters filled with a 0.5-kg-cm layer of gravel over a geotextile fabric covering the bottom of the container to avoid clogging. Soon after, the pots received 22 kg of a Fluvic Neosol with a sandy loam texture. A 15-mm diameter hose was also installed as a drain at the base of each lysimeter, coupled to a plastic container (2 L) to collect the drained water and determine water consumption by the plants. The chemical and physical characteristics of the soil used in the experiment (Table 1) were determined according to Teixeira *et al.* (2017).

Fertilization was carried out according to the recommendation of Novais *et al.* (1991) by applying 100 mg N kg^{-1}, 300 mg P_2O_5 kg^{-1}, and 150 mg K_2O kg^{-1} using urea, monoammonium phosphate, and potassium chloride as the respective sources of nitrogen, phosphorus, and potassium. Fertilization was carried out via topdressing in three applications, starting 10 days after transplanting. Micronutrients were supplied every 15 days, and their application began 20 days after transplanting with the commercial product Micro Rexene® (Mg – 1.2%, B – 0.85%, Zn – 4.2%, Fe – 3.4%, Mn – 3.2%, Cu – 0.5%, and Mo – 0.06%) at the concentration of 0.5 g L^{-1}.

Table 1. Chemical and physical characteristics of the soil used in the experiment before the application of treatments.

Chemical characteristics								
pH H_2O (1:2.5)	OM g kg^{-1}	P (mg kg^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+
						$cmol_c\ kg^{-1}$		
5.58	2.93	39.2	0.23	1.64	9.07	2.78	0.0	8.61
Chemical characteristics				Physical characteristics				
ECse (dS m^{-1})	CEC $cmol_c\ kg^{-1}$	SAR $(mmol\ L^{-1})^{0.5}$	PST %	Granulometric fraction (g kg^{-1})			Moisture (dag $kg^{-1)}$	
				Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
2.15	22.33	0.67	7.34	572.7	100.7	326.6	25.91	12.96

pH – Potential of hydrogen, OM – Organic matter: Walkley-Black wet digestion; Ca^{2+} and Mg^{2+} extracted with 1 M KCl pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc pH 7.0; $Al^{3+}+H^+$ extracted with 0.5 M CaOAc pH 7,0; ECse – Electrical conductivity of the saturation extract; CEC – Cation exchange capacity; SAR – Sodium adsorption ratio of the saturation extract; PST – Percentage of exchangeable sodium; [1, 2] referring to the limits of field capacity and permanent wilting point.

The different water electrical conductivity levels (ECw) were prepared by dissolving sodium chloride (NaCl) in local water supply (ECw = 0.3 dS m^{-1}) while considering the relationship between the ECw and salt concentration (Richards, 1954), according to Equation 1:

$$C\ (mmol_c\ L^{-1}) = 10\ x\ ECw \quad (1)$$

Where:

C = salt concentration to be applied (mmolc L^{-1});

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

At the time of transplanting, the soil moisture content was raised to the level corresponding to the maximum water retention capacity, and irrigation was carried out daily with water of low electrical conductivity (0.3 dS m^{-1}) until 17 DAT. After this period, irrigation was started daily with different salinity levels. The water depth applied was determined based on drainage lysimetry in order to replace the average daily consumption (Equation 2).

$$VC = \frac{VA-VD}{1-LF} \quad (2)$$

Where:

VC - volume consumed (L);

VA – water volume applied on the previous day;

VD – drained volume, quantified in the morning of the next day;

LF – leaching fraction estimated at 0.1, every 10 days, in order to minimize salt accumulation in the root zone.

During the experiment, chemical interventions were carried out with preventive applications of commercial pesticides, e.g., insecticides and fungicides, using a manual compression sprayer for phytosanitary control.

The different concentrations of H_2O_2 were prepared from a stock solution of hydrogen peroxide, obtained by diluting H_2O_2 – 30% in deionized water in each application event. Applications were started 72 hours before irrigation with saline water (15 DAT), according to their respective treatments, using a manual sprayer. The product was applied on the abaxial and adaxial sides in order to completely wet the leaves. A cardboard structure was used to avoid drifting onto neighboring plants. During the experiment period, 2 mL of H_2O_2 was applied per plant.

Chlorophyll *a* fluorescence was measured at 75 DAT, between 7:00 and 10:00 a.m., using leaf tweezers (clips) to determine the initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv) and quantum efficiency of photosystem II (Fv/Fm) after 30 minutes of dark adaptation using a pulse amplitude modulated fluorometer (PAM fluorometer ¬ Model OS5p, Opti Science/Hudson, NY, USA).

Growth was determined at 75 DAT by determining the number of leaves (NL), considering those with a minimum length of 3 cm; plant height (PH) was measured from the base of the plant to the insertion of the apical meristem; stem diameter (SD) was determined at 2 cm from the ground with a digital caliper. Leaf area (LA) was measured by following the methodology of Fideles Filho *et al.* (2010), according to Equation 3:

$$LA = \sum 0.7254 \ (X)^{2.08922} \quad (3)$$

Where:

LA – leaf area per plant (cm^2);

X - length of the midrib of the respective leaf (cm).

