



## Hydrogen peroxide as a saline stress attenuator in okra (*Abelmoschus esculentus* L.)

Tracy Amélia Pereira Lopes<sup>1</sup>  Lauriane Almeida dos Anjos Soares<sup>2\*</sup>   
Geovani Soares de Lima<sup>3</sup>  Iara Almeida Roque<sup>3</sup>  Luderlândio de Andrade Silva<sup>3</sup>   
André Alisson Rodrigues da Silva<sup>3</sup>  Saulo Soares da Silva<sup>3</sup>  Pedro Dantas Fernandes<sup>3</sup> 

<sup>1</sup>Programa de Pós-graduação em Engenharia Agrícola, Universidade Federal Rural de Pernambuco (UFRPE), Recife, PE, Brasil.

<sup>2</sup>Programa de Pós-graduação em Horticultura Tropical, Universidade Federal de Campina Grande (UFCG), 58840-000, Pombal, PB, Brasil. E-mail: laurispo.agronomia@gmail.com. \*Corresponding author.

<sup>3</sup>Programa de Pós-graduação em Engenharia Agrícola, Universidade Federal de Campina Grande (UFCG), Campina Grande, PB, Brasil.

**ABSTRACT:** Okra is a vegetable that stands out for its low production cost, high yield, high nutritional and value and socioeconomic importance in income generation, especially in family farming. In this context, the present study evaluated the physiology, production and water use efficiency of okra under irrigation with saline water and exogenous application of hydrogen peroxide. The treatments were distributed in a randomized block design, in a  $5 \times 3$  factorial arrangement, with five levels of electrical conductivity of irrigation water – ECw (0.3; 1.3; 2.3; 3.3 and 4.3 dS m<sup>-1</sup>) and three concentrations of hydrogen peroxide – H<sub>2</sub>O<sub>2</sub> (0, 25 and 50 μM), with five replicates, totaling 75 plants. Irrigation with ECw above 0.3 dS m<sup>-1</sup> negatively affected stomatal conductance, transpiration, number of fruits, total production and water use efficiency of okra cv. Clemson Americano 80. Hydrogen peroxide at concentration of 22 μM mitigated the effects of salt stress on CO<sub>2</sub> assimilation rate, number of fruits, average fruit weight and total production of okra cv. Clemson Americano 80. Water use efficiency is favored by H<sub>2</sub>O<sub>2</sub> application at concentration of 12 μM, especially in plants irrigated with ECw of 0.3 dS m<sup>-1</sup>.

**Key words:** Salinity, H<sub>2</sub>O<sub>2</sub>, acclimatization.

## Peróxido de hidrogênio como atenuante do estresse salino em quiabeiro (*Abelmoschus esculentus* L.)

**RESUMO:** O quiabeiro é uma hortaliça que se destaca pelo baixo custo de produção, elevada produtividade, alto valor nutricional e pela importância socioeconômica na geração de renda, principalmente na agricultura familiar. Nesse contexto, o presente trabalho teve como objetivo avaliar a fisiologia, a produção e a eficiência no uso da água de quiabeiro sob irrigação com águas salinas e aplicação exógena de peróxido de hidrogênio. Os tratamentos foram distribuídos no delineamento de blocos casualizados, em arranjo fatorial  $5 \times 3$ , sendo cinco níveis de condutividade elétrica da água de irrigação (0,3; 1,3; 2,3; 3,3 e 4,3 dS m<sup>-1</sup>) e três concentrações de peróxido de hidrogênio (0, 25 e 50 μM), com cinco repetições, totalizando 75 plantas. A irrigação com CEa acima de 0,3 dS m<sup>-1</sup> afetou negativamente a condutância estomática, a transpiração, o número de frutos, a produção total e a eficiência no uso da água do quiabeiro cv. Clemson Americano 80. O peróxido de hidrogênio na concentração de 22 μM amenizou os efeitos do estresse salino sobre a taxa de assimilação de CO<sub>2</sub>, número de frutos, peso médio de frutos e produção total de quiabeiro cv. Clemson Americano 80. A eficiência no uso da água é beneficiada pela aplicação de peróxido de hidrogênio na concentração de 12 μM, sobretudo nas plantas irrigadas com CEa de 0,3 dS m<sup>-1</sup>.

**Palavras-chave:** Salinidade, H<sub>2</sub>O<sub>2</sub>, aclimação.

## INTRODUCTION

Okra (*Abelmoschus esculentus* L.) is a fast growing annual crop, cultivated mainly for its tender fruits, being widely grown in tropical and subtropical regions in different countries from Africa to Asia, southern Europe and America (DURAZZO et al., 2019; ISLAM et al., 2019). Okra is an important vegetable in the human diet, being a source of carbohydrates, proteins, fats, minerals, and vitamins (MENDONÇA et al., 2022). It is a vegetable widely produced in Brazil, with about 128,460 tons of okra produced in 2017, and the Northeast is the second largest producer, with 32,337 tons, which corresponds to 25.1% of the national production (IBGE, 2017).

The semi-arid region of northeastern Brazil provides favorable edaphoclimatic conditions for okra production, because of its rusticity and tolerance to high temperatures; however, due to water restrictions in terms of quality and quantity (SOARES et al., 2018; SOARES et al. 2020), it is necessary to perform irrigation using waters with high levels of salts, commonly found in surface and underground water sources, leading to losses in fruit production and quality (LIMA et al., 2020).

The use of saline water can cause osmotic imbalances and ionic, nutritional and oxidative damage, resulting from the excessive accumulation of reactive oxygen species (ROS), such as superoxide radical (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and

hydroxyl radical (OH), compromising plant growth and physiological processes (MORAIS et al., 2020, SILVA et al., 2021). However, the effects of salt stress may vary on each crop, depending on irrigation and fertilization management, edaphoclimatic conditions, species or even development stages (LIMA et al., 2016; SOARES et al., 2021).

In this context, alternatives have been sought to mitigate the effects caused by salt stress on plants, among which the exogenous application of hydrogen peroxide ( $H_2O_2$ ) at low concentrations has proven to be promising in the acclimatization of plants to salt stress (SEMIDA, 2016, SILVA et al., 2019; ANDRADE et al., 2022) In addition,  $H_2O_2$  acts as a signaling molecule in plants under biotic and abiotic stresses, favoring greater accumulation of soluble proteins and carbohydrates that can act as organic solutes, performing the osmotic adjustment of plants under salt stress, thus allowing greater water absorption (ANDRADE et al., 2019; DANTAS et al., 2021; SILVA et al., 2021).

