

## HYDROGEN PEROXIDE AS SALT STRESS ATTENUATOR IN SOUR PASSION FRUIT<sup>1</sup>

JAILTON GARCIA RAMOS<sup>2\*</sup>, VERA LÚCIA ANTUNES DE LIMA<sup>2</sup>, GEOVANI SOARES DE LIMA<sup>2</sup>, FRANCISCO JEAN DA SILVA PAIVA<sup>2</sup>, MARIANA DE OLIVEIRA PEREIRA<sup>2</sup>, KHEILA GOMES NUNES<sup>2</sup>

**ABSTRACT** - Sour passion fruit is a fruit crop widely cultivated throughout Brazil, due to its adaptation to the tropical climate. However, in semi-arid regions its development is limited by the high concentration of soluble salts in the waters commonly used in irrigation. In this context, exogenous application of low concentrations of hydrogen peroxide can attenuate the damage caused by salt stress. The objective of this study was to evaluate electrolyte leakage, photosynthetic pigments and photochemical efficiency in sour passion fruit cv. BRS Rubi do Cerrado under irrigation with saline waters and foliar application of hydrogen peroxide. A completely randomized design was used, with treatments arranged in split plots, corresponding to five levels of irrigation water salinity - EC<sub>w</sub> (0.6; 1.2; 1.8; 2.4 and 3.0 dS m<sup>-1</sup>) and four concentrations of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> (0; 15; 30 and 45 μM of H<sub>2</sub>O<sub>2</sub>) in plots and subplots, respectively. Irrigation with saline water reduced chlorophyll *a* and total chlorophyll contents of sour passion fruit plants cv. BRS Rubi do Cerrado, at 240 days after transplanting. Hydrogen peroxide at concentration of 15 μM stimulated chlorophyll *a* and total chlorophyll biosynthesis and, at 45 μM, relieved the effect of 3.0 dS m<sup>-1</sup> water salinity on electrolyte leakage in the leaf blade of sour passion fruit. Salt stress did not affect the initial, maximum, variable fluorescence and quantum efficiency of photosystem II of sour passion fruit cv. BRS Rubi do Cerrado.

**Keywords:** *Passiflora edulis* Sims. Reactive oxygen species. Photochemical efficiency. Oxidative stress.

## PERÓXIDO DE HIDROGÊNIO COMO ATENUANTE DO ESTRESSE SALINO EM MARACUJAZEIRO-AZEDO

**RESUMO** - O maracujazeiro-azedo é uma fruteira amplamente cultivada em todo Brasil, devido sua adaptação ao clima tropical. No entanto, em regiões semiáridas seu desenvolvimento é limitado devido água alta concentração de sais solúveis nas águas comumente utilizadas na irrigação. Neste contexto, a aplicação de baixas concentrações de peróxido de hidrogênio de forma exógena pode atenuar os danos provocados pelo estresse salino. Objetivou-se com esta pesquisa avaliar o extravasamento de eletrólitos, os pigmentos fotossintéticos e a eficiência fotoquímica em maracujazeiro-azedo cv. BRS Rubi do Cerrado sob irrigação com águas salinas e aplicação foliar de peróxido de hidrogênio. Foi utilizado o delineamento inteiramente casualizados, com os tratamentos dispostos em parcelas subdivididas, sendo cinco níveis de salinidade da água de irrigação - CE<sub>a</sub> (0,6; 1,2; 1,8; 2,4 e 3,0 dS m<sup>-1</sup>) e quatro concentrações de peróxido de hidrogênio - H<sub>2</sub>O<sub>2</sub> (0; 15; 30 e 45 μM de H<sub>2</sub>O<sub>2</sub>) nas parcelas e subparcelas, respectivamente. A irrigação com água salina reduziu os teores de clorofila *a* e *total* das plantas de maracujazeiro-azedo cv. BRS Rubi do Cerrado, aos 240 dias após o transplante. O peróxido de hidrogênio na concentração de 15 μM estimulou a biossíntese de clorofila *a* e *total* e de 45 μM aliviou o efeito da salinidade da água de 3,0 dS m<sup>-1</sup> sobre o extravasamento de eletrólitos no limbo foliar do maracujazeiro-azedo. O estresse salino não afetou a fluorescência inicial, máxima, variável e eficiência quântica do fotossistema II do maracujazeiro-azedo cv. BRS Rubi do Cerrado.

**Palavras-chave:** *Passiflora edulis* Sims. Espécie reativa de oxigênio. Eficiência fotoquímica. Estresse oxidativo.

\*Corresponding author

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<sup>2</sup>Academic Unit of Agricultural Engineering, Universidade Federal de Campina Grande, Campina Grande, PB, Brazil; jailtonbiossistemas@gmail.com – ORCID: 0000-0003-4402-6555, antuneslima@gmail.com –ORCID: 0000-0001-7495-6935, geovani.soares@pq.cnpq.br – ORCID: 0000-0001-9960-1858, je.an\_93@hotmail.com –ORCID: 0000-0001-7603-4782, marianapereira.agri@gmail.com – ORCID: 0000-0003-4243-8150, kheilagomesnunes@gmail.com –ORCID: 0000-0003-1520-843X.