Fruit harvest began at 59 DAT and continued until 82 DAT by removing fruits that had the typical green color of ripe fruits. The average fruit length (AFL) measured from the base to the apex of the fruit and the average fruit diameter (AFD) were evaluated using a digital caliper. Subsequently, the fruits were dried to constant weight in an air circulation oven at 65ºC, after which they were taken to a precision balance (0.01g), where the average dry fruit weight (ADF, g per plant) and total dry fruit weight (TDF, g per plant) were determined.

At 82 DAT, the plants were collected and separated into different parts (leaves, stems, and roots); subsequently, the parts were packed in paper bags and taken to an air circulation oven at 65ºC until reaching constant weight. After this period, the material was weighed on a semi-analytical balance, obtaining the phytomass of leaves (DPL, g per plant), stem (DPS, g per plant), and roots (DPR, g per plant), the sum of which resulted in the total dry phytomass (TDP, g per plant).

The cumulative water consumption (WC, L per plant) was calculated from the sum of daily water consumption per experimental unit, considering the volume applied and drained during the experiment.

The data obtained were subjected to analysis of variance (F-test) at a probability level of 0.05. In cases of significance, linear and quadratic polynomial regression were performed for the water electrical conductivity levels and H_2O_2 concentrations using the statistical software SISVAR (Ferreira, 2019). In cases of significant interaction between factors, response surface graphs were created using the software SigmaPlot Version 12.5.

3. RESULTS AND DISCUSSION

There was a significant effect of water salinity on the maximum (Fm) and variable (Fv) fluorescence of okra plants (Table 2). Hydrogen peroxide concentrations and the interaction between factors (SL × H_2O_2) did not significantly influence the chlorophyll *a* fluorescence of

okra plants at 75 DAT.

Table 2. Summary of the analysis of variance for initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv) and quantum efficiency of photosystem II (Fv/Fm) of okra cv. Clemson Americano 80 cultivated with saline water and concentrations of hydrogen peroxide 75 days after transplanting (DAT).

Sources of variation	GL	Mean squares			
		F_0	Fm	Fv	Fv/Fm
Saline levels (SL)	4	326.97^{ns}	10600.82*	15755.9*	0.0001^{ns}
Linear regression	1	246.40^{ns}	663.60^{ns}	17767.04	0.0000^{ns}
Quadratic regression	1	768.58^{ns}	32875.02**	34830.09	0.0000^{ns}
Hydrogen peroxide (H_2O_2)	2	756.52^{ns}	214.86^{ns}	341.45^{ns}	0.0000^{ns}
Interaction (SL × H_2O_2)	8	239.48^{ns}	2738.63^{ns}	6075.64^{ns}	0.0000^{ns}
Blocks	4	117.72^{ns}	5822.69^{ns}	12877.7^{ns}	0.0000^{ns}
CV (%)		3.71	3.07	4.63	0.93
Mean		442.73	2027.11	1565.63	0.7809

ns, *, **, respectively non-significant and significant at $p \leq 0.05$ and ≤ 0.01; CV= coefficient of variation; GL = degree of freedom.

The maximum fluorescence of okra plants (Figure 1A) achieved the maximum estimated value under the ECw of 2.2 dS m^{-1} (2052.24), decreasing from this level. The lowest value occurred in plants grown under the ECw of 4.3 dS m^{-1} (1997.89). The increase in Fm is related to the reduction of the damage caused by salt stress, increasing the plant's capacity to transfer energy for the formation of NADPH, ATP, and reduce ferredoxin, in addition to favoring the assimilation of CO_2 in the biochemical phase of photosynthesis (Figueiredo *et al.*, 2019).

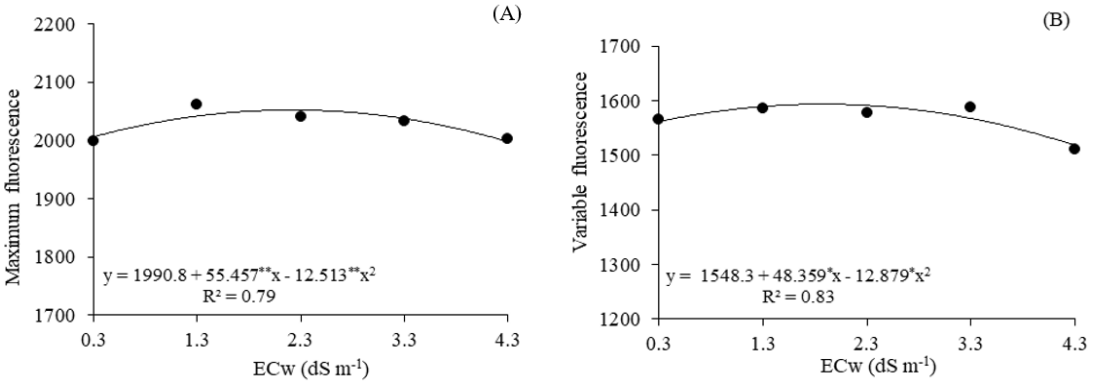

Figure 1. Maximum fluorescence - Fm (A) and variable fluorescence - Fv (B) of the okra cv. Clemson Americano 80 as a function of irrigation water salinity – ECw 75 days after transplanting.

