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Hydrogen peroxide and saline nutrient solution in hydroponic zucchini culture

Peróxido de hidrogênio e solução nutritiva salina no cultivo hidropônico de abobrinha italiana

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Highlights

Hydroponics is a viable technology for using brackish water in water-scarce regions.
Hydrogen peroxide induces the production of organic compounds, helping plant growth.
Nutrient solution salinity reduces photosynthetic pigments and plant growth.

Abstract

Saline water has been used in irrigation due to the limited availability of fresh water, especially in arid and semi-arid regions. However, the use of this type of water can affect crop growth and development. Studies have tested the use of chemical conditioners to minimize the negative effects of salinity on plants. In this scenario, the present study examined the role of hydrogen peroxide in mitigating the negative effects of salt stress on zucchini plants grown in a hydroponic system. The study was carried out in a greenhouse in Pombal - PB, Brazil. The NFT (nutrient film technique) hydroponic system was employed. A randomized complete experimental design was set up with a 4 × 4 factorial arrangement consisting of four levels of electrical conductivity in the nutrient solution (ECns: 2.1 [control], 3.6, 5.1, and 6.6 dS m^{-1}) and four concentrations of hydrogen peroxide (H_2O_2: 0, 20, 40, and 60 µM), in three replicates. Foliar spraying with H_2O_2 at 60 and 40 µM associated with the nutrient solution of 2.1 dS m^{-1} increased stem diameter and root length, respectively, at 47 days after transplanting. However, at ECns higher than 2.1 dS m^{-1}, the application of H_2O_2 at the concentrations of 60 and 40 µM intensified salt stress, reducing stem diameter and root

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length. Nutrient solution salinity levels above 2.1 dS m^{-1} reduce photosynthetic pigments; the number of leaves; leaf area; the length of the main branch; and the dry biomass of stems, leaves, and roots of zucchini plants. Chlorophyll b and carotenoid contents are the variables most sensitive to changes in salinity levels.
Key words: Cross-tolerance. *Cucurbita pepo* L.. ROS. Salinity.

Resumo

A água salina vem sendo utilizada na irrigação devido à disponibilidade limitada de água doce, principalmente em regiões áridas e semiáridas. No entanto, o uso deste tipo de água pode afetar o crescimento e desenvolvimento das culturas. Alguns estudos têm testado o uso de condicionadores químicos a fim de minimizar os efeitos negativos provocados pela salinidade nas plantas. Nesse contexto, este estudo teve como objetivo avaliar o papel do peróxido de hidrogênio como mitigador dos efeitos negativos do estresse salino em plantas de abobrinha italiana cultivadas em sistema hidropônico. O trabalho foi conduzido em casa de vegetação, em Pombal – PB. O sistema de cultivo utilizado foi o hidropônico tipo NFT - Técnica de Fluxo Laminar de Nutriente. O delineamento experimental foi inteiramente casualizados, em esquema fatorial 4 × 4, sendo quatro níveis de condutividade elétrica da solução nutritiva - CEsn (2,1 (controle); 3,6; 5,1 e 6,6 dS m^{-1}), e quatro concentrações de peróxido de hidrogênio – H_2O_2 (0; 20; 40 e 60 µM), com 3 repetições. A pulverização foliar de H_2O_2 na concentração de 60 e 40 µM associada a solução nutritiva com condutividade elétrica de 2,1 dS m^{-1} promoveu aumento no diâmetro do caule e comprimento da raiz, respectivamente, aos 47 dias após o transplantio. Contudo, em soluções nutritivas com condutividade elétrica superior a 2,1 dS m^{-1} a aplicação de H_2O_2 em concentrações de 60 e 40 µM intensificou o efeito do estresse salino, reduzindo o diâmetro de caule e o comprimento das raízes, respectivamente. A salinidade da solução nutritiva acima de 2,1 dS m^{-1} reduziu os pigmentos fotossintéticos, o número de folhas, área foliar, o comprimento do ramo principal, fitomassa seca do caule, de folhas e de raiz da abobrinha italiana, sendo os teores de clorofila b e carotenoides as variáveis mais sensíveis.
Palavras-chave: *Cucurbita pepo* L.. EROs. Salinidade. Tolerância cruzada.

Introduction

In the semi-arid region of Brazil, the inadequate management of saline water coupled with low rainfall, high temperatures, and evaporation of soil water is aggravating the accumulation of salts, which can make the soil unproductive. Soils in this region are young and prone to salinization, especially during the water scarcity period, when farmers resort to alternative water sources such as artesian wells and weirs, which typically contain high levels of dissolved salts due to geology and climatic conditions (Santos et al., 2016; Paiva, Rodrigues, Lopes, & Silva, 2019).

An excess of salts in the water and/or soil reduces plant growth and development and restricts water and nutrient absorption due to the partial closure of the stomata. Sodium and chloride accumulation in tissues triggers the effect of toxic ions, which damages the cytoplasm. As a result, biochemical and photosynthetic functions are affected, which can make this process irreversible and lead to plant senescence (Lima et al., 2016; Tavares et al., 2020).

An alternative for the use of saline water with less environmental impact is the implementation of the nutrient film technique (NFT) hydroponic system. Benefits include the possibility of producing all year round, controlling the electrical conductivity of the saline nutrient solution and the pH, reducing chemical pesticide use, increasing water use efficiency, and planning the proper disposal of wastewater (Fernandes et al., 2018).

In the case of zucchini, the tolerance to water salinity in the seedling formation phase is up to 4.0 dS m^{-1} (Amorim, 2015). However, the effect of salts on plant species varies according to the genotype, stage of crop development, climatic conditions, irrigation management, and time of exposure to stress (Bezerra et al., 2018; Sá et al., 2018; Soares et al., 2021). Although there is an indication of the threshold salinity level for the zucchini crop, there is divergence regarding its tolerance. It is worth noting that these threshold salinity levels are adopted for traditional culture in soil. Therefore, research with this vegetable crop under hydroponic conditions, particularly using brackish water, is important to provide sustainability in crop growing to farmers in the semi-arid region of the Brazilian Northeast.

