Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2021v42n4p2253

Foliar application of H_2O_2 as salt stress attenuator in 'BRS Rubi do Cerrado' sour passion fruit

Aplicação foliar de H_2O_2 como atenuante do estresse salino em maracujazeiro-azedo 'BRS Rubi do Cerrado'

Jailton Garcia Ramos[1]*; Geovani Soares de Lima[2]; Vera Lucia Antunes de Lima[2]; Francisco Jean da Silva Paiva[1]; Kheila Gomes Nunes[3]; Mariana de Oliveira Pereira[2]; Pedro Dantas Fernandes[2]; Luciano Marcelo Fallé Saboya[2]

Highlights

Reduction in gas exchange is related to factors of non-stomatal origin.
Irrigation by 3.0 dS m^{-1} salinity water is not viable for passion fruit development.
Using H_2O_2 at concentration of 15 μM is viable as salt stress attenuator.

Abstract

Irrigation with saline water causes a reduction in yield, especially in semi-arid regions. Cultivation strategies have been developed to mitigate salt stress on plants, such as the use of hydrogen peroxide. The objective of this study was to evaluate the attenuating effect of hydrogen peroxide on the gas exchange and growth of 'BRS Rubi do Cerrado' sour passion fruit cultivated under irrigation with saline water. The design was completely randomized in split-plot plots, with water salinity levels ECw (0.6, 1.2, 1.8, 2.4, and 3.0 dS m^{-1}) considered the plots and the concentrations of hydrogen peroxide H_2O_2 (0, 15, 30, and 45 μM) considered the subplots, with three replicates. Gas exchange (stomatal conductance, transpiration, CO_2 assimilation rate, intercellular CO_2 concentration, instantaneous water use efficiency, and instantaneous carboxylation efficiency), and absolute and relative growth rates in stem diameter were evaluated. An increase in irrigation water salinity from 0.6 dS m^{-1} reduced gas exchange, and exogenous application of hydrogen peroxide did not promote a significant effect on gas exchange. However, foliar application of hydrogen peroxide at 15 μM increased the growth of 'BRS Rubi do Cerrado' sour passion fruit.

Key words: *Passiflora edulis.* Salt stress. Hydrogen peroxide. Acclimatization.

[1] Doctoral Scholar of Agricultural Engineering Course, Universidade Federal de Campina Grande, UFCG, Technology and Natural Resources Center, Campina Grande, PB, Brazil. E-mail: E-mail: jgramos2019@gmail.com; je.an_93@hotmail.com

[2] Profs., Post Graduate Program in Agricultural Engineering, Center of Technology and Natural Resources, UFCG, Campina Grande, PB, Brazil. E-mail: geovani.soares@pq.cnpq.br; antuneslima@gmail.com; marianapereira.agri@gmail.com pedrodantasfernandes@gmail.com; lsaboya@hotmail.com

[3] Undergraduate student in Agricultural Engineering, UFCG, Technology and Natural Resources Center, Campina Grande, PB, Brazil. E-mail: kheilagomesnunes@gmail.com

* Author for correspondence

Received: Nov. 24, 2020 - Approved: Apr. 16, 2021

Resumo

A irrigação com águas salinas causa redução na produtividade, principalmente nas regiões semiáridas. Estratégias de cultivo vêm sendo desenvolvidas, a exemplo uso de peróxido de hidrogênio para mitigar o estresse salino sobre as plantas. Objetivou-se avaliar o efeito atenuante do peróxido de hidrogênio sobre as trocas gasosas e o crescimento do maracujazeiro-azedo 'BRS Rubi do Cerrado' cultivado sob irrigação com águas salinas. O delineamento foi inteiramente casualizados em parcelas subdivididas, sendo os níveis de salinidade da água - CEa (0,6, 1,2, 1,8, 2,4 e 3,0 dS m^{-1}) consideradas as parcelas, e as concentrações de peróxido de hidrogênio – H_2O_2 (0, 15, 30 e 45 μM) as subparcelas, com três repetições. Foram avaliadas as trocas gasosas (condutância estomática, transpiração, taxa de assimilação de CO_2, concentração intercelular de CO_2, eficiência instantânea no uso da água e eficiência instantânea de carboxilação), e a taxa de crescimento absoluto e relativo do caule. O aumento na salinidade da água de irrigação a partir da salinidade de 0,6 dS m^{-1} reduziu as trocas gasosas; a aplicação exógena de peróxido de hidrogênio não promoveu efeito significativos sob as trocas gasosas. A aplicação foliar de 15 μM de peróxido de hidrogênio aumentou o crescimento do maracujazeiro-azedo 'BRS Rubi do Cerrado'.
Palavras-chave: *Passiflora edulis* Sims. Estresse salino. Peróxido de hidrogênio. Aclimatação.

Introduction

Sour passion fruit (*Passiflora edulis* Sims) is among the fruit crops of the highest economic relevance in Brazil, especially the South, Southeast, and Northeast regions. The Northeast region occupies a prominent position, representing about 62.31% (375.54 t) of the national production, with the state of Bahia being the largest producer with an area of 15,704 ha and production of 160.90 t. The state of Paraíba occupies the 13th place in the ranking of harvested area (1.045 ha) and production of 10.544 t (Instituto Brasileiro de Geografia e Estatística [IBGE], 2018), a position that is related to the edaphoclimatic conditions of the region, such as inadequate water and soil management.

Although the Northeast region is the largest producer of passion fruit, factors such as temporal and spatial variability of rainfall, high evapotranspiration, and low relative humidity limit the genetic potential of the crop under semi-arid conditions, as these factors directly affect the quantity and quality of the water available for irrigation (Mudo et al., 2020).

In this region, the occurrence of waters with high concentrations of salts is common, standing out as one of the main obstacles to obtaining satisfactory production, due to changes in soil physical, chemical, and hydraulic attributes, such as the action of specific ions (sodium, chloride, and boron) on the gas exchange and growth of plants (Sá et al., 2020).

The main effects of salinity on plants refer to those of osmotic nature and ionic, nutritional, and hormonal imbalance, causing changes in morphology, physiology, and metabolism, which lead to severe losses in agricultural production (Oliveira et al., 2015; Santos et al., 2020).

According to Lima et al. (2015), excess salts in irrigation water can cause changes in the permeability of cell membranes and physiological and biochemical functions of plants, leading to osmotic stress, which results in disturbances in water relations, changes in the absorption and utilization of essential nutrients, as well as the accumulation of toxic ions (sodium and chlorides mainly) in chloroplasts, regardless of the nature of salts.

Considering that the sour passion fruit crop is classified as sensitive to salinity, with a threshold irrigation water electrical conductivity level of 1.3 dS m^{-1} (Ayers & Westcot, 1985), the search for alternatives capable of alleviating the deleterious effects of salt stress should be considered of fundamental importance for the expansion of irrigated fruit growing in the semi-arid region of northeastern Brazil.

Among the strategies, foliar application of hydrogen peroxide (H_2O_2) at low concentrations has been shown to be efficient as an attenuating agent of the deleterious effects of salts on crops (Caverzan et al., 2016; Andrade et al., 2019; Silva et al., 2019; Santos et al., 2020).

