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ORIGINAL ARTICLE

Mitigation of salt stress in passion fruit seedlings with H_2O_2 application[1]

Mitigação do estresse salino em mudas de maracujá com aplicação de H_2O_2

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HIGHLIGHTS:

Hydrogen peroxide favors the physiology and growth of passion fruit seedlings.
Hydrogen peroxide application allows the use of brackish water in the production of passion fruit seedlings.
Methods of application of H_2O_2 have a distinct influence on the physiology and growth of passion fruit seedlings.

ABSTRACT: Hydrogen peroxide (H_2O_2) can be indicated as a strategy to mitigate salt stress in plants, so the objective of this study was to evaluate the effect of different concentrations and methods of application of H_2O_2 as a mitigator of salt stress on the growth, physiology, and quality of seedlings of yellow passion fruit. The experiment was carried out in a protected environment (screened) belonging to UFERSA, in Caraúbas - RN, Brazil, in a randomized block design and analyzed in a 2 × 4 × 3 factorial scheme, with four replications and one plant per plot. The treatments consisted of levels of electrical conductivity of irrigation water - ECw (0.5 and 3.2 dS m^{-1}), H_2O_2 concentrations (0, 12, 24, and 36 μM), and H_2O_2 application methods (M1 = seed soaking, M2 = foliar spraying, and M3 = seed soaking + foliar spraying). Increased levels of salinity in irrigation water negatively affect seedling quality. Hydrogen peroxide at an average concentration of 24 μM mitigates salt stress in passion fruit seedlings cv. BRS Gigante Amarelo. H_2O_2 application to seeds associated with foliar spraying mitigates the effects of salt stress on the absolute growth rate of the leaf area of passion fruit seedlings irrigated with an ECw of 3.2 dS m^{-1}.

Key words: *Passiflora edulis* Sims, irrigation, salinization, hydrogen peroxide

RESUMO: O peróxido de hidrogênio (H_2O_2) pode ser indicado como uma estratégia para mitigar o estresse salino em plantas, por isso o objetivo deste estudo foi avaliar os efeitos das concentrações e métodos de aplicação de H_2O_2 como mitigador do estresse salino sobre o crescimento, fisiologia e qualidade de mudas de maracujá cv. BRS Gigante Amarelo. O experimento foi conduzido em ambiente protegido (telado) pertencente a UFERSA, em Caraúbas - RN, Brasil, em delineamento em blocos casualizados e analisados em esquema fatorial 2 × 4 × 3, com quatro repetições e uma planta por parcela. Os tratamentos consistiram em: condutividade elétrica da água de irrigação - CEa (0,5 e 3,2 dS m^{-1}); concentrações de H_2O_2 (0, 12, 24 e 36 μM); e métodos de aplicação de H_2O_2 (M1 = embebição das sementes, M2 = pulverização foliar e M3 = embebição das sementes + pulverização foliar). O aumento dos níveis de salinidade da água de irrigação afeta negativamente a qualidade das mudas. O peróxido de hidrogênio na concentração de 24 μM mitiga o estresse salino em mudas de maracujazeiro cv. BRS Gigante Amarelo. A aplicação de H_2O_2 em sementes associada à pulverização foliar mitiga os efeitos do estresse salino sobre a taxa de crescimento absoluto da área foliar de mudas de maracujá irrigadas com CEa de 3,2 dS m^{-1}.

Palavras-chave: *Passiflora edulis* Sims, irrigação, salinização, peróxido de hidrogênio

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Introduction

Yellow passion fruit (*Passiflora edulis* Sims) stands out in Brazilian fruticulture due to its economic, social, and nutritional importance and can be cultivated in the different regions of the country, due to soil and climatic conditions favorable to its exploitation (Bezerra et al., 2019).

The Northeast region in Brazil has the largest area and production of passion fruit, with a total of 69.60% of the national production, but the semi-arid climate in most of this region generates edaphoclimatic conditions that minimize passion fruit potential (Souza et al., 2020), mainly due to the low rainfall and high evapotranspiration rates. In this context, irrigation becomes indispensable because it enables sustainable exploitation and increases the yield of agricultural species (Bezerra et al., 2018).

Due to the edaphoclimatic conditions, the water available for irrigation has a high amount of salts, and associated with the high salinity of the soil, it becomes a barrier to the establishment of crops, due to the disturbances caused in the metabolism of the plants; however, the species respond differently under adverse conditions depending on the cultivar, phenological stage, type and concentration of salts (Veloso et al., 2023).

Due to the complexity of plant responses to salt stress, research has been conducted to evaluate the performance of water and salt stress mitigators (Silva Neta et al., 2020). In this context, the exogenous application of hydrogen peroxide (H_2O_2) constitutes an alternative to reduce the problems resulting from excess of salts (Silva et al., 2024) and may induce the acclimatization of plants. Santos et al. (2019), working with H_2O_2, found that its use favored the growth of plants that were under salt stress.

In this context, the present study aimed to evaluate the effect of different concentrations and methods of application of H_2O_2 as a mitigator of salt stress on the growth, physiology, and quality of seedlings of yellow passion fruit.

Material and Methods

The experiment was carried out from September to November 2022 in a protected environment (environment with 70% shade and measuring 9 × 4 × 2.5 m), belonging to the Universidade Federal Rural do Semiárido - UFERSA, located in Caraúbas, Rio Grande do Norte - Brazil, whose geographic coordinates are 5°46’23” S and 37°34’ 12” W, and an altitude of 144 m.

The experiment was set up in a randomized block design with treatments arranged in a 2 × 4 × 3 factorial scheme, referring to two levels of electrical conductivity of irrigation water - ECw (0.5 and 3.2 dS m^{-1}), four concentrations of hydrogen peroxide - H_2O_2 (0, 12, 24, and 36 μM) and three methods of application of H_2O_2 (M1 = seed imbibition, M2 = foliar spraying, and M3 = seed imbibition + foliar spraying), with four replicates and one plant per plot.

The values of electrical conductivity of water chosen were based on a study conducted by Silva et al. (2019), and the solutions were prepared to have an equivalent proportion of 7:2:1 for Na:Ca:Mg, respectively, through the dissolution of NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ in local-supply water (0.5 dS m^{-1}), considering the relationship between electrical conductivity of water - ECw (dS m^{-1}) and concentration of salts - C ($mmol_c$ L^{-1}) (Richards, 1954), according to Eq. 1:

$$C \approx 10 \times ECw \qquad (1)$$

Hydrogen peroxide concentrations and application time were established based on results reported by Silva et al. (2019) and Veloso et al. (2021). The solutions were prepared by diluting H_2O_2 in distilled water, whereas the 0 μM treatment was obtained using only distilled water.

Before sowing, the seeds from the imbibition and imbibition + foliar spraying treatments underwent a pre-treatment with hydrogen peroxide, where they were soaked in solutions with concentrations of 12, 24, and 36 μM of H_2O_2, for a period of 24 hours; in turn, the seeds from the 0 μM H_2O_2 treatment were soaked in distilled water, and the concentrations were obtained by diluting 35% pure H_2O_2 in deionized water.

At 23 days after sowing (DAS), H_2O_2 began to be applied by foliar spraying with a sprayer every 15 days, in a total of three applications. Initially, an average volume of 3 mL of the solution was applied, which was increased according to the size of the plants, being adjusted to 5 mL, and support was used to prevent the product from drifting.