The variable fluorescence (Fv) of okra plants also showed a quadratic behavior (Figure 1B), with the maximum estimated value of 1593.68 being achieved at the ECw of 1.9 dS m^{-1}. On the other hand, the minimum value of 1518.11 was achieved in plants subjected to irrigation with 4.3 dS m^{-1} (Figure 1B). Under salt stress conditions, deficiency in the photoreduction of quinone A (QA) and in the flow of electrons between photosystems may occur, probably due to the damage caused to the photosynthetic apparatus as a result of water stress caused by the osmotic effect, with the imbibition forces of plant roots becoming low in relation to the water retention forces in the soil due to excess salts (Cova *et al.*, 2021).

There was a significant effect of the interaction between factors (SL × H_2O_2) on plant height and okra stem diameter at 75 DAT. Water salinity levels significantly influenced all growth variables measured (Table 3). Hydrogen peroxide concentrations had a significant effect only on the number of leaves and leaf area of okra.

Table 3. Summary of the analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL), and leaf area (LA) of the okra cv. Clemson Americano 80 cultivated with saline water and hydrogen peroxide concentrations 75 days after transplanting (DAT).

Sources of variation	GL	Mean squares			
		PH	SD	NL	LA
Salinity levels (SL)	4	1674.68**	28.14**	259.05**	2296649.65**
Linear regression	1	6415.74**	108.22**	1008.80**	6681759.64**
Quadratic regression	1	212.00**	1.00^{ns}	2.976^{ns}	2141843.89**
Hydrogen peroxide (H_2O_2)	2	12.97^{ns}	0.47^{ns}	52.92**	1368.75^{ns}
Interaction (SL × H_2O_2)	8	68.50**	3.54**	14.00^{ns}	280458.11^{ns}
Blocks	4	10.28^{ns}	0.30^{ns}	13.58^{ns}	390656.05*
CV (%)		5.19	5.71	17.61	15.17
Mean		62.10	16.91	15.96	2426.35

ns, *, **, respectively non-significant and significant at p ≤0.05 and p ≤ 0.01; CV= coefficient of variation; GL – degree of freedom.

The plant height (Figure 2A) of okra reached the maximum estimated value under the ECw of 0.3 dS m^{-1} (77.92 cm) and foliar application of 50 µM of H_2O_2, being 27.6 cm higher than the lowest value (50.32 cm) obtained under irrigation with 4.3 dS m^{-1} and at a concentration of 0 µM of H_2O_2. When the plants received irrigation with 1.8 dS m^{-1} and 50 µM of H_2O_2, this decrease was only 13.57 cm in relation to the highest value found. The beneficial effects of hydrogen peroxide may be related to its role as an abiotic stress signal in plants, activating the defense system and favoring adaptation to adverse conditions (Souza *et al.*, 2019).

