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Use of hydrogen peroxide in acclimatization of melon to salinity of irrigation water[1]

Uso do peróxido de hidrogênio na aclimatação do melão à salinidade da água de irrigação

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HIGHLIGHTS:
Hydrogen peroxide (15 µmol L^{-1}) increases transpiration, stomatal conductance, and net CO_2 assimilation in melon plants.
High concentrations (15 µmol L^{-1}) of hydrogen peroxide in plants irrigated with 5.0 dS m^{-1} salinity reduce melon fruit production.
Hydrogen peroxide at 6.35 and 10.23 µmol L^{-1} attenuates the deleterious effects of salt stress in melon plants.

ABSTRACT: In the semi-arid region of northeastern Brazil, soil and climate conditions can increase the risk of soil salinization, particularly when poor-quality water is used for irrigation. Therefore, techniques that improve the yields of melon culture under adverse conditions, such as salinity, are of great relevance to the production sector. The objective of this study was to evaluate the effectiveness of hydrogen peroxide (H_2O_2) in acclimatizing melon trees subjected to irrigation water with different salinity levels. The treatments consisted of irrigation water with two electrical conductivities (0.3 and 5.0 dS m^{-1}) and four concentrations of H_2O_2 (0, 5, 10, and 15 µmol L^{-1}). The experimental design used was randomized blocks, arranged in a 2 × 4 factorial scheme, with four replicates and four plants per plot. Increase in salinity of irrigation water reduced the growth, gas exchange, and production of melon plants. However, H_2O_2, at a concentration of 6.35 µmol L^{-1}, yielded improvements in physiology, growth, and production, in addition to reducing the deleterious effects of saline stress on melon production.

Key words: *Cucumis melo* L., physiology, saline water

RESUMO: No semiárido do Nordeste brasileiro as condições edafoclimáticas podem favorecer riscos de salinização do solo, principalmente quando se utiliza água de má qualidade para irrigação. Nesse contexto, técnicas que possibilitem melhorias nos rendimentos da cultura do melão em condições adversas, como a salinidade, são, portanto, de grande relevância para o setor produtivo. Logo, o objetivo deste estudo foi avaliar a eficácia do peróxido de hidrogênio (H_2O_2) na aclimatação do meloeiro submetido a diferentes níveis salinos na água de irrigação. Os tratamentos consistiram de duas condutividades elétricas da água de irrigação (0,3 e 5,0 dS m^{-1}) e quatro concentrações de H_2O_2 (0, 5, 10 e 15 µmol L^{-1}). O delineamento experimental utilizado foi o de blocos casualizados, em esquema fatorial 2 × 4, com quatro repetições e quatro plantas por parcela. O aumento da salinidade da água de irrigação reduz o crescimento, as trocas gasosas e a produção do meloeiro. No entanto, o H_2O_2, na concentração de 6,35 µmol L^{-1} proporcionou melhorias na fisiologia, crescimento e produção, além de reduzir os efeitos deletérios do estresse salino na produção de melão.

Palavras-chave: *Cucumis melo* L., fisiologia, água salina

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Introduction

Melon (*Cucumis melo* L.) belongs to the Cucurbitaceae family and is an olericultural crop cultivated in several regions of the world, owing to its wide adaptability and high socioeconomic expression (Kesh & Kaushik, 2021). Brazil is prominent in the export of this species, with the northeast region responsible for more than half of the national production (Silva et al., 2024).

However, in semi-arid regions, such as Northeast Brazil, soil and climate conditions can increase the risk of soil salinization, particularly when poor quality water is used for irrigation. The amount of salt in irrigation water can impair the physicochemical properties of soils and reduce crop yields according to their degree of tolerance (Sousa et al., 2018).

The melon tree is considered moderately tolerant to salinity, presenting a threshold in terms of electrical conductivity of the saturation extract (ECe) of approximately 2.2 dS m^{-1} (Terceiro Neto et al., 2014). Values above this threshold may result in significant production loss. Excess salts decrease the water potential, restrict the absorption of water, and cause the accumulation of toxic ions, mainly Na^+ and Cl^-.