Another alternative that can enable the use of water with high salt contents is the exogenous application of hydrogen peroxide (H_2O_2). Hydrogen peroxide acts as a metabolic signal and, as such, it is capable of triggering reactions related to the mitogen-activated protein kinase (MAPK) pathway, thus inducing tolerance to different stresses in plants through the expression of genes associated with the defense system (Saxena, Srikanth, & Chen, 2016). The effect of hydrogen peroxide on crops under salt stress, either via seed pretreatment or foliar spray, has been studied in crops such as maize (E. M. da Silva, Lacerda, Medeiros, Souza, & Pereira, 2016), passion fruit (Silva et al., 2021b), and soursop (Veloso et al., 2021; A. A. R. da Silva, Capitulino, Lima, Azevedo, & Veloso, 2021a). Nonetheless, similar studies for the zucchini crop or the family *Cucurbitaceae* are rare.

In view of the foregoing, this study was undertaken to examine the role of H_2O_2 as an attenuator of the negative effects of salt stress on the growth and photosynthetic pigments of zucchini cv. Caserta cultivated in an NFT hydroponic system.

Material and Methods

The study was carried out from July to August 2020 in a greenhouse belonging to the Center of Agri-Food Science and Technology (CCTA) at the Federal University of Campina Grande (UFCG), in Pombal, PB, Brazil (6°46'13" S, 37°48'6" W, 184 m above sea level). Figure 1 illustrates the average maximum and minimum temperature and relative humidity of air data during the experimental period.

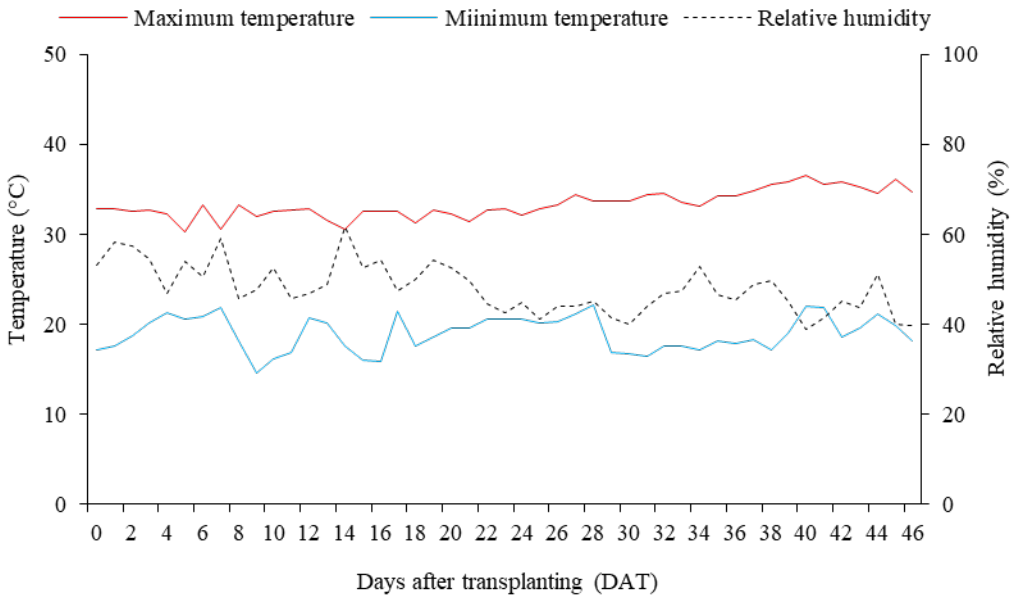

Figure 1. Mean data of maximum and minimum temperatures and relative humidity of air outside the greenhouse during the experiment.

Treatments consisted of four levels of electrical conductivity in the nutrient solution (ECns: 2.1 [control], 3.6, 5.1, and 6.6 dS m^{-1}) and four concentrations of hydrogen peroxide (H_2O_2: 0, 20, 40, and 60 µM) applied by foliar spray, in a completely randomized design with a 4 × 4 factorial arrangement and three replicates. Due to the lack of studies with H_2O_2 in the zucchini cv. Caserta crop and other vegetables, the concentrations used in this experiment were based on a study with cashew (L. de P. Souza et al., 2019) and soursop (Veloso et al., 2020), while the salt levels in the nutrient solution were based on the assay of Putti, Silva, Silva, Gabriel and Klar (2018).

The nutrient film technique (NFT) hydroponic system was employed, which was made with a polyvinyl chloride (PVC) tube of 100 mm in diameter and six meters in length. In the channels, plants were spaced 0.50 m apart and treatments 1.0 m apart.

The channels were supported on 0.60-m-high trestles with an inclination of 4% to allow the flow of the nutrient solution. At the lowest level of each bench in the hydroponic system was a 150-L polyethylene box to collect and lead the nutrient solution to the channels. The nutrient solution was injected into the hidroponic channels by a pump with a power of 35 W, at a flow rate of 3 L min^{-1}. A timer was used to program the circulation of the nutrient solution, adopting an intermittent hourly flow of 30 min. The nutrient solution used was that proposed by Hoagland and Arnon (1950). The chemical composition of nutrient solution is shown in Table 1, which resulted in an electrical conductivity of 2.1 dS m^{-1}.

Table 1
Chemical and physical attributes of the soil used in the experiment, before the application of treatments

Element	Complete solution mg L^{-1}	Fertilizer	Nutrient solution g L^{-1}
N	210	KH_2PO_4	136.09
P	31	KNO_3	101.10
K	234	$Ca(NO_3)_2.4H_2O$	236.15
Ca	200	$MgSO_4.7H_2O$	246.49
Mg	48	H_3BO_3	3.10
S	64	$MnSO_4.4H_2O$	1.70
B	0.5	$ZnSO_4.7H_2O$	0.22
Mn	0.5	$CuSO_4.5H_2O$	0.75
Zn	0.05	$(NH_4)_6Mo_7O_{24}.4H_2O$	1.25
Cu	0.02	$FeSO_4$	13.9
Mo	0.01	EDTA – Na	13.9
Fe	5	-	-
Na	1.2	-	-
Cl	0.65	-	-

Before sowing, the seed coats were removed, then sown in 50-mL polyethylene containers containing vegetable sponge and arranged on trays. The vegetable sponges were sanitized with hypochlorite (2 to 2.5%), washed, and dried in the open air. In the germination phase, until the emergence of the first true leaf (ten days after sowing), a nutrient solution at 50% of the recommended concentration was used. After the emergence of the first true leaf, the sponge was removed and seedlings were transplanted to the hydroponic system, where a nutrient solution at full concentration (100%) was used according to the treatments.