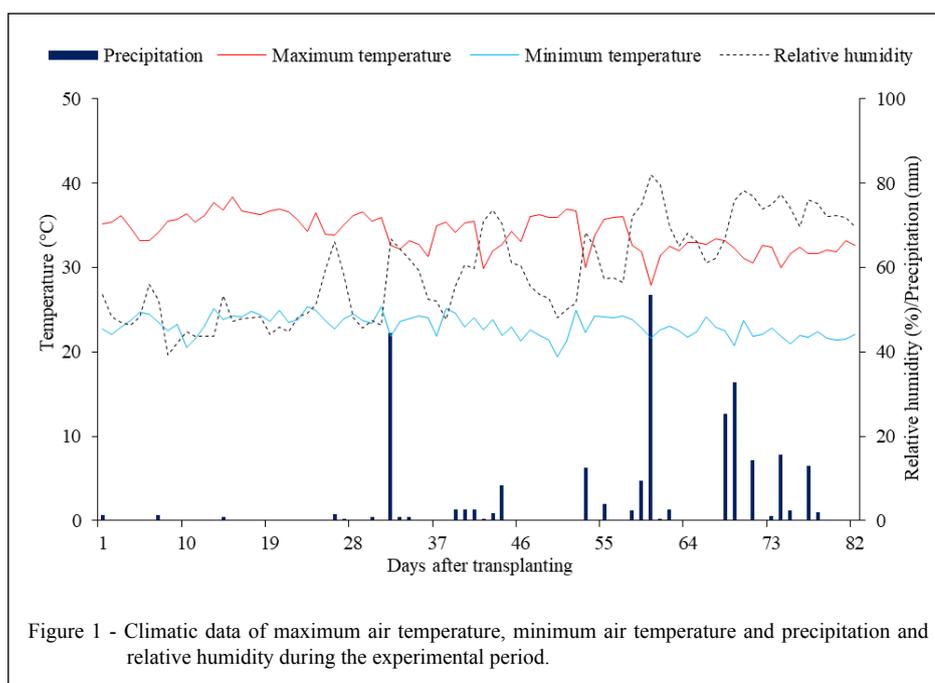
However, there is no information in the literature on the role of this reactive oxygen species in the mitigation of salt stress effects on okra under the semi-arid conditions of northeastern Brazil. In this context, the present research evaluates the effect of hydrogen peroxide on the physiology, production and efficiency of water use in *Abelmoschus esculentus* L. plants under irrigation with saline water.

## MATERIALS AND METHODS

The experiment was carried out under 70% shading conditions from December 2020 to March 2021, at the Center for Science and Agri-Food Technology - CCTA of the Federal University of Campina Grande - UFCG, located in the municipality of Pombal, Paraíba, Brazil, at the geographic coordinates 6°46'13" S, 37°48'06" W and altitude of 193 m. Data on maximum and minimum air temperature, precipitation and relative humidity during the experimental period are shown in figure 1.

The experimental design used was randomized blocks, in a  $5 \times 3$  factorial arrangement, referring to five levels of electrical conductivity of water - ECw (0.3; 1.3; 2.3; 3.3 and 4.3  $dS\ m^{-1}$ ) and three concentrations of hydrogen peroxide -  $H_2O_2$  (0, 25 and 50  $\mu M$ ) with five replicates and one plant per plot. Due to few studies with  $H_2O_2$  in vegetables, the concentrations used were based on studies carried out with zucchini (DANTAS et al., 2021), passion fruit (RAMOS et al. 2021) and passion fruit (Silva et al., 2019), while salinity levels were based on SOARES et al. (2020).

Two seeds of *Abelmoschus esculentus* L. cv. Clemson Americano 80 were sown at 0.5 cm depth in plastic trays with 162 cells, with capacity of 50 ml. The substrate used was obtained by mixing soil, sand and cattle manure in the proportion of 2:1:1, respectively; in this phase, the plants were irrigated daily with low-



salinity water (0.3 dS m<sup>-1</sup>) and, subsequently, thinning was performed, leaving only one plant per cell.

At 20 days after sowing (DAS), when the plants reached 10 cm in height and had two pairs of true leaves, they were transplanted to pots adapted as drainage lysimeters with 20 L capacity, which received a 3-cm-thick layer of crushed stone above a geotextile which covered the base of the container, to prevent clogging by soil material. At the base of each container, a 15-mm-diameter hose was installed, as a drain, connected to a plastic container (2 L) to collect drained water. Then, the pots received 22 kg of a *Neossolo Flúvico* (Fluvent) with sandy loam texture, whose physical and chemical characteristics were determined according to TEIXEIRA et al. (2017): Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Al<sup>3+</sup> + H<sup>+</sup> = 9.07, 2.78, 1.64, 0.23 and 8.61 cmol<sub>c</sub> kg<sup>-1</sup>, respectively; pH (1:2.5 soil water suspension) = 5.58; EC<sub>se</sub> = 2.15 dS m<sup>-1</sup>; organic matter = 2.93 dag kg<sup>-1</sup>; sand, silt and clay = 572.7, 100.7 and 326.6 g kg<sup>-1</sup>, respectively; moisture content at 33.42 and 1519.5 kPa = 25.91 and 12.96 dag kg<sup>-1</sup>, respectively.

Fertilization with NPK (100 mg N kg<sup>-1</sup> soil; 300 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> soil and 150 mg K<sub>2</sub>O kg<sup>-1</sup> soil) was performed according to the recommendation of NOVAIS et al. (1991), using urea, monoammonium phosphate and potassium chloride as sources, respectively, applied as top-dressing, split into three portions, with the first fertilization at 10 days after transplanting (DAT). Micronutrients were supplied every two weeks, starting at 20 DAT with the commercial product Micro Rexene<sup>®</sup> containing: Mg - 1.2%; B - 0.85%; Zn - 4.2%; Fe - 3.4%; Mn - 3.2%; Cu - 0.5% and Mo - 0.06%. The pots were arranged in single rows at spacing of 1.5 m between rows and 1.0 m between plants in the row.

The treatments began to be applied at 20 DAT. Hydrogen peroxide applications were performed every 15 days, in the late afternoon, totaling four applications, with an average volume of 250 mL per plant. Foliar applications were performed on the abaxial and adaxial sides of the leaves, using a manual sprayer with capacity of 1 L.

The water with the lowest electrical conductivity (0.3 dS m<sup>-1</sup>) was obtained from the public supply system of Pombal-PB and the other EC<sub>w</sub> levels were prepared from the dissolution of sodium chloride (NaCl) considering the relationship between EC<sub>w</sub> and the concentration of salts (mmol<sub>c</sub> L<sup>-1</sup> ≈ 10 × EC<sub>w</sub>) (RHOADES et al., 2000).

Prior to transplantation, the soil moisture content was increased to the level corresponding to the maximum water holding capacity, and irrigation

were carried out daily with water of low electrical conductivity (0.3 dS m<sup>-1</sup>) until 20 DAT. After this period, irrigation began to be performed with the different salinity levels, at one-day interval, and the applied depth was determined based on the water balance to replace the average daily consumption by plants, with a leaching fraction of 10% every 15 days, obtained by dividing the value of the volume to be applied (mL) by 0.9, to promote the leaching of excess salts supplied by irrigation water from the root zone.

During the experiment, all preparations and phytosanitary practices recommended for the crop were performed, monitoring the emergence of pests and diseases and adopting control measures when necessary.