## INTRODUCTION

Sour passion fruit (*Passiflora edulis* Sims) is a crop that adapts well to the conditions of cultivation in the Brazilian semi-arid region; however, with the great temporal and spatial variability of the rains, it is necessary to use irrigation and the water used for it often comes from artesian wells that have high salinity. Passion fruit is classified as sensitive to salinity, that is, it has salinity threshold of only 1.3 dS m<sup>-1</sup> for electrical conductivity of the soil saturation extract, which enables the use of water with maximum electrical conductivity of 0.9 dS m<sup>-1</sup>. The national production of sour passion fruit in 2019 was 593,429 ton; the Northeast accounted for 64.50% of this production, Bahia was the largest national producer (168,457 ton), and Paraíba occupied the 13th place in the national ranking, with a production of 9,967 ton (IBGE, 2019).

It is a crop well known and exploited throughout the world and can be consumed in various ways, being highly valued and easy to trade. Its cultivation is a source of job and income generation for many family farmers. The Northeast region has great potential in the exploitation of this crop, mainly due to its edaphoclimatic characteristics. As sour passion fruit is a crop well known and accepted by the consumer market, its cultivation has been growing throughout the country, thus generating employment and income.

A limiting factor for the production of this fruit crop in the semi-arid region of northeastern Brazil is the occurrence of low rainfall, temporal and spatial variability, and high temperatures and evapotranspiration (LIMA et al., 2016; SANTOS et al., 2016). In this region, water scarcity makes it impossible to grow fruit crops under a rainfed regime (SILVA et al., 2016), so irrigation becomes necessary. However, the occurrence of water sources with high concentrations of salts is a barrier to the expansion of irrigated agriculture (BEZERRA et al., 2018).

The use of saline water in irrigation can cause deleterious effects on plants, by reducing water availability due to the decrease in the osmotic potential of the soil solution, leading to stomatal closure and compromising transpiration and photosynthesis (SILVA et al., 2015). In addition, the use of waters with high concentrations of salts causes photoinhibition, photooxidation in chloroplasts, enzyme inactivation, degradation of photosynthetic pigments and lipid peroxidation of membranes (ASHRAF; HARRIS, 2013).

Considering that most crops are sensitive to the presence of salts either in irrigation water or in soil, it is necessary to conduct research to investigate the technical and economic feasibility of strategies capable of minimizing the deleterious effects of salinity on plants (FREIRE et al., 2014). Thus,

hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which is a reactive oxygen species capable of causing oxidation of lipid and proteins, DNA changes and modulation of gene expression, has been studied as an attenuating agent of abiotic stresses in plants (FERNANDO; SOYSA, 2015), functioning as a signaling molecule to mediate stress responses (LI et al., 2016).

Andrade et al. (2019) investigated the effect of irrigation water salinity (0.7 to 2.8 dS m<sup>-1</sup>) and exogenous application of hydrogen peroxide (0 to 60 µM) on the passion fruit variety 'Guinezinho' and observed a higher number of leaves in plants irrigated using water with electric conductivity of 1.5 dS m<sup>-1</sup> and under H<sub>2</sub>O<sub>2</sub> application of 40 µM, with reduction as irrigation water salinity increased.

Silva et al. (2019) observed that, when the soursop cv. 'Morada Nova' is irrigated using water with electrical conductivity of up to 3.5 dS m<sup>-1</sup> and is under exogenous application of H<sub>2</sub>O<sub>2</sub> (0 to 100 µM) via seed soaking and foliar spraying, the application of 25 and 50 µM attenuated the deleterious effects of salinity on stomatal conductance, CO<sub>2</sub> assimilation rate and chlorophyll *a* contents.

In this context, the objective of this study was to evaluate the electrolyte leakage, photosynthetic pigments and photochemical efficiency of sour passion fruit cv. BRS Rubi do Cerrado under irrigation with saline waters and foliar application of hydrogen peroxide.

## MATERIAL AND METHODS

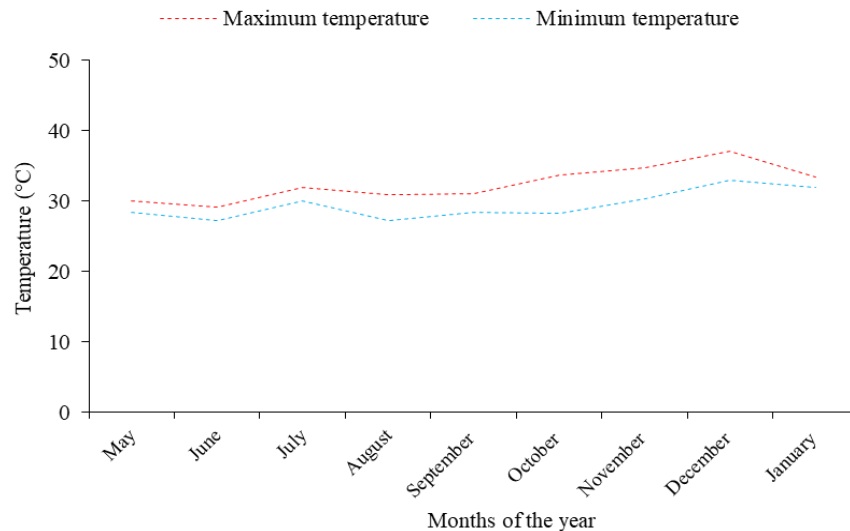
The experiment was conducted between May 2019 and January 2020 in an arch-type greenhouse, 30 m long and 21 wide, with ceiling height of 3.0 m, 150-micron low-density polyethylene cover, sides covered with white screen, with openings of 4 mm x 7 mm, belonging to the Academic Unit of Agricultural Engineering – UAEAg of the Federal University of Campina Grande - UFCG, in Campina Grande, Paraíba, Brazil (7°15'18" S, 35°52'28" W and average altitude of 550 m). The average monthly temperature inside the greenhouse was monitored throughout the experiment with a digital thermometer (Figure 1).