The saline solutions used in the experiment were obtained by adding chloride salts of sodium (NaCl), calcium ($CaCl_2.2H_2O$), and magnesium ($MgCl_2.6H_2O$) to the nutrient solution prepared in municipal supply water of Pombal- PB. These salts were incorporated at the equivalent ratio of 7:2:1, respectively, which is the proportion of Na, Ca, and Mg commonly found in water used for irrigation in the semi-arid region of Northeast Brazil (Medeiros, 1992).

The nutrient solution was replaced completely every eight days to ensure the presence of nutrients at the proper concentration and proportion. Electrical conductivity and the pH were checked daily and the solution was adjusted whenever necessary by adding supply water with an ECw of 0.3 dS m^{-1}, always keeping ECns according to the established treatments and the pH between 5.5 and 6.5 through the addition of 0.1 M KOH or 0.1 M HCl. The plants were staked vertically, and phytosanitary treatments were carried out when necessary.

Although studies have indicated that the reconstitution of the nutrient solution is viable for commercial cultivation, under experimental conditions with multiple factors, such as treatments, there is a need to ensure that the nutrient solution maintains the same characteristics throughout the experimental period so that the observed effects are attributed only to the treatments applied. Because plants generally absorb more water than nutrients, hence there is a tendency for ECns to increase over time, requiring the replenishment of evapotranspired water with supply water to maintain the ECns. Moreover, the absorbed proportion of each nutrient from the nutrient solution may vary depending on the stage of plant development and environmental conditions. Thus, to ensure that all nutrients are in adequate concentrations and proportions and to maintain ionic balance in the solution, it is important to change the nutrient solution (Jones, 1982).

The H_2O_2 stock solution was obtained by diluting 30% H_2O_2 in deionized water, which was then stored in plastic containers lined with aluminum foil and preserved in an air-conditioned environment at a temperature < 23 °C. Before preparing solution, the concentration of H_2O_2 was checked because of the ease of degradation in the presence of light, by reading in a spectrophotometer at 240 nm. After transplanting, the plants received exogenous application of hydrogen peroxide via foliar spray, according to treatment, starting at dusk. This was done manually, with a sprayer, aiming to completely wet the leaves (abaxial and adaxial faces). The procedure was performed at 10-day intervals from 48 h after transplanting and 72 h before the beginning of the application of saline nutrient solution, according to their respective treatments, in a total of three applications. The average volume sprayed on the zucchini leaves was 12 mL plant^{-1} in each application. During the application of the treatments, a cardboard structure was used to prevent drift to the neighboring plants.

At 35 days after transplanting (DAT), the following photosynthetic pigments were evaluated: chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), total chlorophyll (Chl *T*), and carotenoids (Car), according to the methodology of Lichtenthaler (1987). This step involved the use of three discs of plant tissue collected from the third leaf of a branch located in the middle region of the crown. The discs were immersed in 80% acetone and stored in the dark for 48 h in hermetically sealed tubes. The obtained extracts were read in a spectrophotometer with absorbance wavelengths (ABS) of 470, 646, and 663 nm. The Chl *a*, Chl *b*, Chl *T*, and carotenoid contents were estimated using Eq. 1, 2, 3, and 4, and expressed in mg g^{-1} of fresh weight (FW).

$$\text{Chl } a = 12.21\ \text{ABS}_{663} - 2.81\ \text{ABS}_{646} \quad (1)$$

$$\text{Chl } b = 20.13\ \text{ABS}_{646} - 5.03\ \text{ABS}_{663} \quad (2)$$

$$\text{Car} = (1000\ \text{ABS}_{470} - 1.82\ \text{Cl}a - 85.02\ \text{Cl}\ b)/198 \quad (3)$$

$$\text{Chl } T = 17.3\ \text{ABS}_{646} + 7.18\ \text{ABS}_{663} \quad (4)$$

At 47 DAT, plant growth was evaluated based on the length of the main branch, determined by the distance between the neck and the insertion of the apical meristem. Total leaf area (LA, cm^2) was determined by Eq, 5, using the width (W) of leaves that were larger than 5 cm, following Fialho, Dalvi, Corrêa, Kuhlcamp and Effgen (2011):

$$LA = \sum 47.3647+0.6211W^2 \quad \text{..... (5)}$$

The number of leaves was determined by counting. Stem diameter (SD, mm) was measured at a height of 5 cm, above the hydroponic system, using a digital caliper. Root length (RL, cm) was measured using a graduated ruler, as the distance from the root-shoot junction to the apex of the largest root, after careful removal of the root system from the hydroponic profile. Root volume (RV, cm^3) was measured according to Basso (1999), by placing the roots in a graduated cylinder containing a known volume of water; the volume was obtained as a direct response, by difference.

After the biometric measurements, the plants were collected and the different parts were separated, packed in labeled paper bags, and dried in an forced ventilated oven at 65 ºC until constant weight. Later, the dry biomass of leaves (LDB), stems (SDB), and roots (RDB) was determined by weighing on a semi-analytical scale with a precision of 0.001 g.

Data were subjected to analysis of variance by the F test at the 0.05 probability level. When significant, polynomial regression analysis (linear and quadratic) was performed for ECns and the H_2O_2 concentrations, using SISVAR - ESAL statistical software (Ferreira, 2014). Due to the heterogeneity of photosynthetic pigment data, as checked by tests of normality and homogeneity of variances, data referring to the contents of photosynthetic pigments (Chl *a*, Chl *b*, Chl *T*, and carotenoids) were transformed into √x.

Results and Discussion

Nutrient solution salinity significantly affected the levels of photosynthetic pigments (Chl *a*, Chl *b*, Chl *T*, and carotenoids) (Table 2). While, neither H_2O_2 nor the interaction between factors (ECns × H_2O_2) significantly influenced any of the analyzed variables of zucchini cv. Caserta, at 35 DAT.