Prior to sowing, soil moisture was increased to the level corresponding to field capacity with public-supply water (0.5 dS m^{-1}), and irrigation with low-salinity water continued until 27 DAS. Sowing was performed with five seeds per bag, planted at 1 cm depth. At 22 DAS, thinning was done, leaving only the most vigorous plant.

Irrigation with saline water began at 28 DAS, maintaining soil moisture at a level corresponding to maximum water retention capacity in all experimental units. Irrigation was performed daily, applying in each plastic bag a volume of water to keep the substract at moisture content close to field capacity, and the applied volume was determined according to the water requirement of the plants, estimated by the water balance by subtracting the volume drained from the volume applied in the previous irrigation, plus a leaching fraction of 10% every 10 days to avoid excessive accumulation of salts in the root zone.

Fertilization with nitrogen, phosphorus, and potassium was carried out according to Novais et al. (1991), applying the equivalent of 100 mg N, 300 mg P_2O_5, and 150 mg K_2O kg^{-1} of soil, in the forms of urea (45% of N), monoammonium phosphate (52% of P_2O_5 and 11% of N), and potassium chloride (60% of K_2O), respectively. Potassium was applied as basal dose, while N and K were topdressed, via fertigation, at 30 DAS, using manual irrigation. A micronutrient solution at a concentration of 1.0 g L^{-1} of the commercial product Dripsol® micro, containing Mg (1.1%), Zn (4.2%), B (0.85%), Fe (3. 4%), Mn (3.2%), Cu (0.5%), and Mo (0.05%) was applied at 30 and 50 DAS on the adaxial and abaxial surfaces of the leaves, using a knapsack sprayer.

Phytosanitary control was preventive and/or curative when there was an incidence of any pest or disease. Invasive plants were manually uprooted whenever needed.

Passion fruit growth was evaluated at 38 and 68 DAS, by determining: the number of leaves (NL), by counting the green leaves of each plant, considering leaves with a fully open blade; stem diameter (SD), determined using a digital caliper at 3 cm height from the plant collar; plant height (PH), considering the distance between the collar and the point of insertion of the youngest leaf, in cm; and leaf area (LA) (Eq. 2), obtained according to Cavalcante et al. (2011):

$$LA \equiv 5.71 + 0.647X \tag{2}$$

where:
LA - leaf area (cm^2); and,
X - product of leaf length by leaf width (cm).

These data (NL, SD, PH, and LA) were then used to calculate the absolute growth rates during the observed period (between 38 and 68 DAS) of the number of leaves (AGR_{NL}), stem diameter (AGR_{SD}), plant height (AGR_{PH}), and leaf area (AGR_{LA}) of passion fruit seedlings (Eq. 3) according to the methodology of Benincasa (2003):

$$AGR = \frac{A2 - A1}{t2 - t1} \tag{3}$$

where:
A1 and A2 - variable under study (NL, SD, PH, or LA), obtained at the beginning and end of the study period; and,
t2 - t1 - time difference between observations, in days.

At the end of the experimental period (68 DAS), a destructive evaluation of the plants was carried out to obtain data concerning the fresh mass of leaves (LFM), stem (STFM), root (RFM), and total (TFM) per plant, using a precision scale. Subsequently, the parts of the plants (roots, leaves, and stem) were placed separately in paper bags and dried in a forced air circulation oven at 65 °C until reaching constant mass to obtain dry mass. Seedling quality was determined using the Dickson Quality Index (DQI) (Eq. 4), through the formula of Dickson et al. (1960):

$$DQI = \frac{(TDM)}{\left(\frac{PH}{SD}\right) + \left(\frac{SHDM}{RDM}\right)} \tag{4}$$

where:
DQI - Dickson quality index;
TDM - total dry mass (g);
PH - plant height (cm);
SD - stem diameter (mm);
SHDM - shoot dry mass (g); and,
RDM - root dry mass (g).

Physiological evaluations for gas exchange were performed at 62 days after sowing, when the seedlings were ready for transplanting, based on the following variables: CO_2 assimilation rate (A) (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs) (mol H_2O m^{-2} s^{-1}), transpiration (E) (mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration (Ci) (μmol CO_2 m^{-2} s^{-1}), and instantaneous water use efficiency (WUEi) (A/E) [(μmol CO_2 m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$], measured with an LCPro infrared gas analyzer (IRGA) with a constant light source of 1.200 μmol of photons m^{-2} s^{-1}, on the third leaf of the plant, counted from the apex.

The collected data were subjected to analysis of variance, with the F test (at 0.01 and 0.05 probability levels), and when significant, regression analysis was performed for the H_2O_2 concentrations. Means of the factors salinity levels of irrigation water and methods of application of H_2O_2 were compared by Tukey test (at 0.01 and 0.05 probability levels), using the statistical program SISVAR/UFLA (Ferreira, 2011).

Results and Discussion

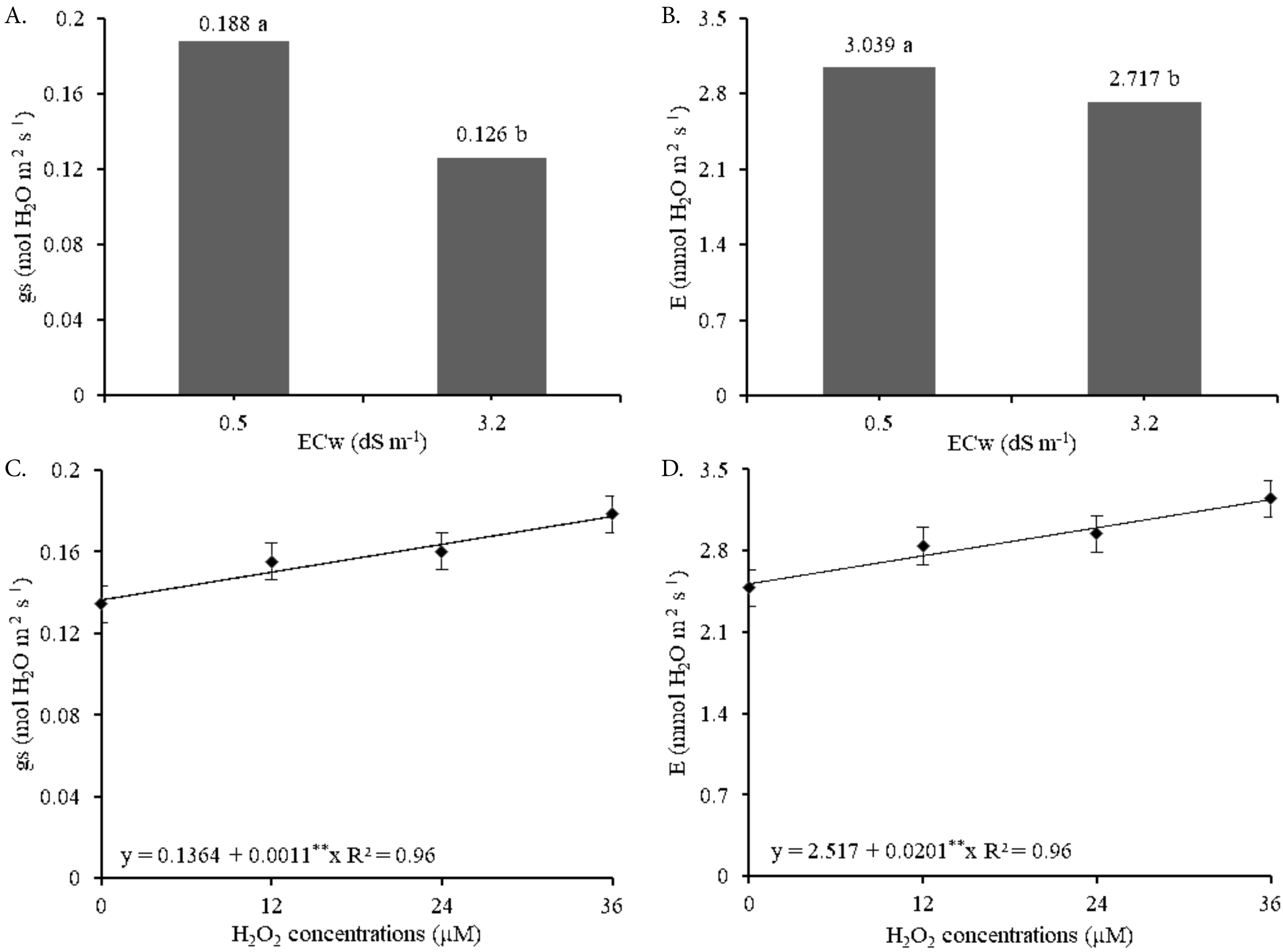
It can be seen in Table 1 that there was a significant individual effect of salinity levels in irrigation water on stomatal conductance (gs), transpiration (E), CO_2 assimilation rate (A), and instantaneous water use efficiency (WUEi). For the concentrations of hydrogen peroxide, there was an isolated effect on gs, E, A, and internal CO_2 concentration (Ci). The SL × H_2O_2 interaction had a significant effect on A, Ci, and WUEi. The H_2O_2 × M interaction promoted a significant effect on the Ci of passion fruit seedlings at 62 DAS.