The use of hydrogen peroxide (H_2O_2) exogenously in small amounts in plants appears to be a potential alternative for acclimatization to saline stress. H_2O_2 stimulates the activation of the antioxidative defense system, functioning as an important intracellular signal for the activation of stress responses and plant defense pathways that promote cross-tolerance (Silva et al., 2016).

Techniques that make it possible to improve the yield of melon trees under adverse conditions, such as salinity, are of great relevance to the production sector. Thus, the objective of this study was to evaluate the effectiveness of H_2O_2 in acclimatizing melon trees to different salinity levels in irrigation water.

Material and Methods

The experiment was conducted in a greenhouse located at the Federal University of Campina Grande, at the Center of Sciences and Agrifood Technology, in Pombal, PB, Brazil (coordinates: 06° 47' 03" S, 37° 48' 08" W, altitude of 194 m).

The climate conditions of the protected environment during the experiment were monitored using a digital thermo-hygrometer model HT-208 (ICEL-Manaus) to record air temperature with a maximum of 39.21 °C and minimum of 31.82 °C, as well as maximum relative air humidity of 80.30% and minimum of 60.15%. The mean photosynthetically active radiation, measured using a ceptometer, was 1317 μmol m^{-2} s^{-1}.

The treatments were composed of two levels of irrigation water (0.3 and 5.0 dS m^{-1}) and four concentrations of H_2O_2 (0, 5, 10, and 15 μmol L^{-1}). The experimental design used was randomized blocks in a 2 × 4 factorial scheme, with four replicates and four plants per plot. The salinity levels were obtained using supply water (0.3 dS m^{-1}), and sodium chloride was added to obtain a level of 5.0 dS m^{-1}. The salinity levels were based on the threshold water salinity of melon (2.2 dS m^{-1}) (Ayers & Westcot, 1999), selecting a low salinity level and another level above the threshold. Due to the absence of research on H_2O_2 in melon, the concentrations used in the present assay were based on studies carried out with zucchini (Dantas et al., 2022) with adaptations.

The H_2O_2 used in this study was prepared from a pure peroxide (99%) dilution. Applications were performed 7 and 15 days after germination of the plants, at the end of the afternoon, with the aid of a manual sprayer. The solution was applied directly to the leaves until they were completely wet, as recommended by Santos et al. (2018).

The 'Gold Mine' yellow melon hybrid was used for the experiment. Pots with a capacity of 8 L were filled with sieved soil (mesh 2). The pots were distributed at 1.20 m × 0.5 m spacing. The soil used for the experiment was classified as Entisol clayey texture (coarse sand = 290; fine sand = 150; silt = 170; and clay = 390 g kg^{-1}). The results of chemical analysis of the soil, before the experiment was begun were as follows: pH in H_2O (1:2.5) = 5.8; P = 58.5 mg dm^{-3} and K = 0.19 $cmol_c$ dm^{-3}; Na = 0.12 $cmol_c$ dm^{-3}; Ca = 4.0 $cmol_c$ dm^{-3}; Mg = 0.8 $cmol_c$ dm^{-3}; Al = 0.0 $cmol_c$ dm^{-3}; H + Al = 6.63 $cmol_c$ dm_{-3}; SB = 4.99; CTC effective = 4.99 $cmol_c$ dm^{-3} and ECe 0.6 dS m^{-1}.

Sowing was performed by placing three seeds per pot at a depth of 0.5 cm. When the plants reached the stage of two definitive leaves, thinning was performed, leaving one plant per pot.

Irrigation was carried out daily in the morning and afternoon according to the water requirements of the plants, and in the first 15 days after sowing (DAS), the plants were irrigated only with saline water of 0.3 dS m^{-1}. The volume of water to be applied was determined by drainage lysimetry. After this period, in addition to the saline treatment, an adequate nutrient solution by Hoagland & Arnon (1950) was used at 75% of the total strength while maintaining a pH of approximately 5.6 (Table 1).