Table 2
Summary of analysis of variance for chlorophyll a (Chl *a*, mg g^{-1} FW), chlorophyll b (Chl *b*, mg g^{-1} FW), total chlorophyll (Chl *T*, mg g^{-1} FW), and carotenoids (Car, mg g^{-1} FW) of zucchini cultivar Caserta cultivated with saline nutrient solution (ECns) and exogenous application of hydrogen peroxide (H_2O_2) in a hydroponic system, at 35 days after transplanting

Source of variation	DF	Mean square			
		Chl *a*[1]	Chl *b*[1]	Chl *T*[1]	Car[1]
Saline nutrient solution (ECns)	3	130.47**	17.02**	234.05**	21.83**
Linear regression	1	372.53**	33.33**	534.26**	45.14**
Quadratic regression	1	5.63^{ns}	15.03*	123.59*	15.79**
Hydrogen peroxide (H_2O_2)	3	3.90^{ns}	0.38^{ns}	10.39^{ns}	1.50^{ns}
Linear regression	1	3.61^{ns}	0.96^{ns}	5.90^{ns}	1.46^{ns}
Quadratic regression	1	4.21^{ns}	0.00^{ns}	11.42^{ns}	1.98^{ns}
Interaction (ECns × H_2O_2)	9	18.44^{ns}	1.08^{ns}	19.36^{ns}	1.37^{ns}
CV		14.88	15.23	14.69	14.03

ns, *, **: not significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively; CV: coefficient of variation. [1]Data transformed into √x.

Chlorophyll a levels in the zucchini plants decreased linearly, by 6.91%, with each unit increase in ECns levels (Figure 2A). Total chlorophyll and Chl *b* levels, however, fitted the quadratic model (Figure 2B and 2C), with maximum estimated values of 4.89 and 22.96 mg g^{-1} FW, respectively, obtained when the plants were cultivated with ECns of 2.1 dS m^{-1}. The plants subjected to the ECns of 6.6 dS m^{-1} had their Chl *a*, Chl *b*, and Chl *T* levels reduced by 6.48, 2.23, and 8.91 mg g^{-1} FW, respectively, relative to those under nutrient solution with the lowest salinity level. In plants sensitive to salt stress, the accumulation of salts in leaf tissues normally results in chlorophyll degradation. As a result, photosynthetic activity and the production of plant pigmentation proteins are restricted due to the activation of the chlorophyllase enzyme (Nóbrega et al., 2020).

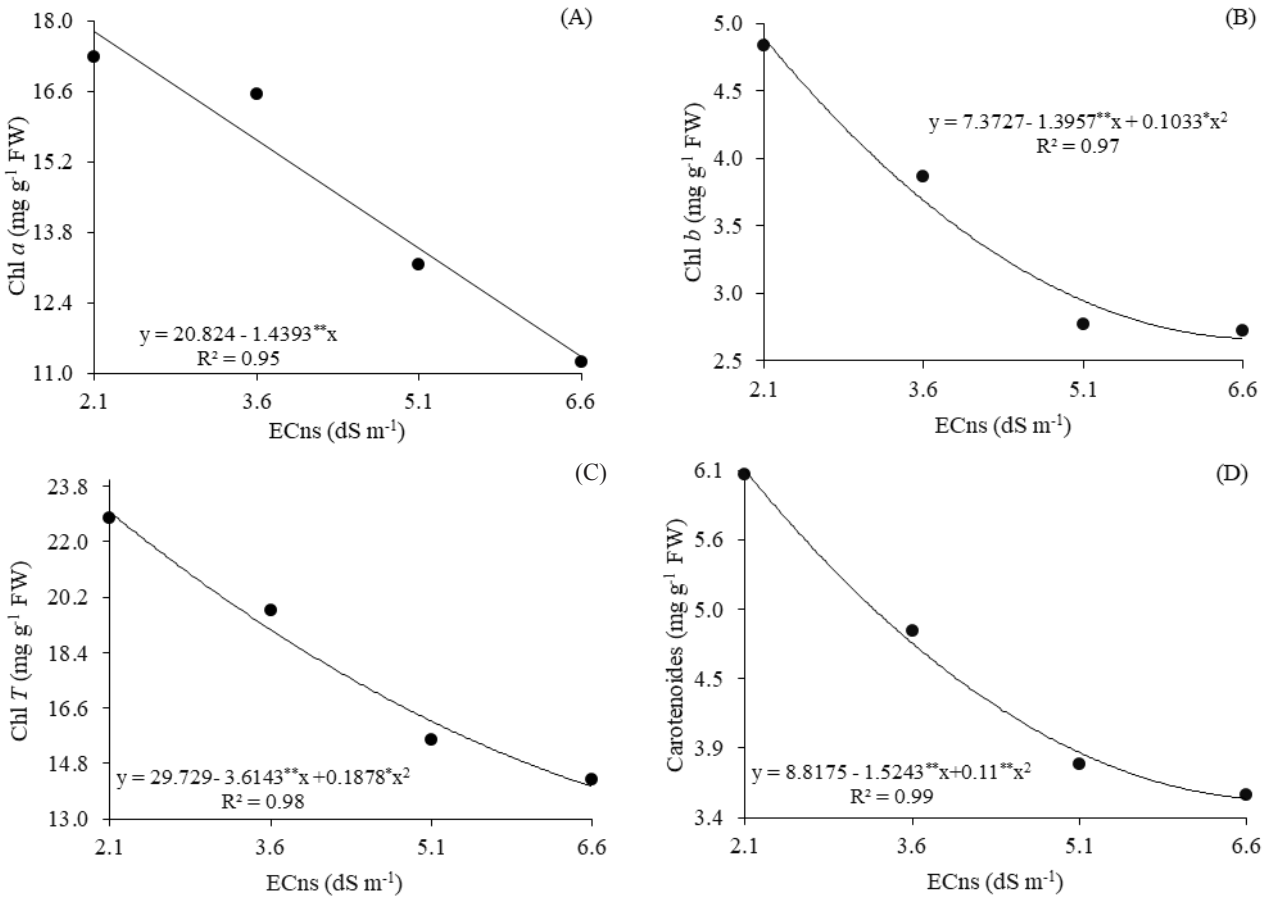

Figure 2. Chlorophyll a (Chl *a*; A), chlorophyll b (Chl *b*; B), total chlorophyll (Chl *T*; C), and carotenoid (D) contents of zucchini cv Caserta as a function of salt levels in the nutrient solution (ECns), in hydroponic cultivation, at 35 days after transplanting.