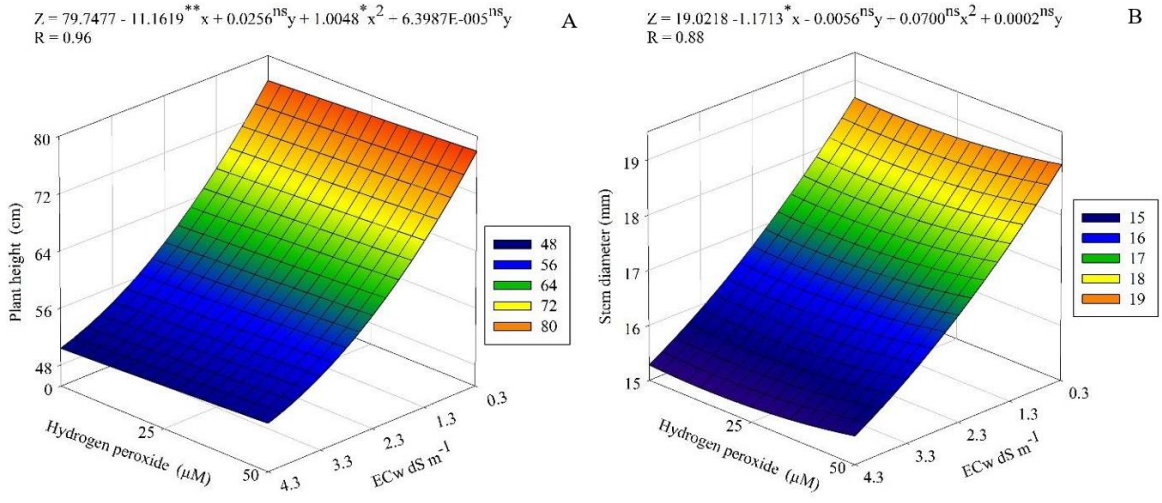

Figure 2. Plant height (A) and stem diameter (B) of the okra cv. Clemson Americano 80 as a function of the interaction between irrigation water salinity – ECw and hydrogen peroxide concentrations – H_2O_2, 75 days after transplanting.
X and Y – Electrical conductivity of irrigation water – ECw and hydrogen peroxide concentrations – H2O2, respectively; ns, *, **, non-significant and significant at p ≤ 0.05 and 0.01 by the F-test.

Irrigation with 0.3 dS m^{-1} and foliar hydrogen peroxide application at a concentration of

50 µM resulted in the highest value (18.91 mm) of stem diameter (SD) in okra plants (Figure 2B). When the plants received irrigation with an ECw of 4.3 dS m^{-1} and a concentration of 12.5 µM of H_2O_2, they showed a SD of 15.24 mm, representing a reduction of 19.41% compared to the highest estimated value. The foliar application of hydrogen peroxide at adequate concentrations can favor greater accumulation of proteins and soluble carbohydrates that act in the osmotic adjustment of plants under salt stress, thus allowing greater water and nutrient uptake and, consequently, greater plant growth (Aragão *et al.*, 2023).

The number of leaves (NL) decreased linearly with the increase in the water electrical conductivity levels (Figure 3A), with reductions of 11.82% per unit increase in the ECw. When comparing the NL of plants subjected to an ECw of 4.3 dS m^{-1} to those irrigated with the lowest salinity, a reduction of 50.9% was observed. Reductions in the number of leaves in plants subjected to salt stress are considered a protection and/or acclimatization strategy to high salinity, minimizing water losses through transpiration and maintaining a high water potential in the cell (Nascimento *et al.*, 2019).

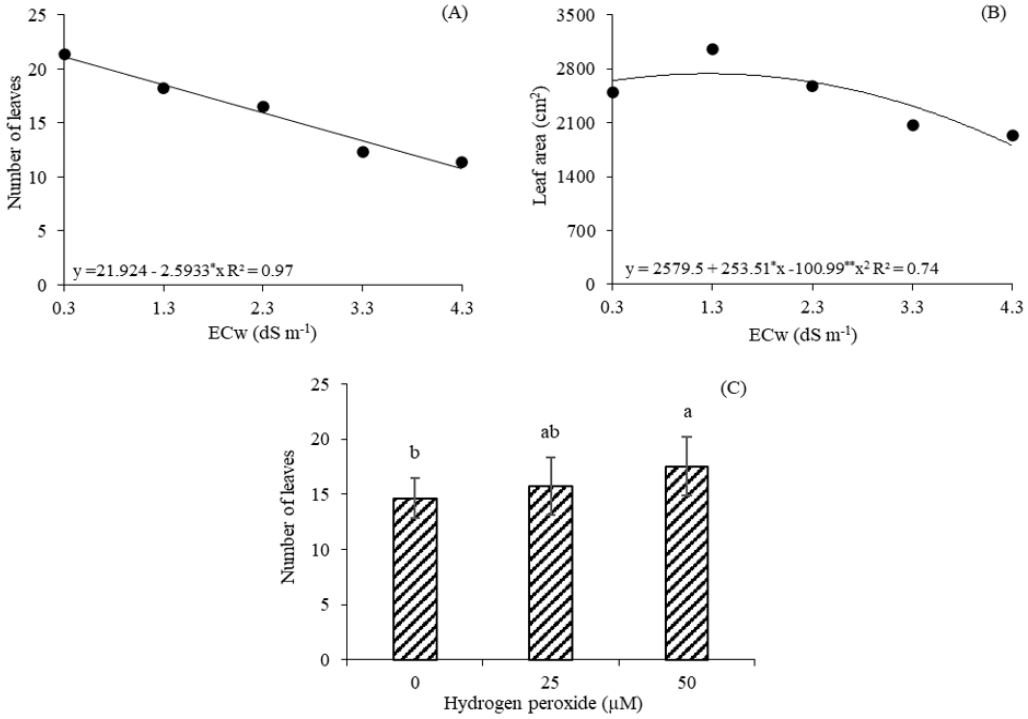

Figure 3. Number of leaves (A) and leaf area (B) of the okra cv. Clemson Americano 80 as a function of irrigation water salinity– ECw and number of leaves as a function of hydrogen peroxide concentrations - H_2O_2 75 days after transplanting.
Equal letters indicate that the H_2O_2 means do not differ by the Tukey test at $p<0.05$. The bars represent the standard error of the mean (n = 5).

