# GAS EXCHANGE AND HYDROPONIC PRODUCTION OF ZUCCHINI UNDER SALT STRESS AND H<sub>2</sub>O<sub>2</sub> APPLICATION<sup>1</sup>

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**ABSTRACT** - Knowledge about the effect of chemical conditioners on the reduction of the deleterious effects caused by salinity on crops is of great importance for the expansion of the cultivation of vegetable crops such as zucchini in the semi-arid region of the Northeast. In this context, the objective of the present study was to evaluate the effect of the foliar application of hydrogen peroxide as a mitigator of salt stress on the gas exchange, production, and postharvest fruit quality of zucchini cultivated in a hydroponic system. The study was conducted in NFT-type (Nutrient Film Technique) hydroponic system in a greenhouse, in Pombal – PB, Brazil. The experimental design was completely randomized, in a 4 × 4 factorial scheme, corresponding to four levels of electrical conductivity of the nutrient solution - ECns (2.1 (control); 3.6; 5.1 and 6.6 dS m<sup>-1</sup>), and four concentrations of hydrogen peroxide - H<sub>2</sub>O<sub>2</sub> (0; 20; 40 and 60  $\mu$ M), with three replicates. Nutrient solution with electrical conductivity above 2.1 dS m<sup>-1</sup> caused a reduction in gas exchange and the total number of fruits of zucchini. An increase in nutrient solution salinity levels increased the total soluble solids content of the fruits and the initial fluorescence of zucchini. Under conditions of nutrient solution salinity above 2.1 dS m<sup>-1</sup>, hydrogen peroxide could not mitigate the effects of salt stress. Application of 20  $\mu$ M of H<sub>2</sub>O<sub>2</sub> when the plants were grown in a nutrient solution of 2.1 dS m<sup>-1</sup> promoted higher total fruit weight and basal diameter of the fruits.

Keywords: Cucurbita pepo L.. Saline solution. Hydrogen peroxide.

## TROCAS GASOSAS E PRODUÇÃO HIDROPÔNICA DE ABOBRINHA ITALIANA SOB ESTRESSE SALINO E APLICAÇÃO DE H<sub>2</sub>O<sub>2</sub>

**RESUMO** - O conhecimento do efeito de condicionadores químicos na redução dos efeitos deletérios provocados pela salinidade nas culturas é de grande importância para a expansão do cultivos tais como abobrinha na região semiárida do Nordeste. Nesse contexto, no presente estudo objetivou-se avaliar o efeito da aplicação foliar de peróxido de hidrogênio como atenuador do estresse salino sob as trocas gasosas, a produção e a qualidade pós-colheita de frutos de abobrinha italiana cultivada em sistema hidropônico. O trabalho foi desenvolvido em sistema hidropônico tipo Técnica de Fluxo de Nutrientes em casa de vegetação, em Pombal – PB. O delineamento experimental foi o inteiramente casualizado, 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<sup>-1</sup>), e quatro concentrações de peróxido de hidrogênio – H<sub>2</sub>O<sub>2</sub> (0; 20; 40 e 60  $\mu$ M), com três repetições. A solução nutritiva com condutividade elétrica acima de 2,1 dS m<sup>-1</sup> promoveu redução nas trocas gasosas e no número total de frutos de abobrinha italiana. A elevação dos níveis salinos da solução nutritiva aumentou o teor de sólidos solúveis totais dos frutos e a fluorescência inicial de abobrinha italiana. Em condições de salinidade da solução nutritiva acima de 2,1 dS m<sup>-1</sup>, o peróxido de hidrogênio não conseguiu atenuar os efeitos do estresse salino. A aplicação de 20  $\mu$ M de H<sub>2</sub>O<sub>2</sub> quando as plantas foram cultivadas em solução nutritiva de 2,1 dS m<sup>-1</sup> promoveu maior peso total dos frutos e diâmetro basal dos frutos.

Palavras-chave: Cucurbita pepo L.. Solução salina. Peróxido de hidrogênio.

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## **INTRODUCTION**

Zucchini (*Cucurbita pepo* L.) is one of the most consumed vegetables, among the most socioeconomically important for Brazil, being traditionally cultivated by small producers (GRANGEIRO et al., 2020). It is a vegetable with high nutritional quality, standing out as a source of Ca, P, Fe, and fibers (OLIVEIRA et al., 2013).

In the Northeast region, low rainfall and high evapotranspiration restrict agriculture during the drought season. Thus, it is necessary to look for alternative water sources, such as underground waters, which generally have high concentrations of salts and, in many cases, make conventional cultivation in soil impossible. In this context, the use of cultivation systems that enable the use of these resources, such as hydroponics, stands out in importance (LIMA et al., 2020a).