At 50 DAS, net CO_2 assimilation (A), stomatal conductance (gs), transpiration (E), intercellular CO_2 concentration (C_i), instantaneous water use efficiency (WUE = A/E), and instantaneous carboxylation efficiency (iCE = A/Ci) were evaluated on the fifth leaf counted from the apex of the branch to the base, using an infrared gas analyzer LCpro+ (Analytical Development, Kings Lynn, UK) with a constant light source of 1200 μmol m^{-2} s^{-1} photons. Growth characteristics were assessed at 62 DAS. The leaf area (LA) was obtained by relating the dry mass of eight leaf discs of known area (11.28 cm^2) removed from intermediate leaves, with the dry mass of the leaves; dry mass of the aerial part (DMAP) comprising leaves and stems was obtained by drying the material in an oven with air circulation at 65 °C for 72 hours. For the fruit production (P), all the fruits of each treatment were analyzed to obtain their average mass.

Table 1. Concentrations of nutrients in the nutrient solution of Hoagland & Arnon (1950)

Nutrients	N	P	K	Ca	Mg	S	Fe	Mn	B	Cu	Zn	Mo
Concentration (mmol L^{-1})	15	1	6	5	2	2	0.05	0.01	0.05	0.003	0.0008	0.001

The data of the evaluated variables were subjected to the F test at $p \leq 0.05$, using analysis of variance. For the salinity factor, the mean values were compared using Tukey's test, and the concentrations of H_2O_2 were subjected to regression analysis. Statistical analyses were performed using SISVAR software (Ferreira, 2014).

Results and Discussion

There was a significant interaction between salinity levels and H_2O_2 concentrations for A, C_i, E, gs, LA, DMAP, and P per plant (Table 2).