The treatments consisted of five levels of electrical conductivity of irrigation water - ECw (0.6; 1.2; 1.8; 2.4 and 3.0 dS m<sup>-1</sup>) and four concentrations of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> (0; 15; 30 and 40 µM). The experimental design adopted was completely randomized in split plots, with the levels of electrical conductivity of irrigation water considered the plots and the concentrations of hydrogen peroxide considered the subplots, with three replicates. Water salinity levels and H<sub>2</sub>O<sub>2</sub> concentrations were established based on a study conducted by Andrade et al. (2019), who found that the 20 µM concentration promoted the highest values for variable and maximum fluorescence and carotenoid

content, constituting an alternative to induce acclimatization of sour passion fruit.

The seeds were obtained from the cv. BRS Rubi do Cerrado provided by the orchard of the Federal Institute of Paraíba - IFPB, campus of Sousa, PB. The fruits of this cultivar are round, with a predominantly red, purplish or yellowish rind,

weighing from 120 to 300 g, with soluble solids content ranging from 13° to 15° Brix, averaging 14° Brix, juice yield around 35%, higher resistance to transport, strong yellow pulp color, greater amount of vitamin C, and resistance to diseases such as those caused by viruses and bacteria, and scabs (EMBRAPA, 2012).



**Figure 1.** Maximum and minimum monthly average temperatures inside the greenhouse during the experimental period.

The seedlings were grown in plastic bags with capacity for 3 kg (35 × 10 cm), filled with substrate in the 84:15:1 ratio of soil (volume based), washed sand and earthworm humus, respectively. The moisture content of the substrate was increased to the level corresponding to the maximum water holding capacity through the weighing lysimetry principle (BERNARDO; SOARES; MANTOVANI, 2013), and then 4 seeds were sown at a depth of 1.0 cm in each bag of seedlings. The water used was local-supply water from the Paraíba State Supply and Sewage Company, with electrical conductivity of 0.4 dS m<sup>-1</sup>.

At 10 days after emergence, thinning was performed leaving only one plant per bag of seedlings. At 70 days after emergence, that is, when the plants had tendrils, the seedlings were transplanted to pots that functioned as drainage lysimeters. Each lysimeter was perforated at the base and a drain was installed; above each drain, a nonwoven geotextile (Bidim) was placed to avoid clogging. The lower end of each drain was connected to a plastic container to collect the drained water, in order to determine the water consumption by the plant and electrical conductivity of the soil solution (EC).

The spacing adopted was 2.20 m between rows and 1.50 m between plants, using the vertical

trellis system with smooth wire N° 14 installed inside the greenhouse, at 2.40 m height from the floor and 1.60 m height from the soil of the lysimeter.

The lysimeters (height of 70 cm, bottom diameter of 57 cm and upper diameter of 57 cm) were filled with 0.5 kg of crushed stone N° 0 and 250 kg of soil classified as *Luvisolo crômico* (Alfisol) (EMBRAPA, 2018), from Alagoa Nova, PB. The soil was collected at 0-30 cm depth (A horizon), before the experiment started, and was characterized for chemical and physical-hydraulic attributes (Table 1) according to the methodology of Teixeira et al. (2017). Before the transplanting, the moisture content of the soil contained in the lysimeters was increased to the level corresponding to the maximum water holding capacity through the drainage lysimetry principle (BERNARDO; SOARES; MANTOVANI, 2013).

Basal fertilization was performed according to the recommendation of São José (2000), applying 250 g of single superphosphate (18.9% P<sub>2</sub>O<sub>5</sub>) and 100 g of potassium chloride (60% K<sub>2</sub>O) monthly until the beginning of flowering; at the beginning of this stage, nitrogen and potassium fertilization was performed monthly, according to the methodology proposed by Santos (2001), using urea (45.9% N) as nitrogen source and potassium chloride (60% K<sub>2</sub>O) as potassium source.

**Table 1.** Chemical and physical-hydraulic characteristics of the soil, before application of the treatments.

Chemical characteristics									
pH (H <sub>2</sub> O) (1:2.5)	OM dag kg <sup>-1</sup>	P (mg kg <sup>-1</sup> )	K <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup> + H <sup>+</sup>	ESP (%)	EC <sub>se</sub> (dS m <sup>-1</sup> )
(cmol <sub>c</sub> kg <sup>-1</sup> )									
5.90	1.36	6.80	0.22	0.16	2.60	3.66	1.93	1.87	1.0
Physical-hydraulic characteristics									
PSF (dag kg <sup>-1</sup> )			Textural class	Moisture (kPa)		AW	Total porosity %	BD	PD
Sand	Silt	Clay		33.42	1519.5				
73.29	14.21	12.50	SL	11.98	4.32	7.66	47.74	1.39	2.66

OM – Organic matter: Walkley-Black Wet Digestion; Ca<sup>2+</sup> and Mg<sup>2+</sup> extracted with 1 M KCl at pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted with 1 M NH<sub>4</sub>OAc at pH 7.0; Al<sup>3+</sup> and H<sup>+</sup> extracted with 0.5 M CaOAc at pH 7.0; ESP - Exchangeable sodium percentage; EC<sub>se</sub> - Electrical conductivity of the soil saturation extract; SL - Sandy Loam; AW - Available water; BD - Bulk density; PD - Particle density; PSF - Particle-size fraction.