Salinity has a negative effect on plants and depending on the cultivated species, the losses are irremediable, compromising the yield and quality of the product, making its commercialization unfeasible (SANTOS et al., 2020). Under conditions of salt stress, plants accumulate sodium and chloride ions in excess, responsible for different physiological and biochemical changes, including ionic imbalance and decreased leaf water potential, inducing the production of reactive oxygen species (KOTAGIRI; KOLLURU, 2017).

High concentrations of salts can also cause membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition, and metabolic dysfunction, which ultimately leads to plant death (ASTANEH et al., 2018). Due to damage to the photosynthetic apparatus, reduced growth, and loss in production, it is relevant to use stress attenuators to minimize the effect of salts on crops. Among the products, research reports that under conditions of salt stress, the use of hydrogen peroxide can stimulate development in crops of socioeconomic importance for Brazil (SOUZA et al., 2019; SILVA et al., 2019a, b).

Hydrogen peroxide is a reactive oxygen species that plays a key role in the process of acclimatization of plants to various stress conditions such as salinity (SILVA et al., 2020) and drought (HOSSAIN; FUJITA, 2013).  $H_2O_2$  induces the production of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase), proteins and compounds that regulate various mechanisms under stress conditions, improving water and nutrient absorption, photosynthetic efficiency, and contributing to the maintenance of ionic homeostasis and redox of plants (CARVALHO et al., 2011).

In a study testing the role of  $H_2O_2$  in the

mitigation of salt stress, Silva et al. (2019a, b) concluded that the deleterious effects caused by irrigation water salinity were mitigated by the exogenous application of hydrogen peroxide at a concentration of 20  $\mu$ M. In the zucchini plant, Dantas et al. (2021) also observed that the exogenous application of H<sub>2</sub>O<sub>2</sub> at the concentration of 40  $\mu$ M mitigated the effect of salt stress on the instantaneous carboxylation efficiency of plants grown in the hydroponic system under nutrient solution salinity of 2.1 dS m<sup>-1</sup>.

The form of cultivation associated with the use of chemical conditioners can be an alternative to obtain satisfactory production with the reduction of the negative impacts of salts on plants. Hydroponic cultivation is an important alternative in the production of vegetables, due to the possibility of having greater control over production factors, especially the management of water and nutrients, besides making it possible to produce throughout the year in a greenhouse, hence being an advantageous cultivation practice for the Northeastern semi-arid conditions (LOUREIRO et al., 2019). Moreover, in the hydroponic cultivation (soilless), only the osmotic potential of the solution is considered, disregarding the matric potential that is observed in conventional cultivation in soil, so this system becomes appropriate to reduce the socioeconomic impacts caused by water scarcity in semi-arid regions (COSTA et al., 2020).

Despite the nutritional and socioeconomic importance of the zucchini crop, results of the studies with this vegetable in hydroponic cultivation with saline nutrient solution are incipient in the literature. In this context, the objective of the present study was to evaluate the effect of hydrogen peroxide concentrations as a mitigator of salt stress on gas exchange, production, and postharvest fruit quality of zucchini cultivated in a Nutrient Film Technique hydroponic system.

## MATERIAL AND METHODS

The experiment was carried out in a greenhouse between July and August 2020, at the Center of Science and Agrifood Technology (CCTA) of the Federal University of Campina Grande (UFCG), in Pombal - PB, Brazil, at the geographic coordinates: 6°46'13" South latitude and 37°48'6" West longitude, at a mean altitude of 184 m.

The data of maximum and minimum temperature and relative humidity of air during the experimental period were obtained at the climatological station of the Irrigated Perimeter of São Gonçalo, Sousa - PB, and are presented in Figure 1.



Figure 1. Average data of maximum and minimum temperature and relative humidity of air during the experimental period.

A completely randomized design was used, in a 4 × 4 factorial scheme, referring to four levels of salinity of the nutrient solution - ECns (2.1; 3.6; 5.1 and 6.6 dS m<sup>-1</sup>), and four concentrations of hydrogen peroxide -  $H_2O_2$  (0; 20; 40 and 60  $\mu$ M) applied by foliar spraying, with 3 replicates. Due to the absence of research with  $H_2O_2$  in vegetables, the concentrations used in the present assay were based on studies carried out with cashew (SOUZA et al., 2019) and soursop (VELOSO et al., 2020). The salinity levels of the nutrient solution were based on the work of Putti et al. (2018) with the zucchini crop.

The nutrient solution used was according to the recommendation of Hoagland and Arnon (1950), whose nutrient concentrations (Table 1) after preparation in the local-supply water (0.3 dS m<sup>-1</sup>) resulted in electrical conductivity of 2.1 dS m<sup>-1</sup>.