Hydrogen peroxide is a reactive oxygen species (ROS) capable of oxidizing membrane lipids, denaturing proteins, and reacting with DNA, causing mutations and acclimatization of the plant to abiotic stress (Bhatla & Lal, 2016). It is considered a signaling molecule because, through the process of previous exposure of an individual to a certain type of stress, H_2O_2 induces metabolic changes responsible for increasing its tolerance to exposure to stress from the production of enzymes that protect crops from the negative effects of irrigation water salinity (Kao, 2014).

Because of the above, the objective of this study was to evaluate the exogenous application of hydrogen peroxide as an attenuator of salt stress on the gas exchange and growth of 'BRS Rubi do Cerrado' sour passion fruit.

Material and Methods

The experiment was conducted between May 2019 and January 2020 under conditions of an arched greenhouse with a length of 30 m, a width of 21 m, and a ceiling height of 3.0 m with a 150-micron low-density polyethylene cover, belonging to the Academic Unit of Agricultural Engineering UAEAg of the Federal University of Campina Grande UFCG, Campina Grande, Paraíba, Brazil (7°15′18″ S, 35°52′28″ W and an average altitude of 550 m).

The treatments resulted from the combination of two factors: five levels of electrical conductivity of irrigation water - ECw (0.6, 1.2, 1.8, 2.4, and 3.0 dS m^{-1}) and four concentrations of hydrogen peroxide - H_2O_2 (0; 15; 30 and 40 μM). The experimental design was completely randomized in split plots, with ECw levels considered plots and H_2O_2 concentrations considered subplots, with three replicates. The ECw levels and H_2O_2 concentrations were established based on a study conducted by Andrade (2018).

Seeds of 'BRS Rubi do Cerrado' sour passion fruit obtained from the orchard of the Federal Institute of Science and Technology of Paraíba IFPB, Sousa campus, were used in the experiment. 'BRS Rubi do Cerrado' passion fruits have a predominantly red, purplish, or yellowish peel, weighing from 120 to 300 g (average 170 g per fruit), with a rounded shape, a soluble solids content of 13 to 15° Brix (average 14° Brix), juice yield around 35%, higher resistance to transport, strong yellow color in the pulp (higher ascorbic acid content), and resistance to diseases such as those caused by viruses, bacteria, and scab (Empresa Brasileira de Pesquisa Agropecuária [EMBRAPA], 2014).

Seedlings were prepared in plastic bags with a capacity of 3 dm^3, filled with a substrate in the proportion of 84% of soil, 15% of washed sand, and 1% of organic matter (earthworm humus). The moisture content of the substrate was raised to the level corresponding to its maximum holding capacity and then sowing was performed by placing 4 seeds per bag at 3 cm depth. The seedlings were irrigated daily based on the principle of weighing lysimetry (Bernardo, Soares, & Mantovani, 2009). At 70 days after emergence, when tendrils emerged, the seedlings were transplanted to the drainage lysimeters.

The pots were adapted as drainage lysimeters with 250 L capacity; each lysimeter was drilled at the base and connected to a drain, above which a nonwoven geotextile (Bidim) was placed to prevent clogging by soil. The end of each drain outside the pot was connected to a plastic container to collect the drained water, estimating the water consumption of plants based on the balance of the volume applied in the irrigation minus the volume drained 24 h after irrigation.

The lysimeters were filled with a 0.5 kg layer of crushed stone number 0, followed by 250 kg of soil material classified as *Luvissolo crômico* (Alfisol) (EMBRAPA, 2018) collected in the municipality of Alagoa Nova, PB. The soil was collected at 0–30 cm depth (A horizon), before the experiment, and its chemical and physical attributes (Table 1) were determined according to the methodology proposed by (Teixeira, Donagemma, Fontana, & Teixeira, 2017).

The levels of electrical conductivity of irrigation water were prepared in such a way to have an equivalent proportion of 7:2:1 in the Na:Ca:Mg ratio, from the salts NaCl, $CaCl_2 \cdot 2H_2O$, and $MgCl_2 \cdot 6\ H_2O$, respectively, adjusting them to the concentrations of the available public-supply water, which is the proportion of salts commonly found in the water bodies of the semi-arid region. Irrigation waters were prepared considering the relationship between ECw and the concentration of salts according to Richards (1954), as shown in Eq. 1:

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

where C = Concentration of salts to be applied ($mmol_c\ L^{-1}$) and

ECw = Electrical conductivity of water ($dS\ m^{-1}$).

Table 1
Chemical and physical-hydraulic characteristics of the soil used in the experiment, before the application of the treatments

Chemical characteristics									
pH (H_2O) (1:2, 5)	OM (dag kg^{-1})	P (mg kg^{-1})	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+} + H^+	ESP (%)	ECse (dS m^{-1})
			....		(cmolc kg^{-1})	....			
5.90	1.36	6.80	0.22	0.16	2.60	3.66	1.93	1.87	1.0
Physical-hydraulic									
Size fraction (g kg^{-1})			Textural class	Water (kPa)		AW	Total porosity (%)	AD	PD
Sand	Silt	Clay		33.42	1519.5	....		(kg dm^{-3})	
732.9	142.1	125.0		....	(dag kg^{-1})				
732.9	142.1	125.0	SL	11.98	4.32	7.66	47.74	1.39	2.66

OM - organic matter: Walkley-Black Wet Digestion; Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted with 1 M NH_4OAc at pH 7.0; Al^{3+} and H^+ extracted with 0.5 M CaOAc at pH 7.0; SL - sandy loam; AW - available water; AD - apparent density; PD - particle density.

Irrigation with saline water was performed 15 days after transplantation; during this period, the plants were irrigated with public-supply water of low electrical conductivity (0.6 dS m^{-1}), whose chemical characteristics are presented in Table 2.

Table 2
Chemical characteristics of water of lowest electrical conductivity used in the experiment

EC	pH	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cl^{-}	CO_3^-	HCO_3^-	SAR
(dS m^{-1})	-				($mmol_c$ L^{-1})				($mmol_c$ L^{-1})$^{0.5}$
0.40	7.67	0.69	1.34	1.19	0.13	1.50	0.10	1.53	1.10

EC—electrical conductivity; CO_3^{--} calcium carbonate; HCO_3^{--} sodium bicarbonate; SAR-sodium adsorption ratio.

After transplanting, irrigation was performed daily, applying in each lysimeter the volume corresponding to that obtained by the water balance, and the volume of water to be applied to the plants was determined by Eq. 2:

$$VI=\frac{(Va\text{-}Vd)}{(1\text{-}LF)} \quad . \qquad (2)$$

where VI = Volume of water to be used in the next irrigation event (mL),

Va = volume applied in the previous irrigation event (mL),

Vd = volume drained (mL), and

LF = leaching fraction of 0.15.

The concentrations of H_2O_2 were prepared in deionized water in each application event since its degradation in the presence of light is rapid; at the end of each application, the volume used in each treatment was quantified. During the crop development period until the beginning of flowering, each plant received on average 63.75 mL of hydrogen peroxide solution in each application. To avoid the drift of H_2O_2 solution, the plants of each treatment were isolated using plastic curtains.

