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Hydrogen peroxide in the acclimation of yellow passion fruit seedlings to salt stress[1]

Peróxido de hidrogênio na aclimatação de mudas de maracujazeiro amarelo ao estresse salino

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HIGHLIGHTS:
Hydrogen peroxide mitigates the effects of water salinity on the gas exchange of yellow passion fruit.
Saline stress inhibits the emergence and relative growth in height of passion fruit plants.
High concentration of hydrogen peroxide intensifies the effect of salt stress on passion fruit.

ABSTRACT: The objective of the present study was to evaluate the effect of exogenous application of hydrogen peroxide on the emergence, growth and gas exchange of yellow passion fruit seedlings subjected to salt stress. The experiment was conducted in pots (Citropote®) under greenhouse conditions, in the municipality of Campina Grande, PB, Brazil. Treatments were distributed in a randomized block design, in a 4 x 4 factorial arrangement, with four levels of electrical conductivity of irrigation water (0.7, 1.4, 2.1 and 2.8 dS m^{-1}) associated with four concentrations of hydrogen peroxide (0, 25, 50 and 75 μM), with four replicates and two plants per plot. Irrigation using water with electrical conductivity above 0.7 dS m^{-1} negatively affects the emergence and growth of passion fruit. Hydrogen peroxide concentrations between 10 and 30 μM induce the acclimation of passion fruit plants to salt stress, mitigating the deleterious effects of salinity on the relative growth rate in stem diameter and leaf area, stomatal conductance, transpiration, CO_2 assimilation rate and instantaneous carboxylation efficiency. Irrigation water salinity combined with hydrogen peroxide concentrations above 30 μM causes reduction in passion fruit growth and physiology.

Key words: *Passiflora edulis* Sims f. *flavicarpa* Degener, saline waters, mitigation

RESUMO: Objetivou-se com o presente trabalho, avaliar o efeito da aplicação exógena de peróxido de hidrogênio sobre a emergência, o crescimento e as trocas gasosas de mudas de maracujazeiro amarelo submetido ao estresse salino. O estudo foi conduzido em citropotes sob condição de casa de vegetação, no município de Campina Grande, PB. Os tratamentos foram distribuídos em delineamento de blocos casualizados, em arranjo fatorial 4 x 4, sendo quatro valores de condutividade elétrica da água de irrigação (0,7; 1,4; 2,1 e 2,8 dS m^{-1}) associados a quatro concentrações de peróxido de hidrogênio (0, 25, 50 e 75 μM), com quatro repetições e duas plantas por parcela. A irrigação com água acima de 0,7 dS m^{-1} afeta negativamente a emergência e o crescimento do maracujazeiro. Concentrações de peróxido de hidrogênio entre 10 e 30 μM induz a aclimatação das plantas ao estresse salino, mitigando os efeitos deletérios da salinidade sobre a taxa de crescimento relativo em diâmetro de caule e área foliar, condutância estomática, transpiração, taxa de assimilação de CO_2 e eficiência instantânea da carboxilação. A salinidade da água de irrigação aliada a concentrações de peróxido de hidrogênio acima de 30 μM causa redução no crescimento e na fisiologia do maracujazeiro amarelo.

Palavras-chave: *Passiflora edulis* Sims f. *flavicarpa* Degener, águas salinas, mitigação

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Introduction

Yellow passion fruit (*Passiflora edulis* Sims f. *flavicarpa Degener*) is among the most economically important fruit crops in Brazil, accounting for about 95% of commercial orchards, with 60% of all production located in the North and Northeast regions. Such relevance, besides being related to the vigor of fruits, yield, flavor of the juice and its medicinal and ornamental characteristics, is also associated with the favorable edaphoclimatic conditions for the exploitation of the crop (Bezerra et al., 2016; Araújo et al., 2017, Silva et al., 2019a).

Although the Northeast region is a major producer of yellow passion fruit, the growth and production of this crop are limited by the high levels of salts commonly found in the springs of the semi-arid region. For being classified as a glycophyte, excess salts in irrigation water have severe consequences on the emergence and initial growth of passion fruit, resulting from the deleterious action of salts on the physiological and biochemical mechanisms of plants (Freire & Nascimento, 2018).

However, it is possible to consider that the viable production of passion fruit seedlings in the semi-arid region of Northeastern Brazil can be optimized through alternatives that mitigate the negative effect of salt stress on plants. In this scenario, studies conducted using hydrogen peroxide in the acclimation of plants to salt stress are alternatives to increase their capacity to survive adverse conditions (Silva et al., 2019b).

Among the acclimation processes, the pretreatment of plants with low concentrations of H_2O_2 has been shown to be promising. Gondim et al. (2011), working with maize plants, concluded that spraying with hydrogen peroxide induced acclimation of plants to salt stress. Souza et al. (2019), working with cashew, verified that the use of the hydrogen peroxide concentration of 20 μM promoted the highest phytomass accumulation and quality of cashew rootstocks.

In view of the above, the objective of this study was to evaluate the effect of exogenous application of hydrogen peroxide on the emergence, growth and gas exchange of passion fruit seedlings cultivated under different levels of irrigation water salinity.

