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The Influence of H_2O_2 Application Methods on Melon Plants Submitted to Saline Stress

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Abstract

The salinity in irrigation water is one of the most important causes to decline cultivated plants yield. The H_2O_2 application has shown efficiency as a stimulator and activator for antioxidative defense system in plants submitted to biotic and abiotic stresses. The objective of this study was to evaluate methods for hydrogen peroxide application as a strategy to minimize the effects of saline stress on melon plants. The experiment was designed in complete randomized blocks and set in 2 × 4 factorial scheme, consisting two levels for irrigation water salinity (S1 = 0.3 and S2 = 2.0 dS m^{-1}) and four methods for hydrogen peroxide application (15 mM), (T1 = no peroxide application, T2= imbibition of seeds, T3 = at sowing, T4 = Foliar spraying), with five repetitions. It was evaluated the following variables at 58 days after transplanting: plant height, stem diameter, number of leaves, number of flowers, shoot dry mass, root dry mass and total dry mass. The results showed that salinity affected the growth, biomass accumulation and plant quality severely, with the highest losses promoted by the electrical conductivity of 2.0 dS m^{-1}.

Keywords: *Cucumis melo* L., hydrogen peroxide, salinity

1. Introduction

The melon cultivation (*Cucumis melo* L.) has increased significantly in the last years in Brazil, representing one of the tropical fruits with greater commercial interest, and its major producers are Rio Grande do Norte and Ceará states (Faria, Lima, Ciqueira, Rezende, & Gomes, 2015),which guarantee to Brazil the highest global production of melon.

Despite this plant has a wide adaptability to the Northeast region, its cultivation in the world's semi-arid areas presents a risk, mainly due to the scarcity of water resources related to the low rainfall intensity, besides the irregularity of the rains and high evaporation, which require the use of poor quality water, usually rich in salts, mainly sodium (Medeiros, Barbosa, Medeiros, Rocha, & Silva, 2010).

The search for alternatives that attenuate the effect of salinity stress on plants is increasing, aiming to promote the acclimatization of plants to survive in adverse conditions. This process consists to expose the plant previously to some stressful conditions, resulting in metabolic changes that culminate in increased tolerance to stress exposure (Gondim, Gomes Filho, Marques, & Prisco, 2011). Among acclimatization processes for salinity stress, plant pretreatment with small amounts of H_2O_2 has shown promise.

According to Silva, Lacerda, Medeiros, Souza, and Pereira (2016) the use of H_2O_2 represents a viable alternative as an attenuator of effects that salts promote on plants under salinity stress. It happens because hydrogen peroxide boosts the intracellular region to activate plant defense responses to stress caused by excess salts, promoting cross-tolerance (Mittler, 2002).

Uchida, Jagendorf, Hibino and Takabe (2002), studying rice and, Azevedo Neto, Prisco, Éneas Filho, Medeiros, and Gomes Filho (2005) corn, verified that the pretreatment of seedlings with H_2O_2 in nutrient solution induced

plant acclimatization to the salinity. Gondim, Gomes Filho, Marques, and Prisco (2011), working with corn from pretreated seeds with hydrogen peroxide and submitted to salinity and Wahid, Perveen, Gelani, and Basra (2007), with wheat from seeds also pretreated with H_2O_2, observed that pretreatment conferred salinity tolerance on plants.

In this context, the aim of this study was to evaluate methods of applying hydrogen peroxide as a strategy to mitigate the effects promoted by salinity stress in *Cucumis melo* L.

2. Material and Methods

The experiment was carried out under field conditions at the Center of Science and Agro-Food Technology from the Federal University of Campina Grande (CCTA/UFCG), Campus of Pombal, Paraíba, Brazil (06º46′13″ S, 37º48′06″ W and altitude of around 242 m). Based on the classification of Köppen the predominant climate is the Bsh (semi-arid) hot and dry, with rains around 700 mm per year (Nóbrega et al., 2018).