Elements	mg $L^{-1}$ - Complete solution	Fertilizers	g L <sup>-1</sup> of nutrient solution
Ν	210	KH <sub>2</sub> PO <sub>4</sub>	136.09
Р	31	KNO3	101.10
Κ	234	Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	236.15
Ca	200	MgSO <sub>4</sub> .7H <sub>2</sub> O	246.49
Mg	48	$H_3BO_3$	3.10
S	64	MnSO <sub>4</sub> .4H <sub>2</sub> O	1.70
В	0.5	ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.22
Mn	0.5	CuSO <sub>4</sub> .5H <sub>2</sub> O	0.75
Zn	0.05	(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> .4H <sub>2</sub> O	1.25
Cu	0.02	FeSO <sub>4</sub>	13.9
Мо	0.01	EDTA – Na	13.9
Fe	5		
Na	1.2		
Cl	0.65		

 Table 1. Chemical composition of nutrients present in the complete nutrient solution of the Hoagland and Arnon (1950), used in the hydroponic cultivation of zucchini.

The saline nutrient solutions used in cultivation were prepared by adding sodium chloride (NaCl), calcium chloride (CaCl<sub>2</sub>.2H<sub>2</sub>O), and magnesium chloride (MgCl<sub>2</sub>.6H<sub>2</sub>O) salts, in the equivalent proportion of 7:2:1, respectively. This is the proportion of Na, Ca, and Mg commonly found

in the waters used for irrigation in the semi-arid region of Northeastern Brazil (MEDEIROS, 1992).

The hydrogen peroxide stock solution was obtained by diluting  $H_2O_2 - 30\%$  in deionized water, stored in plastic containers covered with aluminum foil, and stored at low temperature (<12 °C).

The seeds used were certified and were acquired at an agricultural products store in Pombal - PB. Before sowing, the seed coat was removed and then the seeds were sown in 50-mL polyethylene containers containing small pieces of vegetable sponge, arranged in trays. Before sowing, the vegetable sponges were sanitized with hypochlorite (2.5%), washed, and dried outdoors. From germination until the emergence of the first true leaf (ten days after sowing), a nutrient solution with 50% strength was used. After the first leaf emerged, the vegetable sponge was removed and the seedlings were placed in the hydroponic profiles, using the nutrient solution of full concentration (100%).

The hydroponic system used was Nutrient Film Technique - NFT type, made with polyvinyl chloride (PVC) pipe with 100 mm in diameter and six meters in length, spaced by 0.40 m. In the channels, the spacing was 0.50 m between plants and 1.0 m between treatments (subsystems), and cells for planting had a diameter of 54.17 mm.

The channels were supported on 0.60-m-high sawhorses with a 4% inclination for the nutrient solution to flow. At the lowest part of each bench of the hydroponic system, there was a 150-L polyethylene container to collect and conduct the nutrient solution back to the channels. The nutrient solution was injected into the cultivation channels by a 35 W pump, at a flow rate of 3 L min<sup>-1</sup>. Nutrient solution circulation was programmed by a timer, with an intermittent flow of 15 min every hour during the day and night.

The pH and electrical conductivity of the nutrient solution were checked daily, to maintain the ECns according to the established treatments and the pH between 5.5 and 6.5. When necessary, the ECns was adjusted by adding local-supply water with electrical conductivity - ECw of 0.3 dS m<sup>-1</sup>, and the pH was adjusted by adding 0.1 M KOH or HCl. In addition, the total nutrient solution was replaced at eight-day intervals.

After 48 hours of transplantation (period of acclimatization of plants in the nutrient solutions) and 72 hours before the beginning of the application of saline nutrient solutions, hydrogen peroxide was applied according to treatment. The applications were performed at 17:00 h, manually, using a sprayer, aiming to moisten the total area of the zucchini leaves (adaxial and abaxial sides), applying on average 12 mL per plant, at an interval of 10 days, in a total of three applications. A cardboard structure was used to avoid the drift of treatments between plants. The plants were grown with vertical staking to leave the stem upright with the aid of nylon ribbon. The flowers were artificially pollinated using a brush to transfer pollen grains from anthers to the stigma of different plants. Pollination of zucchini plants started at 6 a.m. due to the receptivity of the stigma and the viability of the pollen grain. After fertilization of the flower, there was no control of the number of fruits per plant. Phytosanitary treatments were performed according to the need for pest and/or disease control.

At 35 days after transplantation (33 days after H<sub>2</sub>O<sub>2</sub> application), gas exchanges were evaluated in the leaves of the middle third, fully expanded and free of diseases and/or pest attacks, through stomatal conductance - gs (mol  $H_2O m^{-2} s^{-1}$ ), transpiration - E (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), CO<sub>2</sub> assimilation rate - A( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and internal CO<sub>2</sub> concentration - Ci ( $\mu$ mol mol<sup>-1</sup>) with the aid of a portable infrared carbon dioxide analyzer (IRGA), model LCPro + Portable Photosynthesis System<sup>®</sup> (ADC BioScientific Limited, UK), irradiation of 1200 µmol photons m<sup>-2</sup> s<sup>-1</sup>, an airflow of 200 mL min<sup>-1</sup>, and atmospheric  $CO_2$  concentration. After data collection, the instantaneous water use efficiency - $WUEi - A/E [(\mu mol m^{-2} s^{-1})(mmol H_2O m^{-2} s^{-1})^{-1})$  and instantaneous carboxylation efficiency - CEi - A/Ci  $[(\mu mol m^{-2} s^{-1})(\mu mol mol^{-1})^{-1}]$  were quantified.