The electrical conductivity of irrigation water of 3.2 dS m^{-1} caused reductions of 32.98% (0.062 mol H_2O m^{-2} s^{-1}) in stomatal conductance (Figure 1A) and 10.60% (0.322 mmol H_2O m^{-2} s^{-1}) in transpiration (Figure 1B) when compared to plants subjected

Table 1. Summary of the analysis of variance for stomatal conductance (gs), transpiration (E), CO_2 assimilation rate (A), internal CO_2 concentration (Ci), and instantaneous water use efficiency (WUEi) of passion fruit seedlings cv. BRS Gigante Amarelo as a function of different electrical conductivities of irrigation water combined with concentrations of hydrogen peroxide (H_2O_2) and methods of application of H_2O_2, at 62 days after sowing

Source of variation	Mean squares				
	gs	E	A	Ci	WUEi
Salinity level (SL)	0.070^{**}	1.869^{*}	525.096^{**}	1770.125^{ns}	40.741^{**}
H_2O_2 concentrations (H_2O_2)	0.006^{*}	1.805^{**}	83.346^{**}	2230.939^{*}	1.710^{ns}
Linear regression	0.017^{**}	5.222^{**}	231.521^{**}	5945.469^{**}	2.557^{ns}
Quadratic regression	0.000^{ns}	0.014^{ns}	5.098^{ns}	666.125^{ns}	2.462^{ns}
Methods of application H_2O_2 (M)	0.001^{ns}	0.210^{ns}	6.104^{ns}	997.389^{ns}	1.255^{ns}
Interaction (SL × H_2O_2 × M)	0.001^{ns}	0.206^{ns}	6.180^{ns}	828.167^{ns}	2.806^{ns}
Interaction (SL × H_2O_2)	0.001^{ns}	0.312^{ns}	32.364^{*}	2273.125^{*}	5.355^{*}
Interaction (SL × M)	0.000^{ns}	0.038^{ns}	2.342^{ns}	1819.500^{ns}	0.172^{ns}
Interaction (M × H_2O_2)	0.009^{ns}	0.347^{ns}	6.997^{ns}	2034.092^{**}	1.865^{ns}
Block	0.006^{ns}	8.569^{**}	10.757^{ns}	490.389^{ns}	35.164^{**}
CV (%)	25.78	20.83	17.16	13.94	19.32

ns, *,** respectively not significant, significant at $p \leq 0.05$ and significant at $p \leq 0.01$; CV- coefficient of variation



Vertical bars represent the standard error of the mean (n=4). Means followed by different letters indicate a significant difference between treatments by the Tukey test at $p \leq 0.05$

Figure 1. Stomatal conductance - gs (A) and transpiration - E (B) of passion fruit seedlings cv. BRS Gigante Amarelo as a function of salinity of irrigation water (A and B) and H_2O_2 concentrations (C and D), at 62 days after sowing

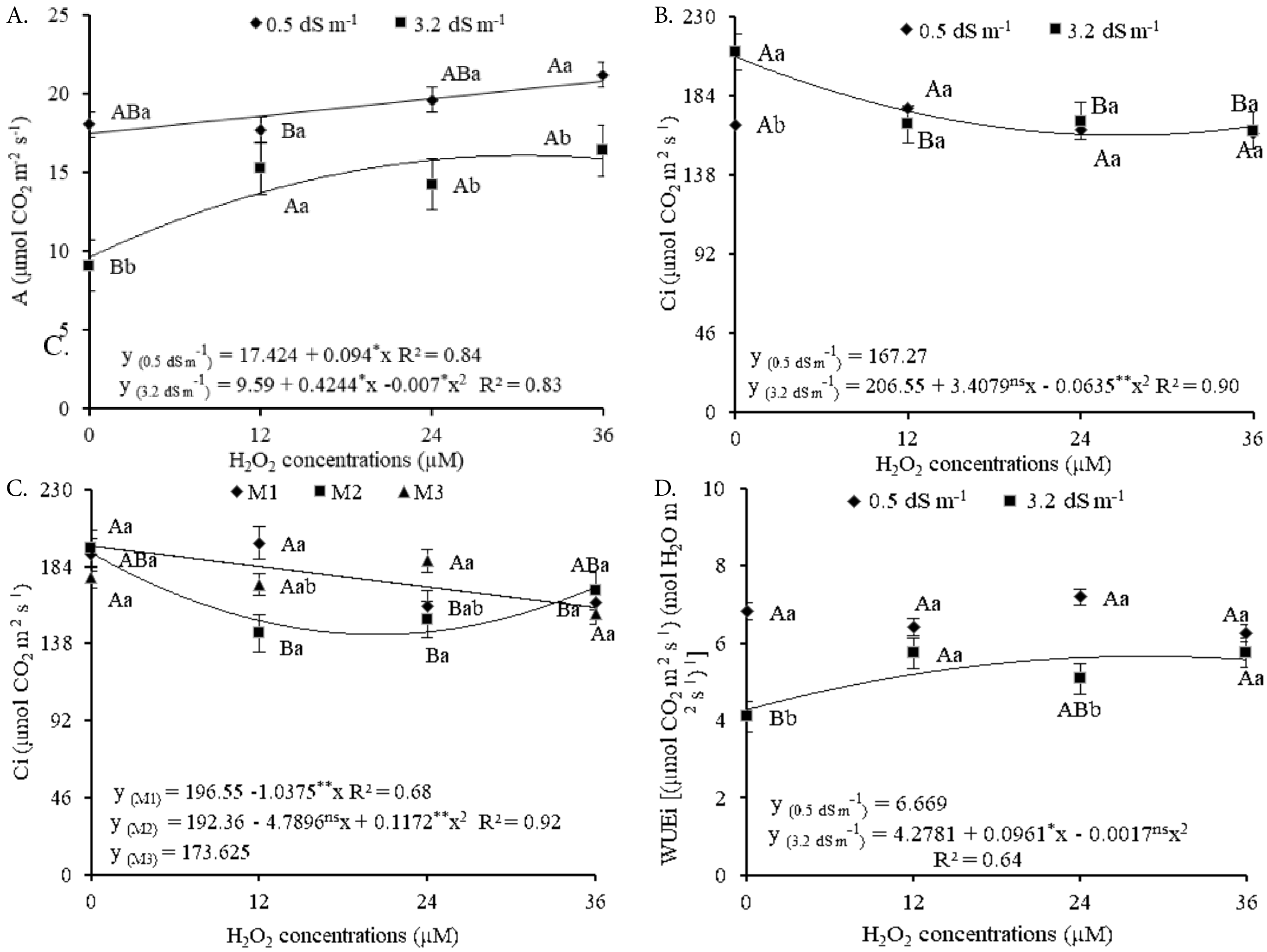
to the lowest salinity (0.5 dS m^{-1}), which had the highest values of gs (0.188 mol H_2O m^{-2} s^{-1}) and E (3.039 mmol H_2O m^{-2} s^{-1}). According to Pacheco et al. (2021), plants subjected to salt stress tend to close their stomata, and a series of mechanisms are activated in response to changes in stomatal conductance and transpiration, as seen in this experiment.