The hydrogen peroxide concentrations were applied by foliar spraying on the adaxial and abaxial sides, at 15-day intervals, starting at 15 days after the beginning of irrigation with salinized water until the flowering stage, using a manual sprayer for PET bottle, with an adjustable1 cm metal conical nozzle, service pressure of 300 Psi and flow rate of 1.1 L min^{-1}.

The applications were performed on the leaves from 17h00, due to the lower incidence of light. The spacing adopted was 2.20 m between rows and 1.50 m between plants, using the vertical trellis system with smooth wire Nº14 installed inside the greenhouse, at 2.40 m height from the floor and 1.60 m from the soil of the lysimeter.

When the plants reached 10 cm above the trellis, their apical bud was pruned, aiming to stimulate the production of secondary branches, which were grown one to each side up to the length of 1.10 m, and a new pruning was performed for the production of tertiary branches and, consequently, formation of the curtains, that is, the productive branches. The tertiary branches were pruned at 30 cm height from the soil. After pruning, the Bordeaux mixture was applied to heal the injuries and prevent diseases (Mazaro, Mongnabosco, Citadin, Paulus, & Gouvea, 2013).

Basal fertilization was performed according to the recommendation of São José et al. (2000), applying 250 g of single superphosphate (18.9% P_2O_2) and 100 g of potassium chloride (60% K_2O), monthly until the beginning of flowering. After reaching this stage of development, nitrogen and potassium fertilization were performed monthly, according to the methodology proposed by Santos (2001), using urea (45.9% N) and potassium chloride (60% K_2O) as sources of nitrogen and potassium, respectively.

A ratio of 1N:1K was used in the crop formation stage, taking as reference 10 g of nitrogen; from the beginning of flowering, the N dose was raised to 20 g and the K dose to 30 g, increasing the N:K ratio to 1:1.5. Micronutrient fertilization was performed according to EMBRAPA (2010) at 15 day intervals after transplanting (DAT), by spraying the plants with a solution containing 2.5 g L^{-1} of commercial fertilizer with the following characteristics: N (15%); P_2O_5 (15%); K_2O (15%); Ca (1%); Mg (1.4%); S (2.7%); Zn (0.5%); B (0.05%); Fe (0.5%); Mn (0.05%); Cu (0.5%), and Mo (0.02%).

At 120 DAT, the gas exchange parameters stomatal conductance - *gs* (mol m^{-2} s^{-1} of H_2O), transpiration - *E* (mmol m^{-2} s^{-1} of H_2O), CO_2 assimilation rate - *A* (μmol m^{-2} s^{-1}), and intercellular CO_2 concentration - *Ci* (μmol m^{-2} s^{-1}) were measured in the third fully expanded leaf counted from the apical bud using the gas exchange meter "LCPro+" (ADC BioScientific Ltda.). These variables were then used to determine the instantaneous water use efficiency - *WUEi* (*A/E*) [(μmol m^{-2} s^{-1}) (mol m^{-2} s^{-1} of $H_2O)^{-1}$) and the instantaneous carboxylation efficiency - CEi (A/Ci) [(μmol m^{-2} s^{-1}) (μmol $mol^{-1})^{-1}$].

Biomass accumulation was determined at 240 DAT. The leaves were placed in paper bags and identified, for removal of the stem. All tertiary branches were removed, leaving only the primary and secondary ones, the latter with a length of 1.5 m.

After collection, the samples were kept in a forced air circulation oven at 63 °C until they reached constant weight; after that, leaf dry biomass (LDB) and stem dry biomass (StDB) were determined, and shoot dry biomass (ShDB) was obtained by summing LDB and StDB.

Plant growth was measured between 30 and 180 DAT based on the absolute growth rate (AGR) and relative growth rate (RGR) in stem diameter, according to the methodology proposed by Benincasa (2003), as presented in Eq. 3 and 4, respectively:

$$\mathrm{AGR} = \frac{\mathrm{A2} - \mathrm{A1}}{\mathrm{T2} - \mathrm{T1}} \quad (3)$$

$$\mathrm{RGR} = \frac{\ln(\mathrm{A2}) - \ln(\mathrm{A1})}{\mathrm{T2} - \mathrm{T1}} \quad (4)$$

where: AGR = absolute growth rate (mm per day),

RGR = relative growth rate (mm mm^{-1} per day),

A2 = plant growth at time T2 (mm), A1 = plant growth at time T1 (mm) *T2 - T1* = time difference between samplings (days), and *ln* = natural logarithm.

The Shapiro-Wilk test was applied to the obtained data to verify the normality of the distribution. Then, the data were subjected to analysis of variance by F test at $p \leq 0.05$ and, when significant, linear or quadratic polynomial regression analysis was performed, using the statistical program SISVAR ESAL (Ferreira, 2019). When heterogeneity occurred in the data, verified by the values of the coefficient of variation, they were subjected to descriptive exploratory analysis, with square root transformation.

Results and Discussion

The interaction between irrigation water salinity levels and H_2O_2 concentrations did not significantly influence ($p \leq 0.01$) any of the variables evaluated at 120 days after transplanting (Table 3). Similarly, the H_2O_2 concentrations did not cause a significant effect ($p \leq 0.01$) on any of the variables measured. However, salinity levels significantly ($p \leq 0.01$) affected the intercellular CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance (*gs*), CO_2 assimilation rate (A), instantaneous water use efficiency (*WUEi*), and instantaneous carboxylation efficiency (*CEi*) of sour passion fruit.

The increase in the electrical conductivity of irrigation water resulted in a reduction of 5.54% (11.75 μmol m^{-2} s^{-1}) in the intercellular CO_2 concentration (*Ci*) at the salinity level of 1.8 dS m^{-1}, in relative terms. Moreover, there was an increase of 19.52% (48.58 μmol m^{-2} s^{-1}) when 'BRS Rubi do Cerrado' passion fruit plants were irrigated using water with a salinity level of 3.0 dS m^{-1} at 120 DAT (Figure 1A). According to the regression equation, the maximum estimated value of 204.26 μmol m^{-2} s^{-1} was verified when plants were irrigated with water of 1.34 dS m^{-1}. In relative terms, when comparing the *Ci* of plants subjected to water salinity of 3.0 dS m^{-1} to the value of those subjected to the lowest ECw level (0.6 dS m^{-1}), there was an increase of 36.83 μmol m^{-2} s^{-1}.

The increase in intercellular CO_2 concentration may be related to a reduction in CO_2 assimilation rate, since RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase) has a lower CO_2 consumption, indicating that carbon was not efficiently captured by PEP-carboxylase (phosphoenolpyruvate carboxylase), probably due to the low availability of substrate (ATP and NADPH) for activation and regeneration of the enzyme from the photochemical phase of photosynthesis, leading to the reduction of CO_2 carboxylation and increase in its internal concentration (Sá et al., 2017). Figueiredo et al. (2019), when evaluating the physiological responses of mulungu under saline conditions (ECw ranging from 0.5 to 9.0 dS m^{-1}) and application of salicylic acid, observed that the increase in salinity levels promoted an increase in the internal CO_2 concentration and stated that this effect was related to lower consumption of CO_2 by the RuBisCO enzyme.