In the same period, chlorophyll *a* fluorescence parameters were measured in leaves of the middle third, fully expanded, and pre-adapted to the dark for 30 minutes. The parameters evaluated were: initial fluorescence (F<sub>0</sub>), maximum fluorescence (Fm), variable fluorescence (Fv), and quantum efficiency of PSII (Fv/Fm) using the Opti science OS5p pulse-modulated fluorometer. The light pulse used was low intensity-modulated red (0.03  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) and then a saturated actinic light pulse (>6000  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>).

Fruit harvest began at 28 days and ended at 47 days after transplanting, harvesting ripe and unripe fruits, with a length of up to 20 cm according to the methodology of Delfim and Mauch et al. (2017). After harvest, the total number of fruits (TNF) was determined and then the fruits were weighed on a semi-analytical scale to obtain the total production per plant (TP), with the result expressed in g per plant. The basal diameter of the fruit (BDF) was measured at the end of the largest thickness of the fruit using a digital caliper, and the result was expressed in mm.

The diameter of the lower middle third (DLMT) was evaluated at the end with the smallest thickness of the fruit using a digital caliper, and the result was expressed in mm. The firmness of the basal diameter (FBD) and the firmness of the lower middle third (FLMT) were determined by two readings on the fruit at equidistant points, with a McCormick FT 327 analog penetrometer (8-mm-diameter tip), and the results were expressed in Newton (N).

To determine the postharvest quality, three

fruits were cut and crushed in the blender to obtain the extract. After crushing, 50 mL was collected to determine total soluble solids (TSS) and hydrogen potential (pH). Total soluble solids were determined in the pulp extract of the peeled fruits in triplicates, using a digital refractometer with automatic temperature compensation, which was calibrated with distilled water always when necessary, expressing the results in °Brix. The hydrogen potential was determined with direct reading in the extract of the fruit with peel (without adding water), in triplicate, using a benchtop pH meter calibrated with the buffer solution of pH 4 and 7.

The results were subjected to analysis of variance by Fisher test (F) at 0.05 probability level and, when significant, polynomial regression analysis (linear and quadratic) was performed for the ECns and hydrogen peroxide concentrations, using the statistical program SISVAR - ESAL.

## **RESULTS AND DISCUSSION**

The interaction between factors, the electrical conductivity of the nutrient solution (ECns) and the application of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) did not significantly affect the gas exchange of zucchini (Table 2). As a single factor, ECns had a significant effect on stomatal conductance, transpiration, CO<sub>2</sub> assimilation rate, internal CO<sub>2</sub> concentration, instantaneous water use efficiency, and instantaneous carboxylation efficiency. On the other hand, hydrogen peroxide did not influence any of the variables analyzed at 35 DAT. Dantas et al. (2021) in a study evaluating the effects of saline nutrient solution and exogenous application of hydrogen peroxide on the gas exchange of zucchini, also found that the H<sub>2</sub>O<sub>2</sub> concentrations did not influence any of the variables analyzed.

**Table 2**. Results of Fisher test (F) for the gas exchange of zucchini plants cultivated with saline nutrient solution (ECns) and exogenous application of hydrogen peroxide  $(H_2O_2)$  in a hydroponic system.

SV	F test						
51	gs	Ε	Α	Ci	WUEi	CEi	
ECns	**	**	**	**	**	**	
Linear	**	**	**	**	**	**	
Quadratic	ns	ns	**	ns	**	**	
$H_2O_2$	ns	ns	ns	ns	ns	ns	
Linear	ns	ns	ns	ns	ns	ns	
Quadratic	ns	ns	ns	ns	ns	ns	
$ECns \times H_2O_2$	ns	ns	ns	ns	ns	ns	
Overall Mean	0.42	5.27	29.27	184.76	5.66	0.16	
CV (%)	12.58	5.97	11.80	15.84	13.57	26.04	

gs - stomatal conductance (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), E – transpiration (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), A - CO<sub>2</sub> assimilation rate (µmol m<sup>-2</sup> s<sup>-1</sup>), Ci - internal CO<sub>2</sub> concentration (µmol mol<sup>-1</sup>), WUEi - instantaneous water use efficiency [(µmol m<sup>-2</sup> s<sup>-1</sup>) (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>], CEi - instantaneous carboxylation efficiency [(µmol m<sup>-2</sup> s<sup>-1</sup>) (µmol mol<sup>-1</sup>)<sup>-1</sup>]. <sup>ns, \*\*</sup> \*\* respectively not significant and significant at p < 0.05 and < 0.01; SV - Source of variation; CV- coefficient of variation.