In the crop formation stage, the 1N:1K ratio was used, based on a reference of 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. Fertilization with micronutrients was performed according to the recommendation of Costa et al. (2008) at 15-day intervals after transplanting, by spraying the plants with a solution containing 2.5 g L<sup>-1</sup> of commercial fertilizer with the following characteristic: N (15%), P<sub>2</sub>O<sub>5</sub> (15%), K<sub>2</sub>O (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%). Throughout the experiment, each plant received 250 g of single superphosphate, 150 g of urea and 200 g of potassium chloride.

The levels of electrical conductivity of the irrigation water were prepared with Na:Ca:Mg in the equivalent proportion of 7:2:1, using the salts NaCl, CaCl<sub>2</sub>·2H<sub>2</sub>O and MgCl<sub>2</sub>·6H<sub>2</sub>O, adjusting them to the concentrations of the available supply water. This is a proportion of salts commonly found in the water bodies of the semi-arid region of northeastern Brazil. The irrigation waters were prepared considering the relationship between EC<sub>w</sub> and the concentration of salts according to Richards (1954), as shown in Equation 1:

$$Q=640 \times EC_w \quad (1)$$

Where:

Q = Quantity of salts to be applied (mg L<sup>-1</sup>);

EC<sub>w</sub> = Electrical conductivity of water (dS m<sup>-1</sup>)

After transplanting, irrigation was performed daily according to Rhoades, Kandiah and Mashali (2000), applying in each lysimeter a volume of water corresponding to that obtained by the water balance, determined by Equation 2:

$$VI = \frac{(V_a - V_d)}{(1 - LF)} \quad (2)$$

Where:

VI = volume of water to be used in the next

irrigation event (mL);

V<sub>a</sub> = volume applied in the previous irrigation event (mL);

V<sub>d</sub> = volume drained (mL) and LF = leaching fraction of 0.15.

The salinized waters were stored in five boxes with capacity for 500 L, one for each salinity level, protected from the weather and covered with a lid to reduce evaporation and contamination by external agents. The salinized waters began to be applied at 15 days after transplanting and, during this period, the plants were irrigated with water of low electrical conductivity (0.4 dS m<sup>-1</sup>).

Hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> concentrations were prepared with deionized water. At the end of each application, the volume used in each treatment was quantified, and an average volume of 63.75 mL of H<sub>2</sub>O<sub>2</sub> was applied per plant, in each application event, until the beginning of flowering. To avoid drift of the H<sub>2</sub>O<sub>2</sub> solution, the plants of each treatment were isolated using plastic curtains.

Hydrogen peroxide concentrations were applied by foliar spraying (on the adaxial and abaxial sides) from 17:00 h, due to the lower incidence of light, because its degradation in the presence of light is very fast, using a manual sprayer, with 1-cm-diameter adjustable metal conical nozzle, operating pressure of 2.07 MPa, and flow rate of 1.1 L min<sup>-1</sup> at 15-day intervals, starting at 15 days after the beginning of irrigation with the salinized waters until the flowering stage of the crop.

When the plants reached 10 cm above the trellis, their apical bud was pruned, aiming to stimulate the growth of secondary branches, which were trained one to each side up to a length of 1.10 m, and then a new pruning was performed to stimulate the growth of tertiary branches and, consequently, the formation of curtains and production of inflorescences. Pruning of the tertiary branches was performed at 30 cm from the soil to avoid their contact with the soil and the possible attack of pests or diseases. During pruning,

Bordeaux mixture was applied to promote healing of injuries (MAZARO et al., 2013).

At 240 days after transplanting, photosynthetic pigments were determined after collecting three fully expanded leaves, in the third order of three tertiary branches, and taking them to the Plant Physiology Laboratory of UFCG. Nine discs were collected to quantify the contents of chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), total chlorophyll (*Chl total*) and carotenoids (*Car*), according to the methodology proposed by Arnon (1949). From the extracts, the chlorophyll concentration was determined in the solutions using a spectrophotometer at absorbance wavelength (ABS) (470, 646, and 663 nm), according to Equations 3, 4 and 5.

$$\text{Chlorophyll } a \text{ (Chl } a) = (12.21 \times \text{ABS}_{663}) - (2.81 \times \text{ABS}_{646}) \quad (3)$$

$$\text{Chlorophyll } b \text{ (Chl } b) = (20.13 \times \text{ABS}_{646}) - (5.03 \times \text{ABS}_{663}) \quad (4)$$

$$\text{Carotenoids (Car)} = \frac{[(1000 \times \text{ABS}_{470}) - (1.82 \text{ Chl } a - 85.02 \text{ Chl } b)]}{198} \quad (5)$$

The values obtained for chlorophyll *a*, chlorophyll *b* and carotenoids contents in the leaves were expressed in milligram per gram of fresh matter ( $\text{mg g}^{-1}$  FM).