Table 3
Summary of the analysis of variance for intercellular CO_2 concentration (*Ci*), transpiration (*E*), stomatal conductance (*gs*), CO_2 assimilation rate (*A*), instantaneous water use efficiency (*WUEi*) and instantaneous carboxylation efficiency (*CEi*) of 'BRS Rubi do Cerrado' sour passion fruit irrigated with saline waters and under foliar application of hydrogen peroxide, 120 days after transplanting (DAT)

Source of variation	DF	Mean squares					
		Ci	*E*	*gs*	*A*	*WUEi*	*CEi*
Salinity levels (SL)	4	4319.94**	4.13**	0.004*	67.71**	5.38**	0.001**
Linear regression	1	9955.40**	12.94**	0.006**	198.79**	2.36^{ns}	0.005**
Quadratic regression	1	6012.05*	0.038^{ns}	0.002^{ns}	62.21**	18.09**	0.0007*
Residual 1	8	612.02	0.13	0.0003	0.85	0.30	0.00006
Hydrogen peroxide (H_2O_2)	3	671.40^{ns}	0.57^{ns}	0.002^{ns}	3.19^{ns}	1.57^{ns}	0.0001^{ns}
Linear regression	1	211.68^{ns}	0.69^{ns}	0.001^{ns}	2.08^{ns}	0.004^{ns}	0.00002^{ns}
Quadratic regression	1	6.66^{ns}	0.07^{ns}	0.0006^{ns}	7.37^{ns}	1.39^{ns}	0.0004^{ns}
Interaction (SL x H_2O_2)	12	2103.27^{ns}	0.31^{ns}	0.0006^{ns}	3.57^{ns}	0.92^{ns}	0.0002^{ns}
Residual 2	30	1324.50	0.40	0.0010	4.47	1.96	0.00013
CV 1 (%)		11.16	15.72	23.22	13.07	17.39	23.27
CV 2 (%)		16.57	27.55	39.64	29.98	44.11	35.25

^{ns} Not significant; *,** significant at 0.05 and 0.01, respectively; DF-degrees of freedom; CV-coefficient of variation.

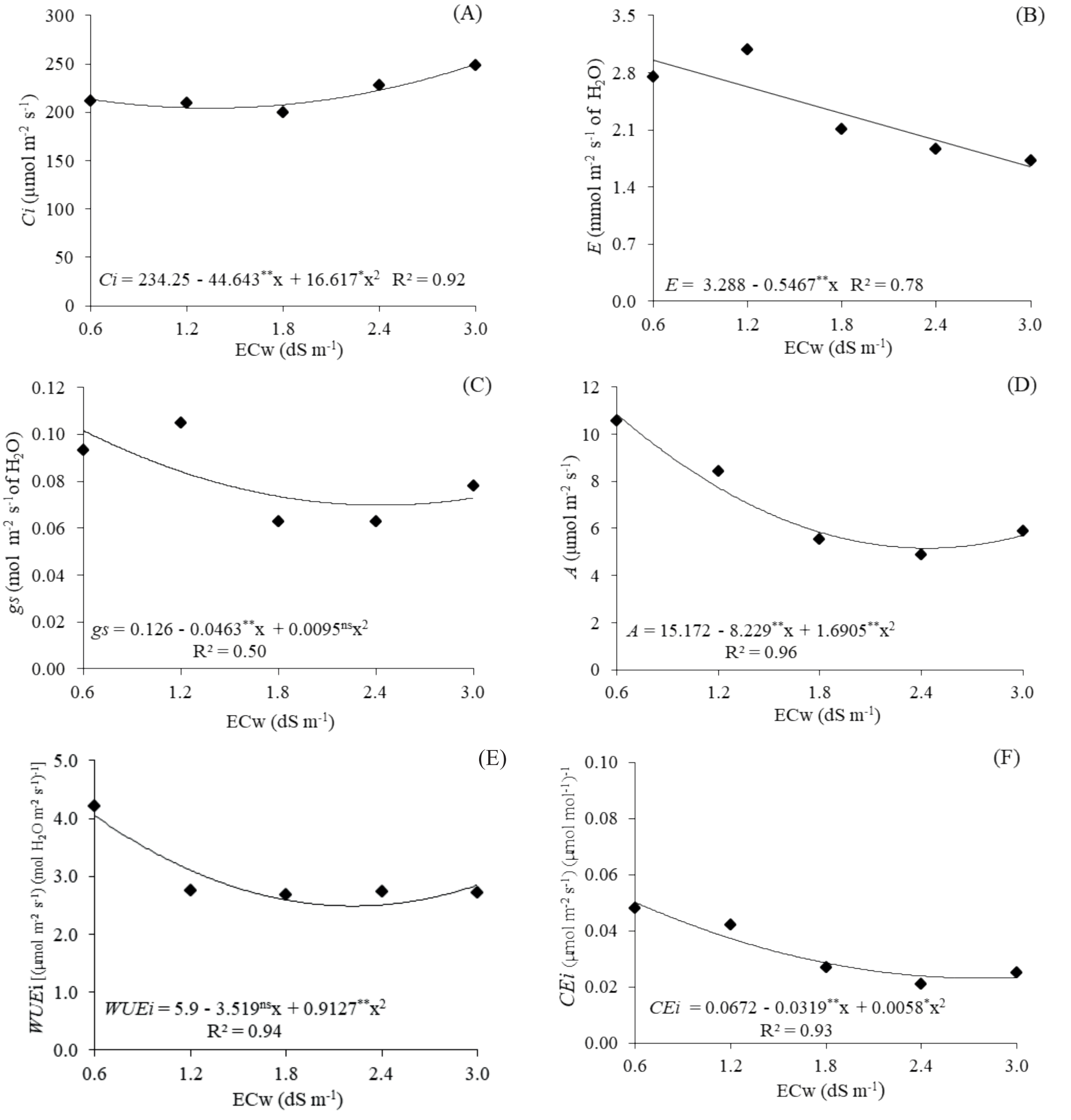

Figure 1. Intercellular CO_2 concentration- *Ci* (A), transpiration- *E* (B), stomatal conductance - *gs* (C), CO_2 assimilation rate - *A* (D), instantaneous water use efficiency - *WUEi* (E), and instantaneous carboxylation efficiency - *CEi* (F) of 'BRS Rubi do Cerrado' sour passion fruit as a function of the levels of water electrical conductivity - ECw, 120 days after transplanting. **; * Significant at 1 and 5% probability levels, respectively.

There was a decrease in the transpiration of 'BRS Rubi do Cerrado' sour passion fruit as a function of irrigation with saline water (Figure 1B), and data showed decreasing linear behavior, with a reduction of 18.66% (0.56 mmol m^{-2} s^{-1} of H_2O) per unit increase in ECw. When comparing the transpiration of plants irrigated with water of the lowest salinity level (0.6 dS m^{-1}) with that of plants under the highest salinity level (3.0 dS m^{-1}), there was a reduction of 1.03 mmol m^{-2} s^{-1} of H_2O.

The reduction in transpiration occurred due to stomatal closure and the decrease in the total soil water potential. Stomatal conductance is responsible for the influx and efflux of CO_2 and water through the stomata, and the smaller the opening, the greater the stomatal resistance, resulting in a decrease in transpiration (Sousa, Gheyi, Brito, Xavier, & Furtado, 2016). Corroborating this study Dias, Lima, Gheyi, Soares and Fernandes (2020) evaluated the gas exchange of white fiber cotton plants cv. 'BRS 368 RF' under salinity of irrigation water (0.7, 2.2, 3.7, 5.2, and 6.7 dS m^{-1}) and verified a reduction of 8.96% in their transpiration as a function of the unit increment in the electrical conductivity of irrigation water.