Stomatal conductance decreased linearly with the increase in ECns, with a reduction of 5.30% per unit increment in ECns (Figure 2A). When comparing the plants subjected to ECns of 6.6 dS m<sup>-1</sup> with those in the control treatment, there was a reduction of 26.84%. In plants grown under salt stress, the strategy to mitigate water losses to the atmosphere and maintain high water status is the partial or total closure of stomata, which also contributes to the lower absorption of toxic ions such as Na<sup>+</sup> and Cl<sup>-</sup> (DIAS et al., 2019). The negative effect of water salinity was also verified by Melo et al. (2017) in irrigated bell pepper, which showed a decrease in stomatal conductance with saline water above 3.0 dS m<sup>-1</sup>.

Leaf transpiration of zucchini showed reductions of 6.63% per unit increment in the

electrical conductivity of the nutrient solution (Figure 2B). Reduction in the transpiration rate is directly linked to the decrease in stomatal conductance, because with less opening of the stomata there will be a decline in transpiration, restricting the loss of water from the leaf to the atmosphere in the form of vapor, hence reducing plant dehydration (LIMA et al., 2017). These results are similar to those found by Melo et al. (2017) in a study evaluating gas exchange in bell peppers cultivated with saline solutions (0, 1, 3, 5, 7, and 9 dS m<sup>-1</sup>), which showed a reduction in transpiration as water salinity increased, with the lowest value of  $3.58 \mu$ mol mol<sup>-1</sup> observed in plants grown under 9.0 dS m<sup>-1</sup>.

For the  $CO_2$  assimilation rate (Figure 2C), a quadratic behavior was verified, with the highest

estimated value of 31.76  $\mu$ mol mol<sup>-1</sup> in plants that received the saline nutrient solution with 5.3 dS m<sup>-1</sup>, while the lowest value of 24.97  $\mu$ mol mol<sup>-1</sup> was found with ECns of 2.1 dS m<sup>-1</sup> (control). The lower CO<sub>2</sub> assimilation rate in plants grown under lower ECns is attributed not only to stomatal closure, which restricts the  $CO_2$  diffusion in the substomatal chamber and consequently the net photosynthetic rate but also to non-stomatal factors, such as limitations of enzymatic activity (ALAM et al., 2015).



Figure 2. Stomatal conductance - gs (A), transpiration - E (B), CO<sub>2</sub> assimilation rate - A (C), and internal CO<sub>2</sub> concentration - Ci (D) of zucchini plants as a function of saline nutrient solution - ECns in hydroponic cultivation.

The internal  $CO_2$  concentration decreased with the increase in ECns, with a loss of 4.04% per unit increase of salinity (Figure 2D). This reduction implies that the  $CO_2$  fixed in the mesophyll cell is being consumed in the synthesis of sugars during photosynthesis (DIAS et al., 2018), which was confirmed by the increase observed in the  $CO_2$ assimilation rate. In a study with watermelon cv. Crimson Sweet using a mixture of soil and organic compost, in a 1:1 ratio, Ribeiro et al. (2020) observed that the increase in irrigation water salinity reduced the internal  $CO_2$  concentration, leading to the lowest value under water with electrical conductivity of 4.0 dS m<sup>-1</sup>.

For instantaneous water use efficiency of zucchini (Figure 3A), the highest estimated value of 6.16 [( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>)( $\mu$ mol mol<sup>-1</sup>)<sup>-1</sup>] was obtained in plants subjected to ECns of 3.2 dS m<sup>-1</sup>. The lowest value of 4.76 [( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>)( $\mu$ mol mol<sup>-1</sup>)<sup>-1</sup>] was

verified in plants under ECns of 6.6 dS  $m^{-1}$ , with a reduction of 22.72%.

According to Sá et al. (2019), plants try to overcome osmotic stress and reduce the absorption of toxic ions by reducing stomatal conductance and transpiration to increase water use efficiency and the relative water content in their leaves. However, this mechanism was not sufficient to increase the water use efficiency in zucchini under saline conditions.