Photosynthetic efficiency was determined using an Opti Science OS5p pulse-modulated fluorometer; first, the Fv/Fm protocol was used to determine the fluorescence induction variables: initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv = Fm - Fo) and quantum efficiency of photosystem II (Fv/Fm); this protocol was performed after adaptation of the leaves to the dark for a period of 30 min, using a clip of the device, in order to ensure that all the primary acceptors are oxidized, that is, the reaction centers are open (MONTEIRO et al., 2018).

Electrolyte leakage (EL) in the leaf blade was also determined according to the methodology proposed by Scotti-Campos et al. (2013) by the relationship between the initial (Ci) and final (Cf) electrical conductivity, according to Equation 6.

$$\text{EL} = \frac{C_i}{C_f} \times 100 \quad (6)$$

Where:

EL = Electrolyte leakage in the leaf blade (%);

Ci = initial electrical conductivity ( $\text{dS m}^{-1}$ );

Cf = final electrical conductivity ( $\text{dS m}^{-1}$ ).

Shapiro-Wilk test was applied to the obtained data to verify the assumption of normality. Next, the data were subjected to analysis of variance by the F test at  $p \leq 0.05$  and, when significant, linear and quadratic polynomial regression analysis was performed, using the statistical program SISVAR ESAL (FERREIRA, 2019). When heterogeneity

occurred in the data, verified through the values of the coefficient of variation, they were subjected to exploratory analysis, with transformation to square root.

## RESULTS AND DISCUSSION

Chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid contents were significantly affected ( $p \leq 0.05$ ) by the salinity levels of irrigation water and by the foliar application of hydrogen peroxide -  $\text{H}_2\text{O}_2$ . The interaction between the factors (SL  $\times$   $\text{H}_2\text{O}_2$ ) significantly affected ( $p \leq 0.05$ ) the chlorophyll *b* and carotenoid contents of sour passion fruit (Table 2).

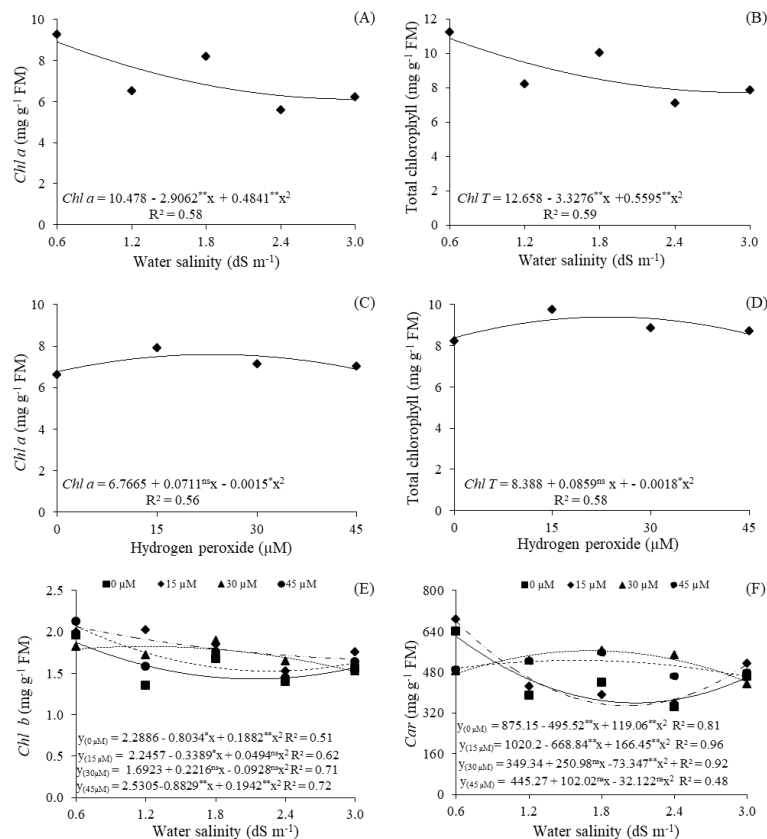
The increase in the electrical conductivity of irrigation water reduced the chlorophyll *a* contents in the sour passion fruit cv. BRS Rubi do Cerrado and, according to the regression equation (Figure 2A), there was a quadratic reduction, with maximum and minimum values estimated at 8.91 and 6.11  $\text{mg g}^{-1}$  FM, respectively, in plants irrigated using waters with electrical conductivity of 0.6 and 3.0  $\text{dS m}^{-1}$ . In relative terms, in the comparison of plants irrigated with water of higher salinity level (3.0  $\text{dS m}^{-1}$ ) with those that received the lowest ECw level (0.6  $\text{dS m}^{-1}$ ), there was a reduction of 32.72% (3.03  $\text{mg g}^{-1}$  FM) in *Chl a* content. The reduction in chlorophyll *a* content can be attributed to the increase in the activity of the chlorophyllase enzyme, which acts by degrading photosynthesizing pigments (FREIRE et al., 2013). Lima et al. (2020), when studying the contents of photosynthetic pigments in passion fruit cv. BRS Rubi do Cerrado irrigated using water with electrical conductivity of 0.3 to 3.5  $\text{dS m}^{-1}$  and under two doses of potassium -  $\text{K}_2\text{O}$  (50 and 100% of the recommendation) in a protected environment, concluded that chlorophyll *a* synthesis was significantly reduced with the use of 3.5  $\text{dS m}^{-1}$  water.