The leaf area (LA) of okra plants was also negatively affected by water salinity (Figure 3B), with the maximum estimated value of LA (2738.39 cm^2) obtained in plants grown under the ECw of 1.3 dS m^{-1}, decreasing from this point onwards and reaching the minimum value of 1802.28 cm^2 under the ECw of 4.3 dS m^{-1}. The leaf area reduction constitutes one of the initial responses of plants to salt stress and may be related to cellular inhibition and leaf surface expansion (Lima *et al.*, 2017). Under these conditions, plants delay the emission of leaves (Figure 4A) and deactivate part of their leaf area through leaf abscission, in an attempt to reduce water loss through transpiration (Dias *et al.*, 2017).

Hydrogen peroxide (H_2O_2) at a concentration of 50 µM (Figure 3C) resulted in a number of leaves (NL) 16.43% higher than plants grown in the absence of H_2O_2 (0 µM). However,

when comparing the NL of plants that received foliar application of 25 and 50 µM of H_2O_2, it appears that there was no significant difference between them. The application of H_2O_2 favors the activation of physiological responses that allow the accumulation of proteins, soluble carbohydrates, and NO_3^-, inducing cell division and increasing the emission of leaves (Gondim *et al.*, 2011).

There was a significant effect of the interaction between salinity levels and hydrogen peroxide (SL × H_2O_2) on the stem (DPS), root (DPR) and total (TPM) dry matter of okra plants (Table 4) 82 days after transplanting. The water salinity levels significantly affected all variables analyzed. The hydrogen peroxide concentrations significantly influenced the DPL, DPR, and TDP of okra plants at 82 DAT.

Table 4. Summary of the analysis of variance for dry phytomass of leaves (DPL), stem (DPS), roots (DPR) and total dry phytomass (TDP) of the okra cv. Clemson Americano 80 cultivated with saline water and hydrogen peroxide concentrations 82 days after transplanting (DAT).

Sources of variation	GL	Mean squares			
		DPL	DPS	DPR	TDP
Salinity levels (SL)	4	480.87**	593.06**	1163.05**	6454.04**
Linear regression	1	1911.16**	2340.13**	4608.83**	25593.68**
Quadratic regression	1	9.62^{ns}	30.83*	22.68^{ns}	180.05*
Hydrogen peroxide (H_2O_2)	2	61.38**	35.26^{ns}	122.27**	477.21**
Interaction (SL × H_2O_2)	8	5.31^{ns}	26.99**	103.80**	236.85**
Blocks	4	4.40^{ns}	4.19^{ns}	13.87^{ns}	38.05**
CV (%)		9.36	14.00	15.64	9.77
Mean		21.57	18.71	17.31	57.60

ns, *, **, respectively non-significant at significant at $p \leq 0.05$ and $p \leq 0.01$; CV= coefficient of variation; GL = degree of freedom.

The dry phytomass of okra leaves (DPL) was negatively affected by the increase in water electrical conductivity (Figure 4A), with a reduction of 11.98% per unit increase in the ECw. A reduction of 49.72 % (14.27 g per plant) was observed when comparing the DPL of plants subjected to the ECw of 4.3 dS m^{-1} to those under the lowest electrical conductivity (0.3 dS m^{-1}). The reduction in DPL is related to the lower emission of leaves observed in this study under salinity conditions as excess salts reduce the free energy status of water in the root environment and the availability of water and nutrients to plants and compromise meristematic activity and cell elongation, consequently restricting phytomass accumulation in the leaves (Shankar *et al.*, 2016; Silva *et al.*, 2020).

When analyzing the effects of Hydrogen peroxide (H_2O_2) concentrations on okra leaf dry matter (DPL) (Figure 4B), it was observed that plants subjected to foliar application of 50 µM of H_2O_2 had an NL 8.51 and 13.28% higher than those under 0 and 25µM of H_2O_2, respectively. These results are related to the effect of the highest H_2O_2 level (50 µM) on leaf emission, indicating the role of hydrogen peroxide in inducing the production of several enzymes, e.g., catalase, superoxide dismutase, glutathione reductase, and peroxidase, which are associated with plant detoxification (Das and Roychoudhury, 2014). Pereira *et al.* (2022) evaluated the effects of irrigation with saline water (ECw varying from 0.3 and 5.0 dS m^{-1}) and four concentrations of H_2O_2 (0, 5, 10, and 15 µmol L^{-1}) on melon and found that the highest dry phytomass values of the shoot part were 78.62 and 46.34 g per plant under ECw values of 0.3 and 5.0 dS m^{-1} and foliar application of 9.11 and 8.35 µmol L^{-1} of H_2O_2.