Material and Methods

The study was conducted from June to August 2017 in 8-dm^3 polypropylene pots (Citropote®), under greenhouse conditions, at the Center for Technology and Natural Resources of the Federal University of Campina Grande (CTRN/UFCG), located in Campina Grande, PB, Brazil, at the geographical coordinates 7° 15’ 18” S latitude, 35° 52’ 28” W longitude and average altitude of 550 m.

The treatments resulted from the combination, in a 4 x 4 factorial arrangement, of levels of electrical conductivity of irrigation water - ECw (0.7, 1.4, 2.1 and 2.8 dS m^{-1}) and concentrations of hydrogen peroxide - H_2O_2 (0, 25, 50 and 75 μM), distributed in the randomized block design, with four replicates. The experimental unit consisted of two plants.

The seeds of yellow passion (*Passiflora edulis* Sims *f. flavicarpa* Degener) used in the study came from fruits of a commercial orchard located in the municipality of Nova Floresta, PB, obtained from plants subjected to mass selection, standardized based on vigor and health. This genotype is commonly known as *Guinezinho* due to the spots on the rind similar to those existing on the feathers of a bird locally known as ‘galinha Guiné’ (Helmeted guineafowl - *Numida meleagris*) (Medeiros et al., 2016).

The irrigation waters with the respective levels of electrical conductivity - ECw were prepared by dissolving the salts NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$, in the equivalent ratio of 7:2:1, respectively, in water from the local supply (ECw = 1.10 dS m^{-1}), based on the relationship between ECw and the concentration of salts (mmol$_c$ L^{-1} = 10 * ECw dS m^{-1}) (Rhoades et al., 2000). This proportion of salts is commonly found in sources of water used for irrigation, in small properties in the Northeastern Brazil, while the level of 0.7 dS m^{-1} was obtained by diluting water from the local supply in rainwater (ECw = 0.02 dS m^{-1}).

The pots were filled with 6.0 kg of an air-dried substrate composed of: soil (84%), sand (15%) and humus (1%) on mass basis. The soil used in the experiment was classified as *Neossolo Regolítico* (Psamments - United States, 2014) of sandy loam texture collected in the 0-20 cm layer, from the rural area of Lagoa Seca, PB, properly pounded to break up clods and sieved. Its physical and chemical characteristics were determined according to the methodology proposed by Teixeira et al. (2017), showing the following results: Ca^{2+}, Mg^{2+}, Na^+, K^+, Al^{3+}+H^+ = 2.60; 3.66; 0.16; 0.22 and 1.93 cmol$_c$ kg^{-1}, respectively; pH (water 1:2.5) = 5.9; ECse = 1.0 dS m^{-1}; organic matter = 1.36 dag kg^{-1}; sand, silt and clay = 732.9, 142.1, and 125.0 g kg^{-1}, respectively; apparent density = 1.39 kg dm^{-3}; moisture content at 33.42 and 1519.5 kPa = 11.98 and 4.32 dag kg^{-1}, respectively.

Prior to sowing, the soil moisture content was increased until reaching field capacity using the respective water of each treatment. After sowing, irrigation was performed daily, applying in each pot a volume of water to maintain soil moisture close to field capacity. The applied volume was determined according to the water requirement of the plants, estimated by water balance, subtracting the volume drained from the volume applied in the previous irrigation, plus a leaching fraction of 0.10 to avoid excessive accumulation of salts in the root zone. The frequency of leaching fraction application was 15 days.

Hydrogen peroxide (H_2O_2) concentrations were established according to a study conducted by Panngom et al. (2018), whose dilution was performed in deionized water. The seeds underwent a pre-treatment with H_2O_2 in which they were soaked in a solution with the concentrations of the respective treatments for 24 hours. Seeds of the control treatment (0 μM) were soaked in distilled water for the same period. Sowing was performed after the previously described treatment, by planting five seeds at 3 cm depth, equidistantly distributed. At 20 days after emergence (DAE), thinning was performed in order to leave only one plant per pot, selecting the one with the best vigor.

Top-dressing fertilization with nitrogen, potassium and phosphorus was performed based on the recommendation of Novais et al. (1991), applying 1.33 g of urea, 1.50 g of potassium chloride and 3.60 g of monoammonium phosphate, equivalent to 100, 150 and 300 mg kg^{-1} of the substrate of N, K and P, respectively, in four applications via fertigation, at intervals of 15 days, the first application being performed at 15 days after sowing (DAS). To meet the need for micronutrients, the plants were sprayed with a nutrient solution containing 2.5 g L^{-1} of the following composition: [(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%); Mo (0.02%)], 30, 45 and 60 DAS. foliar sprays (adaxial and abaxial faces) were manually performed between 17:00 and 17:30 hours with the respective hydrogen peroxide solutions using a sprayer.

Treatment effects on passion fruit seedlings were determined through the emergence speed index (ESI), seedling emergence percentage (EP), relative growth rate in plant height (RGR_{PH}), stem diameter (RGR_{SD}) and leaf area (RGR_{LA}); and gas exchange variables: stomatal conductance (gs), transpiration (E), CO_2 assimilation rate (A), intercellular CO_2 concentration (Ci), instantaneous carboxylation efficiency (CEi) and instantaneous water use efficiency (WUEi).