Melon seeds of Hales Best Jumbo variety were used. The treatments were arranged in a randomized block design, and set in a 2 × 4 factorial scheme, corresponding to two irrigation water salinity levels (S1 = 0.3 and S2 = 2.0 dS m^{-1}) and four methods of hydrogen peroxide application (15 mM), T1 = no peroxide application (control); T2 = imbibition of seeds; T3 = at sowing; T4 = Foliar spraying, with five repetitions containing two plants placed in perforated rectangular plastic pots, to aid in the flow of excess water. The pots had the capacity of 3 dm^3 which were filled with soil from a campus area, whose chemical characteristics are set out in Table 1.

Table 1. Soil chemical characteristics used to fill the bags

Textural class	Apparently density (EC)	Total porosity	Organic matter	$pH_{(H_2O)}$	P	Sorptive complex			
						Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
	g cm^{-3}	%	g kg^{-1}		mg dm^{-3}	---------- $cmol_c$ dm^{-3} ----------			
Sandy Soil	-	-	4.79	6.5	563.6	1.2	0.71	0.07	143.6

The hydrogen peroxide solution (H_2O_2) was prepared by the dilution of 99% pure peroxide. In the treatment which the seeds were imbibed (T2), they were placed in beakers, containing water concentration of 15 mM, remaining for 8 hours of imbibition. The seeds were then collected and seeded in expanded polystyrene trays of 160 cells filled with Basaplant® commercial substrate, placing two seeds per cell.

At the same time, seeds corresponding to the other treatments were sown, and the peroxide solution (15mM) was applied immediately after sowing in the tray corresponding to the treatment (T3) as water from first irrigation, moistening the substrate.

Seedlings were produced under shading conditions at 50% of shading. After the seedling emergence, thinning was done, leaving only one plant per cell. The application of H_2O_2 by foliar spraying corresponding to the treatment (T4), was done with only one application at 10 days after the plant emergence at the end of the afternoon, using a hand sprayer.

The transplanting was carried out when the seedlings had two definitive leaves about 12 days after sowing, placing two plants per bag, which were conducted until 28 days after transplanting, where a plant was removed.

Irrigations were conduced daily in the morning and afternoon according to the water requirement of plants. Until twenty days after sowing (8 days after transplanting), the seedlings received water with low electrical conductivity, 0.3 dS m^{-1}, and from this period on, water was applied with different levels of conductivity.

The saline water (2.0 dS m^{-1}) was prepared by adding sodium chloride (NaCl) to the water which had electrical conductivity of 0.3 dS m^{-1} until reaching the desired electrical conductivity, then it was measured by a portable conductivity meter automatically corrected to 25 °C. After that, the water was then stored in a plastic tank with the capacity of 60 liters duly closed, avoiding evaporation and contamination with materials that could compromise its quality.

The plant fertilization was performed by irrigation water using the standard nutrient solution of Hoagland and Arnon (1950) at 50%. The scarification on substrate was necessary and the weeding was provided when in need.

At 60 days after transplanting (DAT), the following growth parameters were evaluated:

Plant Height (PH): it was established by measuring the main stem of the plants from the soil surface until the insertion of the last visible leaf (apical meristem) using a graduated ruler, with results expressed in cm;

Stem Diameter (SD): obtained with the aid of a digital caliper, measured at the height of the plant neck. Results were expressed in mm;

Number of leaves (NL): counting the number of leaves completely developed;

Number of flowers (NF): obtained by the counting daily the number of flowers completely open in the first hours of the morning;

Shoot, root and total dry mass: carried out by separating the parts at the height of the plant neck and placing them into Kraft paper bags and packing them in a forced air circulation oven at 65 ° C until reaching constant weight. Then the material was weighed in a precision analytical balance. The total dry mass was obtained by the sum of shoot and root dry masses. The results were expressed in g plant^{-1};

Shoot/root dry mass ratio: performed by dividing shoot dry mass by root dry mass values;

Dickson quality index: obtained according to the relation among plant height, neck diameter, shoot dry mass and root dry mass, as shown in Equation 1 (Dickson, Leaf, & Hosner 1960).

$$DQI = TDM/(PH/SD)/(SDM/RDM) \quad (1)$$

Where, DQI = Dickson quality index; TDM = total dry mass; SDM = shoot dry mass; RDM = root dry mass.