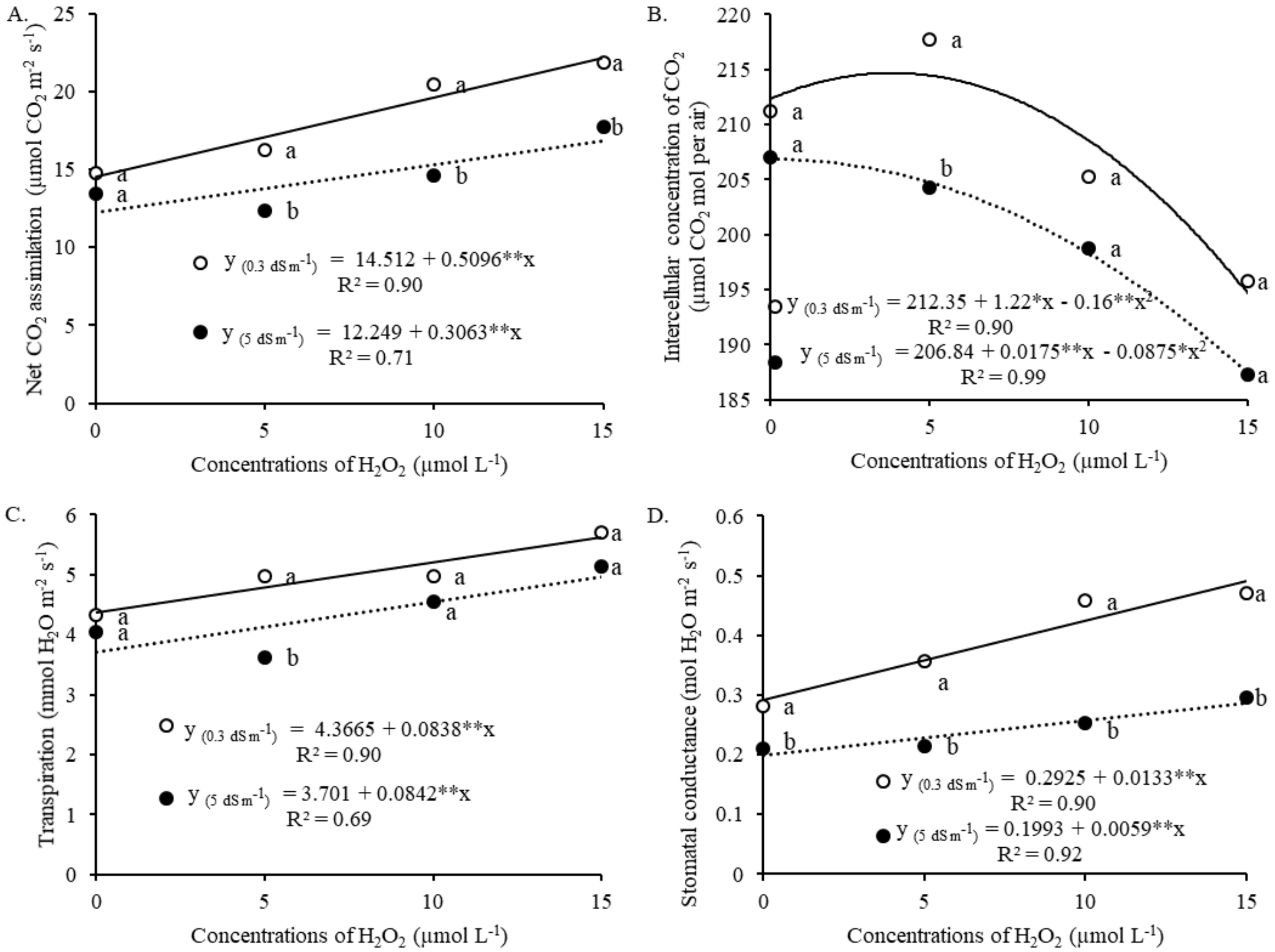
In contrast, iCE and WUE were not significantly influenced by either factor studied, possibly because they are mathematical relationships, such as the ratio between the carbon dioxide assimilation rate and intracellular CO_2 concentration (iCE), as well as the ratio between the CO_2 assimilation rate and conductance (WUE), as observed by Sousa et al. (2018).

For A, a linear behavior was observed with an increase of 52.61 and 37.5% between the lowest (0.0 μmol L^{-1}) and highest (15.0 μmol L^{-1}) concentrations of H_2O_2 when irrigated with water at 0.3 and 5.0 dS m^{-1}, respectively (Figure 1A). Increments in photosynthesis by H_2O_2 priming can be related to carbon fixation enzyme activity, PSII efficiency, and protection

Table 2. Summary of the F test for the net CO_2 assimilation (A), intercellular CO_2 concentration (Ci), transpiration (E), stomatal conductance (gs), instantaneous carboxylation efficiency (iCE), water use efficiency (WUE), leaf area (LA), dry mass of the aerial part (DMAP), and fruit production (P) per plant in melon plants subjected to 0.3 and 5.0 dS m^{-1} levels of salinity of irrigation water and various H_2O_2 concentrations

Source of variation	F test								
	A	Ci	E	gs	iCE	WUE	LA	DMAP	P
Blocks	ns	ns	ns	ns	ns	ns	ns	ns	ns
Salinity (S)	**	**	**	**	ns	ns	**	**	**
Hydrogen peroxide (H_2O_2)	**	**	**	**	ns	ns	*	*	*
Interaction (S × H_2O_2)	**	*	*	*	ns	ns	*	*	*
CV (%)	6.26	3.54	8.79	13.26	7.49	6.54	23.10	15.57	16.06

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



* and **: significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test, respectively

Figure 1. Net CO_2 assimilation (A), intercellular concentration of CO_2 (B), transpiration (C), and stomatal conductance (D) in melon plants in function of H_2O_2 concentrations for 0.3 and 5.0 dS m^{-1} levels of salinity of irrigation water

of cellular organelles, such as chloroplasts (Araújo et al., 2021). Analysis of the interaction for salinity levels at each concentration of H_2O_2 showed that the net photosynthesis of melon plants irrigated with electrical conductivity of water (ECw) of 0.3 dS m^{-1} was significantly higher than that of melon plants subjected to water salinity of 5 dS m^{-1}.