Seedling emergence percentage was obtained by daily counting the number of emerged seedlings, until their establishment, adopting the criterion of epicotyl appearance on the surface of the container. These data were used to determine ESI (seedlings d^{-1}) using Eq.1, presented by Carvalho & Nakagawa (2000)

$$\mathrm{ESI}\left(\text{seedlings d}^{-1}\right)=\frac{\Sigma_1}{N_1}+\frac{\Sigma_2}{N_2}+\ldots+\frac{\Sigma_n}{N_n} \quad (1)$$

where:

Σ_1, Σ_2, ... Σ_n - number of seedlings emerged, respectively, in the first, second, ... and last counts; and,

N_1, N_2, ... N_n - number of days between sowing and the first, second, ... and last counts, respectively.

The relative growth rates of passion fruit seedlings were measured in the period from 35 to 60 DAS, based on the mean values of plant height, stem diameter and leaf area. The relative growth rates were obtained according to the methodology contained in Benincasa (2003) expressed by Eq. 2.

$$\mathrm{RGR}=\frac{\left(\ln A_2-\ln A_1\right)}{\left(T_2-T_1\right)} \quad (2)$$

where:

RGR - relative growth rate (mm mm^{-1} d^{-1}; cm cm^{-1} d^{-1}; or cm^2 cm^{-2} d^{-1});

A_1 - value of the variable at time T_1;

A_2 - value of the variable at time T_2; and,

ln - natural logarithm.

Gas exchange was measured at 45 DAS by determining stomatal conductance (mol H_2O m^{-2} s^{-1}), transpiration (mmol H_2O m^{-2} s^{-1}), CO_2 assimilation rate (μmol m^{-2} s^{-1}) and internal CO_2 concentration (μmol m^{-2} s^{-1}), evaluated in the third fully expanded leaf, from the apex, with the portable gas exchange meter "LCPro+" from ADC BioScientific Ltda. These data were then used to calculate instantaneous water use efficiency (WUEi) (A/E) [(μmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1}) and instantaneous carboxylation efficiency (CEi) (A/Ci) [(μmol m^{-2} s^{-1}) (μmol mol^{-1}]$^{-1}$.

The collected data were subjected to analysis of variance and, when significant, linear and quadratic polynomial regression analysis was performed, using the statistical program SISVAR (Ferreira, 2019).

Results and Discussion

According to the summary of the analysis of variance (Table 1), there was a significant effect of the interaction between the studied factors (SL x H_2O_2) on the relative growth rate in stem diameter (RGR_{SD}) and leaf area (RGR_{LA}). The salinity levels of irrigation water influenced ($p \leq 0.01$) all variables analyzed, except for the relative growth rate in leaf area (RGR_{LA}). Hydrogen peroxide concentrations, when analyzed alone, influenced only the relative growth rate in stem diameter (RGR_{SD}) in the period from 35 to 60 days after sowing.

Emergence speed index was negatively affected by the increase in irrigation water salinity (Figure 1A) and, according to the regression equation, there was a reduction of 7.65% per unit increase in ECw. When comparing the ESI of seedlings cultivated under ECw of 2.8 dS m^{-1} with the values of those that received the lowest salinity level (0.7 dS m^{-1}), there was decrease of 16.98% (0.0483 seedlings d^{-1}). Similar results were obtained by Ribeiro et al. (2016) in yellow passion fruit plants under salt stress (0.23 to 4.5 dS m^{-1}), with a reduction of 21.09% in ESI under the highest salinity level when compared with lowest salinity level.

Table 1. Summary of the analysis of variance for emergence speed index (ESI), emergence percentage (EP) and relative growth rates of plant height (RGR_{PH}), stem diameter (RGR_{SD}) and leaf area (RGR_{LA}) of yellow passion fruit irrigated with saline waters and subjected to hydrogen peroxide concentrations

Source of variation	DF	Mean squares				
		ESI	EP	RGR_{PH}	RGR_{SD}	RGR_{LA}
Salinity levels (SL)	3	0.0073^{**}	859.37^{**}	0.0013^{**}	$13 \times 10^{-5**}$	0.0001^{ns}
Linear regression	1	0.0211^{**}	1531.25^{**}	0.0033^{**}	0.0002^{**}	3×10^{-5ns}
Quadratic regression	1	0.0009^{ns}	976.56^{ns}	0.0001^{ns}	2×10^{-5ns}	2×10^{-5ns}
Hydrogen peroxide (H_2O_2)	3	0.0015^{ns}	156.25^{ns}	2×10^{-5ns}	$6 \times 10^{-5**}$	6×10^{-5ns}
Linear regression	1	0.0043^{ns}	281.25	0.0003^{ns}	0.0001^{ns}	4×10^{-5ns}
Quadratic regression	1	0.0001^{ns}	156.25^{ns}	0.0001^{ns}	1×10^{-6ns}	1×10^{-5ns}
Interaction (SL x H_2O_2)	9	0.0027^{ns}	269.09^{ns}	0.0002^{ns}	$6 \times 10^{-5**}$	$23 \times 10^{-5*}$
Blocks	3	0.0029^{ns}	52.08^{ns}	0.009^{ns}	3×10^{-5ns}	0.0001^{ns}
Residue	45	0.0016	149.31	0.0001	1×10^{-5}	1×10^{-5}
CV (%)		15.32	13.32	15.92	15.16	9.57