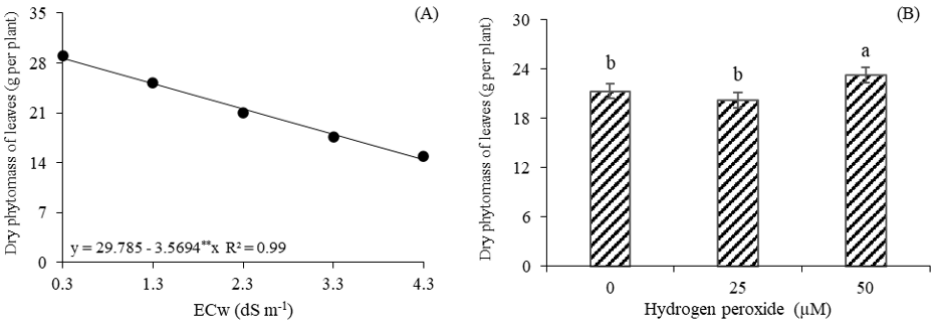

Figure 4. Dry phytomass of leaves of the okra cv. Clemson Americano 80 as a function of irrigation water salinity - ECw (A) and hydrogen peroxide concentrations - H_2O_2 (B) 82 days after transplanting.
Equal letters indicate that the H_2O_2 means do not differ by the Tukey test at $p<0.05$. The bars represent the standard error of the mean (n = 5).

Irrigation with low salinity water (ECw of 0.3 dS m^{-1}) and foliar application of H_2O_2 at a concentration of 50 µM provided greater accumulation of dry phytomass in the stem (DPS) (28.71 g per plant). On the other hand, the water salinity of 4.3 dS m^{-1} and the concentration of 18.75 µM of H_2O_2 resulted in a lower DPS (10.54 g per plant), i.e., a reduction of 34.69% compared the plants grown under the ECw of 0.3 dS m^{-1} and 50 µM H_2O_2 (Figure 5A). For Bienert *et al.* (2006), hydrogen peroxide can act as a secondary messenger in cells by increasing the flow of Ca^{2+} ions and modifying the pattern of proteins and gene expression. Andrade *et al.* (2019) evaluated four concentrations of hydrogen peroxide (0, 20, 40, and 60 µM) in sour passion fruit under salt stress and observed beneficial effects with foliar application of 20 µM of H_2O_2.

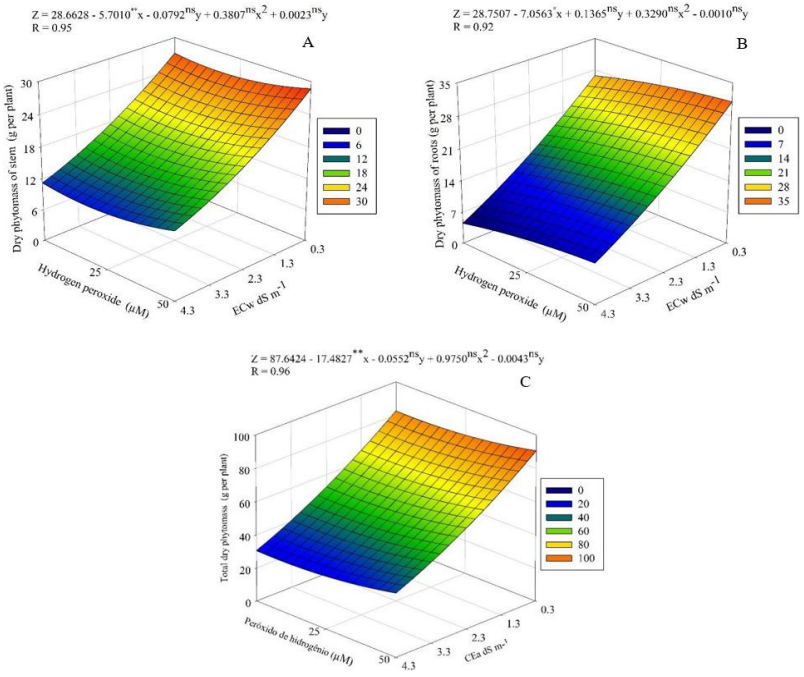

Figure 5. Dry phytomass of stem (A), roots (B) and total dry phytomass (C) of the okra cv. Clemson Americano 80 as a function of the interaction between irrigation water salinity - ECw and hydrogen peroxide concentrations - H_2O_2 82 days after transplanting. X and Y – Electrical conductivity of irrigation water– ECw and hydrogen peroxide concentrations – H_2O_2, respectively; ^{ns}, *, ** significant at $p \leq 0.05$ and 0.01 by the F-test.

For root dry phytomass (DPR), the maximum estimated value (31.02 g per plant) was obtained in plants grown under the ECw of 0.3 dS m^{-1} and foliar application of 50 µM of H_2O_2. On the other hand, the minimum value of 4.49 g per plant was reached in plants grown under the water salinity of 4.3 dS m^{-1} and foliar application of 0 µM H_2O_2 (Figure 5B). The restriction in water uptake due to the osmotic effect causes stomatal closure, reducing carbon accumulation in the cell and resulting in less photosynthesis, consequently decreasing phytomass accumulation (Silva *et al.*, 2023).