The data were submitted to analysis of variance and and when significant, the Tukey test was applied at 5% probability using statistical software named Sisvar® version 5.6 (Ferreira, 2014).

3. Results and Discussion

The growth for plant height, stem diameter, number of leaves and flowers got the influence of water salinity and hydrogen peroxide applications, which plants submitted to the salinity of 2.0 dS m^{-1} (Table 2) presented severe reductions. This effect must be originated from the reduction of the osmotic potential promoted by the accumulation of salts in the soil, inhibiting plant ability to grow and produce (Munns &Tester, 2008; Sá et al., 2018).

In a study carried out by Araújo et al. (2016), evaluating the initial growth and tolerance in melon cultivars under water salinity, verified there was a growth reduction for height, stem diameter and number of leaves, decreasing in the order of 12.38%, 8.17% and 19.37%, respectively, according to the increase in electrical conductivity unit of irrigation water.

In addition, it was noticed that plant height, stem diameter and number of leaves hydrogen peroxide, regardless of the application method, did not promote attenuating salinity effects when compared to the control, showing that hydrogen peroxide did not mitigate the effects promoted by saline stress (Table 2).

This effect may arise from a response to the high level of H_2O_2 (15 mM), promoting metabolic disturbances during plant growth. Carvalho et al. (*Oryza sativa* L.), with pretreated rice seeds, verified that 10 μM of H_2O_2, promoted membrane damage, due to the high peroxidation rate of lipids which compound this structure.

Table 2. Mean values for plant height (PH), stem diameter (SD), number of leaves (NL) and number of flowers (NF) of C*ucumis melo* L., according to the methods of hydrogen peroxide application and irrigation water salinity

Methods of H_2O_2 application	Analyzed variables			
	PH (cm)		SD (mm)	
	0.3 dS m^{-1}	2.0 dS m^{-1}	0.3 dS m^{-1}	2.0 dS m^{-1}
Control	36.7 Aa	14.3 Bab	7.31 Aa	5.26 Ba
Imbibition of seeds	32.7 Aa	14.9 Ba	7.19 Aa	6.06 Ba
At sowing	33.6 Aa	10.5 Bb	7.02 Aa	5.50 Ba
Foliar spraying	35.5 Aa	13.0 Bab	7.30 Aa	5.86 Ba
Means	23.9		6.44	
CV (%)	9.81		7.02	
SD	2.34		0.45	
Methods of H_2O_2 application	Analyzed variables			
	NL		NF	
	0.3 dS m^{-1}	2.0 dS m^{-1}	0.3 dS m^{-1}	2.0 dS m^{-1}
Control	26 Ab	12 Ba	6.0 Aa	1.0 Ba
Imbibition of seeds	24 Ac	10 Bab	3.0 Ab	2.0 Ba
At sowing	30 Aa	8.0 Bb	4.0 Ab	0.0 Bb
Foliar spraying	26 Ab	12 Ba	6.0 Aa	0.0 Bb
Means	18.7		2.85	
CV (%)	8.10		23.2	
SD	1.51		0.66	

Note. Means followed by the same lowercase letter in the column and capital letter in the line do not differ from each other at 5% probability by the Tukey test.

Hydrogen peroxide influenced the number of leaves, and results showed that plants whose treatment was during sowing under salinity of 0.3 dS m^{-1} were those that presented the largest number of leaves (Table 2). However, plants produced under the salinity level of 2 dS m^{-1} were not efficient when compared to the control treatment, presenting high decrease in the number of leaves due to the increase of the stress caused by salts.