Intercellular concentration of CO_2, which was adjusted to the quadratic polynomial model, exhibited decreases from the concentrations of 3.81 and 0.1 μmol L^{-1} to the salinity levels of 0.3 and 5.0 dS m^{-1}, respectively (Figure 1B). The C_i was significantly higher in plants treated with 0.3 dS m^{-1}, only in those wherein 5 μmol L^{-1} of H_2O_2 was used; the other H_2O_2 concentrations did not significantly influence this variable between salt levels.

The reduction in the CO_2 influx may be associated with several factors. However, the accumulation of salts in the apoplast, the toxic effect of ions, or the reduction of the internal water potential has been proposed to initially promote a decrease in the process of CO_2 fixation. This process may also have been accelerated by the accumulation of reactive oxygen species but not by interrupting photosynthetic activity (Ahanger et al., 2019). With an increase in photosynthetic activity, the plant consumes more CO_2, and when its influx decreases significantly, there can be a limitation in the substomatic cavity, and consequently, a decrease in the activity of the enzyme ribulose 1.5-bisphosphate carboxylase oxygenase (RuBisCO) (Souza et al., 2016). This limitation of CO_2 fixation in the Calvin cycle by RuBisCO in plants under stress conditions reduces the oxidation of NADPH. When this occurs, the reduced ferredoxin electron that would be transferred to $NADP^+$ goes to O_2 and forms a superoxide radical ($O_2^{\bullet-}$), which in excess can cause photooxidation (Barbosa et al., 2014).

For E, it was found that the concentration of 15 μmol L^{-1} H_2O_2 resulted in an increase of 28.51 and 34.04% over the concentration of 0.0 μmol L^{-1}, for the salinity levels of 0.3 and 5.0 dS m^{-1}, respectively (Figure 1C). At 5 μmol L^{-1} H_2O_2, E values were significantly higher in plants irrigated with water having 0.3 dS m^{-1} salinity level compared to those of plants irrigated with water having 5 dS m^{-1} salinity level.