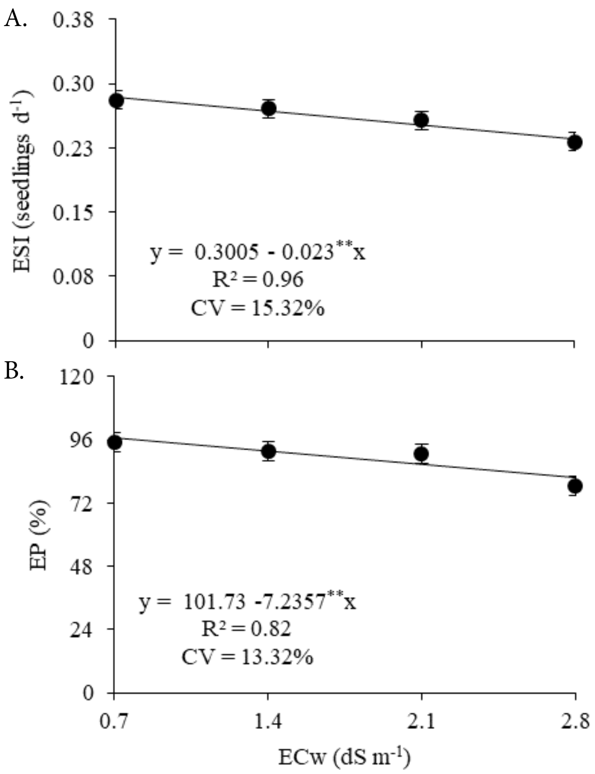
$^{ns, *, **}$ - Not significant, significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test, respectively

The emergence percentage of yellow passion fruit seedlings also decreased linearly as a function of the increase in ECw levels and, according to the regression equation (Figure 1B), there were reductions of 7.11% per unit increase in ECw, that is, seedlings under irrigation with the highest salinity level (2.8 dS m^{-1}) had a decrease of 15.72% in EP compared to those subjected to ECw of 0.7 dS m^{-1}. Beserra et al. (2014), evaluating the effects of irrigation water salinity on the emergence of yellow passion fruit seedlings, verified a reduction from 86.2 to 32.6% in emergence percentage, due to the use of water of lower (0.30 dS m^{-1}) and higher (4.0 dS m^{-1}) salinity.

The reductions in ESI and EP under saline stress can be attributed to the reduction of osmotic potential caused by the concentration of soluble salts in the soil, which directly affect water absorption by plants, thus contributing to making the germination process unviable; in addition, the toxic effect caused by the accumulation of Na^+ and Cl^- ions promotes changes in the metabolic processes of germination and, in extreme cases, death of the embryo may occur due to the excess of these toxic ions (Ibrahim, 2016; Belmehdi et al., 2018).

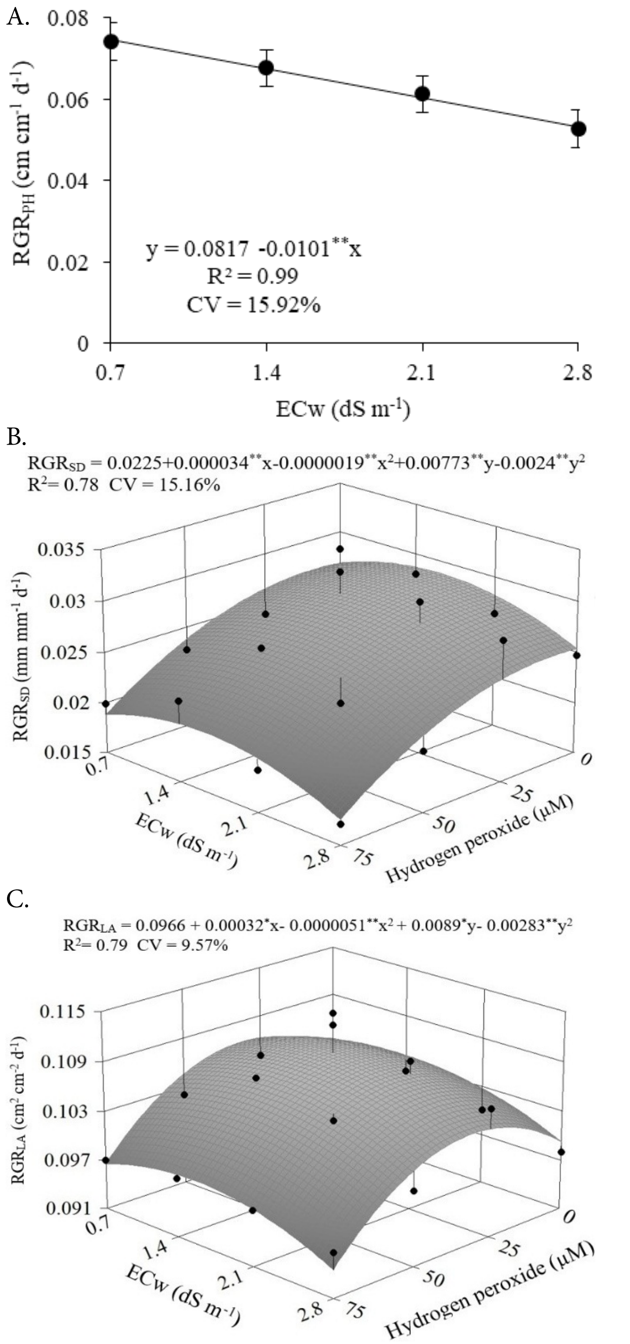
The increase in irrigation water salinity negatively affected the relative growth rate in plant height (RGR_{PH}), and the regression equation (Figure 2A) showed a reduction of 12.36% per unit increase in water salinity; thus, plants irrigated with water of 2.8 dS m^{-1} had a reduction of 28.42% in RGR_{PH} compared to those irrigated with water of 0.7 dS m^{-1}, that is, the height of plants irrigated with the lowest salinity level increased 0.012 cm cm^{-1} per day more than that of plants cultivated with the highest salinity level, in the period between 35 and 60 DAS.