Gas exchange was measured at 75 DAT based on stomatal conductance - *g<sub>s</sub>* (mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), internal CO<sub>2</sub> concentration - *C<sub>i</sub>* (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>), transpiration - *E* (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) and CO<sub>2</sub> assimilation rate - *A* (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>). These data were then used to determine the instantaneous water use efficiency - *WUE<sub>i</sub>* (*A/E*) [(μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>] and instantaneous carboxylation efficiency - *CE<sub>i</sub>* [(μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) (μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>], with an infrared gas analyzer - IRGA (LCpro - SD model from ADC BioScientific, UK).

At 59 DAT, fruits began to be harvested manually as they showed the typical green color of ripe fruits, over a period of 23 days. The number of fruits per plant (NFP), average fruit length (AFL), average fruit weight (AFW) and total production per plant (TPP) were recorded. Fruit length was measured from the tip of the fruit to the insertion point of its peduncle. The fruits obtained in each harvest were weighed on a precision scale (0.01 g) to obtain the fresh matter. The average production per plant was obtained considering the number of plants during the harvest period. Water use efficiency - *WUE* (g L<sup>-1</sup>) was determined through the relationship between production (total production per plant) and accumulated water consumption until the end of the production cycle.

The data obtained were subjected to analysis of variance (F test) at 0.05 and 0.01 probability levels and, in cases of significance, linear and quadratic polynomial regression analysis was performed for the levels of electrical conductivity of irrigation water and means comparison test (Tukey) was performed for H<sub>2</sub>O<sub>2</sub> concentrations using the statistical software SISVAR (FERREIRA 2019).

## RESULTS AND DISCUSSION

There was a significant effect of water salinity levels (SL) on stomatal conductance (*g<sub>s</sub>*),

transpiration ( $E$ ) and  $\text{CO}_2$  assimilation rate ( $A$ ) of *Abelmoschus esculentus* L. plants (Table 1). Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) concentrations significantly affected all variables studied. Only the instantaneous water use efficiency ( $WUE_i$ ) of *Abelmoschus esculentus* L., at 75 days after transplanting, was not significantly influenced by the interaction between factors ( $\text{SL} \times \text{H}_2\text{O}_2$ ).

Stomatal conductance (Figure 2A) decreased with increasing water salinity in plants, regardless of  $\text{H}_2\text{O}_2$  concentration, and it is also verified that the increase in  $\text{H}_2\text{O}_2$  concentration intensified the deleterious effects of salinity on  $g_s$ . While plants irrigated with ECw of  $0.3 \text{ dS m}^{-1}$  and subjected to a concentration of  $0 \text{ }\mu\text{M}$   $\text{H}_2\text{O}_2$  obtained the highest value of  $g_s$  ( $0.387 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), plants irrigated with ECw of  $4.3 \text{ dS m}^{-1}$  and cultivated with  $\text{H}_2\text{O}_2$  at concentration of  $50 \text{ }\mu\text{M}$  had the lowest  $g_s$  ( $0.312 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), corresponding to a reduction of 19.3% ( $0.075 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) compared to the highest value of  $g_s$ .

Stomatal closure occurred because  $g_s$  is dependent on stomatal cells, that is, when plants cannot absorb water from the soil, their mechanism of functioning is affected, due to the osmotic effect. Stomatal closure is a strategy to prevent water loss to the environment and maintain high water status in the cell (DIAS et al., 2019). Decrease in  $g_s$  in plants grown under water salinity has also been observed in other crops, such as cowpea (OLIVEIRA et al., 2017) and zucchini (DANTAS et al., 2021).

$\text{H}_2\text{O}_2$  concentrations up to  $25 \text{ }\mu\text{M}$  promoted an increase in the internal  $\text{CO}_2$  concentration, regardless of the electrical conductivity of irrigation water (Figure 2B). Plants subjected to a concentration of  $25 \text{ }\mu\text{M}$  and irrigated with water of  $2.1 \text{ dS m}^{-1}$  obtained the highest value of  $C_i$  ( $166.3 \text{ }\mu\text{mol CO}_2$

$\text{m}^{-2} \text{ s}^{-1}$ ). Plants irrigated with ECw of  $2.1 \text{ dS m}^{-1}$  and subjected to  $\text{H}_2\text{O}_2$  concentration of  $25 \text{ }\mu\text{M}$  increased their  $C_i$  by 13.7% ( $20.1 \text{ }\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) compared to those cultivated with ECw of  $2.1 \text{ dS m}^{-1}$  and without  $\text{H}_2\text{O}_2$  application ( $0 \text{ }\mu\text{M}$ ). It is also verified that the increase in ECw combined with  $\text{H}_2\text{O}_2$  at concentrations above  $25 \text{ }\mu\text{M}$  reduced  $C_i$ , and a value of  $137.6 \text{ }\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  was recorded in plants irrigated with ECw of  $4.3 \text{ dS m}^{-1}$  and subjected to  $\text{H}_2\text{O}_2$  concentration of  $50 \text{ }\mu\text{M}$ .

DANTAS et al. (2021), when evaluating gas exchange in Italian zucchini cultivated under salinity of nutrient solutions in hydroponic system and foliar application of  $\text{H}_2\text{O}_2$ , also verified that the internal  $\text{CO}_2$  concentration decreased with the increase in salinity levels. Decrease in  $C_i$  is indicative that the  $\text{CO}_2$  fixed in the mesophyll cell is being consumed in the synthesis of sugars during the photosynthetic process (DIAS et al., 2019).