According to the regression equations, the increasing concentrations of H_2O_2 promoted linear increments in gs (Figure 1C) and E (Figure 1D) of 0.80 and 0.79%, respectively, per unit increase in H_2O_2 concentration, resulting in increases of 29.03% (gs) and 30.03% (E) in plants that received the highest concentration (36 μM) compared to those that were subjected to H_2O_2 dose of 0 μM. Other studies indicate that pretreatment with H_2O_2 may increase stomatal conductance (Terzi et al., 2014) and photosynthesis, hence promoting greater growth, dry mass production, and quality of seedlings of passion fruit, as observed in the present study.

The CO_2 assimilation rate (A) was affected by the interaction between the factors (SL × H_2O_2) and, according to the regression equation (Figure 2A), it was observed that, at the lowest salinity (0.5 dS m^{-1}), there was linear behavior with an increment of 0.54% per unit increase in H_2O_2 concentration, increasing by 19.42% (3.07 μmol CO_2 m^{-2} s^{-1}) in plants that received the highest H_2O_2 concentration compared to those under the lowest H_2O_2 concentration. For the highest salinity (3.2 dS m^{-1}), there was quadratic behavior, and the maximum mean (16.020 μmol CO_2 m^{-2} s^{-1}) was observed in plants that received the concentration of 36 μM. According to Ramos et al. (2022), pre-treatment with an adequate level of H_2O_2 creates a modulation of several physiological processes, such as photosynthesis, and also the best efficacy of metabolic pathways that act on the detoxification of ROS, in response to the most diverse stressful factors such as salinity, water deficit, presence of heavy metals, and high and low temperatures.

The interaction between the factors (SL × H_2O_2) promoted a significant effect on the internal CO_2 concentration (Ci) of passion fruit at 62 DAS and, according to regression equation (Figure 2B), there was no significant influence of the increase in H_2O_2 concentrations on plants irrigated with ECw of 0.5 dS m^{-1}, which had an average value of 167.27 μmol CO_2 m^{-2} s^{-1}. For plants irrigated with brackish water (3.2 dS m^{-1}), there was a quadratic behavior, and the highest value (209.11 μmol CO_2 m^{-2} s^{-1}) was obtained in those not subjected to H_2O_2. The concentrations of 12, 24, and 36 μM showed no significant differences within the salinity levels.

The decrease in the internal CO_2 concentration, observed in plants subjected to an H_2O_2 concentration of 36 μM and irrigated with water of ECw 3.2 dS m^{-1}, was consistent



M1 - Seed imbibition; M2 - Foliar spraying; M3 - Seed imbibition + foliar spraying. For the SL x H_2O_2 interaction, means followed by different letters indicate a significant difference between treatments by Tukey test at p≤0.05, lowercase letters indicate the behavior of salinity levels within each H_2O_2 concentration and uppercase letters indicates the behavior of doses of H_2O_2 at the same water salinity level. For the M × H_2O_2 interaction, uppercase letters indicate the behavior of the methods of application within doses of H_2O_2. Vertical bars represent the standard error of the mean (n=4)

Figure 2. CO_2 assimilation rate - A (A), internal CO_2 concentration - Ci (B), and instantaneous water use efficiency - WUEi (D) as a function of the interaction between the factors SL × H_2O_2, and internal CO_2 concentration - Ci (C) as a function of the interaction between the factors H_2O_2 × M of passion fruit seedlings cv. BRS Gigante Amarelo, at 62 days after sowing

since the CO_2 assimilation rate (Figure 2A) increased with the application of 36 μM at the same salinity level. Thus, it indicates that the carbon present in the substomatal chamber was used by photosynthesis in the production of photoassimilates (Dalastra et al., 2014) since the doses of H_2O_2 promote higher gs (Figure 1C) without restricting the entry of CO_2 into the leaf mesophyll.

For the internal CO_2 concentration of the plants subjected to the H_2O_2 application method M1, there was a linear decrease of 0.53% per unit increase in the concentration of hydrogen peroxide, which resulted in a decrease of 19% at the highest level compared to the lowest H_2O_2 concentration (Figure 2C). The M2 method of H_2O_2 application promoted the maximum value of 192.36 μmol CO_2 m^{-2} s^{-1} under the application of 0 μM H_2O_2. It is also verified that there were no significant differences in the Ci of plants subjected to the M3 method, which obtained an average of 173.625 μmol CO_2 m^{-2} s^{-1}. These results indicate that the method of application by foliar spraying (M2) was more efficient because there was no restriction on CO_2 entry through the stomata, as observed in the results for gs (Figure 1C). Higher Ci values in plants that received M1 and M3 can be attributed to the non-use of CO_2 in the photosynthetic process since there was no interference from stomatal factors (Pinheiro et al., 2022).

The instantaneous water use efficiency of passion fruit was affected by the interaction between the factors SL × H_2O_2 and, according to the regression equation (Figure 2D), the highest WUEi values were observed in plants irrigated with ECw of 0.5 dS m^{-1}, which did not differ statistically under different concentrations of H_2O_2. However, it can be seen that plants under ECw of 3.2 dS m^{-1} showed a quadratic behavior, and the highest value, equal to 5.75 [(μmol CO_2 m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1})$^{-1}$], was observed in plants that received the concentration of 36 μM. Instantaneous water use efficiency is a variable that measures the amount of carbon fixed per unit of water that is lost; when the flow of water vapor into the atmosphere decreases as the stomata close, it consequently restricts the entry of CO_2, reducing photosynthesis, and consequently affecting water use efficiency (Veloso et al., 2022). However, in plants under ECw of 3.2 dS m^{-1}, the increase in H_2O_2 concentrations promoted an increase in WUEi and mitigated the effect of salt stress, leading to no significant differences in the WUEi of plants under different levels of ECw at the highest concentration of H_2O_2.