Irrigation with waters of different electrical conductivity levels reduced the stomatal conductance (*gs*) of sour passion fruit cv. 'BRS Rubi do Cerrado' and, according to the regression equation (Figure 1C), there was an estimated minimum value of 0.07 mol m^{-2} s^{-1} of H_2O in plants irrigated with water of 2.37 dS m^{-1}. When comparing the gs of plants irrigated with water of 3.0 dS m^{-1} to that of plants that received ECw of 0.6 dS m^{-1}, there was a reduction of 0.015 mol m^{-2} s^{-1} of H_2O. Stomatal closure is one of the first defense mechanisms of the plant to maintain its cell water potential, aimed at reducing the excessive absorption of salts from the soil. According to Y. L. Melo et al. (2020), high salt contents cause deleterious effects on stomatal opening due to the increase in the resistance to CO_2 diffusion resulting from the reduction in the stomatal opening, or limitations through inhibition of metabolism in biochemical reactions.

As observed for gs (Figure 1C), the CO_2 assimilation rate *A* of sour passion fruit (Figure 1D) decreased quadratically, and its maximum estimated value (10.54 μmol m^{-2} s^{-1}) was obtained in plants irrigated with ECw of 0.6 dS m^{-1}. When comparing the CO_2 assimilation rate of plants irrigated with water of the highest salinity level (3.0 dS m^{-1}) to that of plants that received the lowest level of ECw (0.6 dS m^{-1}), there was a reduction of 4.63 μmol m^{-2} s^{-1}. Reduction in CO_2 assimilation rate may be associated with lower carbon assimilation in the substomatal chamber, which directly affects the photosynthetic complex of the plant. Salinity, by limiting stomatal conductance, and as the entry of CO_2 into the leaf mesophyll, also reduces the CO_2 assimilation rate due to the decrease in the partial pressure of this gas in the intercellular spaces, besides reducing the loss of water in the form of vapor through the stomata and increasing leaf blade temperature (Lima et al., 2017).

The instantaneous water use efficiency was reduced by irrigation water salinity (Figure 1E) and, based on the regression equation, there was a minimum *WUEi* value of 1.96 [(μmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$] when plants were irrigated using water with estimated ECw of 2.51 dS m^{-1}, the quadratic model indicated that the highest value of *WUEi* {4.21 [(μmol m^{-2} s^{-1}) (mol m^{-2} s^{-1} of H_2O)$^{-1}$]} was obtained in plants cultivated with water of lowest salinity level (0.6 dS m^{-1}). When comparing the instantaneous water use efficiency of plants irrigated with ECw of 0.6 dS m^{-1} to that of plants that received the water of highest salinity (3.0 dS m^{-1}), there was a reduction of 16.38% [(0.69 μmol m^{-2} s^{-1}) (mmol H_2O m^{-2} s^{-1})$^{-1}$]. Silva et al. (2019), when studying the gas exchange of passion fruit seedlings cv. 'Guinezinho' irrigated with saline water (0.7 to 2.8 dS m^{-1}) and under foliar application of hydrogen peroxide (0 to 75 μM), found that the highest *WUEi* of 6.62 [(μmol m^{-2} s^{-1}) (mol m^{-2} s^{-1} H_2O)$^{-1}$] was obtained when plants were irrigated using water with a salinity of 0.7 dS m^{-1}.

The increase in irrigation water salinity quadratically reduced the instantaneous carboxylation efficiency (Figure 1F), with the highest value [0.048 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$] being obtained in plants irrigated with the lowest salinity level (0.6 dS m^{-1}), while the lowest CEi [0.025 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$] was obtained when plants were irrigated with water of 3.0 dS m^{-1}. In comparative terms, there was a reduction of 47.91% {(0.023 μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$} of the instantaneous carboxylation efficiency in plants irrigated with the highest salinity level (3.0 dS m^{-1}) compared to those that received the water of lowest electrical conductivity (0.6 dS m^{-1}). According to the regression equation (Figure 1F), the lowest CEi {(0.024 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$} was obtained when plants were cultivated under water salinity estimated at 2.75 dS m^{-1}, decreasing sharply from this level of ECw. In plants grown under salt stress conditions, the reduction of *CEi* may be related to metabolic restrictions in the Calvin cycle (Sousa et al., 2016).

Melo, Souza, Duarte, Cunha and Santos (2017) when evaluating the gas exchange of bell pepper grown under different water salinity levels (0, 1, 3, 5, 7, and 9 dS m^{-1}) using two sources of salts: NaCl and a mixture of NaCl, KCl, $CaCl_2$, and $MgCl_2$, observed an increase in water use efficiency of 37.36% in plants irrigated with a salinity level of 9.0 dS m^{-1} in comparison to the control treatment (without the addition of NaCl).

Andrade et al. (2019), when studying the effect of salinity (0.7 to 2.8 dS m^{-1}) on the gas exchange of passion fruit under foliar application of hydrogen peroxide (0 to 60 μM), verified reductions in instantaneous carboxylation efficiency resulting from the osmotic effect caused by excess salts in irrigation water, due to the increase in the concentration of salts in the soil, which ends up compromising the absorption of water by the roots, leading passion fruit plants to reduce their stomatal opening as a way to avoid water loss to the atmosphere, consequently reducing transpiration and photosynthetic rate.

The salinity levels (SL) of irrigation water significantly ($p \leq 0.05$) affected leaf dry biomass (LDB), stem dry biomass (StDB), and shoot dry biomass (ShDB) of sour passion fruit, at 240 days after transplantation (DAT) and absolute growth rate in stem diameter (AGR_{SD}) at 180 DAT. The concentrations of H_2O_2 (H_2O_2) and the interaction between the factors (SL × H_2O_2) significantly affected all analyzed variables of 'BRS Rubi do Cerrado' sour passion fruit (Table 4).

Table 4

Summary of the analysis of variance for leaf dry biomass (LDB), stem dry biomass (StDB), shoot dry biomass (ShDB), at 240 days after transplanting (DAT), and absolute growth rate in stem diameter (RGR_{SD}) and relative growth rate in stem diameter (RGR_{SD}), at 180 days after transplanting, of 'BRS Rubi do Cerrado' sour passion fruit irrigated with saline waters and under foliar application of hydrogen peroxide

Source of variation	DF	Mean squares				
		LDB	StDB	ShDB	RGR_{SD}	RGR_{SD}
Salinity levels (SL)	4	11589.15**	5040.41**	23648.08**	0.00027**	$0.1x10^{-6n}$
Linear regression	1	3701.40**	18981.20**	39446.52**	0.0002^{ns}	$0.01x10^{-5ns}$
Quadratic regression	1	2195.31**	433.47**	4579.81**	0.0002*	$0.01x10^{-5ns}$
Residual 1		391.73	18.39	465.01	0.000051	0.000007
Hydrogen peroxide (H_2O_2)	3	1673.39**	5040.41**	1292.75*	0.00043**	$0.3x10^{-5}$**
Linear regression	1	589.70^{ns}	190.89*	1451.64*	0.0001^{ns}	$0.3x10^{-6ns}$
Quadratic regression	1	4404.80**	391.93**	2168.88**	0.00111**	$0.1x10^{-5ns}$
Interaction (SL x H_2O_2)	12	4280.24**	2506.81**	7747.77**	0.00012**	$0.2x10^{-5}$**
Residual 2	30	171.16	40.77	222.77	0.000067	0.000004
CV 1 (%)		11.77	4.67	8.30	20.75	34.44
CV 2 (%)		7.78	6.96	5.74	23.67	26.95

^{ns} Not significant; *,** significant at 0.05 and 0.01, respectively; DF - degrees of freedom; CV - coefficient of variation.