The total dry mass (TDP) of okra plants achieved the maximum value (90.54 g per plant) under irrigation with 0.3 dS m^{-1} and foliar application of 50 µM of H_2O_2. The lowest value (30.31 g per plant) was achieved in plants irrigated with water at 4.3 dS m^{-1} and hydrogen peroxide at a concentration of 6.25 µM (Figure 5C). The toxicity of specific ions (Na^+, Cl^-, and B) occurs due to the absorption of saline water by plant roots, causing cell disruption and metabolic changes in plants and reducing growth and phytomass (Ali *et al.*, 2021).

There was a significant effect of the interaction between factors (SL × H_2O_2) only for the total dry fruit weight (TDF) (Table 5). The salinity levels significantly influenced water consumption (WC). On the other hand, the hydrogen peroxide concentrations significantly affected the average dry fruit weight (TDF) of okra plants.

Table 5. Summary of the analysis of variance for total dry fruit weight (TDF), average dry fruit weight (ADF), water consumption (WC), average fruit diameter (AFD), and average fruit length (AFL) of the okra cv. Clemson Americano 80 cultivated with saline water and hydrogen peroxide concentrations 82 days after transplanting (DAT).

Sources of variation	GL	Mean squares				
		TDF	ADF	WC	AFD	AFL
Salinity levels (SL)	4	42299.95**	0.2076^{ns}	142.001**	3.6310^{ns}	0.789^{ns}
Linear regression	1	165812.43**	0.2046^{ns}	523.089**	0.7790^{ns}	1.626^{ns}
Quadratic regression	1	549.08**	0.0082^{ns}	30.121**	3.5152^{ns}	0.016^{ns}
Hydrogen peroxide (H_2O_2)	2	3669.32**	0.8674**	0.636^{ns}	3.4776^{ns}	1.428^{ns}
Interaction (SL × H_2O_2)	8	3300.10**	0.1466^{ns}	2.555^{ns}	3.0261^{ns}	1.024^{ns}
Blocks	4	842.41*	0.0836^{ns}	1.266^{ns}	6.4506^{ns}	1.387^{ns}
CV (%)		10.51	14.84	5.59	9.57	9.41
Mean		140.92	1.90	25.24	21.08	12.34

ns, *, **, respectively non-significant at significant at $p \leq 0.05$ and $p \leq 0.01$; CV= coefficient of variation; GL = degree of freedom.

The total weight of the dry fruit (TDF) decreased with increasing salinity levels (Figure 6), with the maximum value (18.39 g per plant) being achieved in plants irrigated with 0.3 dS m^{-1} and no H_2O_2 (0 µM). It should be noted that the increase in salinity levels associated with the increase in H_2O_2 concentrations intensified the deleterious effects of salt stress on okra, with irrigation with 4.3 dS m^{-1} and a concentration of 50 µM of H_2O_2 resulting in a reduction of 66.82% compared to the plants grown under the ECw of 0.3 dS m^{-1}. Azeem *et al.* (2017) explain that a reduction in photosynthetic efficiency in okra plants, which are sensitive to the effects of saline stress, decreases fruit growth and biomass production. Similar results were found by Soares *et al.* (2020) in research with okra and irrigation with saline water (ECw ranging from 0.3 to 4.3 dS m^{-1}), in which they found that higher salinity levels reduce fruit weight, and the highest value of 33 .64 g for average fruit weight was achieved in plants irrigated with an ECw of 2.4 dS m^{-1} and under hydroponic conditions (nutrient solution EC levels of 2.1, 3.6, 5.1, and 6.6 dS m^{-1}), Mendonça *et al.* (2022a) observed a 3.82% reduction in the average weight of okra fruits per unit increase in the EC of the nutrient solution.

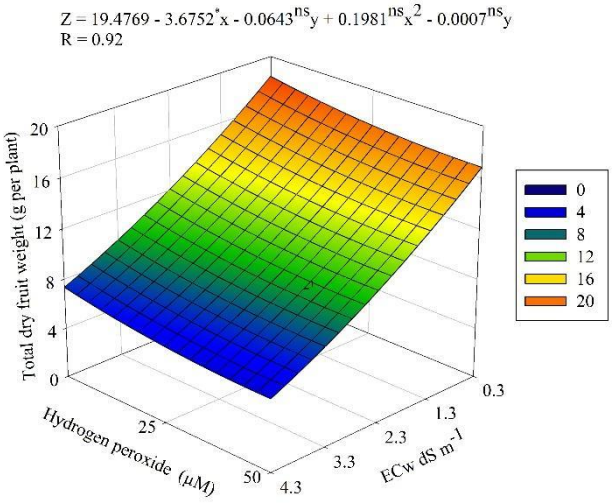

Figure 6. Total dry fruit weight (TDF) of the okra cv. Clemson Americano 80 as a function of the interaction between salinity levels of irrigation water - ECw and concentrations of hydrogen peroxide – H_2O_2 82 days after transplanting.
X and Y – Electrical conductivity of irrigation water– ECw and hydrogen peroxide concentrations – H2O2, respectively; ns,*, **, non-significant and significant at $p \leq 0.05$ and 0.01 by the F-test.