Similar results were obtained by Bezerra et al. (2016) in yellow passion fruit plants under salt stress caused by waters



** - Significant at $p \leq 0.01$ by F test

Figure 1. Emergence speed index - ESI (A) and emergence percentage - EP (B) of yellow passion fruit plants as a function of the electrical conductivity of irrigation water - Ecw



X and Y - H_2O_2 concentration and ECw, respectively; *,** - Significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test

Figure 2. Relative growth rate in plant height - RGR_{PH} (A) as a function of electrical conductivity of irrigation water - ECw; relative growth rate in stem diameter - RGR_{SD} (B) and relative growth rate in leaf area - RGR_{LA} (C) of yellow passion fruit plants as a function of the interaction between ECw and hydrogen peroxide concentrations, in the period from 35 to 60 DAS

with salinity ranging from 0.3 to 8.0 dS m^{-1}, where reductions in RGR_{PH} were attributed to water deficit caused by excess soluble salts in the root zone, which results in reduction of turgor, leading to decrease in cell expansion and consequently in plant growth rate.

Wanderley et al. (2018) observed a significant reduction in the height of passion fruit seedlings under salt stress (irrigating with waters varying from 0.3 to 3.1 dS m^{-1}), demonstrating the sensitivity to salinity of the growth in height of the seedlings.

The interaction between the electrical conductivity of irrigation water and H_2O_2 concentrations significantly influenced the relative growth rate in stem diameter. The regression equation (Figure 2B) showed that plants irrigated with water of 1.6 dS m^{-1} and subjected to a H_2O_2 concentration of 15 µM obtained the highest RGR_{SD} (0.029 mm mm^{-1} d^{-1}). It can also be observed that RGR_{SD} decreased when using H_2O_2 concentrations above 15 µM, regardless of the electrical conductivity of irrigation water used; the lowest RGR_{SD} (0.017 mm mm^{-1} d^{-1}) was obtained in plants irrigated with water of 2.8 dS m^{-1} and subjected to a H_2O_2 concentration of 75 µM, corresponding to a reduction of 41.38% (0.012 mm mm^{-1} d^{-1}) when compared to plants with highest RGR_{SD}.

The relative growth rate in leaf area of yellow passion fruit was also affected by the interaction between the studied factors (SL x H_2O_2) and, according to regression equation (Figure 2C), plants irrigated with water of 1.6 dS m^{-1} and subjected to treatment with H_2O_2 concentration of 30 µM had the highest RGR_{LA} (0.1086 cm^2 cm^{-2} d^{-1}). However, RGR_{LA} decreased under the use of H_2O_2 concentrations above 30 µM, regardless of the electrical conductivity of irrigation water; the lowest RGR_{LA} (0.0946 cm^2 cm^{-2} d^{-1}) was obtained in plants irrigated with water of 2.8 dS m^{-1} and subjected to H_2O_2 concentration of 75 µM, corresponding to a reduction of 12.89% (0.014 cm^2 cm^{-2} d^{-1}) when compared to plants with highest RGR_{LA}.

The beneficial effect of hydrogen peroxide on the relative growth rate in stem diameter and leaf area of yellow passion fruit plants can be attributed to the fact that, when applied at appropriate concentrations, H_2O_2 stimulates some physiological processes such as CO_2 assimilation rate and stomatal conductance, thus leading to improvement of vegetative growth. In addition, the increase in plant growth with the application of hydrogen peroxide may have occurred due to greater absorption of water and nutrients, including essential ions for plant growth, such as N, P and K (Farouk & Amira, 2018).

However, it can be noted that at high concentrations hydrogen peroxide was harmful to the RGR_{SD} and RGR_{LA} of yellow passion fruit, with the lowest values obtained at the concentration of 75 µM. According to Farooq et al. (2017), hydrogen peroxide is the most stable reactive oxygen species in cells and, at high concentrations, it can spread rapidly through the subcellular membrane, thus resulting in oxidative damage to the cell membrane.

The interaction between salinity levels and H_2O_2 caused a significant effect on stomatal conductance (gs), transpiration (E), CO_2 assimilation rate (A) and instantaneous carboxylation efficiency (CEi) (Table 2). The salinity of irrigation water influenced all the variables analyzed, except the internal CO_2 concentration (Ci). In relation to the hydrogen peroxide concentrations, there were significant effects on gs, E and WUEi. Unlike the results found in the present study, Andrade et al. (2019) analyzed gas exchange and growth of passion fruit under irrigation with saline water and H_2O_2 application and verified that the interaction between factors did not significantly interfere in the gas exchange variables analyzed at 61 and 96 DAT, and there was no significant effect ($p > 0.05$) of H_2O_2 concentrations.