For instantaneous carboxylation efficiency -*CEi* (Figure 3B), plants under a saline nutrient solution of 5.0 dS m<sup>-1</sup> achieved the highest value, 0.18 [( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) ( $\mu$ mol mol<sup>-1</sup>)<sup>-1</sup>], while plants subjected to ECns of 2.1 dS m<sup>-1</sup> expressed the lowest value, 0.12 [( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) ( $\mu$ mol mol<sup>-1</sup>)<sup>-1</sup>], corresponding to a reduction of 33.33% compared to plants that achieved the highest *CEi*. The increase in instantaneous carboxylation efficiency is related to the increase in CO<sub>2</sub> assimilation and can confirm that  $CO_2$  assimilation occurs even with the reduction in internal  $CO_2$  concentration because this behavior is influenced by non-stomatal factors, caused by nonenzymatic inhibition (JACINTO JÚNIOR et al., 2019). The interaction between the factors (ECns  $\times$  H<sub>2</sub>O<sub>2</sub>) and H<sub>2</sub>O<sub>2</sub> concentrations did not significantly influence any of the variables analyzed. However, there was a significant effect of the saline nutrient solution (ECns) only for the initial fluorescence (F<sub>0</sub>) of zucchini, at 35 days after transplanting (Table 3).



Figure 3. Instantaneous water use efficiency - *WUEi* (A) and instantaneous carboxylation efficiency - *CEi* (B) of zucchini plants as a function of saline nutrient solution - ECns in hydroponic cultivation.

**Table 3**. Results of Fisher test (F) for initial fluorescence ( $F_0$ ), maximum fluorescence (Fm), variable fluorescence (Fv), and potential quantum efficiency of PSII (Fv/Fm) of zucchini plants cultivated with saline nutrient solution (ECns) and exogenous application of hydrogen peroxide ( $H_2O_2$ ) in a hydroponic system.

SV	F test					
5 V -	$F_0$	Fm	Fv	Fv/Fm		
ECns	*	ns	ns	ns		
Linear	**	ns	ns	ns		
Quadratic	ns	ns	ns	ns		
$H_2O_2$	ns	ns	ns	ns		
Linear	ns	ns	ns	ns		
Quadratic	ns	ns	ns	ns		
$ECns \times H_2O_2$	ns	ns	ns	ns		
Overall Mean	513.10	2242.64	1729.53	0.77		
CV (%)	7.03	8.03	9.32	2.17		

ns, \*\*\* respectively not significant and significant at p < 0.05 and < 0.01; SV - Source of variation; CV- coefficient of variation.

The initial fluorescence showed a positive linear behavior as the salinity of the nutrient solution increased, with the highest value of 530.63 observed in plants irrigated with ECns of 6.6 dS m<sup>-1</sup> (Figure 4). The parameter  $F_0$  is defined as the fluorescence emission when the reaction centers are open and quinone A (QA) is fully oxidized (AZEVEDO NETO et al., 2011). An increase in F<sub>0</sub> indicates a change in the reaction center of photosystem II (PSII) that reduces the electron transport capacity, through the dissociation of the PSII antenna complex and its respective reaction center, motivated by photoinhibition (CINTRA et al., 2020). Unlike the

results obtained in this study, Lima et al. (2019) evaluated the photochemical efficiency of cotton under salt stress conditions in soil and observed that the increase in water salinity from 5.1 to 9.1 dS  $m^{-1}$  resulted in a decrease in the initial, maximum, and variable fluorescence of the plants.

There was a significant effect of the interaction between saline nutrient solution (ECns) and application of hydrogen peroxide  $(H_2O_2)$  for total production per plant, the basal diameter of the fruit, the diameter of the lower middle third of the fruits, firmness of the lower middle third and total soluble solids (Table 4). As single factors, there were

significant effects of saline nutrient solution and hydrogen peroxide on the number of fruits and fruit pH. Putti et al. (2018), when evaluating the effect of brackish water (ECw: 0, 1.25, 2.5, 3.75, and  $5.0 \text{ dS m}^{-1}$ ) on the zucchini crop, also verified that the yield per plant and fruit diameter and length were significantly influenced by ECw.



Figure 4. Initial fluorescence of zucchini plants as a function of saline nutrient solution - ECns in hydroponic cultivation.

**Table 4**. Results of Fisher test (F) for total production per plant (TP - g per plant), total number of fruits (TNF), the basal diameter of the fruits (BDF - mm), diameter of the lower middle third of the fruits (DLMT - mm), firmness of the basal diameter (FBD - N), firmness of the lower middle third (FLMT - N), total soluble solids ( $TSS - {}^{\circ}Brix$ ) and hydrogen potential (pH) of zucchini plants cultivated with saline nutrient solution (ECns) and exogenous application of hydrogen peroxide ( $H_2O_2$ )in a hydroponic system.