This decrease may be attributed to the effects promoted by salinity, resulting in physiological disturbances which affect plant development and growth (Neves et al., 2018). The high content of salts in soil or irrigation water promotes reduction in absorption capacity, transport and assimilation of nutrients, and consequently, promotes losses in photosynthetic rates, transpiration and stomatal conductance (Silva et al., 2010, 2014; Oliveira et al., 2017).

The application of hydrogen peroxide did not affect shoot dry mass (Table 3). On the other hand, the salinity affected the accumulation of shoot biomass severely, occurring reduction up to 27.6%, highlighting the deleterious effects promoted by the saline stress. This effect may occur as a result of reduced photosynthetic capacity due to salt toxicity and the nutritional imbalance, reducing the transport and accumulation of photoassimilates in shoot. In a study by N. Sivritepe, H. O. Sivritepe, and Eris (2003), salt-induced damages may not only occur by osmotic and oxidative effects, but also by toxic effects and nutrient deficiency that reflect on photosynthesis and consequently on plant growth.

The salts contained in irrigation water also influenced root dry mass, and results showed the most aggressive effects in the salinity of 2 dS m^{-1} (Table 3). Regarding hydrogen peroxide application, in the salinity of 0.3 dS m^{-1}, imbibed seeds and the application at sowing improved root dry mass.

This attenuating effect promoted in plants in the salinity of 0.3 dS m^{-1} may be due to the action of H_2O_2, since it acts with a signal molecule in plants under stress conditions (Petrov & Breusegem, 2012), inducing the plant defense system, promoting the activation of antioxidant enzymes, mitigating the effects promoted by the salts (Carvalho et al., 2011). However, this behavior does not set as increasing the salinity level.

The total dry mass accumulation was reduced due to the increase in salinity, reaching losses of 26%, evidencing the deleterious effects promoted by excess salts (Table 3). This effect is related to the reduction of plant capacity to absorb and transport water and nutrients, as well as the toxicity promoted by the salts.

Similar results were obtained by Freitas, Figueirêdo, Porto Filho, Costa, and Cunha (2014) who evaluated the growth of cultivar Orange Flesh melon under different salinity levels, and observed reductions in dry mass accumulation, which must be related to the decrease of the plant photosynthetic capacity, through ionic interactions promoted by excess sodium salts, as analyzed in other studies (Silva et al., 2014; Soares et al., 2015). Besides the reduction in photoassimilate accumulation by its lower production, there is more waste of energy by the plant because of reduction in osmotic potential, which decreases water availability for plant growth (Taiz, Zeiger, Møller, & Murphy, 2017).

Table 3. Shoot dry mass (SDM), root dry mass (RDM) and total dry mass (MST) of *Cucumis melo* L., according to the methods of hydrogen peroxide application and irrigation water salinity

Methods of H_2O_2 application	Analyzed variables					
	SDM (g plant^{-1})		RDM (g plant^{-1})		TDM (g plant^{-1})	
	0.3 dS m^{-1}	2.0 dS m^{-1}	0.3 dS m^{-1}	2.0 dS m^{-1}	0.3 dS m^{-1}	2.0 dS m^{-1}
Control	5.34 Aa	2.49 Ba	0.68 Ab	0.27 Ba	6.03 Ab	2.76 Ba
Imbibition of seeds	5.92 Aa	2.57 Ba	1.55 Aa	0.37 Ba	7.47 Aa	2.93 Ba
At sowing	6.49 Aa	1.96 Ba	1.29 Aa	0.14 Ba	7.77 Aa	2.10 Ba
Foliar spraying	5.89 Aa	1.79 Ba	0.76 Ab	0.24 Ba	6.64 Aab	2.03 Ba
Means	4.06		0.66		4.72	
CV (%)	18.6		27.2		16.7	
SD	0.76		0.17		0.78	

Note. Means followed by the same lowercase letter in the column and capital letter in the line do not differ from each other at 5% probability by the Tukey test.