The increase in the electrical conductivity of irrigation water reduced the leaf dry biomass of 'BRS Rubi do Cerrado' passion fruit, which decreased when 30 μM was applied, compared to those that received the other concentrations (Figure 2A). According to the regression equation, plants under application of 30 μM showed a minimum leaf dry biomass of 164.37 g per plant when irrigated with water of electrical conductivity of 2.69 dS m^{-1}. In relative terms, as salinity increased from 0.6 dS m^{-1} to 3.0 dS m^{-1}, there was a reduction of 17.71% (34.54 g per plant). In turn, the application of the concentration of 15 μM was able to mitigate the deleterious effects of irrigation water salinity, with a maximum value of 249.27 g per plant for the highest salinity level (3.0 dS m^{-1}).

Ciências Agrárias
SEMINA

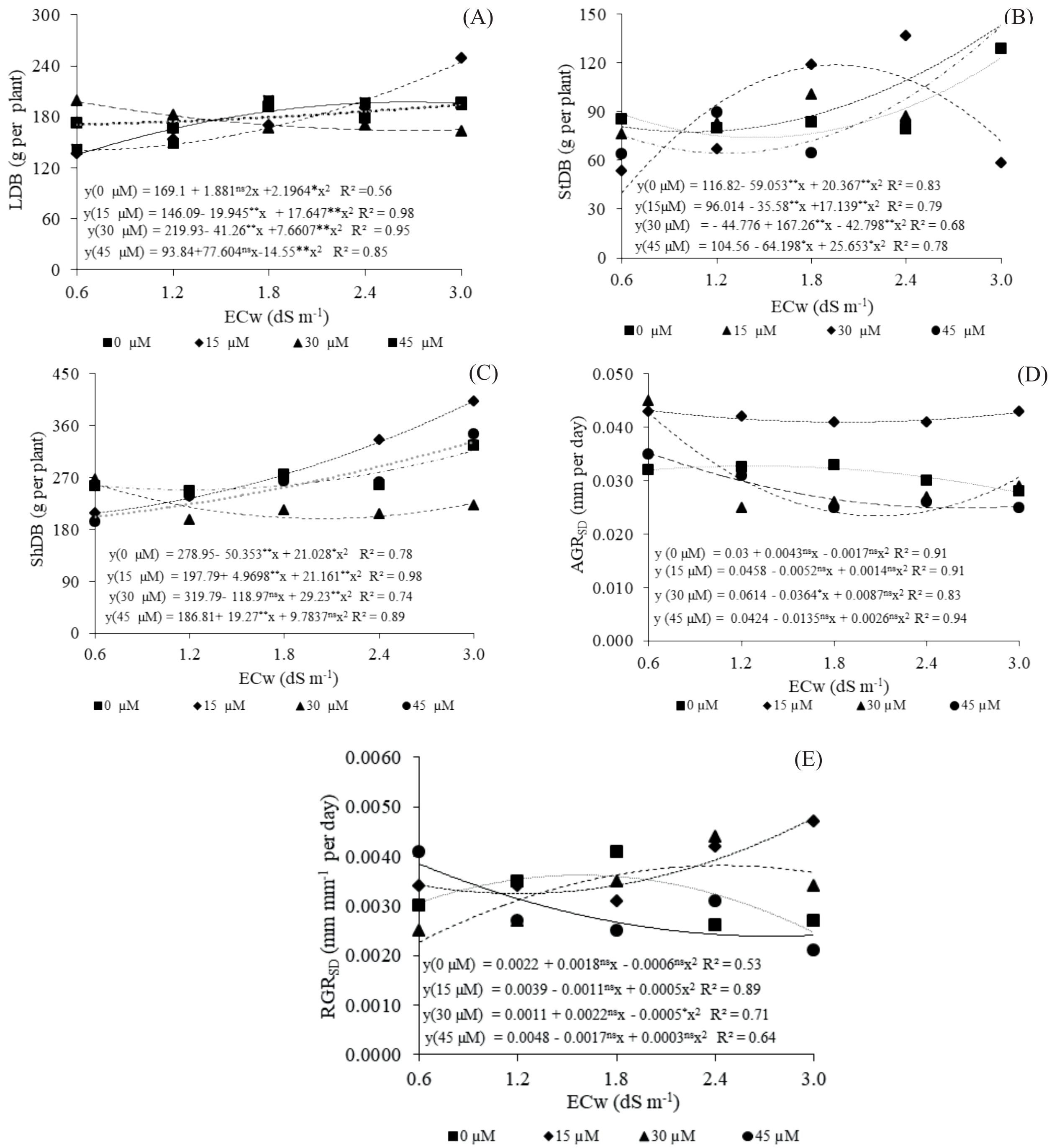

Figure 2. Leaf dry biomass - LDB (A), stem dry biomass - StDB (B), shoot dry biomass - ShDB (C) at 240 DAT, the absolute growth rate of the stem - AGR_{SD} (D) and relative growth rate of the stem - RGR_{SD} (E) at 180 DAT, of 'BRS Rubi do Cerrado' sour passion fruit plants as a function of the interaction between the levels of water electrical conductivity - ECw and the concentrations of hydrogen peroxide. **; * Significant at 1 and 5% probability levels, respectively.

It was possible to verify that the highest accumulation of stem dry biomass (152.41 g per plant) was obtained in plants irrigated with the highest salinity level (3.0 dS m^{-1}) and that received a concentration of 15 μM (Figure 2B). The lowest value of StDB (53.79 g per plant) was observed when plants received the water of the lowest salinity level (0.6 dS m^{-1}) and were sprayed with hydrogen peroxide at a concentration of 30 μM. It was found that the highest value of StDB (77.54 g per plant) was obtained when plants received the water of 1.04 dS m^{-1} and the concentration of 15 μM. In relative terms, a reduction of 59.25% (90.31 g per plant) was observed when comparing plants that received a concentration of 30 μM to those that received 15 μM and was irrigated with the highest salinity level. Thus, it can be inferred that excessive application of H_2O_2 can cause damage to crop growth (Figure 2B).

Irrigation with saline water and the foliar application of hydrogen peroxide influenced shoot dry biomass (Figure 2C), and the highest value of ShDB (401.68 g per plant) occurred when plants were irrigated with the highest salinity level (3.0 dS m^{-1}) under foliar application of 15 μM. The mathematical model that best fitted the data was the quadratic one, and the highest value of shoot dry biomass (401.68 g per plant) was obtained in plants subjected to ECw of 3.0 dS m^{-1} and that received an H_2O_2 concentration of 15 μM L^{-1}.