SV	F test							
	TP	TNF	BDF	DLMT	FBD	FLMT	TSS	pН
ECns	**	**	**	**	ns	**	**	**
Linear	**	**	**	**	ns	**	*	**
Quadratic	ns	ns	ns	ns	ns	ns	**	ns
$H_2O_2$	ns	**	ns	ns	ns	ns	ns	**
Linear	ns	**	ns	ns	ns	ns	ns	*
Quadratic	ns	ns	ns	ns	ns	ns	ns	ns
$ECns \times H_2O_2 \\$	**	ns	*	**	ns	**	*	ns
Overall Mean	1454.77	4.21	58.40	41.74	56.51	62.06	4.35	6.26
CV (%)	26.35	21.33	5.94	5.19	6.51	4.90	5.63	5.36

 $^{ns,\, *.}$  \*\* respectively not significant and significant at p<0.05 and p<0.01; CV= coefficient of variation.

Under conditions of low salinity of the nutrient solution (2.1 dS m<sup>-1</sup>), the application of  $H_2O_2$  at the concentration of 19 µM promoted higher total production per plant (2443.60 g per plant), indicating a positive effect of hydrogen peroxide on plants cultivated in nutrient solution (Figure 5). When applied at adequate concentration, H<sub>2</sub>O<sub>2</sub> can enzymatic activate the antioxidant defense mechanism (catalase and peroxidase) in plants, reducing the negative effect of reactive oxygen species (KILIC; KAHRAMAN, 2016). On the other hand, when the plants were grown in the nutrient solution of the highest salinity (ECns =  $6.6 \text{ dS m}^{-1}$ ), the application of H<sub>2</sub>O<sub>2</sub> was less effective, with the lowest total production per plant (671.95 g per plant) at  $H_2O_2$  dose of 60  $\mu$ M. Thus, it can be verified that the increase in salinity reduced the total production per plant, intensified by the application of hydrogen peroxide, which causes oxidative effect.

The number of fruits of zucchini (Figure 6A) decreased linearly with the increase in the nutrient solution salinity levels, by 5.86% per unit increment in ECns. In a comparison between plants grown in nutrient solutions of 2.1 and 6.6 dS m<sup>-1</sup>, there was a decrease of 30.10% in the number of fruits. Excess salts in the water lead to a decrease in cell expansion due to lower cell turgor, consequently affecting plant production (KAUSHAL; WANI, 2016). Lima et al. (2016), in a study evaluating the growth and production of 'All Big' bell pepper as a function of irrigation with saline waters (0.6 and 3.0 dS m<sup>-1</sup>), observed a marked reduction in the number of fruits per plant when using ECw of 3.0 dS m<sup>-1</sup>.



**Figure 5**. Total production per plant - TP of zucchini plants in hydroponic cultivation, as a function of the interaction between saline nutrient solution (ECns) and exogenous application of hydrogen peroxide ( $H_2O_2$ ). X and Y correspond to ECns and  $H_2O_2$  concentrations.



Figure 6. Total number of fruits of zucchini as a function of saline nutrient solution - ECns (A), and hydrogen peroxide -  $H_2O_2$  (B) in hydroponic cultivation.

Regarding the application of hydrogen peroxide, a linear reduction was noted for the number of fruits (Figure 6B), which decreased by 23.60% when comparing the control treatment  $(0 \mu M)$  with plants that received H<sub>2</sub>O<sub>2</sub> concentration of 60 µM. Probably the reduction in the number of fruits was caused by the accumulation of reactive oxygen species (ROS) produced by the mitochondria, which may lead to programmed cell death, coordinated by metabolism to eliminate unnecessary cells, acting as a defense mechanism under stress conditions, resulting in a negative effect on the development, photosynthesis and reproductive organs of the plant (SYCHTA; SŁOMKA; KUTA, 2021).

For the basal diameter of the fruits (Figure 7A), the nutrient solution salinity of 2.1 dS  $m^{-1}$ 

promoted the highest value (63.21 mm) when plants were subjected to a concentration of 28 µM. On the other hand, the lowest BDF of 54.08 mm was recorded in the fruits of plants grown under the concentration of 60 µM and nutrient solution salinity of 6.6 dS m<sup>-1</sup>. In Figure 7B, for the control treatment  $(2.1 \text{ dS m}^{-1})$  the increase in H<sub>2</sub>O<sub>2</sub> concentration led to a more marked reduction in the middle third diameter of the fruits than in plants cultivated in a more saline nutrient solution (6.6 dS  $m^{-1}$ ). The concentration of 20 µM may have contributed to plant metabolism along with hormones and signaling molecules, activating the production of organic compounds and antioxidant enzymes to minimize the effect of stress on the plant, reflecting on fruits (SOHAG et al., 2020).





**Figure 7**. Basal diameter of the fruits - BDF (A), the diameter of the lower middle third - DLMT (B), and firmness of the lower middle third - FLMT (C) of zucchini plants in hydroponic cultivation, as a function of the interaction between the saline nutrient solution (ECns) and exogenous application of hydrogen peroxide ( $H_2O_2$ ). X and Y correspond to ECns and  $H_2O_2$  concentrations, respectively.