The interaction between the factors (SL x H_2O_2) caused a significant effect on the stomatal conductance (gs) of yellow passion fruit, at 45 DAS. Based on the regression equation (Figure 3A), it was verified that the highest stomatal conductance (0.13 mmol H_2O m^{-2} s^{-1}) was obtained in plants subjected to H_2O_2 concentration of 25 µM and irrigated with water of 1.6 dS m^{-1}. On the other hand, the increase in H_2O_2 concentrations from 25 µM led to reductions in stomatal conductance, with lowest gs (0.07 mmol H_2O m^{-2} s^{-1}) obtained in plants subjected to H_2O_2 concentration of 75 µM and irrigated with water of 2.8 dS m^{-1}, corresponding to a reduction of 46.15% (0.06 mmol H_2O m^{-2} s^{-1}) when compared to plants with the highest gs.

According to the regression equation of Figure 3B, referring to the transpiration of yellow passion fruit (E), there was an effect similar to that occurred on gs, that is, plants subjected to H_2O_2 concentration of 25 µM and irrigated with water of 1.6 dS m^{-1} had the highest transpiration (1.91 mmol H_2O m^{-2} s^{-1}). Despite the beneficial effect of hydrogen peroxide at the concentration of 25 µM, it can be noted that at concentrations greater than 25 µM transpiration is reduced and, with the increase in the electrical conductivity of irrigation water, the reduction of E becomes more pronounced, with lowest E (1.05 mmol H_2O m^{-2} s^{-1}) in plants subjected to H_2O_2 concentration of 75 µM and irrigated with water of 2.8 dS m^{-1}, corresponding to a reduction of 45.03% (0.86 mmol H_2O m^{-2} s^{-1}) when compared to plants with the highest E.

Similar results were obtained by Silva et al. (2019c), when evaluating the effect of hydrogen peroxide (0, 25, 50, 75 and 100 µM) on soursop (*Annona muricata* L.) plants under salt stress (0.7 to 3.5 dS m^{-1}), verifying that the exogenous application

Table 2. Summary of the analysis of variance for internal CO_2 concentration (Ci), stomatal conductance (gs), transpiration (E), CO_2 assimilation rate (A), instantaneous carboxylation efficiency (CEi) and instantaneous water use efficiency (WUEi) of yellow passion fruit irrigated with saline waters and subjected to hydrogen peroxide concentrations, at 45 days after sowing

Source of variation	DF	Mean squares					
		Ci	gs	E	A	CEi	WUEi
Salinity levels (SL)	3	2816.60^{ns}	0.0067^{**}	1.50^{**}	115.45^{**}	0.0043^{**}	7.82^{*}
Linear regression	1	7781.51^{ns}	0.0197^{**}	4.09^{**}	345.52^{**}	0.0127^{**}	15.17^{**}
Quadratic regression	1	650.25^{ns}	0.0002^{ns}	0.29^{ns}	0.007^{ns}	0.0002^{ns}	3.59^{ns}
Hydrogen peroxide (H_2O_2)	2	2072.56^{ns}	0.0022^{*}	0.58^{**}	1.61^{ns}	4×10^{-5ns}	13.22^{**}
Linear regression	1	1.01^{ns}	0.0012^{ns}	0.38^{ns}	0.98^{ns}	5×10^{-5ns}	17.01^{*}
Quadratic regression	1	3937.56^{ns}	0.0047^{*}	1.36^{**}	1.45^{ns}	1×10^{-5ns}	21.56^{**}
Interaction (SL x H_2O_2)	9	963.21^{ns}	0.0021^{*}	0.69^{**}	12.08^{**}	0.0003^{*}	2.45^{ns}
Blocks	3	4698.94^{ns}	0.0013^{ns}	0.27^{ns}	5.23_{ns}	5×10^{-5ns}	3.56^{ns}
Residue	45	1158.37	0.0008	0.13	2.44	13×10^{-5}	1.92
CV (%)		16.65	28.96	22.31	16.67	23.59	23.27

ns, *, ** - Not significant, significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test, respectively of H_2O_2 at the concentration of 25 μM promoted an increase in stomatal conductance and transpiration when compared to the control treatment (0 μM).