When analyzing the ShDB values of plants irrigated with the highest salinity level (3.0 dS m^{-1}) as a function of H_2O_2 concentrations of 0 and 30 μM, there were increments of 19.07% (76.62 g per plant) and 44.77% (179.84 g per plant), respectively, in comparison to plants subjected to a concentration of 15 μM. Hydrogen peroxide, when applied at low concentrations, acts as a signaling molecule in such a way to produce antioxidative enzymes to maintain its homeostasis redox. The expression of many antioxidant enzymes is positively correlated with higher levels of tolerance against abiotic stresses; thus, the activation of some enzymes leads to the protection of the plant against oxidative damage (Caverzan et al., 2016).

For the absolute growth rate in stem diameter of sour passion fruit plants (Figure 2D), the fit of the equation showed that the maximum estimated value of 0.0041 mm day^{-1} was obtained under a water salinity of 1.85 dS m^{-1} and an H_2O_2 concentration of 15 μM. In relative terms, it was observed that plants that received irrigation water with the highest salinity level (3.0 dS m^{-1}) and that received H_2O_2 concentrations of 15 and 30 μM had absolute growth rates of 0.043 and 0.029 mm per day, respectively (Figure 2D).

For the relative growth rate in stem diameter (RGR_{SD}), plants that received 15 μM and were irrigated with the lowest salinity level (0.6 dS m^{-1}) showed an increase in RGR_{SD} of 27.66% (0.0013 mm mm^{-1} per day) compared to those subjected to the highest salinity level (3.0 dS m^{-1}) (Figure 2E). It was verified that the highest RGR_{SD} of 'BRS Rubi do Cerrado' sour passion fruit was 0.0039 mm mm^{-1} per day when plants were irrigated with water of 0.6 dS m^{-1} for a concentration of 15 μM. Possibly, hydrogen peroxide at the concentration of 15 μM acted as a sensor of changes at plasma membrane/cell wall level through the production of antioxidative agents, which attenuated the deleterious effects of irrigation water salinity, also acting in the increase of the production of organic solutes via roots, which may have favored osmotic adjustment and the balance of water and nutrients in the plant (G. M. Melo, 2016).

Bezerra, Pereira, Silva and Raposo (2016) when investigating the effect of different salinity levels of irrigation water (0.3, 2.0, 4.0, 6.0, and 8.0 dS m^{-1}) on two passion fruit genotypes ('BRS Sol do Cerrado' and 'Redondo Amarelo'), highlighted that the increase in the electrical conductivity of irrigation water reduced the absolute growth rate by 51.05% with the increase in water salinity from 0.3 to 8.0 dS m^{-1}.

Hydrogen peroxide has been considered a signaling and regulating molecule of the expression of some genes in cells that act in the production of antioxidative enzymes, being one of the main reactive oxygen species produced by plants when affected by biotic and abiotic stresses. It acts as a key molecule in the Third Principle of the Redox Code, which is: "Redox detection through activation/deactivation cycles of H_2O_2 production linked to NAD and NADP systems to support the spatial-temporal organization of key processes" of oxidation or production of antioxidant enzymes such as superoxide dismutase, for example, which acts in the degradation of superoxide (Jones & Sies, 2015).

Silva et al. (2019), when evaluating the growth of 'Guinezinho' passion fruit as a function of irrigation with saline waters (0.7, 1.4, 2.1, and 2.8 dS m^{-1}) and foliar application of hydrogen peroxide (0, 25, 50, and 75 μM), observed that hydrogen peroxide at the concentration of 25 μM mitigated the negative effects of ECw 1.4 dS m^{-1} on transpiration, CO_2 assimilation rate, internal CO_2 concentration, plant height, and leaf area.

Conclusion

Irrigation with saline waters negatively affects the gas exchange of 'BRS Rubi do Cerrado' sour passion fruit, and transpiration is the most sensitive variable to salt stress.

Foliar application of hydrogen peroxide at 15 μM increases leaf dry biomass, stem dry biomass, and shoot dry biomass, as well as the absolute and relative growth rates of 'BRS Rubi do Cerrado' sour passion fruit irrigated with a salinity of up to 3.0 dS m^{-1}.

Foliar application of hydrogen peroxide (H_2O_2) at 15 μM mitigated salt stress on the growth of 'BRS Rubi do Cerrado' sour passion fruit, at 120 days after transplantation.

References

Andrade, E. M. G. (2018). *Águas salinas e aplicação foliar de peróxido de hidrogênio no cultivo de maracujazeiro amarelo.* Tese de doutorado, Universidade Federal de Campina Grande, Campina Grande, PB, Brasil. Recuperado de http://dspace.sti.ufcg.edu.br:8080/jspui/handle/riufcg/3589

Andrade, E. M., Lima, G. S. de, Lima, V. L. A., Silva, S. S. da, Gheyi, H. R., & Silva, A. A. R. da. (2019). Gas exchanges and growth of passion fruit under saline water irrigation and H_2O_2 application. *Revista Brasileira de Engenharia Agrícola e Ambiental, 23*(12), 945-951. doi: 10.1590/1807-1929

Ayers, R. S., & Westcot, D. W. (1985). *Water quality for agriculture.* Rome: Food and Agriculture Organization of the United Nations.

Benincasa, M. M. P. (2003). *Análise de crescimento de plantas: noções básicas.* Jaboticabal, SP: FUNEP.

Bernardo, S., Soares, A. A., & Mantovani, E. C. (2009). *Manual de irrigação* (8a ed., 2a Reimp.). Viçosa, MG: UFV.

Bezerra, J. D., Pereira, W. E., Silva, J. M. da, & Raposo, R. W. C. (2016). Crescimento de dois genótipos de maracujazeiro-amarelo sob condições de salinidade. *Revista Ceres, 63*(4), 502-508. doi: 10.1590/0034-737X201663040010

Bhatla, S. C., & Lal, A. M. (2016). *Plant physiology, development and metabolism.* Singapore: Springer.

Caverzan, A., Casassola, A., & Brammer, S. P. (2016). Antioxidant responses of wheat plants under stress. *Genetics and Molecular Biology, 39*(1), 1-6. doi: 10.1590/1678-4685-GMB-2015-0109

Dias, A. S., Lima, G. S. de, Gheyi, H. R., Soares, L. A. dos A., & Fernandes, P. D. (2020). Growth and gas exchanges of cotton under water salinity and nitrogen-potassium combination. *Revista Caatinga, 33*(2), 470-479. doi: 10.1590/1983-21252020v33n219rc

Empresa Brasileira de Pesquisa Agropecuária (2010). *Recomendações de calagem e adubação para maracujazeiro.* Recuperado de https://ainfo.cnptia.embrapa.br/digital/bitstream/CPAC-2010/30292/1/ doc-223.pdf

Empresa Brasileira de Pesquisa Agropecuária (2014). *BRS Rubi do Cerrado: hibrido de maracujazeiro-azedo de frutos avermelhados e amarelos para indústria e mesa* (2a ed.). Recuperado de http://www. cpac.embrapa.br/publico/usuarios/uploads/lancamentobrsrubidocerrado/brsrc.pdf