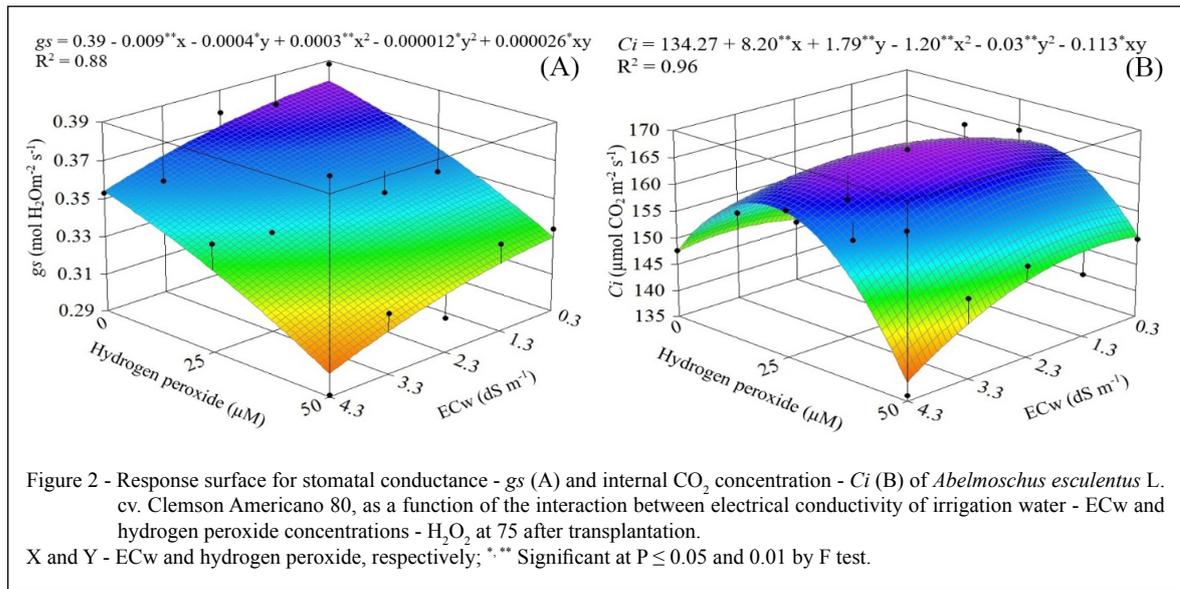
Effect similar to that observed on  $g_s$  (Figure 2A) was verified for the transpiration rate (Figure 3A), i.e., the increase in the electrical conductivity of irrigation water reduced  $E$ , regardless of  $\text{H}_2\text{O}_2$  concentration. It is also observed that the increase in  $\text{H}_2\text{O}_2$  concentration intensifies such reduction, with the lowest value of  $E$  ( $3.73 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) obtained in plants irrigated with ECw of  $4.3 \text{ dS m}^{-1}$  and subjected to  $\text{H}_2\text{O}_2$  concentration of  $50 \text{ }\mu\text{M}$ . The reduction of transpiration may be related to the low water potential in the roots caused by osmotic stress, which leads to physiological changes in plants, such as stomatal closure, to avoid dehydration of leaves due to the loss of water to the atmosphere (LIMA et al., 2016).

Foliar spraying of  $\text{H}_2\text{O}_2$  at concentration of  $22 \text{ }\mu\text{M}$  resulted in the mitigation of the deleterious

Table 1 - Summary of the analysis of variance for stomatal conductance ( $g_s$ ), transpiration ( $E$ ), internal  $\text{CO}_2$  concentration ( $C_i$ ),  $\text{CO}_2$  assimilation rate ( $A$ ) instantaneous water use efficiency ( $WUE_i$ ) and instantaneous carboxylation efficiency ( $CE_i$ ) of *Abelmoschus esculentus* L. cv. Clemson Americano 80 cultivated with saline waters and hydrogen peroxide concentrations, at 75 days after transplanting.

Sources of variation	DF	Mean squares					
		$g_s^1$	$E$	$C_i$	$A^1$	$WUE_i$	$CE_i^1$
Salinity levels (SL)	4	0.003*	0.28*	160.61 <sup>ns</sup>	16.23*	0.76 <sup>ns</sup>	0.001 <sup>ns</sup>
Linear Regression	1	0.01**	0.66**	77.76 <sup>ns</sup>	33.15*	0.008 <sup>ns</sup>	0.01**
Quadratic Regression	1	0.001 <sup>ns</sup>	0.40*	17.14 <sup>ns</sup>	14.36 <sup>ns</sup>	2.59*	0.0006 <sup>ns</sup>
Hydrogen peroxide ( $\text{H}_2\text{O}_2$ )	2	0.01**	1.53**	1070.56*	74.87**	2.27*	0.008**
Interaction (SL $\times$ $\text{H}_2\text{O}_2$ )	8	0.002*	0.35**	1172.79**	19.59**	0.62 <sup>ns</sup>	0.003**
Blocks	4	0.001 <sup>ns</sup>	0.42	520.74 <sup>ns</sup>	4.11 <sup>ns</sup>	0.51 <sup>ns</sup>	0.001 <sup>ns</sup>
CV (%)		10.35	6.92	11.16	7.83	9.48	13.88
Mean		0.34	4.33	154.08	30.76	7.13	0.20

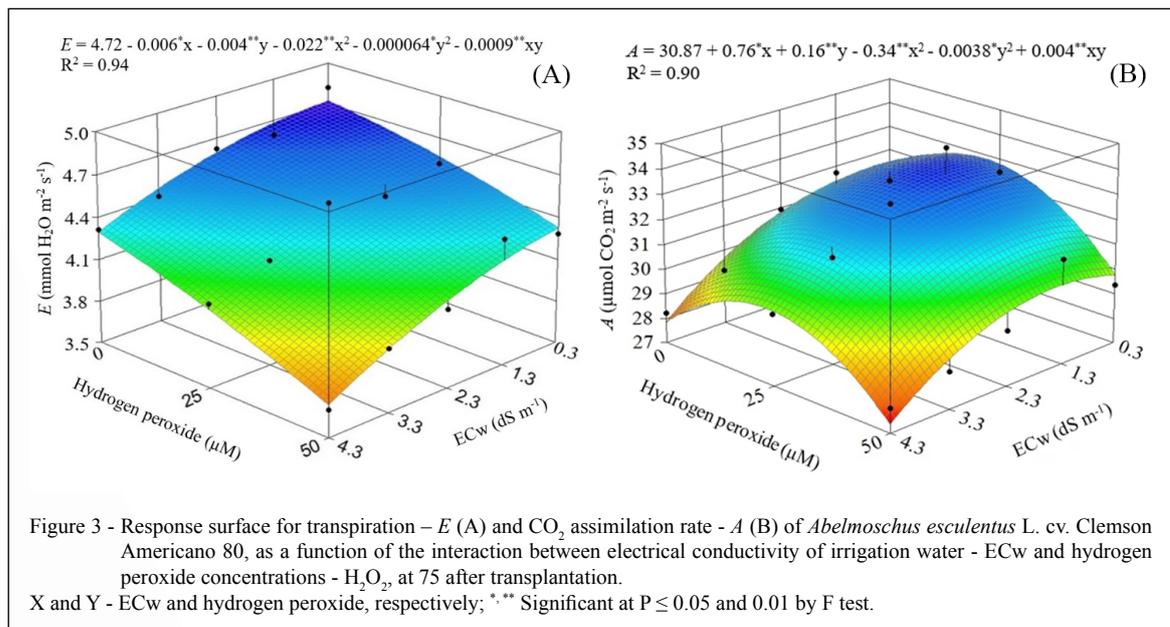
<sup>ns</sup>, \*, \*\*, respectively not significant and significant at  $P \leq 0.05$  and  $P \leq 0.01$ ; CV= coefficient of variation; DF = degrees of freedom; <sup>1</sup>data transformed into  $\sqrt{x}$ .