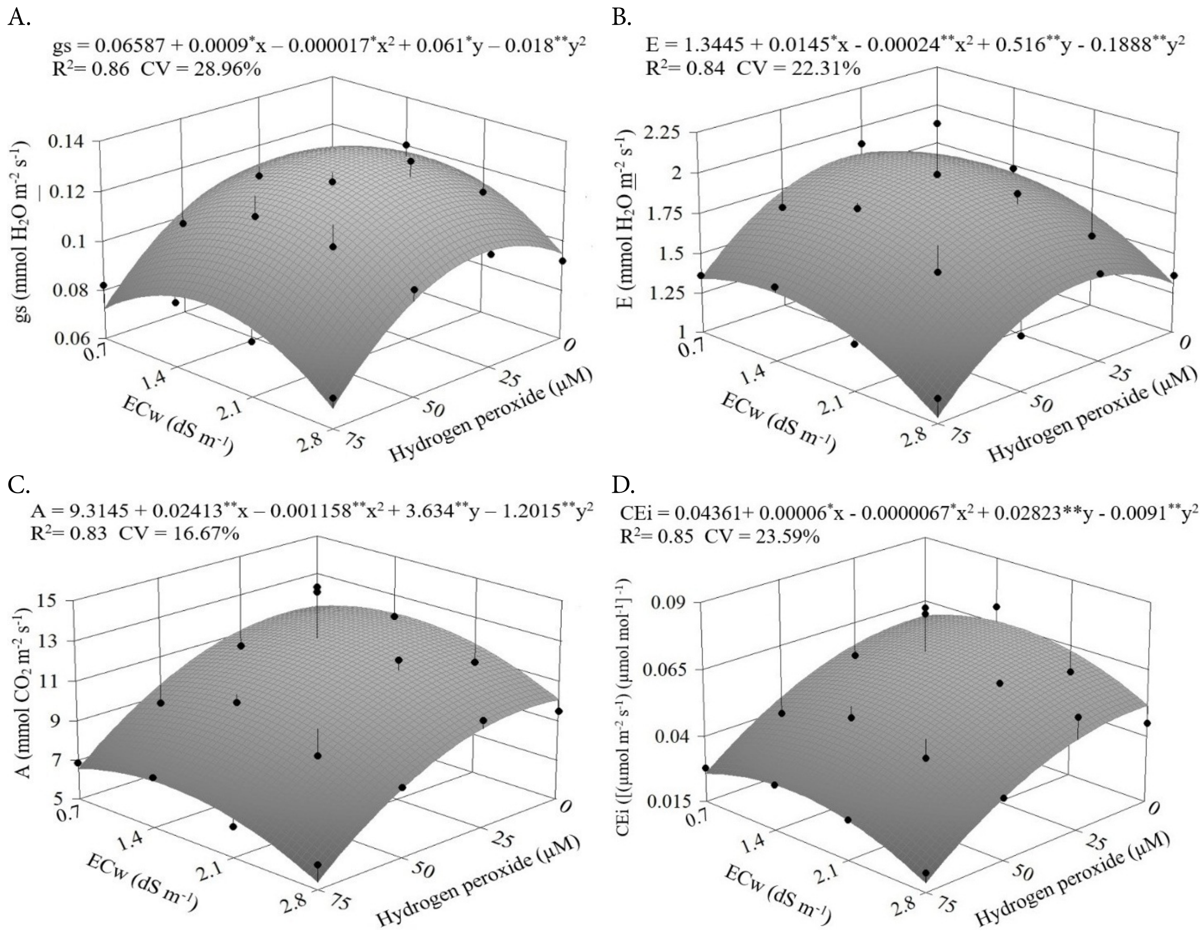
The beneficial effect of hydrogen peroxide up to the concentration of 25 μM observed on the stomatal conductance and transpiration of yellow passion fruit may occur due to the defense mechanisms developed by the plant itself, inducing the defense system of antioxidant enzymes, minimizing the deleterious effects of salinity (Carvalho et al., 2011). According to Azevedo Neto et al. (2005), exogenous application of H_2O_2 before exposure to salt stress induces salinity tolerance by activating the defense system of antioxidant enzymes such as superoxide dismutase, catalase, guaiacol peroxidase and ascorbate peroxidase.

The CO_2 assimilation rate (A) and the instantaneous carboxylation efficiency (CEi) were also affected by the interaction between the factors (SL x H_2O_2) and, according to the regression equations (Figures 3C and D), plants subjected to H_2O_2 concentration of 10 μM and irrigated with water of 1.6 dS m^{-1} reached the highest A (12.18 mmol CO_2 m^{-2} s^{-1}) and CEi (0.065 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$). The lowest CO_2 assimilation rate (7.07 mmol CO_2 m^{-2} s^{-1}) and CEi (0.018 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$) were obtained in plants subjected to a H_2O_2 concentration of 75 μM and irrigated with water of 2.8 dS m^{-1}, corresponding to a reduction of 41.95% (5.11 mmol CO_2 m^{-2} s^{-1}) for CO_2 assimilation rate and 72.31% (0.047 (μmol m^{-2} s^{-1}) (μmol mol^{-1})$^{-1}$) in instantaneous carboxylation efficiency when compared to plants with the highest A and CEi, respectively.

Induction of tolerance through exogenous application of H_2O_2 at concentration of 10 μM on CO_2 assimilation rate and instantaneous carboxylation efficiency may occur through the modulation of the detoxification processes of reactive oxygen species (Wang et al., 2014) and also through the regulation of stomata (Gondim et al., 2013), since gs (Figure 3A) was higher in plants subjected to hydrogen peroxide up to a concentration of 25 μM.