Empresa Brasileira de Pesquisa Agropecuária (2018). *Sistema brasileiro de classificação de solos* (5a ed.). Recuperado de https://www.embrapa.br/solos/sibcs

Ferreira, D. F. (2019). SISVAR: a computer analysis system to fixed effects split plot type designs. *Revista Brasileira de Biometria, 37*(4), 529-535. doi: 10.28951/rbb.v37i4.450

Figueiredo, F. R. A., Lopes, M. de F. Q., Silva, R. T. da, Nóbrega, J. S., Silva, T. I. da, & Bruno, E. de L. A. (2019). Respostas fisiológicas de mulungu submetida a estresse salino e aplicação de ácido salicílico. *Irriga, 24*(3), 662-675. doi: 10.15809/irriga.2019v24n3p662-675

Instituto Brasileiro de Geografia e Estatística (2018). *Produção agrícola municipal.* Recuperado de https://sidra.ibge.gov.br/tabela/5457#resultado

Kao, C. H. (2014). Role of hydrogen peroxide in rice plants. *Crop, Environment & Bioinformatics, 11*(1), 1-10. doi: 10.30061/CEB.201403_11(1).0001

Jones, D. P., & Sies, H. (2015). The redox code. *Antioxidants & Redox Signaling, 23*(9), 734-746. doi: 10.10 89/ars.2015.6247

Lima, G. S. de, Gheyi, H. R., Nobre, R. G., Soares, L. A. dos A., Xavier, D. A., & Santos, J. A., Jr. (2015). Water relations and gas exchange in castor bean irrigated with saline water of distinct cationic nature. *African Journal of Agricultural Research, 10*(1), 1581-1594. doi: 10.5897/AJAR2015.9606

Lima, G. S. de, Dias, A. S., Gheyi, H. R., Soares, L. A. dos A., Nobre, R. G., Pinheiro, F. W. A.,

& Silva, A. A. R. da (2017). Gas exchanges and production of colored cotton under salt stress and nitrogen fertilization. *Bioscience Journal, 33*(6), 1495-1505.

Mazaro, S. M., Mangnabosco, M. C., Citadin, I., Paulus, D., & Gouvea, A. de. (2013). Produção e qualidade de morangueiro sob diferentes alterações da calda bordalesa, sulfocálcica e supermagro biofertilizante. *Semina: Ciências Agrárias, 1*(34), 3285-3294. doi: 10.5433/1679-0359.

Melo, G. M. (2016). *Adaptabilidade fisiológica ao estresse abiótico induzida pela aplicação de Priming in vitro em plantas de cana-de-açúcar.* Tese de doutorado, Universidade Federal Rural de Pernambuco, Recife, PE, Brasil. Recuperado de http://www.tede2.ufrpe.br:8080/tede2/handle/tede2/7283

Melo, H. F. de, Souza, E. R. de, Duarte, H. H. F., Cunha, J. C., & Santos, H. R. B. (2017). Gas exchange and photosynthetic pigments in bell pepper irrigated with saline water. *Revista Brasileira de Engenharia Agrícola e Ambiental, 21*(1), 38-43. doi: 10.1590/1807-1929/agriambi.v21n1p38-

Melo, Y. L., Garcia, R. R., Macêdo, C. E. C. de, Delgado, E. A. O., Maia, J. M., & Ortega-Rodés, P. (2020). Unraveling physiological traits of *Jatropha curcas,* a biodiesel plant, to overcome salinity conditions. *Revista Caatinga, 33*(2), 446-457. doi: 10.1590/1983-21252020v33n217rc

Mudo, L. E. D., Lobo, J. T., Carreiro, D. de A., Cavacini, J. A., Silva, L. dos S., & Cavalcante, I. H. L. (2020). Leaf gas exchange and flowering of mango sprayed with biostimulant in semi-arid region. *Revista Caatinga, 33*(2), 332-340. doi: 10.1590/1983-21252020v33n206rc

Oliveira, F. A. de, Lopes, M. A. C., Sá, F. V. da S., Nobre, R. G., Moreira, R. C. L., Silva, L. de A., & Paiva, E. P. de (2015). Interação salinidade da água de irrigação e substratos na produção de mudas de maracujazeiro amarelo. *Comunicata Scientiae, 6*(4), 471-478. doi: 10.14295/cs.v6i4.982

Richards, L. A. (1954). *Diagnosis and improvement of saline and alkali soils.* (Agriculture Handbook, 60). Washington: U. S. Department of Agriculture.

Sá, F. V. da S., Santos, M. G. dos, Barros, A. P., Jr., Albuquerque, J. R. T. de, Souza, A. R. E. de, & Ribeiro, R. M. P. (2020). Tolerance of peanut (Arachis hypogea) genotypes to salt stress in the initial phase. *Revista Brasileira de Engenharia Agrícola e Ambiental, 24*(1), 37-43. doi: 10.1590/1807-1929/agriambi.v24n1p37-43

Sá, F. V. da S., Gheyi, H. R., Lima, G. S. de, Paiva, E. P. de, Fernandes, P. D., Moreira, R. C. L., Ferreira, M., Neto. (2017). Water relations and gas exchanges of West Indian cherry under salt stress and nitrogen and phosphorus doses. *Journal of Agricultural Science, 9*(10), 168-177. doi: 10.5539/jas.v9 n10p168

Santos, J. B. (2001). *Estudo das relações nitrogênio: potássio e cálcio: magnésio sobre o desenvolvimento vegetativo e produtivo do maracujazeiro amarelo.* Dissertação de mestrado. Universidade Federal da Paraíba, João Pessoa, PB, Brasil.

Santos, L. C. dos, Silva, S. T. de A., Medeiros, C. R. de, Santos, A. V. D. dos, Lima, G. S. de, Severo, P. J. da S., Pereira, J. D. A. (2020). Peróxido de hidrogênio como atenuante do estresse salino na formação de mudas de pitaia vermelha (*hylocereus costaricensis*). *Brazilian Journal of Development, 6*(5), 7295-27308. doi: 10. 34117/bjdv6n5-252

São José, A. R., Rebouças, T. N. H., Pieres, M. M., Angel, D. N., Sousa, I. V. B., Bonfim, M. P. *Maracujá: Práticas de cultivo e comercialização.* Vitória da Conquista: UESB/DFZ, 2000.

Silva, A. A. R. da, Lima, G. S. de., Azevedo, C. A. V. de, Gheyi, H. R., Souza, L. de P., & Veloso, L. L. de S. A. (2019). Gas exchanges and growth of passion fruit seedlings under salt stress and hydrogen peroxide. *Pesquisa Agropecuária Tropical, 49*(1), 1-10. doi: 10.1590/1983-40632019v4955671

Sousa, J. R. M. de, Gheyi, H. R., Brito, M. E. B., Xavier, D. A., & Furtado, G. de F. (2016). Impact of saline conditions and nitrogen fertilization on citrus production and gas exchanges. *Revista Caatinga, 29*(2), 415-424. doi: 10.1590/1983-21252016v29n218rc

Teixeira, P. C., Donagemma, G. K., Fontana, A., & Teixeira, W. G. (2017). *Manual de métodos e análise de solos.* Brasília, GO: Embrapa.