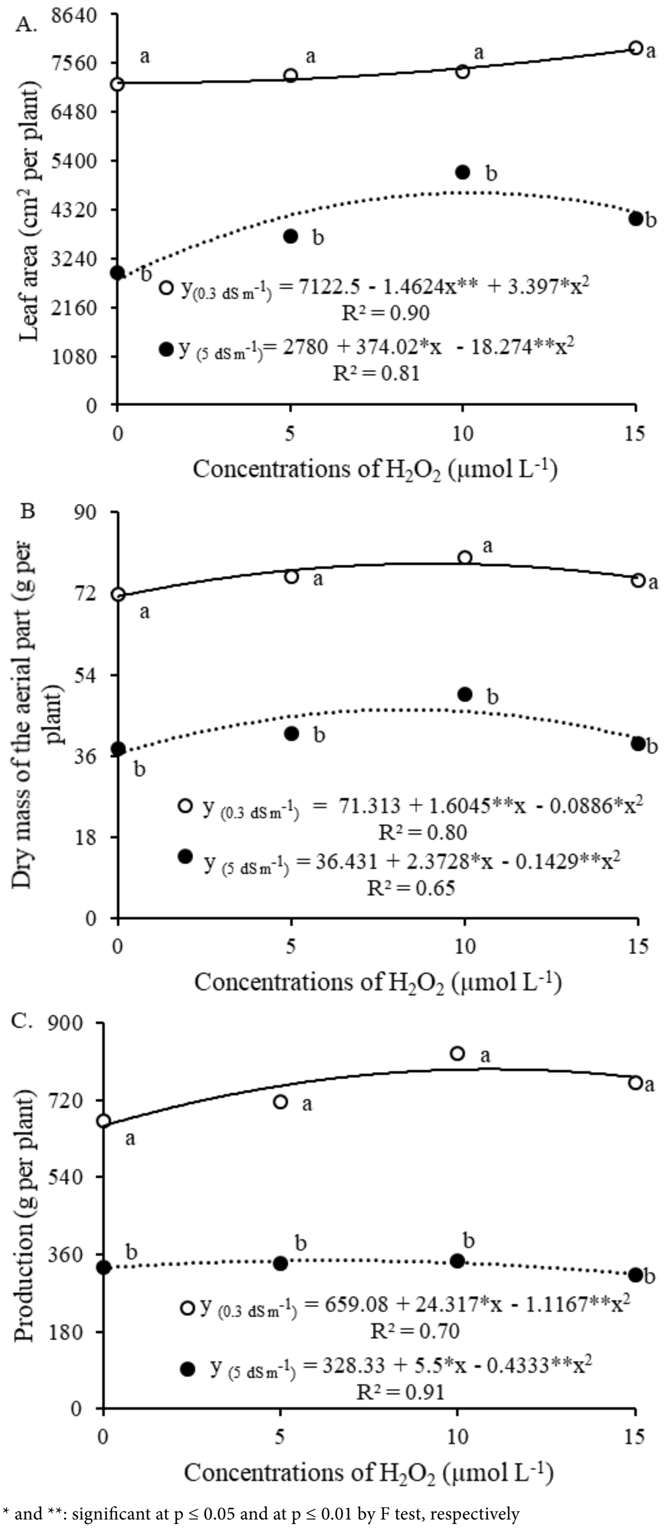
The same was observed for gs, with higher increases (66.78 and 37.68%) for both salinity levels, in the same order as before, using the highest peroxide concentration (Figure 1D). Regarding the interaction of factors, it was observed that plants treated with 0.3 dS m^{-1} showed higher gs than plants treated with 5 dS m^{-1}, at all concentrations of H_2O_2. Lacerda et al. (2022) also observed a reduction in gs in corn plants subjected to 2 dS m^{-1}, at all concentrations of H_2O_2. As E decreases, gs follows the same behavior as that of the plant to avoid water loss in stress situations, as stated by Veloso et al. (2022). This stomatal behavior is responsible for determining the transpiration demand to which leaves are potentially subjected. Under optimal water availability conditions, plants generally have high transpiration rates; therefore, as water becomes scarce for indoor activities, there is usually a reduction in the transpiration rate. While culturing pistachio, Bagheri et al. (2019) found that pre-treatment with H_2O_2 resulted in a better water status under osmotic stress, inducing tolerance and further increasing soluble sugar, proline, and polyamine levels. Thus, it was noticeable in this study that the use of lower concentrations of H_2O_2 in melon culture attenuated salinity, as it avoided the greater implication of the physiological parameters evaluated.

When analyzing the interaction between the electrical conductivity of irrigation water and H_2O_2 concentrations, it was observed that the LA (Figure 2A), DMAP (Figure 2B), and P of melon plants (Figure 2C) subjected to ECw of 0.3 dS m^{-1} differed significantly from that of plants irrigated with water at 5.0 dS m^{-1}. This was due to high saline concentrations reducing melon growth and production (Sousa et al., 2018).