The salinity of the nutrient solution caused the carotenoid contents of the zucchini plants to decrease quadratically (Figure 2D), with a 41.96% reduction when we compare the plants subjected to the ECns of 6.6 dS m^{-1} with those that received the lowest salt level (2.1 dS m^{-1}). The reduction in carotenoid levels is due to the degradation of β-carotene caused by photo-oxidation, which leads to photosynthetic damage and, consequently, a decrease in plant growth (Dias, Lima, Pinheiro, Gheyi, & Soares, 2019). Melo, Souza, Duarte, Cunha and Santos (2017) studied the bell pepper crop irrigated with water of electrical conductivity from 0 to 9 dS m^{-1} in soil under protected environment conditions and also found that increasing water salinity resulted in inhibition of the synthesis of chlorophylls a and b and carotenoids, whose contents decreased by 5.06, 4.79, and 4.41% with each unit increase in ECw, respectively.

At 47 DAT, there was a significant interaction effect between ECns and H_2O_2 only for the SD and RL of zucchini cv. Caserta (Table 3). In the same period, there was an isolated effect of ECns on the other analyzed variables.

Table 3
Summary of analysis of variance for length of the main branch (LMB, cm), number of leaves (NL), total leaf area (LA, cm^2), stem diameter (SD, mm) and root length (RL, cm) of zucchini cv. Caserta cultivated in saline nutrient solution (ECns) and exogenous application of hydrogen peroxide (H_2O_2) in a hydroponic system, at 47 days after transplanting

Source of variation	DF	Mean square				
		LMB	NL	LA	SD	RL
Saline nutrient solution (ECns)	3	649.88**	41.04*	24404.29**	11.31*	335.93**
Linear regression	1	1743.77**	117.61**	67519.17**	30.87*	918.01**
Quadratic regression	1	9.00^{ns}	4.00^{ns}	5646.01^{ns}	0.57^{ns}	25.00^{ns}
Hydrogen peroxide (H_2O_2)	3	58.19^{ns}	12.04^{ns}	4524.16^{ns}	15.75**	171.68**
Linear regression	1	172.57^{ns}	12.01^{ns}	12059.20^{ns}	39.08*	49.61ns
Quadratic regression	1	0.062^{ns}	1.00^{ns}	1499.62^{ns}	7.29^{ns}	462.25**
Interaction (ECns × H_2O_2)	9	39.89^{ns}	19.97^{ns}	4892.88^{ns}	11.47**	85.67*
CV		11.56	11.39	10.54	10.30	5.90

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

The increasing ECns induced a linear decrease in the length of the main branch, number of leaves, and total leaf area of zucchini (Figure 3A, 3B, and 3C), whose minimum values obtained were 57.52 cm, 30.55, and 578.86 cm^2, respectively, in the plants subjected to the ECns of 6.6 dS m^{-1}. These values were 19.59, 10.64, and 13.08% lower, respectively, than those achieved by the plants in control treatment. The decrease in plant growth under salt stress conditions is associated with the reduced water absorption caused by partial stomatal closure, which is induced by osmotic and ionic effects that cause changes in the photosynthetic rate and in plant metabolism, thereby inhibiting growth (E. G. da Silva, Silva, Lima, Silva, & Maia, 2017). In zucchini plants, lower vegetative growth may also be associated with reduced chlorophyll, which compromises photosynthesis and consequently their growth. In a study with two cucumber varieties irrigated with water with ECw ranging from 0.6 to 3.0 dS m^{-1}, Albuquerque et al. (2016) also observed a reduction in plant height and number of leaves with increasing salinity.