As observed for *Chl a* (Figure 2A), the total chlorophyll content of sour passion fruit plants decreased quadratically with increasing salinity of irrigation water (Figure 2B), and the maximum and minimum values were estimated at 10.86 and 7.71  $\text{mg g}^{-1}$  FM, respectively, when the plants were grown under irrigation with ECw of 0.6 and 3.0  $\text{dS m}^{-1}$ . When comparing using water with lower salt concentration (0.6  $\text{dS m}^{-1}$ ) with that of plants that received ECw of 3.0  $\text{dS m}^{-1}$ , there was a decrease of 30.16% (3.39  $\text{mg g}^{-1}$  FM). This reduction in chlorophyll synthesis may be associated with inhibition of chlorophyll biosynthesis and instability of protein complexes caused by the effects of salt stress (HOUIMLI et al., 2010).

**Table 2.** Chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), total chlorophyll (*Chl total*) and carotenoids (*Car*) contents of sour passion fruit cv. BRS Rubi do Cerrado under irrigation with saline waters and foliar application of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub>, at 240 days after transplanting.

Source of variation	DF	Mean squares			
		<i>Chl a</i>	<i>Chl b</i>	<i>Chl total</i>	<i>Car</i>
Salinity levels (SL)	4	27.7**	0.3**	34.6**	71918.1**
Linear regression	1	58.6**	0.9**	74.1**	40785.5**
Quadratic regression	1	5.1**	0.1 <sup>ns</sup>	6.8**	16467.6**
Residual 1	8	0.5	0.01	0.6	374.9
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	3	4.2*	0.1**	5.9**	17583.4**
Linear regression	1	0.1 <sup>ns</sup>	0.05 <sup>ns</sup>	0.2 <sup>ns</sup>	113.4 <sup>ns</sup>
Quadratic regression	1	7.0*	0.2**	10.0**	35133.5**
Interaction (SL × H <sub>2</sub> O <sub>2</sub> )	12	2.2 <sup>ns</sup>	0.06**	2.7 <sup>ns</sup>	19818.5**
Residual 2	30	1.2	0.01	1.4	422.8
CV 1 (%)	-	10.6	6.4	8.7	3.9
CV 2 (%)	-	15.6	7.6	13.5	4.2

<sup>ns</sup>, \*\*, \* respectively not significant, significant at probability levels of  $p \leq 0.01$  and  $p \leq 0.05$ . <sup>1</sup> Data transformed to  $\sqrt{x}$ .



**Figure 2.** Chlorophyll *a* - *Chl a* (A) and total chlorophyll (B) contents of sour passion fruit plants cv. BRS Rubi do Cerrado as a function of irrigation water salinity; *Chl a* (C) and total chlorophyll (D) contents as a function of hydrogen peroxide concentrations; and chlorophyll *b* - *Chl b* (E) and carotenoids - *Car* (F) contents as a function of the interaction between irrigation water salinity and hydrogen peroxide concentrations, at 240 days after transplanting.

Regarding the effects of hydrogen peroxide concentrations on chlorophyll *a* contents of sour passion fruit plants (Figure 2C), the regression showed that the highest estimated value of 7.609 mg g<sup>-1</sup> FM was obtained in plants that received the estimated H<sub>2</sub>O<sub>2</sub> concentration of 24 μM and, from this point on, there was a reduction in *Chl a* contents. In relative terms, plants that received 45 μM of H<sub>2</sub>O<sub>2</sub> increased their chlorophyll contents by 0.162 mg g<sup>-1</sup> FM compared to those that did not receive hydrogen peroxide (0 μM of H<sub>2</sub>O<sub>2</sub>). According to Farooq et al. (2017), hydrogen peroxide is the reactive oxygen species with greatest stability in cells and, at high concentrations, can rapidly diffuse through the subcellular membrane, resulting in oxidative damage to the cell membrane.

Regarding the *Chl total* contents (Figure 2D), it was verified that the exogenous application of 15 μM of H<sub>2</sub>O<sub>2</sub> promoted the highest value (9.74 mg g<sup>-1</sup> FM). According to the regression equation (Figure 2D), the highest *Chl total* content (9.41 mg g<sup>-1</sup> FM) was reached when the H<sub>2</sub>O<sub>2</sub> concentration of 23.86 μM was applied. When comparing the total chlorophyll contents of plants that received foliar application of 45 μM of H<sub>2</sub>O<sub>2</sub> with that of plants subjected to the control treatment (0 μM), there was an increase of 5.61% (0.49 mg g<sup>-1</sup> FM). Despite the action of this reactive oxygen species in the plant defense system in the production of antioxidative biochemical agents such as superoxide dismutase, catalase and ascorbate peroxidase that seek to find redox homeostasis (YAO et al., 2021), its effect on plants depends on the H<sub>2</sub>O<sub>2</sub> concentration applied.

From the moment photooxidation occurs, an irreversible process that directly involves photosynthesizing pigments that absorb light and become excited, producing free radicals such as superoxide and/or hydrogen peroxide, which can destroy the pigments (VERNON; SEEL, 2014), it is believed that the exogenous application of low concentrations of hydrogen peroxide stimulates biochemical defenses of plants, hence producing enzymes such as superoxide dismutase, which endogenously destroys free radicals of H<sub>2</sub>O<sub>2</sub>.