When it comes to hydrogen peroxide application, the plants submitted to application at sowing and imbided seeds showed the largest increases in total biomass accumulation under the salinity of 0.3 dS m^{-1}, while the plants produced under 2.0 dS m^{-1}, were not influenced by H_2O_2 (Table 3). The H_2O_2 application increasing phytotoxic effect of the salts may cause the reduction promoted in plants as the increase of the electric conductivity by irrigation water. Hydrogen peroxide acts as a signal molecule for plant defense, but at high levels it must promote the formation of OH^-, resulting in oxidation and inactivation of antioxidant enzymes (Barbosa, Silva, Willadino, Ulisses, & Câmara 2014).

The relation between shoot dry mass and root dry mass showed the greatest increase for plants produced under the salinity of 2.0 dS m^{-1}, presenting higher ratio for plants that were submitted to the H_2O_2 application at sowing (Table 4). This effect may come from the mechanism of action of H_2O_2, attenuating the salinity effect, enhancing shoot growth of the plants. This higher shoot growth than root system may be a defense mechanism as a response to the luminosity in which the plants were produced (Oliveira et al., 2013). Thus, providing greater plant capacity to promote photosynthesis and, consequently, better shoot biomass accumulation.

Table 4. Dry mass ratio of the shoot/root (DMSR) and quality index of Dickson (DQI) of *Cucumis melo* L., according to the methods of hydrogen peroxide application and irrigation water salinity

Methods of H_2O_2 application	Analyzed variables			
	DMSR		DQI	
	0.3 dS m^{-1}	2.0 dS m^{-1}	0.3 dS m^{-1}	2.0 dS m^{-1}
Control	7.98 Aa	9.64 Ab	0.47 Ab	0.23 Bab
Imbibition of seeds	4.09 Bb	6.97 Ab	0.89 Aa	0.31 Ba
At sowing	5.15 Bab	13.8 Aa	0.79 Aa	0.14 Bb
Foliar spraying	8.31 Aa	8.10 Ab	0.52 Ab	0.21 Bab
Means	8.0		0.44	
CV (%)	27.3		20.7	
SD	2.18		0.09	

Note. Means followed by the same lowercase letter in the column and capital letter in the line do not differ from each other at 5% probability by the Tukey test.

The Dickson quality index: presented the highest values in plants produced under the electrical conductivity of 0.3 dS m^{-1}, resulting in reduction as a function of salinity increase (Table 4). Regarding the hydrogen peroxide application, it was verified that the plants submitted to the treatments with imbided seeds and the application at sowing attenuated the effect of the salts in the level of 0.3 dS m^{-1}, promoting the obtaining of plants with greater force. In the salinity of 2.0 dS m^{-1}, the highest values were obtained by the treatment in which the seeds were submitted to H_2O_2 imbibition, but not statistically differing from control and foliar application.

Increased salinity may have promoted physiological and metabolic disturbances in plant, resulting in less vigorous plants. Higher concentrations of salts promote reduction in osmotic potential and culminate in nutritional imbalance due to the lower absorption capacity, transport and assimilation of the nutrients, causing inhibition of physiological and biochemical processes in plant (Rezende et al., 2018).

4. Conclusions

The salinity affected the growth, biomass accumulation and plant quality of *Cucumis melo* L. The most aggressive effects were verified in plants under the salinity of 2.0 dS m^{-1}.

The application of H_2O_2 in the imbibition of seeds and at sowing mitigated the effect of salts on the conductivity of 0.3 dS m^{-1} for number of leaves, accumulation of root and total dry mass and Dickson quality index.

The use of H_2O_2 at high concentrations in plants under high salinity increases the effect of the stress promoted by the salts.

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