With regard to the effects of H_2O_2 on the average dry fruit weight (Figure 7A), the foliar application of 25 and 50 µM H_2O_2 reduced this parameter by, respectively, 16.79 and 12.94% in plants that received 0 µM H_2O_2. The average dry fruit weight decreased with the application of H_2O_2, probably because it is a reactive oxygen species (ROS) which, depending on its concentration, can act in the oxidation of the lipid membrane, DNA, and other cell components through toxic free radicals (Barbosa *et al.*, 2014; Silva *et al.*, 2019c).

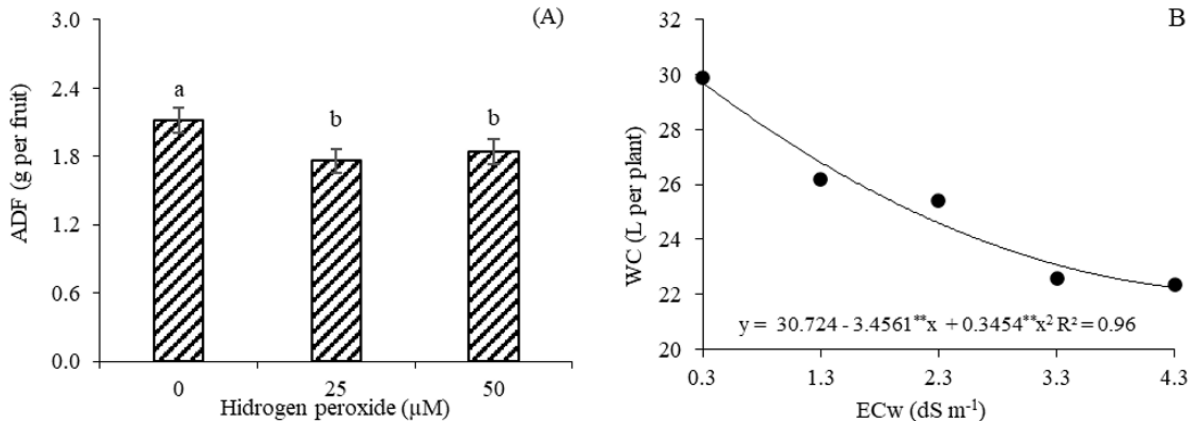

Figure 7. Average dry fruit weight – ADF of the okra cv. Clemson Americano 80 as a function of hydrogen peroxide concentrations, (A) and water consumption – WC of okra plants as a function of irrigation water salinity - ECw (B) 82 days after transplanting.
Equal letters indicate that the H2O2 means do not differ by the Tukey test at $p \leq 0.05$. The bars represent the standard error of the mean (n = 5).

The water consumption of okra (WC) plants decreased quadratically with the increase in the electrical conductivity of irrigation water (Figure 7B). Okra plants irrigated with an ECw of 0.3 dS m^{-1} achieved the highest WC (29.71 L per plant), decreasing sharply from this salinity

level and reaching the lowest WC under the ECw of 4.3 dS m^{-1} (22.24 L per plant). When comparing plants subjected to the ECw of 4.3 dS m^{-1} in relation to those grown under 0.3 dS m^{-1}, there was a reduction in water consumption amounting to 7.46 L per plant. The reduction in water consumption is a reflection of the osmotic effect that reduces water absorption by plants due to high salt concentrations in the soil, making the osmotic potential more negative and leading to greater stomatal resistance and reduced transpiration, thus reducing water uptake by roots (Santos *et al.*, 2022). Ünlükara *et al.* (2008) evaluated five salinity levels of irrigation water (1.5, 2.5, 3.5, 5.0, and 7.0 dS m^{-1}) in okra plants and found a reduction in water consumption of 2.43% per unit increase in the ECw.

4. CONCLUSIONS

Irrigation water salinity levels up to 2.2 dS m^{-1} increase the maximum fluorescence of okra plants 75 days after transplanting.

The foliar application of 50 µM hydrogen peroxide proved to be beneficial for plant height, stem diameter, stem dry matter, root dry matter, and total dry matter of okra when plants were grown in low-salinity water.

The hydrogen peroxide concentrations of 25 and 50 µM increased the number of leaves. However, these concentrations reduced the average weight of the okra dry fruits.

The foliar application with 50 µM hydrogen peroxide had a significant effect on the dry leaf phytomass of the okra cv. Clemson American 80 regardless of the electrical conductivity of irrigation water.

Foliar hydrogen peroxide application at concentrations up to 50 µM intensifies the deleterious effects of salt stress on the total weight of dried okra fruits.

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