The increase in chlorophyll *b* and carotenoid contents in sour passion fruit plants may be related to the physiological defense process of plants against photooxidation to avoid a reduction in photosynthetic efficiency (SILVA et al., 2014). Plants use a number of enzymatic antioxidants (catalase – CAT, ascorbate peroxidase – APX, glutamine reductase – GR) and non-enzymatic antioxidants (ascorbic acid, carotenoids, flavonoids and phenolic compounds) to prevent oxidative damage and maintain the concentrations of reactive oxygen species within a functional range (OZGUR et al., 2013).

Qiao et al. (2021), when studying the effect of the combined treatment of NaCl (85.56, 171.12 and 342.23 mM) and hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> (0.5, 1, 2 and 4 mM) on lipid accumulation in *Monoraphidium* sp. (microalgae), verified that chlorophyll contents decreased with the application of hydrogen peroxide and NaCl, indicating that there was a change in physiological and biochemical metabolism from normal growth to biosynthesis of lipids.

Regarding the chlorophyll *b* contents of sour passion fruit (Figure 2E), plants that received foliar application of H<sub>2</sub>O<sub>2</sub> at concentrations of 15, 30 and 45 μM showed a quadratic reduction, with maximum estimated values in plants irrigated with ECw of 3.0, 1.21 and 2.26 dS m<sup>-1</sup>, respectively. When considering the effect of irrigation water salinity with the foliar application of hydrogen peroxide on chlorophyll *b* contents, it was verified that the foliar application of 45 μM of H<sub>2</sub>O<sub>2</sub> promoted an increase of 22.64% (0.48 mg g<sup>-1</sup> FM) in plants irrigated with water of lower salinity (0.6 dS m<sup>-1</sup>) compared to those receiving the same concentration of hydrogen peroxide under irrigation with water of 3.0 dS m<sup>-1</sup>. It is assumed that the application of hydrogen peroxide at high concentrations associated with irrigation with saline water can induce oxidative stress.

Regarding the carotenoid contents of sour passion fruit plants (Figure 2F), it is verified that the data were described by a quadratic model, whose estimated maximum values (360.04, 347.73, 564.04 and 526.47 mg g<sup>-1</sup> FM) were obtained in plants that received 0, 15, 30 and 45 μM of H<sub>2</sub>O<sub>2</sub> and were irrigated using water with ECw of 2.08, 2.01, 1.71 and 1.58 dS m<sup>-1</sup>, respectively. Plants irrigated with the lowest salinity level (0.6 dS m<sup>-1</sup>) showed the highest contents of carotenoids (689 mg g<sup>-1</sup> FM) for H<sub>2</sub>O<sub>2</sub> concentration of 15 μM. The lowest carotenoid content (1.34 mg g<sup>-1</sup> FM) was observed when sour passion fruit plants cv. BRS Rubi do Cerrado were irrigated with ECw of 1.2 dS m<sup>-1</sup> and subjected to 0 μM of hydrogen peroxide.

It is suggested that, for photosynthetic pigments, hydrogen peroxide at the concentration of 30 μM can act as a signaling molecule of abiotic stress, through the action of enzymatic and non-enzymatic components, promoting an increase in carotenoid contents, induced by the production of β-carotene, which are integrated components of thylakoids, acting in the absorption and transfer of light to chlorophyll (SILVA et al., 2017), since the excess of salts causes imbalances in chloroplast activities, such as reduction in the synthesis of 5-aminolevulinic acid, which is a chlorophyll precursor molecule, inducing an increase in oxidative activity, resulting in the degradation of molecules of photosynthesizing pigments such as chlorophyll *b* (CAVALCANTE et al., 2011).

The salinity of irrigation water significantly affected ( $p \leq 0.05$ ) only electrolyte leakage (EL), while hydrogen peroxide concentrations significantly affected the quantum efficiency of photosystem II

(Fv/Fm). There was interaction between the factors (SL  $\times$  H<sub>2</sub>O<sub>2</sub>), significantly affecting the electrolyte leakage (EL) of sour passion fruit cv. BRS Rubi do Cerrado (Table 3).

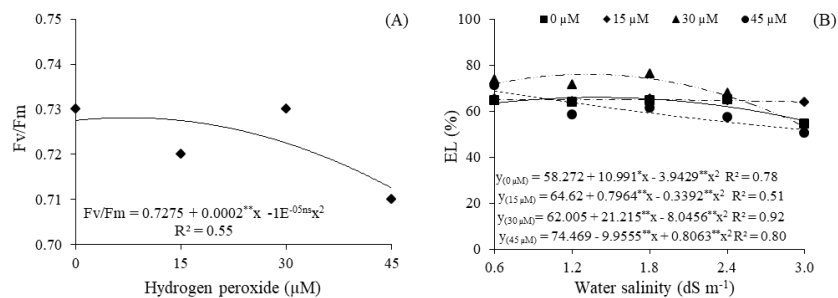
**Table 3.** Summary of the analysis of variance for initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv), quantum efficiency of photosystem II (Fv/Fm), and electrolyte leakage (EL) of sour passion fruit cv. BRS Rubi do Cerrado under irrigation with saline waters and foliar application of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub>, at 240 days after transplanting.