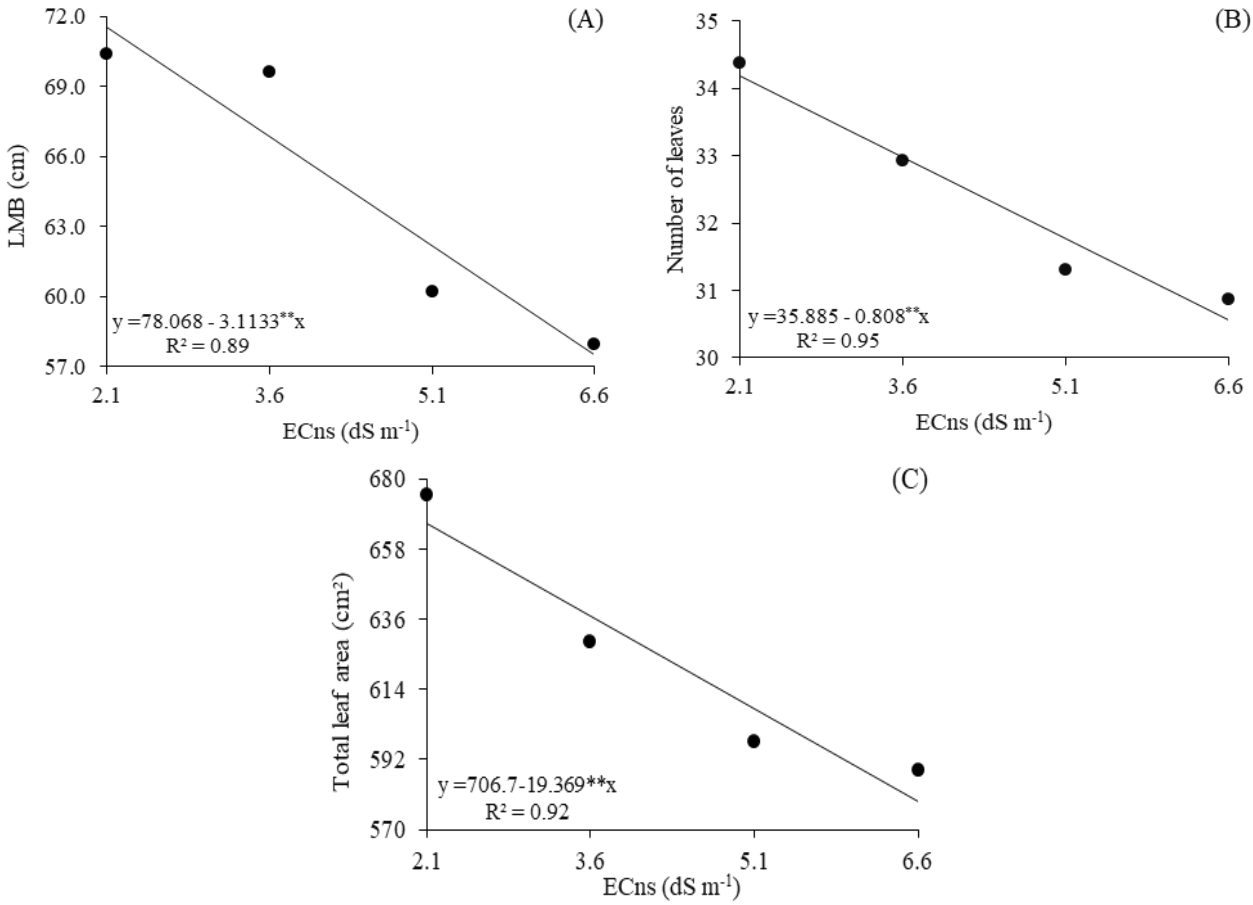

Figure 3. Length of main branch (LMB), number of leaves, and total leaf area of zucchini cv. Caserta as a function of salt levels in the nutrient solution (ECns), in hydroponic cultivation, at 47 days after transplanting.

In the plants subjected to the H_2O_2 concentrations of 0, 40, and 60 µM, stem diameter (Figure 4A) was highest (19.98, 17.58, and 20.17 mm) when they received the ECns of 4.4, 2.1, and 2.1 dS m^{-1}, respectively. The plants that received 20 µM H_2O_2 reached an average SD of 18.76 ± 0.612 mm. The increase in SD, especially in the plants that received 60 µM H_2O_2, indicates that H_2O_2 acted positively on plant growth, with the nutrient solution. E. M. da Silva et al. (2016) stated that H_2O_2 reacts with proteins, DNA, lipid membranes, and soluble carbohydrates, inducing plant defense and thus contributing to growth. However, the saline nutrient solution with electrical conductivity of 6.6 and 4.3 dS m^{-1} interacting with the H_2O_2 concentrations of 40 and 60 µM reduced SD.

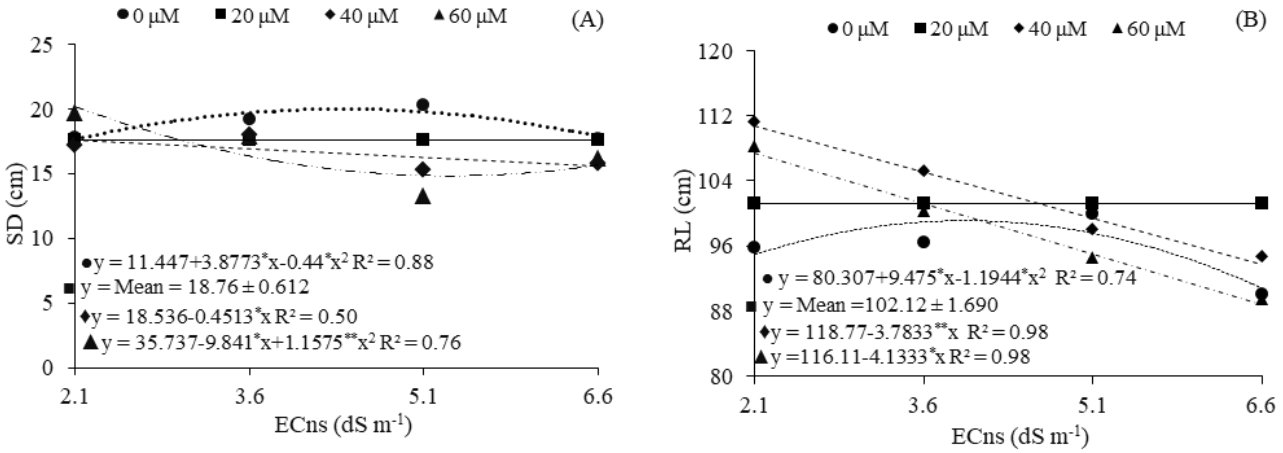

Figure 4. Stem diameter (SD; A) and root length (RL; B) of zucchini cv. Caserta as a function of the interaction between the salt levels in the nutrient solution (ECns) and exogenous application of hydrogen peroxide (H_2O_2) in hydroponic system, at 47 days after transplanting.
Mean ± standard error (n = 4).

The reduction observed in these treatments is due to the increase in salinity, and the negative effect was possibly intensified by high concentrations of H_2O_2, which in turn can contribute to increased oxidative stress (Ransy, Vaz, Lombès, & Bouillaud, 2020). Oxidative stress inhibited the elongation/expansion of cells, because to maintain osmotic and ionic homeostasis, the plant depends on a greater amount of energy, and partial stomatal closure prevents excessive absorption and accumulation of Na^+ and Cl^- in the cells (Moura et al., 2017).

The nutrient solutions with the salt concentrations of 2.1, 2.1, and 4.0 dS m^{-1} provided the greatest root lengths, of 110.82, 107.43, and 99.09 cm, in the zucchini sprayed with H_2O_2 concentrations of 40, 60, and 0 µM (Figure 4B), respectively. On the other hand, the lowest root lengths (90.81, 93.80, and 88.83 cm) occurred at the ECns of 6.6 dS m^{-1} and the H_2O_2 concentrations of 0, 40, and 60 µM, respectively. In plants that received exogenous application of 20 µM H_2O_2, the data did not fit satisfactorily to any regression models, averaging 102.12 ± 1.690 cm. The positive effect on root length observed at the H_2O_2 concentrations of 40 and 60 µM is in line with the descriptions of Khan, Yusuf and Fariduddin (2018), who stated that H_2O_2 induces the production of organic compounds and proteins in plants, whether under stress conditions or absence of stress, acting on biochemical and physiological activity, detoxifying reactive oxygen species (ROS), and causing a positive effect on the cellular functioning, growth, and development of plants.

There was no significant interaction effect between nutrient solution salinity and exogenous application of H_2O_2 on RV, LDB, SDB, or RDB (Table 4). On the other hand, there were isolated effects of nutrient solution salinity on LDB, SDB, and RDB, and of H_2O_2 on the accumulation of LDB and RDB in the zucchini, at 47 DAT (Table 4).