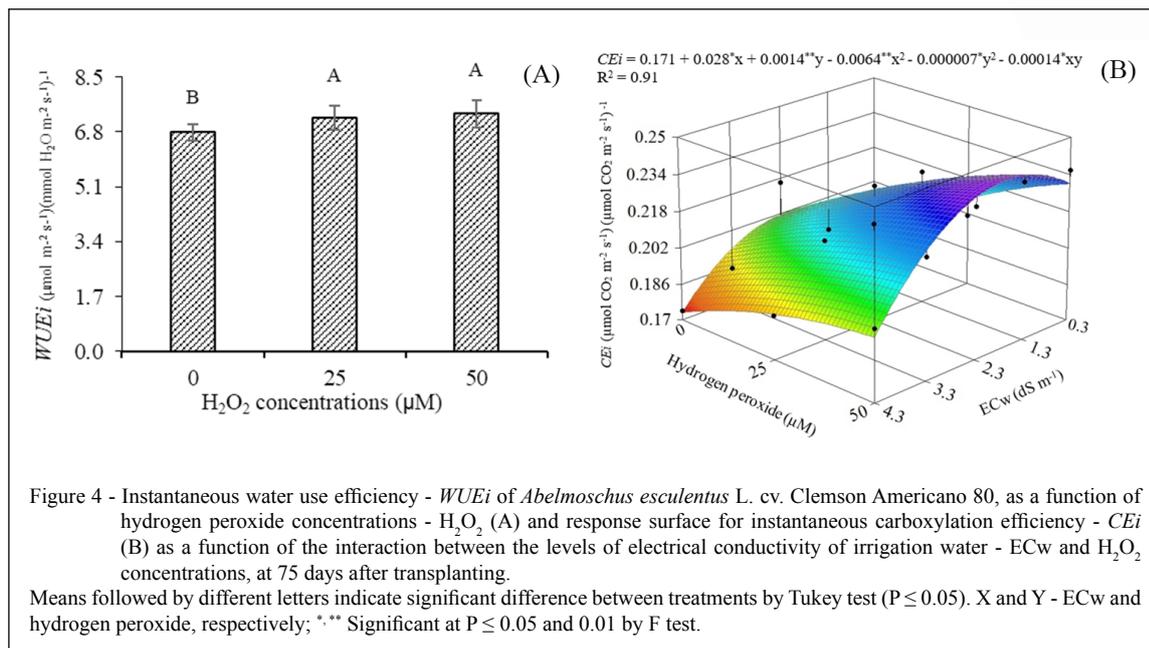


effects of salinity on the  $CO_2$  assimilation rate (Figure 3B). Plants sprayed with  $H_2O_2$  at concentration of 22  $\mu M$  and irrigated with ECw of 1.3  $dS\ m^{-1}$  reached the highest value of  $A$  (33.08  $\mu mol\ CO_2\ m^{-2}\ s^{-1}$ ), which corresponded to an increase of 5.7% (1.80  $mmol\ CO_2\ m^{-2}\ s^{-1}$ ) compared to plants irrigated with the same ECw (1.3  $dS\ m^{-1}$ ) and without  $H_2O_2$  application (0  $\mu M$ ). The beneficial effects of hydrogen peroxide may be related to the fact that it functions as a signaling molecule and acts in the regulation of

several pathways, including responses to salt stress (SEMIDA, 2016; SILVA et al., 2021). It is important to highlight that  $H_2O_2$  is a ROS and that its generation is part of the natural metabolism of plants.

For instantaneous water use efficiency (Figure 4A), plants that received foliar application of  $H_2O_2$  at 25 and 50  $\mu M$  had statically higher  $WUE_i$  compared to those grown without  $H_2O_2$  (0  $\mu M$ ). However, when comparing the  $WUE_i$  values of plants that received 25 and 50  $\mu M$ , it was observed that there was no significant





difference between them. According to CARVALHO et al. (2011), pre-exposure of plants to signaling metabolites such as  $H_2O_2$  may promote an increase in metabolites or antioxidative enzymes and, therefore, may result in better physiological performance.

Regarding the instantaneous carboxylation efficiency of plants (Figure 4B), the increase in  $H_2O_2$  concentration to  $50 \mu M$  promoted an increase in  $CE_i$ , regardless of the electrical conductivity of irrigation water, with the highest value of  $CE_i$  ( $0.241 (\mu mol CO_2 m^{-2} s^{-1})(\mu mol CO_2 m^{-2} s^{-1})^{-1}$ ) obtained in plants irrigated with  $EC_w$  of  $1.8 dS m^{-1}$  and subjected to  $H_2O_2$  concentration of  $50 \mu M$ . However, irrigation with  $EC_w$  above  $1.8 dS m^{-1}$  leads to reduction of  $CE_i$ , and the lowest  $CE_i$  value ( $0.173 (\mu mol CO_2 m^{-2} s^{-1})(\mu mol CO_2 m^{-2} s^{-1})^{-1}$ ) was recorded in plants irrigated with  $EC_w$  of  $4.3 dS m^{-1}$  and without  $H_2O_2$  application ( $0 \mu M$ ).

Reduction in  $CE_i$  is an indication that factors of non-stomatal origin also influenced the photosynthetic activity of plants, such as low activity of the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), probably due to limited availability of substrate (ATP and NADPH) for enzyme activation and regeneration (HUSSAIN et al., 2012).

There was a significant effect of the interaction between the factors ( $SL \times H_2O_2$ ) for number of fruits per plant (NFP), total production per plant (TPP), average fruit weight (AFW) and water use efficiency (WUE) (Table 2). As single factor, there was significant effect of water salinity levels on all variables studied, except average fruit length.

Hydrogen peroxide concentrations, on the other hand, promoted significant differences in plant production, average fruit weight and water use efficiency.

The increase in the electrical conductivity of irrigation water reduced the number of fruits of *Abelmoschus esculentus* L., regardless of the  $H_2O_2$  concentration (Figure 5A). However, plants subjected to  $H_2O_2$  concentration of  $22 \mu M$  and irrigated with  $EC_w$  of  $0.3 dS m^{-1}$  stood out with the highest NFP (9.23 fruits), which corresponded to an increase of 2.92% compared to those irrigated with the same level ( $0.3 dS m^{-1}$ ) and without application of  $H_2O_2$  ( $0 \mu M$ ). The lowest NFP value (3.53 fruits) was obtained in plants irrigated with  $EC_w$  of  $4.3 dS m^{-1}$  and without application of  $H_2O_2$  ( $0 \mu M$ ). The reduction in the number of fruits in *Abelmoschus esculentus* L. plants may be related to their difficulty in absorbing water and nutrients, due to the decrease in the osmotic potential of the soil solution, caused by excess salts (MENDONÇA et al., 2023). Water salinity induced stomatal closure, a situation verified by the reduction in stomatal conductance and changes in  $CO_2$  assimilation rate.