Source of variation	DF	Mean squares				
		Fo	Fm	Fv	Fv/Fm	EL
Salinity levels (SL)	4	10865.5 <sup>ns</sup>	87047.3 <sup>ns</sup>	66784.6 <sup>ns</sup>	0.0007 <sup>ns</sup>	305.5 <sup>**</sup>
Linear regression	1	22715.0 <sup>*</sup>	270750.0 <sup>ns</sup>	146091.4 <sup>ns</sup>	1 $\times$ 10 <sup>-5ns</sup>	1023.8 <sup>**</sup>
Quadratic regression	1	1423.3 <sup>ns</sup>	28029.3 <sup>ns</sup>	24650.1 <sup>ns</sup>	1 $\times$ 10 <sup>-3ns</sup>	86.4 <sup>**</sup>
Residual 1	8	4699.9	61737.2	52213.9	0.001	0.3
Hydrogen peroxide (H <sub>2</sub> O <sub>2</sub> )	3	3517.1 <sup>ns</sup>	56004.1 <sup>ns</sup>	74221.9 <sup>ns</sup>	0.002 <sup>**</sup>	210.2 <sup>**</sup>
Linear regression	1	6093.0 <sup>ns</sup>	134916.8 <sup>ns</sup>	183224.6 <sup>*</sup>	0.004 <sup>**</sup>	0.05 <sup>ns</sup>
Quadratic regression	1	19.3 <sup>ns</sup>	564.3 <sup>ns</sup>	41.6 <sup>ns</sup>	0.0003 <sup>ns</sup>	434.7 <sup>**</sup>
Interaction (SL $\times$ H <sub>2</sub> O <sub>2</sub> )	12	1788.9 <sup>ns</sup>	36915.9 <sup>ns</sup>	27482.4 <sup>ns</sup>	0.0003 <sup>ns</sup>	76.1 <sup>**</sup>
Residual 2	30	2197.5	40455.6	34582.4	0.0005	0.7
CV 1 (%)	-	9.12	9.19	11.64	4.54	0.95
CV 2 (%)	-	6.24	7.44	9.47	2.98	1.35

ns, \*\*, \* respectively not significant, significant at probability levels of  $p \leq 0.01$  and  $p \leq 0.05$ .

Quantum efficiency of photosystem II was also significantly affected by the exogenous application of hydrogen peroxide (Figure 3A). According to the regression equation, there was higher Fv/Fm (0.72) with the application of 10  $\mu$ M of H<sub>2</sub>O<sub>2</sub>. In relative terms, there was a reduction of 2.73% (0.02) in Fv/Fm when the hydrogen peroxide concentration increased from 0 to 45  $\mu$ M. The decrease in Fv/Fm possibly stands out as a defense mechanism to reduce the absorption of light energy and thus decrease the flow of electrons in the electron transport chain (WILLADINO et al., 2011).

According to Seon et al. (2000), the ideal Fv/Fm ratio should be between 0.75 and 0.85, and the photoinhibitory damage to the reaction centers of photosystem II in plants cultivated with high-salinity water also varies according to the phenological stage of the crop. Freire et al. (2014) evaluated the cultivation of yellow passion fruit under irrigation with saline water (ECw: 0.5 and 4.5 dS m<sup>-1</sup>) and observed that the increase in water salt content until the beginning of flowering reduced Fv/Fm from 0.82 to 0.77, with depletion of 6.1%.



**Figure 3.** Quantum efficiency of photosystem II - Fv/Fm (A) of sour passion fruit plants cv. BRS Rubi do Cerrado, as a function of the application of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> and electrolyte leakage - EL (B) as a function of the interaction between the levels of irrigation water salinity - ECw and concentrations of H<sub>2</sub>O<sub>2</sub>, at 240 days after transplantation.



The use of saline waters in the irrigation of the sour passion fruit cv. BRS Rubi do Cerrado with foliar application of hydrogen peroxide led to an increase in electrolyte leakage in the leaf blade and consequently a reduction in cell integrity. By the regression equation (Figure 3B), it can be observed that the lowest EL (64.62%) was obtained when plants were irrigated with water of electrical conductivity estimated at  $1.16 \text{ dS m}^{-1}$  and with foliar application of  $15 \text{ }\mu\text{M}$ . However, the highest EL occurred when hydrogen peroxide was not applied ( $0 \text{ }\mu\text{M}$ ) and under saline water of  $1.38 \text{ dS m}^{-1}$ .

$\text{H}_2\text{O}_2$  is a molecular signaling agent that contributes to regulating the size of the antenna of photosystem II, leading to the long-term acclimatization response of the photosynthetic apparatus under excessive light conditions. Therefore, it is proposed that  $\text{H}_2\text{O}_2$  is a molecule released by the set of molecules of the plastoquinone group, which is one of the main agents through which from its redox state it is an isoprenoid quinone molecule involved in the electron transport chain in light-dependent reactions of photosynthesis, providing regulatory effect on abiotic stress (BORISOVA-MUBARAKSHINA et al., 2015).

## CONCLUSIONS

Irrigation with saline water reduces chlorophyll *a* and total chlorophyll contents of sour passion fruit plants cv. BRS Rubi do Cerrado, at 240 days after transplanting.

Hydrogen peroxide at the concentration of  $15 \text{ }\mu\text{M}$  stimulates chlorophyll *a* and total chlorophyll biosynthesis and, at  $45 \text{ }\mu\text{M}$ , relieves the effect of  $3.0 \text{ dS m}^{-1}$  water salinity on electrolyte leakage in the leaf blade of sour passion fruit.

Salt stress does not affect the initial, maximum and variable fluorescence and quantum efficiency of photosystem II of the sour passion fruit cv. BRS Rubi do Cerrado.

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