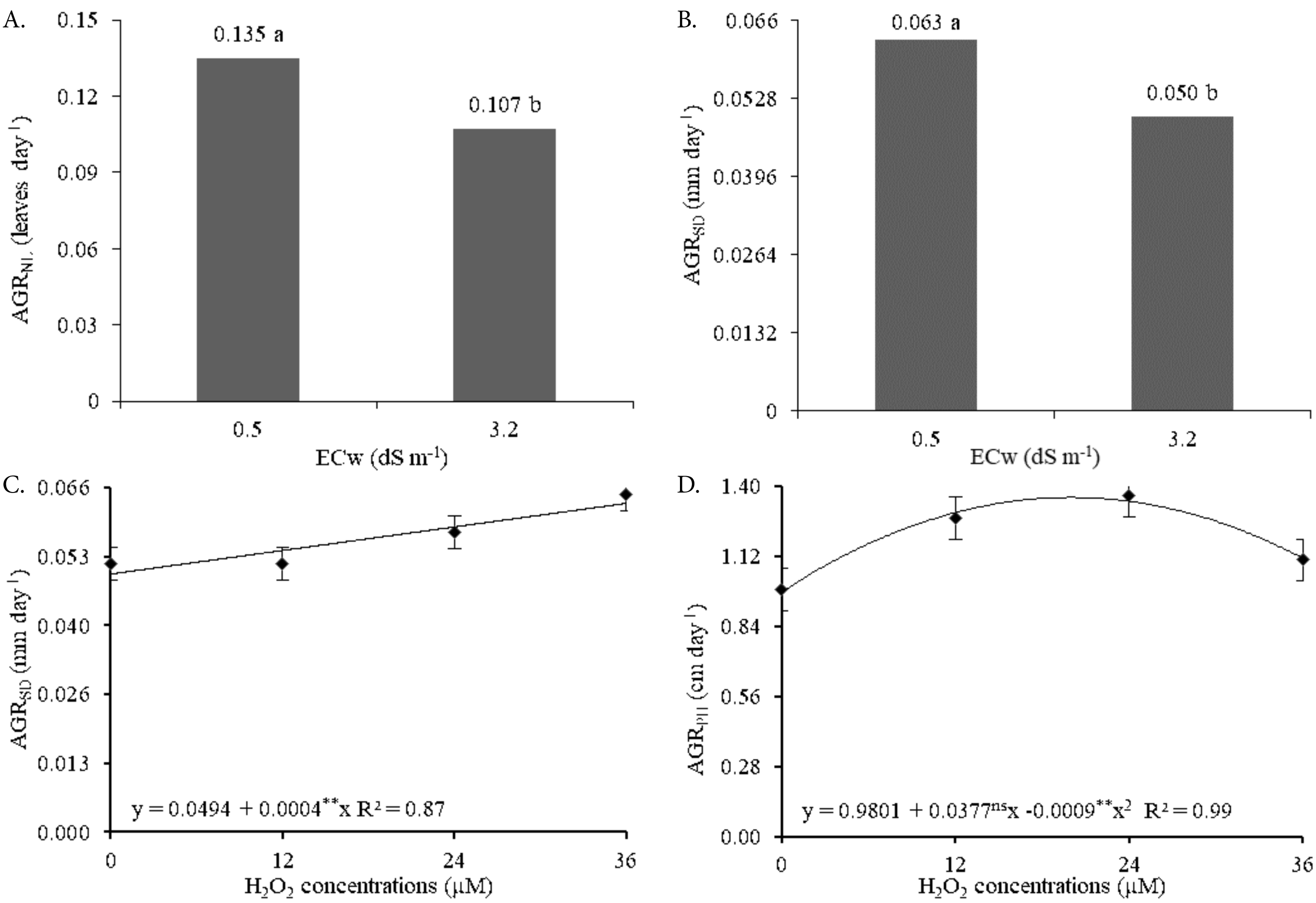
According to the summary of the analysis of variance (Table 2), there was a significant effect of salinity levels on the absolute growth rates of the number of leaves (AGR_{NL}) and stem diameter (AGR_{SD}), between 38 and 68 DAS, of passion fruit seedlings cv. BRS Gigante Amarelo. In turn, the H_2O_2 doses alone significantly affected the absolute growth rates of stem diameter (AGR_{SD}), plant height (AGR_{PH}), and leaf area (AGR_{LA}). A significant effect of the SL × M interaction on AGR_{LA} was also observed between 38 and 68 DAS.

AGR_{NL} and AGR_{SD} were negatively affected by the salinity of irrigation water and, according to the means comparison test (Figures 3A and 3B), the highest values of 0.135 leaves day^{-1} and 0.063 mm day^{-1}, respectively, were obtained in plants cultivated with water of low salinity (0.5 dS m^{-1}), with reductions of 20.75% (0.028 leaves day^{-1}) for AGR_{NL} and 20.64% (0.013 mm day^{-1}) for AGR_{SD}, in plants irrigated with the highest salinity

Table 2. Summary of the analysis of variance for the absolute growth rates of the number of leaves (AGR_{NL}), stem diameter (AGR_{SD}), plant height (AGR_{PH}), and leaf area (AGR_{LA}) of passion fruit seedlings cv. BRS Gigante Amarelo as a function of different electrical conductivities of irrigation water combined with concentrations and methods of application of hydrogen peroxide between 38 and 68 days after sowing

Source of variation	Mean squares			
	AGR_{NL}	AGR_{SD}	AGR_{PH}	AGR_{LA}
Salinity level (SL)	0.0139^{**}	0.0029^{**}	0.0268^{ns}	5.0355^{ns}
H_2O_2 concentrations (H_2O_2)	0.0029^{ns}	0.0007^{**}	0.5087^{**}	5.6095^{*}
Linear regression	0.0089^{ns}	0.0018^{**}	0.1792^{ns}	1.4913^{ns}
Quadratic regression	0.0001^{ns}	0.0002^{ns}	1.3279^{**}	15.3264^{*}
Methods of application H_2O_2 (M)	0.0056^{ns}	0.0003^{ns}	0.0845^{ns}	7.4840^{ns}
Interaction (SL × H_2O_2 × M)	0.0008^{ns}	0.0001^{ns}	0.0335^{ns}	3.3767^{ns}
Interaction (SL × H_2O_2)	0.0021^{ns}	0.0001^{ns}	0.1049^{ns}	1.8457^{ns}
Interaction (SL ×M)	0.0019^{ns}	0.0000^{ns}	0.1229^{ns}	22.3650^{**}
Interaction (H_2O_2 × M)	0.0015^{ns}	0.0002^{ns}	0.0480^{ns}	1.8022^{ns}
Block	0.0037^{ns}	0.0000^{ns}	0.3395^{*}	26.2781^{**}
CV (%)	34.81	21.76	21.89	12.79

ns, *,** respectively not significant, significant at $p \leq 0.05$ and significant at $p \leq 0.01$; CV- coefficient of variation



Vertical bars represent the standard error of the mean (n=4). Means followed by different letters indicate a significant difference between treatments by the Tukey test at $p \leq 0.05$ probability

Figure 3. Absolute growth rate of the number of leaves - AGR_{NL} and stem diameter - AGR_{SD} as a function of salinity of irrigation water (A and B), and as a function of H_2O_2 concentrations (C and D) of passion fruit seedlings cv. BRS Gigante Amarelo between 38 and 68 days after sowing

(3.2 dS m^{-1}) compared to those under low water salinity level. The change in growth may have occurred due to the greater difficulty of water absorption by the roots, tending to induce stomatal closure, restricting the entry of CO_2 that would be used in plant growth (Silva et al., 2020), as observed by the reduction of stomatal conductance (Figure 1A).