Table 4
Summary of analysis of variance for root volume (RV, cm^3), leaf dry biomass (LDB, g $plant^{-1}$), stem dry biomass (SDB, g $plant^{-1}$) and root dry biomass (RDB, g $plant^{-1}$) of zucchini cv. Caserta grown with saline nutrient solution (ECns) and exogenous application of hydrogen peroxide (H_2O_2) in a hydroponic system, at 47 days after transplanting

Source of variation	DF	Mean square			
		RV	LDB	SDB	RDB
Saline nutrient solution (ECns)	3	2687.50^{ns}	3480.14**	102.35**	17.12*
Linear regression	1	7411.25^{ns}	9574.03**	279.09**	50.75**
Quadratic regression	1	100.00^{ns}	127.91^{ns}	14.12^{ns}	0.61^{ns}
Hydrogen peroxide (H_2O_2)	3	3354.16^{ns}	1151.49**	25.36^{ns}	17.29*
Linear regression	1	845.00^{ns}	998.84*	42.04^{ns}	0.51^{ns}
Quadratic regression	1	8556.25^{ns}	2454.70**	17.44^{ns}	38.28**
Interaction (ECns × H_2O_2)	9	3316.66^{ns}	119.89^{ns}	27.35^{ns}	6.92^{ns}
CV		7.06	15.47	17.63	17.38

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

Leaf dry biomass accumulation decreased linearly (Figure 5A) with increasing nutrient solution salinity levels, with a reduction of 5.83% per unit increment in ECns. This means a 30.02% decrease, or 32.81 g $plant^{-1}$, if we compare the plants subjected to the ECns of 6.6 dS m^{-1} with those that received 2.1 dS m^{-1}. The salts present in irrigation water act negatively on the accumulation of plant biomass, as they compromise meristematic activity and cell elongation and expansion and, therefore, the biomass (Wanderley et al., 2020). Lima et al. (2016) also observed a reduction in LDB accumulation when evaluating the growth and production of 'All Big' peppers as a function of irrigation with saline water (ECw of 0.6 and 3.0 dS m^{-1}) in a study conducted in pots using a *Neossolo Regolítico* (*Psamments*). The researchers observed that plants irrigated with water of 0.6 dS m^{-1} showed an increase in LDB of 2.72 g $plant^{-1}$, relative to the average obtained by those that were under an ECw of 3.0 dS m^{-1}.

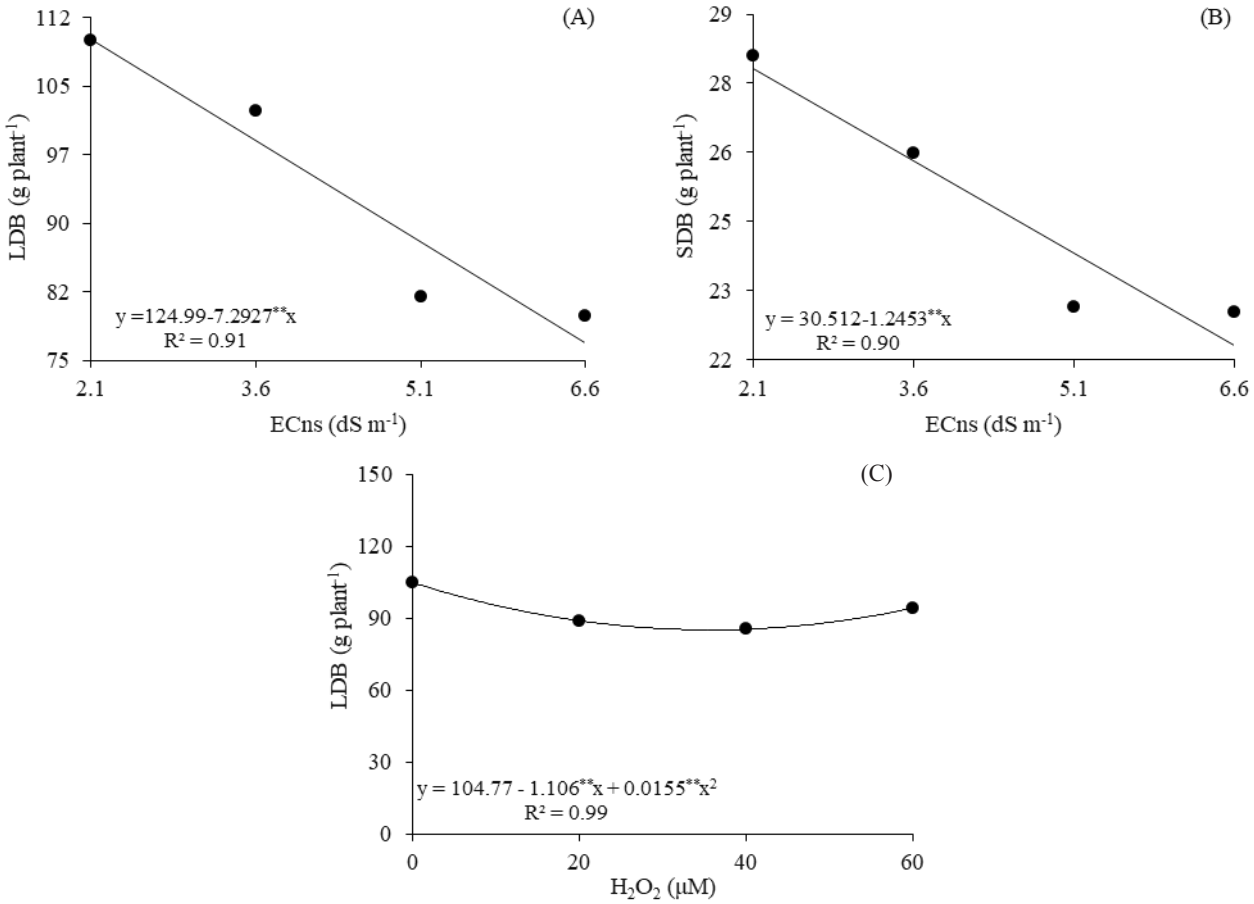

Figure 5. Leaf dry biomass (LDB; A) and stem dry biomass (SDB; B) of zucchini cv. Caserta as a function of salt levels in nutrient solution (ECns) and leaf dry biomass (LDB; C) as a function of exogenous application of hydrogen peroxide in hydroponic culture, at 47 days after transplanting.