The total production per plant (Figure 5B) was also reduced by the increase in the electrical conductivity of irrigation water at all  $H_2O_2$  concentrations. However, foliar spraying of  $H_2O_2$  at the concentration of  $22 \mu M$  promoted the highest TPP ( $224.3 g$  per plant) in plants cultivated with  $EC_w$  of  $0.3 dS m^{-1}$ , decreasing from this salinity level and reaching the lowest value ( $88.4 g$  per plant) under  $EC_w$  of  $4.3 dS m^{-1}$  and  $H_2O_2$  of  $50 \mu M$ . The reduction in plant

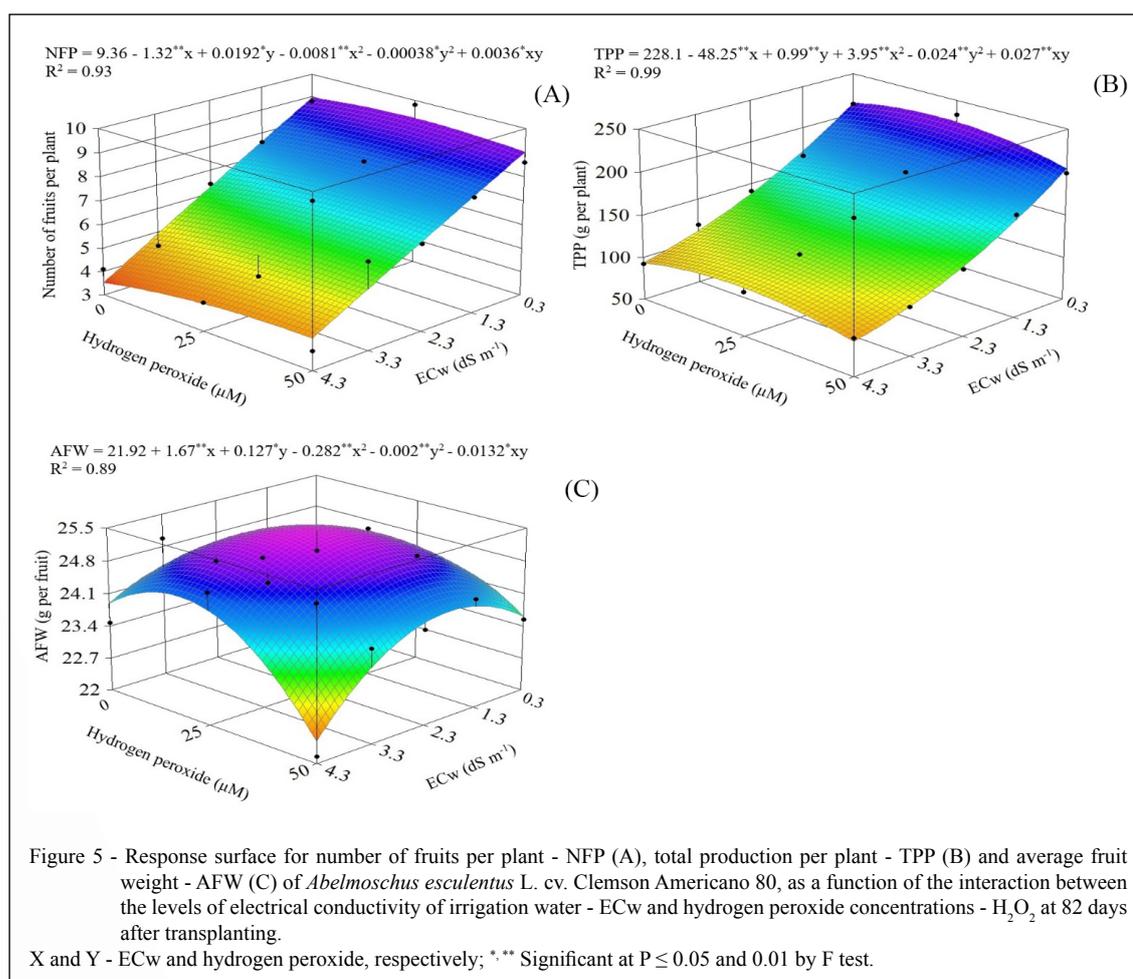
Table 2 - Summary of the analysis of variance for number of fruits per plant (NFP), total production per plant (TPP), average fruit weight (AFW), average fruit length (AFL) and water use efficiency (WUE) of *Abelmoschus esculentus* L. cv. Clemson Americano 80, cultivated with saline waters and hydrogen peroxide concentrations (H<sub>2</sub>O<sub>2</sub>), at 82 days after transplanting.

Sources of variation	---DF---	-----Mean squares-----				
		NFP	TPP	AFW	AFL	WUE
Salinity levels (SL)	4	87.053**	42299.604**	28.945*	0.789 <sup>ns</sup>	31.927**
Linear Regression	1	340.506**	165810.934**	17.967 <sup>ns</sup>	1.626 <sup>ns</sup>	124.619**
Quadratic Regression	1	0.076 <sup>ns</sup>	549.238 <sup>ns</sup>	8.178 <sup>ns</sup>	0.016 <sup>ns</sup>	1.675 <sup>ns</sup>
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	2	0.280 <sup>ns</sup>	3669.260**	62.634**	1.428 <sup>ns</sup>	4.231**
Interaction (SL × H <sub>2</sub> O <sub>2</sub> )	8	1.113**	3300.000**	32.922**	1.024 <sup>ns</sup>	4.011**
Blocks	4	0.886 <sup>ns</sup>	842.520*	7.422 <sup>ns</sup>	1.387 <sup>ns</sup>	1.149 <sup>ns</sup>
CV (%)		9.69	10.51	14.56	9.41	12.64
Mean		6.24	140.92	22.84	12.34	5.45

<sup>ns</sup>, \*\*, respectively not significant and significant at P ≤ 0.05 and ≤ 0.01; CV = coefficient of variation.

production under salt stress conditions is also related to energy diversion to maintain metabolic activities, due to the restriction in the absorption of water and nutrients caused by osmotic and ionic stress, resulting

from the high concentration of salts in the soil solution, especially Na<sup>+</sup> and Cl<sup>-</sup> ions, disorganization of the membrane system and production of reactive oxygen species (LUCENA et al., 2012).



Foliar spraying of  $H_2O_2$  at a concentration of 22  $\mu M$  resulted in the mitigation of the deleterious effects of salinity on the average fruit weight (Figure 5C). Plants sprayed with  $H_2O_2$  at the concentration of 22  $\mu M$  and irrigated with ECw of 2.5  $dS m^{-1}$  reached the highest value of AFW (25.4 g per fruit), which corresponded to an increase of 4.5% (1.1 g per fruit) compared to plants irrigated with the same ECw (2.5  $dS m^{-1}$ ) and without application of  $H_2O_2$  (0  $\mu M$ ). The increase in AFW may be associated with the role of hydrogen peroxide as a signaling molecule under biotic and abiotic stresses. In addition,  $H_2O_2$  acts by inducing the antioxidant enzyme defense system when applied at low concentrations, attenuating the deleterious effects of salinity (PETROV & BREUSEGEM, 2012).

The water use efficiency of plants was negatively affected by the increase in electrical conductivity of irrigation water (Figure 6). Nevertheless, the regression equation showed that the  $H_2O_2$  concentration of 12  $\mu M$  was able to promote an increase in WUE, with the maximum value of 6.51  $g L^{-1}$  obtained in plants irrigated with ECw of 0.3  $dS m^{-1}$  and cultivated with  $H_2O_2$  concentration of 12  $\mu M$ .