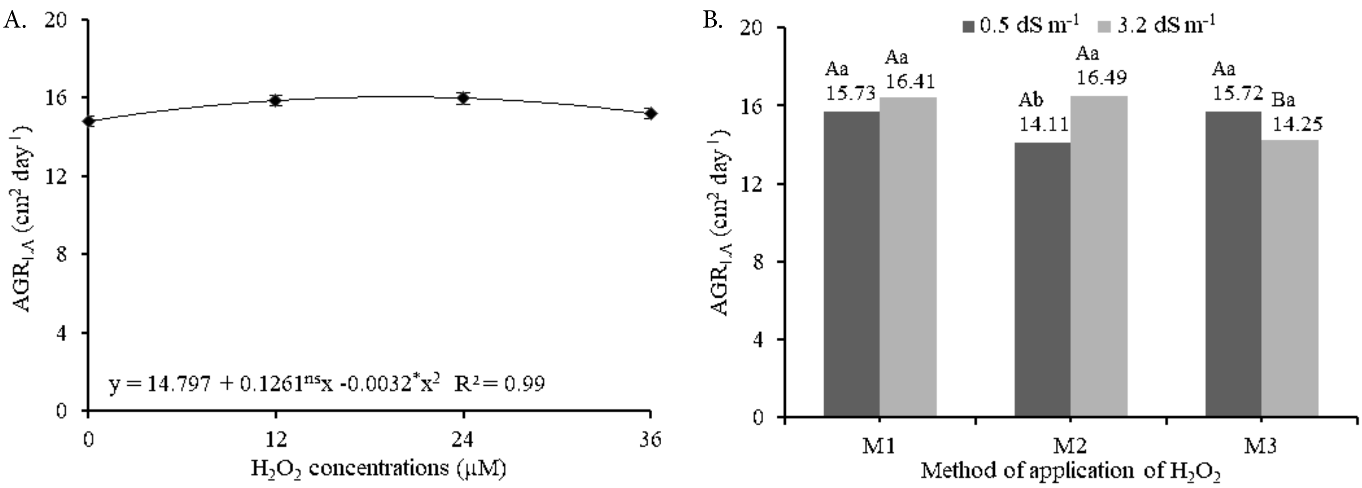
Increasing concentrations of H_2O_2 caused a significant effect on AGR_{SD}, and according to the regression equation (Figure 3C), there was a linear increase of 0.81% per unit increase in H_2O_2 concentration, which resulted in an increase of 29.16% in the AGR_{SD} of plants that received the highest concentration compared to those without application. For AGR_{PH} (Figure 3D) and AGR_{LA} (Figure 4A), the regression equations showed a quadratic behavior in response to the H_2O_2 concentrations, and their highest values were 1.37 cm day^{-1} (AGR_{PH}) and 16.04 cm^2 day^{-1} (AGR_{LA}), obtained with the concentration of 21 and 20 µM, respectively. According to the results obtained for the growth variables, adequate concentrations of H_2O_2 promote the accumulation of proteins, soluble carbohydrates, NO_3^-, and reduction in the Na^+ and Cl^- contents in plants, as they also promote tolerance to stresses and, consequently, contribute to greater absorption of water and nutrients (Gondim et al., 2011) to the point of favoring the growth of yellow passion fruit seedlings. However, H_2O_2 at high concentrations may affect some variables in plants due to its capacity to diffuse easily through subcellular membranes and ultimately cause oxidative damage (Farooq et al., 2017; Silva et al., 2023).

The AGR_{LA} of passion fruit was influenced by the SL x M interaction and, according to the means comparison test (Figure 4B), seedlings that had their leaves sprayed with H_2O_2 by M2 (foliar spraying) method reached the highest value, 16.49 cm^2 day^{-1}, at salinity of 3.2 dS m^{-1}, whereas the methods of application by seed imbibition (M1) and seed imbibition + foliar spraying (M3) did not differ significantly within the salinity levels. It is also observed that M3 was inferior to the other methods in plants irrigated with water of highest salinity (3.2 dS m^{-1}). According to Forman et al. (2010), pre-exposure of plants to signaling metabolites such as H_2O_2 and/or a lighter degree of stress can induce metabolic signaling of the cell and thus cause the organism to show better physiological performance when exposed to more severe stressful conditions. However, it can be observed that, in this study, for the variable AGR_{LA}, the application of H_2O_2 by foliar spraying was more efficient since all plants, regardless of the method of application, received the concentrations of H_2O_2 before being subjected to salt stress. Therefore, M3 was less efficient for this variable, resulting in a reduction of 13.58%, which can be considered acceptable.

According to the summary of the analysis of variance (Table 3), there were significant isolated effects of salinity levels of irrigation water and H_2O_2 concentrations on shoot dry mass (SHDM), root dry mass (RDM), total dry mass (TDM), and Dickson quality index (DQI). The effects of interaction between the factors SL × H_2O_2 on DQI and that of SL × M on RDM were significant at 68 DAS.

There was a reduction in SHDM values with the increase in the salinity of irrigation water (Figure 5A), and the highest value observed, 3.09 g, was obtained in plants irrigated with ECw of 0.5 dS m^{-1}, being 12% higher (0.37 g) than that obtained under ECw of 3.2 dS m^{-1}, that is, despite the decrease in shoot dry mass production, there is a certain tolerance of passion fruit seedlings to salt stress. Based on these results, it is suggested that they may have developed some mechanism in an attempt to maintain their potential more negative than that of the medium and thus 'force' the entry of water, for the proper functioning of the reactions that occur in the cytosol, so that the plant continues to produce dry matter (Simões et al., 2021), because some plant species have developed mechanisms such as osmotic adjustment, compartmentalization of ions in cell vacuoles, as a way to survive under salt stress conditions.