An increase in LA was noted, as a function of the increase in concentrations of H_2O_2 to the salinity of 0.3 dS m^{-1} (Figure 2A). However, for the salinity level of 5.0 dS m^{-1}, a higher value (4694.01 cm^2) was obtained with a concentration of 10.23 μmol L^{-1} of H_2O_2, from which the values started to decrease (Figure 2A). Probably with the highest salinity level, the plant increased the production of reactive oxygen species and began to suffer from oxidative stress; this process was further driven by the high concentration of H_2O_2 applied, which may have overloaded the defense system of the plant, causing significant morphological changes in leaf expansion as a way of trying to survive such adverse conditions. According to Rutschow et al. (2011), low concentrations of H_2O_2 activate signaling sites capable of relieving the most intense stress. However, the accumulation of higher amounts of these reactive oxygen species produces a strong stress signal, which can lead to cell death (Pan et al., 2021). Under normal conditions, plant cells activate molecules that function as antioxidants (e.g., ascorbic acid and glutathione), which promote the elimination of the H_2O_2 molecule by reducing its concentration in water and free oxygen (Ahanger et al., 2019); this logic may explain the fact that plants irrigated with saline water at 0.3 dS m^{-1} respond better to peroxide accumulation.

DMAP was 78.62 and 46.34 g per plant under concentrations of 9.11 and 8.35 μmol L^{-1} of H_2O_2 and 0.3 and 5.0 dS m^{-1} salinity, respectively (Figure 2B). A similar behavior was observed by Oliveira et al. (2014) regarding salinity in the culture of gherkin (*Cucumis sativus* L.) plants, obtaining lower values for this variable in the treatment of 5.0 dS m^{-1}. Exposure to high H_2O_2 concentrations also favored the decline of this variable, corroborating the results of Sathiyaraj et al. (2014), who reported that as concentrations and exposure of ginseng (*Panax ginseng* C.A. Mey.) seedlings to H_2O_2 increased, there was a reduction in growth rates and acclimatization of this species to saline stress; in contrast, pre-treatment with H_2O_2 at lower concentrations promoted greater growth and acclimatization of plants to saline stress by increasing the activity of antioxidative enzymes such as superoxide dismutase, peroxidase, ascorbate peroxidase, catalase, and glutathione reductase.

P per plant increased with the application of H_2O_2. For the water salinity of 0.3 and 5.0 dS m^{-1}, the increment was 791.39 and 345.76 g per plant for 10.89 and 6.35 μmol L^{-1} H_2O_2, respectively (Figure 2C), with successive reductions under higher concentrations of H_2O_2. Similar results regarding decreases caused by increased electrical conductivity of irrigation water were reported by Silva et al. (2014) in pumpkin (*Cucurbita pepo* L.) and by Pereira et al. (2018) in melon.



* and **: significant at $p \leq 0.05$ and at $p \leq 0.01$ by F test, respectively

Figure 2. Leaf area (A), dry mass of the aerial part (B), and production (C) in melon plants as a function of H_2O_2 concentrations for 0.3 and 5 dS m^{-1} levels of irrigation salinity

Terceiro Neto et al. (2013) stated that the losses that occurred in the production of melon under salinity reflect the negative effect of the osmotic potential of saline solution due to several factors, such as toxicity of salts, reduction in CO_2 supply, and changes in enzyme activity and senescence. However, the use of H_2O_2 has proven to be efficient to some extent, avoiding large production losses.

Bagheri et al. (2021) reported that acclimatization to the adverse effects of salt stress in plants treated with H_2O_2 is due to the fact that the same regular osmotic adjustment, by maintaining a low Na^+/K^+ ratio, improves ionic balance, thereby increasing the absorption of K^+ and increasing the levels of sugars and relative water content under saline conditions, consequently improving production.

Conclusions

1. Irrigation with a water electrical conductivity of 5 dS m^{-1} reduced growth and reduced melon production, compared to plants irrigated with 0.3 dS m^{-1}.

2. The increase in H_2O_2 concentration increased E, A, and gs in melon plants irrigated with water having 0.3 and 5 dS m^{-1} of electrical conductivity.

3. The LA and DMAP increased with the application of H_2O_2 up to concentrations of 10.23 and 8.35 μmol L^{-1} of H_2O_2, respectively, in plants irrigated with 5 dS m^{-1}.

4. H_2O_2, at a concentration of 6.35 μmol L^{-1}, reduces the adverse effects of saline stress in the melon plant up to the production stage.

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