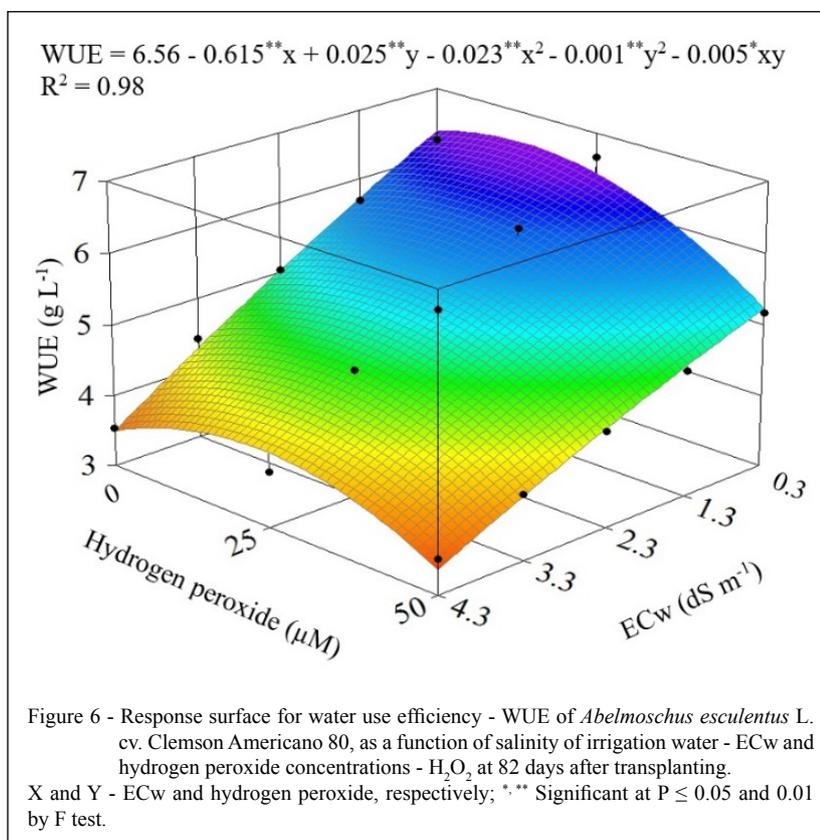
The lowest WUE (3.49  $g L^{-1}$ ) was recorded in plants irrigated with ECw of 4.3  $dS m^{-1}$  and without  $H_2O_2$  application (0  $\mu M$ ).

Thus, it can be affirmed that the plants had a lower capacity to convert the volume of water actually consumed into photoassimilates, which was observed by the accumulation of biomass, due to higher energy expenditure to maintain the biosynthesis of osmotic solutes and the generation of energy necessary for this biosynthesis, in addition to other important processes for the osmotic adjustment of plants, which contribute to their absorption of water and nutrients (LIMA et al., 2020).

## CONCLUSION

Irrigation with ECw above 0.3  $dS m^{-1}$  negatively affects stomatal conductance, transpiration, number of fruits, total production and water use efficiency of *Abelmoschus esculentus* L. cv. Clemson Americano 80.

Hydrogen peroxide at concentration of 22  $\mu M$  mitigates the effects of salt stress on the  $CO_2$  assimilation rate, number of fruits, average



fruit weight and total production of *Abelmoschus esculentus* L. cv. Clemson Americano 80.

Water use efficiency is favored by the application of hydrogen peroxide at concentration of 12  $\mu\text{M}$ , especially in plants irrigated with ECw of 0.3  $\text{dS m}^{-1}$ .

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

## AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

## REFERENCES

- ANDRADE, E. M., G. e tal. Production and postharvest quality of yellow passion fruit cultivated with saline water and hydrogen peroxide. **AIMS Agriculture and Food**, v.4, n.4, p.907-920, 2019. Available from: <<http://dx.doi.org/10.3934/agrfood.2019.4.907>>. Accessed: Dec. 18, 2021. doi: 10.3934/agrfood.2019.4.907.
- ANDRADE, E. M., G. et al. Hydrogen peroxide as attenuator of salt stress effects on the physiology and biomass of yellow passion fruit. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.26, n.8, p.571-578, 2022. Available from: <<https://doi.org/10.1590/1807-1929/agriambi.v26n8p571-578>>. Accessed: Jun. 04, 2023. doi: 10.1590/1807-1929/agriambi.v26n8p571-578.
- CARVALHO, F. E. L. et al. Salt stress acclimation in rice plants induced by  $\text{H}_2\text{O}_2$  pretreatment. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.15, n.4, p.416-423, 2011. Available from: <<https://doi.org/10.1590/S1415-43662011000400014>>. Accessed: Dec. 18, 2021. doi: 10.1590/S1415-43662011000400014.
- DANTAS, M. V. et al. Summer squash morphophysiology under salt stress and exogenous application of  $\text{H}_2\text{O}_2$  in hydroponic cultivation. **Comunicata Scientiae**, v.12, n.1, e3464, 2021. Available from: <<https://doi.org/10.14295/cs.v12.3464>>. Accessed: Dec. 15, 2021. doi: 10.14295/cs.v12.3464.
- DIAS, A. S. et al. Gas exchanges, quantum yield and photosynthetic pigments of west indian cherry under salt stress and potassium fertilization. **Revista Caatinga**, v.32, n.2, p.429-439, 2019. Available from: <<https://doi.org/10.1590/1983-21252019v32n216rc>>. Accessed: Dec. 15, 2021. doi: 10.1590/1983-21252019v32n216rc.
- DURAZZO, A. et al. *Abelmoschus esculentus* (L.): Bioactive components' beneficial properties — Focused on antidiabetic role - For sustainable health applications. **Molecules**, v.24, n.1, p.1-13, 2019. Available from: <<https://doi.org/10.3390/molecules24010038>>. Accessed: Dec. 10, 2021. doi: 10.3390/molecules24010038.
- FERREIRA, D. F. SISVAR: A computer analysis system to fixed effects split plot type designs. **Revista Brasileira de Biometria**, v.37, n.4, p.529-535, 2019. Available from: <<https://doi.org/10.28951/rbb.v37i4.450>>. Accessed: Dec. 10, 2021. doi: 10.28951/rbb.v37i4.450.
- HUSSAIN, S. et al. Physiological analysis of salt stress behavior of citrus species and genera: Low chloride accumulation as an indicator of salt tolerance. **South African Journal of Botany**, v.81, n.1, p.103-112, 2012. Available from: <<https://doi.org/10.1016/j.sajb.2012.06.004>>. Accessed: Dec. 8, 2021. doi: 10.1016/j.sajb.2012.06.004.
- IBGE. Instituto Brasileiro de Geografia. **Censo agropecuário 2017: resultados preliminares**. 2017. Available from: <<https://sidra.ibge.gov.br/tabela/6954>>. Accessed: Nov. 18, 2021.
- ISLAM, M. T. Phytochemical information and pharmacological activities of Okra (*Abelmoschus esculentus*): A literature-based review. **Phytotherapy Research**, v.33, n.1, p.72-80, 2019. Available from: <<https://doi.org/10.1002/ptr.6212>>. Accessed: Jan. 2, 2022. doi: 10.1002/ptr.6212.
- LIMA, G. S. de et al. Irrigação com águas salinas e aplicação de prolina foliar em cultivo de pimentão 'All Big'. **Comunicata Scientiae**, v.7, n.4, p.513-522, 2016. Available from: <<https://doi.org/10.14295/CS.v7i4.1671>>. Accessed: Jan. 12, 2022. doi: 10.14295/CS.v7i4.1671.
- LIMA, G. S. de et al. Saline water irrigation and nitrogen fertilization on the cultivation of colored fiber cotton. **Revista Caatinga**, v.31, n.1, p.151-160, 2018. Available from: <<https://doi.org/10.1590/1983-21252018v31n118rc>>. Accessed: Jan. 3, 2022. doi: 10.1590/1983-21252018v31n118rc.
- LIMA, G. S. de et al. Gas exchanges, growth and production of okra cultivated with saline water and silicon fertilization. **Semina: Ciências Agrárias**, v.41, n.5, p.1937-1950, 2020. Available from: <<http://dx.doi.org/10.5433/1679-0359.2020v41n5supl1p1937>>. Accessed: Feb. 01, 2022. doi: 10.5433/1679-0359.2020v41n5supl1p1937.
- LUCENA, C. C. de et al. Efeito do estresse salino na absorção de nutrientes em mangueira. **Revista Brasileira de Fruticultura**, v.34, n.1, p.297-308, 2012. Available from: <<https://doi.org/10.1590/S0100-29452012000100039>>. Accessed: Feb. 01, 2022. doi: 10.1590/S0100-29452012000100039.
- MENDONÇA, A. J. T. et al. Gas exchange, photosynthetic pigments, and growth of hydroponic okra under salt stress and salicylic acid. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.27, n.9, p.673-681, 2023. Available from: <<http://dx.doi.org/10.1590/1807-1929/agriambi.v27n9p673-681>>. Accessed: Jun. 04, 2023. doi: 10.1590/1807-1929/agriambi.v27n9p673-681.
- MENDONÇA, A. J. T. et al. Salicylic acid modulates okra tolerance to salt stress in hydroponic system. **Agriculture**, v.12, n.10, e1687, 2022. Available from: <<https://doi.org/10.3390/agriculture12101687>>. Accessed: Jun. 04, 2023. doi: 10.3390/agriculture12101687.
- MORAIS, M. B. D. et al. Antioxidative metabolism in sugarcane (Poaceae) varieties subjected to water and saline stress. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.24, n.11, p.776-782, 2020. Available from: <<https://doi.org/10.1590/1807->