For firmness of the lower middle third (Figure 7C), it was verified that plants subjected to ECns of 2.1 dS m<sup>-1</sup> and hydrogen peroxide concentration of 60  $\mu$ M obtained the highest value (68.27 N). On the other hand, the lowest FLMT of 55.01 N was verified in plants grown under hydrogen peroxide concentration of 40  $\mu$ M and ECns of 6.6 dS m<sup>-1</sup>. The increase in fruit firmness when plants were irrigated with the nutrient solution can be attributed to hydrogen peroxide, which acts as an activator of proteins, proline, and enzymes that maximize fruit yield and quality (KHANDAKER; BOYCE; OSMAN, 2012).

The total soluble solids of zucchini fruits increased linearly (1.79% per unit increment) as a function of the electrical conductivity of the nutrient solution (Figure 8A). Total soluble solids is an essential characteristic for products marketed fresh since the increase in the concentration of total soluble solids has a positive correlation with the contents of sugars and organic acids present in the fruit (CANUTO et al., 2010). For Simões et al. (2019), the increase in sugar content occurs as a result of a reduction in the plant's ability to absorb water from the soil. However, the accumulation of sugars can be a strategy to reduce water potential and favor cellular osmotic adjustment. Souza et al. (2020), when evaluating the quality of zucchini fruits, cv. 'Caserta-Italiana', cultivated in pots, using an *Argissolo* (Ultisol) and different levels of

irrigation water salinity, also verified an increase of soluble solids in the fruits, equal to 21.93% (0.68 °Brix) between the ECw of 0.5 and 5.0 dS m<sup>-1</sup>.



**Figure 8**. Total soluble solids - TSS of the pulp of zucchini fruits as a function of saline nutrient solution - ECns (A), and hydrogen potential of the pulp of zucchini fruits as a function of ECns (B) and hydrogen peroxide -  $H_2O_2$  (C) in hydroponic cultivation.

The hydrogen potential (pH) decreased with increasing salinity of the nutrient solution (Figure 8B), a reduction of 1.94% per unit increase in ECns. The highest pH of zucchini fruit pulp (6.46) was obtained under ECns of 2.1 dS m<sup>-1</sup>, showing that the excess of salts in the nutrient solution increased acidity in the fruits. The pH is an indication of the degree of deterioration in fruit quality (NASSER; ZONTA, 2014). The ideal to prevent the proliferation of microorganisms is to obtain fruit pulp with pH below 4.5, as pH values higher than 4.5 require long periods for sterilization of the raw material in thermal processing (MONTEIRO et al., 2008). This result of fruit pulp pH is in line with that found by Araújo et al. (2014), who verified pH variation between 6.58 and 6.74 for zucchini fruits produced with  $K_2O$  doses of 50 and 200 kg ha<sup>-1</sup> as top-dressing. Lima et al. (2020b), when evaluating the effects of irrigation with water of increasing salinity and phosphorus fertilization on the postharvest physicochemical composition of fresh

fruits of West Indian cherry, 'BRS 366 Jaburu', also observed that the increase in ECw levels from 0.6 to  $3.8 \text{ dS m}^{-1}$  reduced the hydrogen potential of the fruits.

Regarding hydrogen peroxide (Figure 8C), the fruit pulp pH decreased with the increase in  $H_2O_2$ concentrations, by 2.13% for each increment of 20  $\mu$ M. Plants subjected to H<sub>2</sub>O<sub>2</sub> application of 60  $\mu$ M reduced the hydrogen potential of the fruits by 6.43% compared to those in the control treatment (0 µM). The decrease in the pH of zucchini pulp possibly occurred because the high concentrations of salts in the nutrient solution reduce the absorption of water and nutrients by plants and cause changes in membrane permeability. Such changes affect water and nutrient balance, hormonal metabolism, gas exchange, and production of reactive oxygen species, which, in excess, can cause damage to lipids, proteins, and DNA, leading to a change in structural function and/or inhibition (ROSSATTO et al., 2017).

### CONCLUSIONS

Nutrient solution with electrical conductivity above 2.1 dS m<sup>-1</sup> caused reductions in gas exchange and the total number of fruits of zucchini plants in hydroponic cultivation.

An increase in salinity levels of the nutrient solution increased the total soluble solids content of the fruits and the initial fluorescence of zucchini.

 $\rm H_2O_2$  does not affect the gas exchange and chlorophyll fluorescence of zucchini plants, at 33 days after its application.

Under conditions of nutrient solution salinity above 2.1 dS m<sup>-1</sup>, hydrogen peroxide was not able to mitigate the effects of salt stress on zucchini plants.

Application of 20  $\mu$ M of H<sub>2</sub>O<sub>2</sub> when plants were grown in a nutrient solution of 2.1 dS m<sup>-1</sup> promoted total fruit weight and basal diameter of zucchini fruits.

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