The dry mass of the zucchini stem also decreased linearly, with a 4.08% decline observed with each unit increment in ECns in nutrient solution (Figure 5B). According to F. M. de Souza et al. (2018), toxic ions restrict water and nutrient absorption, causing a deleterious effect on growth and photosynthesis, which in turn lead to a decrease in biomass. Lucena, Negreiros, Medeiros, Grangeiro, & Marrocos (2011) examined the growth of another curcubit, 'Quetzali' watermelon, grown under field conditions, using water with different salinity levels, and also found that water with higher electrical conductivity (3.98 dS m^{-1}) reduced the accumulation of dry biomass, thus reducing plant growth. The reduction in biomass accumulation may be related to the decrease in chlorophyll levels, the photosynthetic rate of plants, and the diversion of energy destined for growth to activate and maintain membrane integrity, synthesis of organic solutes for osmoregulation, and/or protection of macromolecules and regulation of ionic transport and distribution in various organs within cells (Lima et al., 2020).

Hydrogen peroxide caused LDB to decrease quadratically (Figure 5C), with a

minimum value of 85.04 g plant^{-1} observed for the plants that received application of hydrogen peroxide at an estimated concentration of 35 µM, with a further stability. Hydrogen peroxide is used to stimulate the production of proteins and carbohydrates and induce the plant's defense system, detoxifying ROS, and to contribute to plant growth (Silva et al., 2019).

The RDB of zucchini cv. Caserta (Figure 6A) decreased linearly with increasing salinity in the nutrient solution, with a 3.67% reduction (0.5327 g) per unit increment in ECns. Plants subjected to the ECns of 6.6 dS m^{-1} had their RDB reduced by 17.88% (2.39 g plant^{-1}) relative to those that received 2.1 dS m^{-1}. The reduction in RDB is a reflection of lower root growth with increased salinity. This is a plant tolerance mechanism to reduce the absorption of water with salts and mitigate toxicity (Lima et al., 2021). Putti et al. (2018) evaluated the cultivation of zucchini under salt stress conditions (ECw from 0 to 5 dS m^{-1}) and found a reduction of 1.64 g in the dry biomass of the zucchini root with each unit increment of ECw, at 30 DAT.

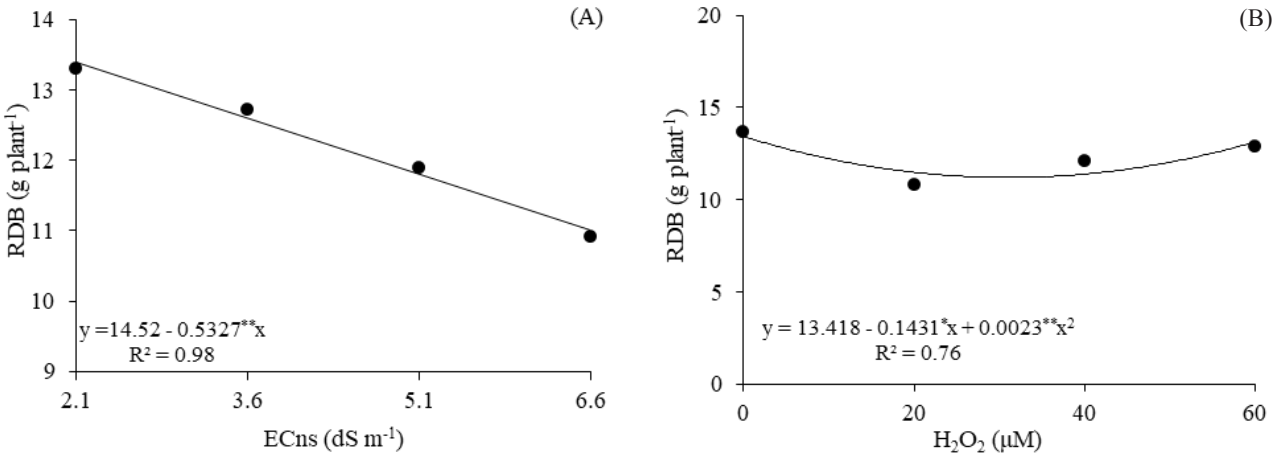

Figure 6. Root dry biomass (RDB) of zucchini plants as a function of the salt levels in the nutrient solution (ECns; A) and hydrogen peroxide (H_2O_2; B) in hydroponic cultivation, at 47 days after transplanting.

Similar to what was observed for LDB (Figure 5C), RDB showed a quadratic reduction with increasing H_2O_2 concentrations (Figure 6B). The maximum reduction was observed in plants subjected to spray with the estimated H_2O_2 concentration of 32 µM (RDB=11.19 g plant^{-1}). From the estimated H_2O_2 concentration of 33 µM, there was an upward trend in RDB, similar to LDB, which led to the accumulation of 13.11 g plant^{-1} under foliar application of 60 µM. Reactive oxygen species, such as hydrogen peroxide, can act as signaling molecules involved in the acclimatization of plants to various abiotic stresses. However, it is still unclear how the generalized increase in ROS and downstream signaling events that occur in response to stressful conditions are coordinated to modify

plant growth and development (Voothuluru et al., 2020), depending on factors such as concentration, method of application, and plant species.

Conclusions

Foliar spray with H_2O_2 at the concentrations of 60 and 40 µM, under nutrient solution with an electrical conductivity of 2.1 dS m^{-1}, increases stem diameter and root length, respectively, in hydroponically grown zucchini, at 47 days after transplanting. However, in nutrient solutions with electrical conductivity greater than 2.1 dS m^{-1}, the application of H_2O_2 at the same concentrations intensifies salt stress, reducing stem diameter and root length.

Nutrient solution salinity levels above 2.1 dS m^{-1} reduce the content of photosynthetic pigments, the number of leaves, leaf area, the length of the main branch, and the dry biomass of stems, leaves, and roots of zucchini. Chlorophyll b and carotenoid contents are the most sensitive variables.

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