- 1929/agriambi.v24n11p776-782>. Accessed: Feb. 01, 2022. doi: 10.1590/1807-1929/agriambi.v24n11p776-782.
- NOVAIS, R. F. et al. Ensaio em ambiente controlado. In: OLIVEIRA, A. J. (ed). **Métodos de pesquisa em fertilidade do solo**. Brasília: Embrapa-SEA. p.189-253. 1991.
- OLIVEIRA, W. J. de et al. Leaf gas exchange in cowpea and CO<sub>2</sub> efflux in soil irrigated with saline water. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.21, n.1, p.32-37, 2017. Available from: <<https://doi.org/10.1590/1807-1929/agriambi.v21n1p32-37>>. Accessed: Jan. 23, 2022. doi: 10.1590/1807-1929.
- PETROV, V. D.; BREUSEGEM, F. V. Hydrogen peroxide: a central hub for information flow in plant cell. **AoB Plants**, v.2012, n.1, p.1-13, 2012. Available from: <<https://doi.org/10.1093/aobpla/pls014>>. Accessed: Jan. 23, 2022. doi: 10.1093/aobpla/pls014.
- RAMOS, J. G. et al. Foliar application of H<sub>2</sub>O<sub>2</sub> as salt stress attenuator in 'BRS Rubi do Cerrado' sour passion fruit. **Semina: Ciências Agrárias**, v.42, n.4, p.2253-2270, 2021. Available from: <<http://dx.doi.org/10.5433/1679-0359.2021v42n4p2253>>. Accessed: Jan. 23, 2022. doi: 10.5433/1679-0359.2021v42n4p2253.
- RHOADES, J. D. et al. **Uso de águas salinas para produção agrícola**. Campina Grande: UFPB, 2000.
- SEMIDA, W. M. Hydrogen peroxide alleviates salt stress in two onion (*Allium cepa* L.) cultivars. **American-Eurasian Journal of Agricultural & Environmental Sciences**, v.16, n.2, p.294-301, 2016. Available from: <<http://dx.doi.org/10.5829/idosi.ajeaes.2016.16.2.12864>>. Accessed: Jan. 13, 2022. doi: 10.5829/idosi.ajeaes.2016.16.2.12864.
- SILVA, A. A. R. da et al. Gas exchanges and growth of passion fruit seedlings under salt stress and hydrogen peroxide. **Revista Pesquisa Agropecuária Tropical**, v.49, n.1, e55671, 2019. Available from: <<https://doi.org/10.1590/1983-40632019v4955671>>. Accessed: Jan. 13, 2022. doi: 10.1590/1983-40632019v4955671.
- SILVA, A. A. R. da et al. Hydrogen peroxide in the acclimation of yellow passion fruit seedlings to salt stress. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.25, n.2, p.116-123, 2021. Available from: <<https://doi.org/10.1590/1807-1929/agriambi.v25n2p116-123>>. Accessed: Jan. 13, 2022. doi: 10.1590/1807-1929/agriambi.v25n2p116-123.
- SOARES, L. A. dos A. et al. Growth and fiber quality of colored cotton under salinity management strategies. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.25, n.2, p.132-138, 2018. Available from: <<https://doi.org/10.1590/1807-1929/agriambi.v22n5p332-337>>. Accessed: Jan. 23, 2022. doi: 10.1590/1807-1929/agriambi.v22n5p332-337.
- SOARES, L. A. dos A. et al. Phytomass and production components of colored cotton under salt stress in different phenological stages. **Revista Brasileira de Engenharia Agrícola e Ambiental**, v.25, n.2, p.132-138, 2021. Available from: <<https://doi.org/10.1590/1807-1929/agriambi.v25n2p132-138>>. Accessed: Jan. 23, 2022. doi: 10.1590/1807-1929/agriambi.v25n2p132-138.
- SOARES, L. A. dos A. et al. Preservation by lactic fermentation and physicochemical characterization of okra produced underwater salinity and potassium fertilization. **Semina: Ciências Agrárias**, v.41, n.6, p.2495-2508, 2020. Available from: <<https://doi.org/10.5433/1679-0359.2020v41n6p2495>>. Accessed: Jan. 23, 2022. doi: 10.5433/1679-0359.2020v41n6p2495.
- TEIXEIRA, P. C. et al. **Manual de métodos de análise de solo**. Rio de Janeiro, Embrapa. 573p. 2017.