Increasing concentrations of H_2O_2 caused increasing linear responses in SHDM (Figure 5B), RDM (Figure 5C), and TDM (Figure 6B) and, according to the regression equations, there were increments of 0.43% (SHDM), 1.22% (RDM), and 0.55% (TDM) per unit increase in H_2O_2 concentration, resulting in increases of 15.76% (0.43 g), 44.27% (0.20 g), and 19.82% (0.63 g), respectively, in plants that received the highest H_2O_2 concentration (36 µM) compared to those that



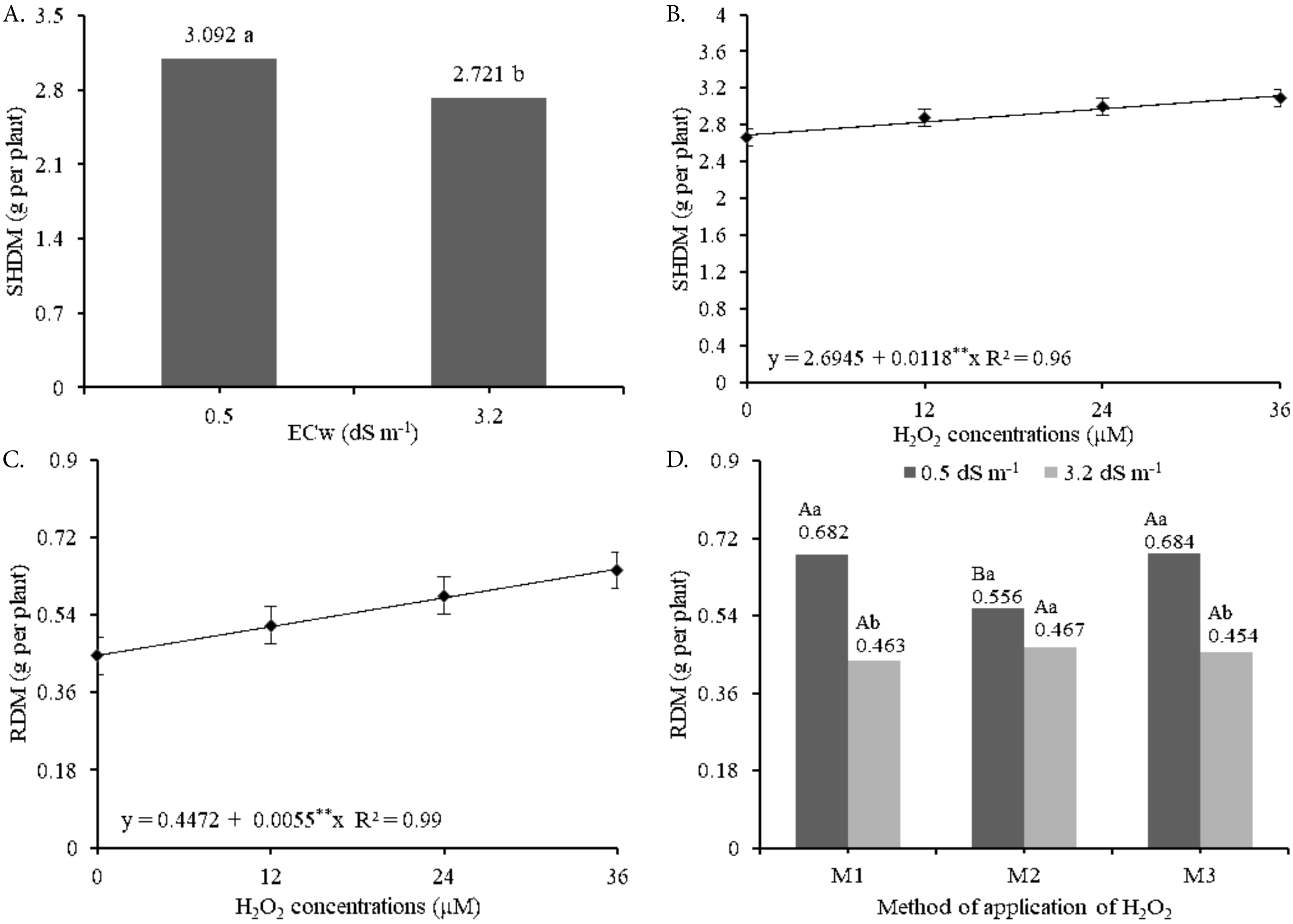
M1 - Seed imbibition; M2 - Foliar spraying; M3 - Seed imbibition + foliar spraying. For the SL x M interaction, means followed by different letters indicate a significant difference between treatments by the Tukey test at p≤0.05, uppercase letters indicate the behavior of the methods of application within the salinity levels and lowercase letters indicate the behavior of each salinity level within the methods of application. Vertical bars represent the standard error of the mean (n=4)

Figure 4. Absolute growth rate of leaf area - AGR_{LA} (A) as a function of H_2O_2 concentrations (A) and as a function of the interaction between the factors water salinity level (SL) and method of H_2O_2 application (M)

Table 3. Summary of the analysis of variance for dry mass of shoot (SHDM), root (RDM) and total dry mass (TDM), and Dickson quality index (DQI) of passion fruit seedlings cv. BRS Gigante Amarelo as a function of different electrical conductivities of irrigation water combined with concentrations of hydrogen peroxide (H_2O_2) and methods of application of H_2O_2 at 68 days after sowing

Source of variation	Mean squares			
	SHDM	RDM	TDM	DQI
Salinity level (SL)	2.4842**	0.6380**	5.6403**	0.0808**
H_2O_2 concentrations (H_2O_2)	0.6259*	0.1331**	1.3278**	0.0155**
Linear regression	1.7986**	0.3988**	3.8912**	0.0333**
Quadratic regression	0.0725ns	0.0006ns	0.0872ns	0.0127**
Methods of application H_2O_2 (M)	0.0841ns	0.0228ns	0.0382ns	0.0003ns
Interaction (SL × H_2O_2 × M)	0.1707ns	0.0205ns	0.2565ns	0.0015ns
Interaction (SL × H_2O_2)	0.1043ns	0.0368ns	0.2187ns	0.0060*
Interaction (SL × M)	0.4240ns	0.0453*	0.7416ns	0.0014ns
Interaction (H_2O_2 × M)	0.1271ns	0.0135ns	0.1900ns	0.0018ns
Block	0.5782ns	0.0812ns	1.0869*	0.0136**
CV (%)	15.91	21.71	16.07	18.35

ns, *,**, respectively, not significant, significant at $p \le 0.05$ and significant at $p \le 0.01$; CV- coefficient of variation