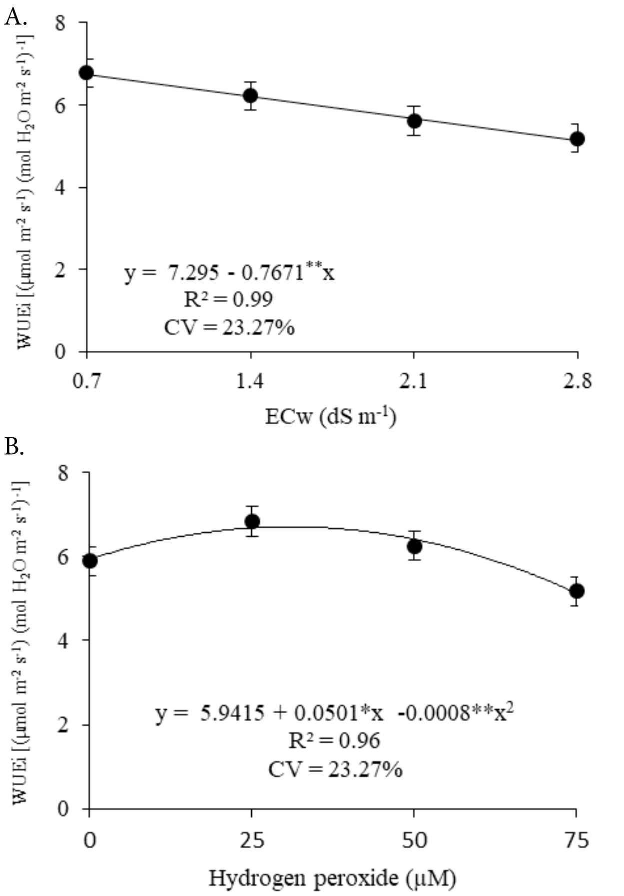
The instantaneous water use efficiency (WUEi) was negatively affected by irrigation water salinity and, according to the regression equation (Figure 4A), the linear model indicates that plants irrigated with ECw of 0.7 dS m^{-1} had the highest WUEi [6.76 μmol m^{-2} s^{-1} (mol H_2O m^{-2} s^{-1})$^{-1}$], while the lowest WUEi [5.15 μmol m^{-2} s^{-1} (mol H_2O m^{-2} s^{-1})$^{-1}$] was verified in plants grown with water of highest salinity level (2.8 dS m^{-1}). When the behavior of this variable is evaluated as a function of the salinity increment in the waters, it was possible to observe a reduction of 23.82% [1.61 μmol m^{-2} s^{-1} (mol H_2O m^{-2} s^{-1})$^{-1}$] between the highest and lowest levels of irrigation water salinity. It can be inferred that the increase in irrigation water salinity directly affects the WUEi of yellow passion fruit plants.

This situation may be related to the osmotic effects of salinity, which contribute to the reduction in the osmotic potential of the soil and, consequently, hampers the absorption of water by



X and Y - H_2O_2 concentration and ECw, respectively; *, ** - Significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test, respectively

Figure 3. Stomatal conductance - gs (A), transpiration - E (B), CO_2 assimilation rate - A (C) and instantaneous carboxylation efficiency - CEi (D) of yellow passion fruit plants as a function of the electrical conductivity of irrigation water and hydrogen peroxide concentration, at 45 days after sowing



**, * - Significant at p ≤ 0.01 and at p ≤ 0.05 by F test, respectively

Figure 4. Instantaneous water use efficiency - WUEi as a function of the electrical conductivity of irrigation water (A) and hydrogen peroxide concentration (B) of yellow passion fruit plants, 45 days after sowing

plants. Sá et al. (2019) also observed a reduction in instantaneous water use efficiency in West Indian cherry (*Malpighia glabra* L.) irrigated with saline water (0.6 to 3.8 dS m^{-1}).

The different concentrations of hydrogen peroxide affected the WUEi at 45 DAS, and the data were described by a quadratic model (Figure 4B), with the maximum estimated value of 6.72 [μmol m^{-2} s^{-1} (mol H_2O m^{-2} s^{-1})$^{-1}$] in plants subjected to 30 μM of hydrogen peroxide, with reduction from this concentration. The minimum value found was 5.2 [μmol m^{-2} s^{-1} (mol H_2O m^{-2} s^{-1})$^{-1}$] in plants under H_2O_2 concentration of 75 μM.

In general, it was observed in this study that the exogenous application of hydrogen peroxide at concentrations between 10 and 30 μM induced acclimation of yellow passion fruit plants to salt stress. However, with applications of H_2O_2 at higher concentrations, a negative effect was observed on the analyzed variables, and this response shows that the application of high concentrations of hydrogen peroxide causes damage to plants, possibly due to changes that occur in their metabolism, especially as a consequence of oxidative stress, leading to restriction of photosynthetic processes (Cattivelli et al., 2008).

Conclusions

1. Irrigation using water with electrical conductivity above 0.7 dS m^{-1} negatively affects the emergence and growth of yellow passion fruit.

2. Pretreatment of passion fruit plants with hydrogen peroxide at concentrations between 10 and 30 μM induces their acclimation to salt stress, mitigating the deleterious effects of salinity on the relative growth rate in stem diameter and leaf area, stomatal conductance, transpiration, CO_2 assimilation rate and instantaneous carboxylation efficiency.

3. Irrigation water salinity combined with hydrogen peroxide concentrations above 30 μM causes reductions in growth and gas exchange of yellow passion fruit plants.

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