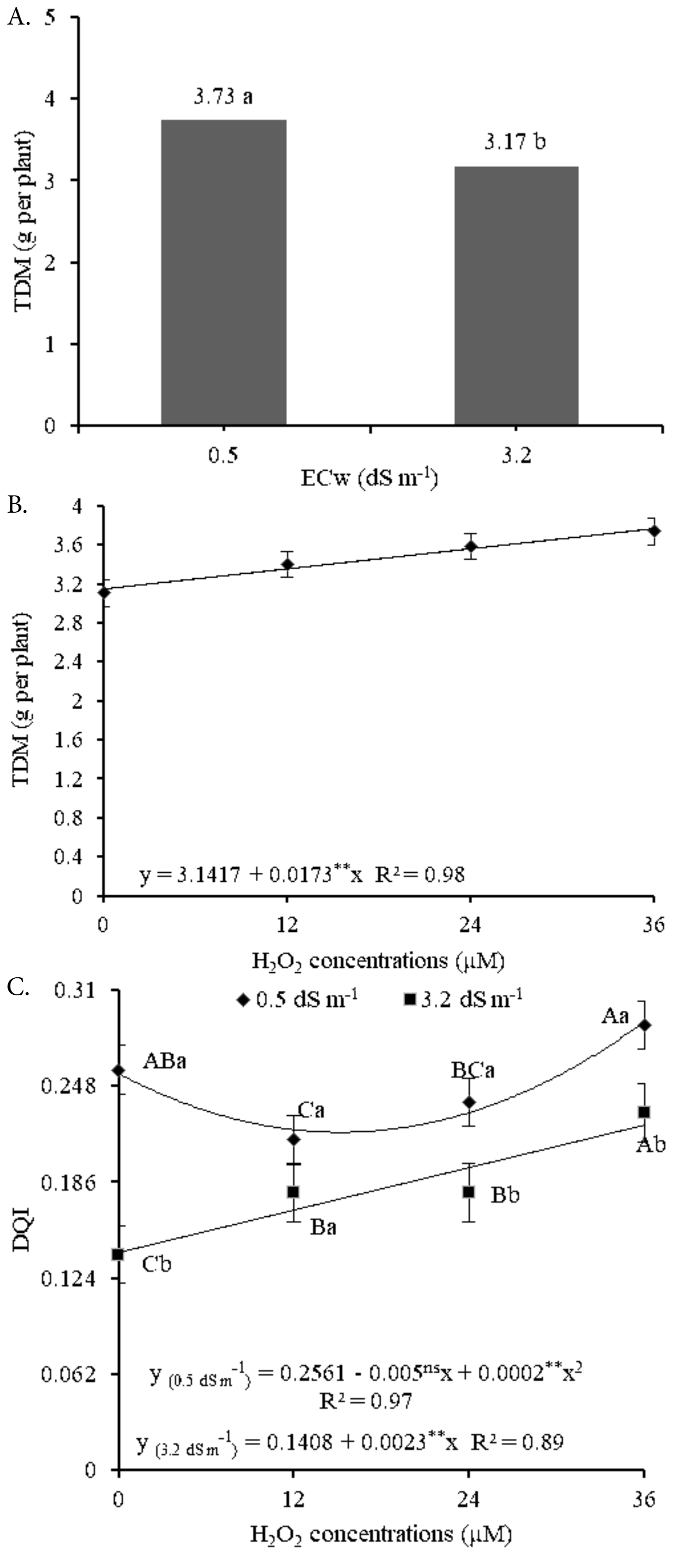


M1 - Seed imbibition; M2 - Foliar spraying; M3 - Seed imbibition + foliar spraying. Means followed by different letters indicate a significant difference between treatments by the Tukey test at $p \le 0.05$, lowercase letters indicate the behavior of each salinity level within the method of application and uppercase letters indicate the behavior of methods of application at the same salinity level. Vertical bars represent the standard error of the mean (n=4)

Figure 5. Shoot dry mass - SHDM (A) as a function of salinity of irrigation water, shoot dry mass - SHDM (B) and root dry mass - RDM (C) as a function of H_2O_2 concentrations, and RDM (D) as a function of the interaction between the factors water salinity level (SL) and method of H_2O_2 application (M)

did not receive H_2O_2. Hydrogen peroxide acts on plants by interacting with hormones, triggering responses in their growth and development to the different environmental conditions to which they are exposed (Wojtyla et al., 2016), besides contributing to accelerating the growth of primary and lateral roots, consequently favoring biomass production, as occurred in the present study.

Root dry mass was significantly affected by the interaction between the factors SL and M (Figure 5D), and in plants irrigated with ECw of 0.5 dS m^{-1} the higher means obtained were 0.682 g and 0.684 g, referring to the method of application by seed imbibition (M1) and seed imbibition + foliar spraying (M3), respectively, which did not differ statistically from each other and were superior to the method of application by



For the SL x H_2O_2 interaction, means followed by different letters indicate a significant difference between treatments by the Tukey test at p≤0.05, lowercase letters indicate the behavior of salinity levels within each H_2O_2 concentration and uppercase letters indicate the behavior of H_2O_2 levels at the same salinity level. Vertical bars represent the standard error of the mean (n=4)

Figure 6. Total dry mass - TDM (A) as a function of salinity of irrigation water and of H_2O_2 concentrations (B), and Dickson quality index - DQI (C) as a function of the interaction between the factors water salinity level (SL) and method of H_2O_2 application (M)

foliar spraying (M2), with an average of 0.556 g. In relation to plants that received water with the highest ECw (3.2 dS m^{-1}), the methods of application of H_2O_2 did not differ from one another and also led to 32.13% (M1), 16% (M2), and 33.62% (M3) lower means compared to plants irrigated with water of low electrical conductivity.

For the methods of application M1 and M3, exposure to H_2O_2 started with the hydration of the seeds. Thus, several processes are initiated in the imbibed seeds, which can be stored as a kind of "stress memory" and can be expressed during later stages of plant development (Santos et al., 2019; Veloso et al., 2021). Furthermore, H_2O_2 acts by interacting with hormones that control germination and production of O_2 for respiration in mitochondria, improving metabolic activity, besides helping to overcome seed coat dormancy, which in turn allows greater water absorption (Ahmad et al., 2013) and consequently may favor RDM production.

There was a reduction in TDM (Figure 6A) due to excess salts, and the highest value was 3.73 g, observed in plants that received water with ECw of 0.5 dS m^{-1}, with a reduction of 15.02% (0.56 g) in plants irrigated with water of 3.2 dS m^{-1}. As observed for the absolute growth rates of the number of leaves and stem diameter (Figures 3A and 3B), the reductions in SHDM (Figure 5A) and TDM (Figure 6A) due to the increase in irrigation water salinity may be related to the decrease in photosynthetic rates, probably due to denaturation of enzymes involved in the carboxylation phase, caused by the toxic effect of high salt concentrations, causing the plant to have difficulty in performing CO_2 fixation, hence reducing the production of photoassimilates that are essential for the increase in biomass (Silva et al., 2023). However, there is a certain tolerance of passion fruit seedlings to salts because, when the ECw was increased by more than six times, there were average reductions of only 17.37% in their growth rates and biomass.

When analyzing the SL × H_2O_2 interaction for the Dickson quality index (DQI) of seedling formation, it was observed from the regression analysis (Figure 6C) that plants under ECw of 0.5 dS m^{-1} showed a quadratic response, whose highest value (0.287) was obtained in those that received the concentration of 36 μM. In turn, for plants irrigated using water with ECw of 3.2 dS m^{-1} the response was linear, with an increase of 1.63% per unit increase in H_2O_2 concentration, resulting in an increase of 58.68% in plants that received the highest H_2O_2 concentration (36 μM) compared to those that did not receive H_2O_2 (0 μM).

Although the seedlings irrigated with brackish water had lower quality than under ECw of 0.5 dS m^{-1}, it can be seen that the increase in hydrogen peroxide applied to the plants attenuated effects of salt stress and promoted better quality seedlings when they were irrigated with water of different salinities. Thus, it is possible to infer that hydrogen peroxide promoted seedlings with the potential to be transplanted in all treatments where irrigation was performed with low-salinity water, especially at the concentration of 36 μM, which led to higher DQI compared to the others, since seedlings with DQI above 0.2 are considered of good quality according to Eloy et al. (2013).

Conclusions

1. Increase in salinity levels of irrigation water negatively affects the quality of the seedlings of passion fruit cv. BRS Gigante Amarelo.

2. Hydrogen peroxide mitigates the effect of salt stress at an average concentration of 24 μM on the CO_2 assimilation rate, internal CO_2 concentration, and quality of seedlings of passion fruit cv. BRS Gigante Amarelo.

3. Application of H_2O_2 in seeds associated with foliar spraying mitigates effects of salt stress on the absolute growth rate of leaf area of passion fruit seedlings irrigated with ECw of 3.2 dS m^{-1}.

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