# Gas exchange, growth, and production of mini-watermelon under saline water irrigation and phosphate fertilization

# Trocas gasosas, crescimento e produção de mini-melancieira irrigada com águas salinas e adubação fosfatada

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#### **Highlights:**

Phosphorus doses did not mitigate the effects of salt stress on gas exchange and growth. The  $CO_2$  assimilation rate was limited by factors of non-stomatal and stomatal origin. Mini-watermelon production is drastically reduced by water salinity.

## Abstract

In the semi-arid region of Northeastern Brazil, due to the occurrence of excess salts, both in the water and soil, plants are constantly exposed to various conditions of abiotic stress. Thus, it is extremely important to identify methods capable of minimizing the effects of salt stress on plants as a way to ensure the expansion of irrigated areas. In this context, the objective of this study was to evaluate the gas exchange, growth, and production of mini-watermelon irrigated with saline waters and fertilized with phosphorus. The experiment was conducted in pots under greenhouse conditions in Pombal, PB, Brazil, using a randomized block design in a 5 x 4 factorial scheme, corresponding to five levels of electrical conductivity of irrigation water—ECw (0.3, 1.3, 2.3, 3.3, and 4.3 dS m<sup>-1</sup>), four phosphorus doses— PD (60, 80, 100, and 120% of the recommendation), and with three replicates. Watermelon plants cv. Sugar Baby were sensitive to water salinity greater than 0.3 dS m<sup>-1</sup>, with more pronounced inhibition of gas exchange, growth, and production. Reduction in the CO, assimilation rate of watermelon plants cv. Sugar Baby was associated with factors of stomatal and non-stomatal origin. Phosphorous doses corresponding to 73 and 88% of the recommended values promoted an increase in the intercellular CO<sub>2</sub> concentration and stem diameter of mini-watermelon plants. P,O, doses ranging from 60 to 120% of the recommendation did not mitigate the effects of salt stress on the cultivation of watermelon cv. Sugar Baby.

Key words: Citrullus lanatus. Salt stress. Semi-arid region.

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#### Resumo

No semiárido do Nordeste brasileiro devido à ocorrência do excesso de sais, tanto na água como no solo, as plantas estão constantemente expostas às diversas condições de estresses abióticos. Assim, é de extrema importância a identificação de alternativas capazes de minimizar os efeitos decorrentes do estresse salino sobre as plantas como forma de garantir a expansão das áreas irrigadas. Neste contexto, objetivou-se com este trabalho avaliar as trocas gasosas, o crescimento e a produção de mini-melancieira irrigada com águas salinas e adubadas com fósforo. A pesquisa foi desenvolvida em vasos sob condições de casa-de-vegetação em Pombal, PB, utilizando-se o delineamento de blocos casualizados em esquema fatorial 5 x 4, correspondendo a cinco níveis de condutividade elétrica da água de irrigação - CEa (0.3; 1,3; 2,3; 3,3 e 4,3 dS m<sup>-1</sup>), quatro doses de fósforo - DP (60; 80; 100 e 120% da recomendação), com três repetições. As plantas de melancieira cv. Sugar Baby foram sensíveis a salinidade da água a partir de 0,3 dS m<sup>-1</sup>, destacando-se inibição nas trocas gasosas, no crescimento e na produção. A redução na taxa de assimilação de CO, nas plantas de melancieira cv. Sugar Baby está associado a fatores de origem estomáticos e não estomáticos. Doses de fosforo correspondente a 73 e 88% da recomendação promoveram aumento na concentração intercelular de CO, e no diâmetro de caule das plantas de minimelancia. Doses de P<sub>2</sub>O<sub>5</sub> variando de 60 a 120% da recomendação não amenizou os efeitos do estresse salino no cultivo da melancieira cv. Sugar Baby.

Palavras-chave: Citrullus lanatus. Estresse salino. Semiárido.

#### Introduction

Belonging to the Cucurbitaceae family, watermelon (Citrullus lanatus) is considered one of the most important vegetables produced and commercialized in Brazil. Its fruits are appreciated for the sweet taste and high water content, and it is considered a medicinal plant with diuretic properties, low caloric value, and is high in vitamins A, C, B1, and B2 (Saraiva et al., 2013). Among the varieties of watermelon, mini-watermelon has stood out in the market because of its potential for export and mainly attracts consumers who make up small families, due to its practicality in terms of transport, its reduced size, and ease of packaging in refrigerators (N. C. Silva et al., 2008).

In the 2018 season, the five largest Brazilian producers in terms of harvested area were the states of Rio Grande do Norte (15,862 ha), Bahia (14,349 ha), Rio Grande do Sul (14,212 ha), São Paulo (10,173 ha) and Tocantins (6,369 ha), corresponding to average yields of 24.68, 11.66, 19.95, 27.68 and 28.13 t ha<sup>-1</sup>, respectively (Instituto Brasileiro de Geografia e Estatística [IBGE], 2018). Although some states in Northeastern Brazil stand out as the largest producers, the cultivation of this vegetable

in areas of the Brazilian semi-arid region is at risk due to the variation in rainfall, resulting from the low intensity of precipitation and high rates of evapotranspiration during most of the year (Araújo et al., 2016).

Due to the scarcity of water in semi-arid areas of Northeastern Brazil, it is essential to adopt the practice of irrigation with saline waters to ensure crop production and expansion of irrigated areas (Alvarenga et al., 2019). Normally, the water sources available for irrigation in this region are small and medium-sized reservoirs and shallow wells, with electrical conductivity ranging from 1.97 to 2.98 dS m<sup>-1</sup> (Medeiros, Lisboa, Oliveira, Silva, & Alves, 2003).

High concentrations of salts in water and/or soil can cause changes in various physiological and metabolic processes in plants (Gupta & Huang, 2014). This is due to the reduction in water availability for plants caused by the osmotic effect of the soil solution; this toxic effect occurs mainly due to the increased concentration of Na<sup>+</sup> and Cl<sup>-</sup> ions and resulting nutritional imbalance, leading to deficiencies in Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, and NO<sub>3</sub><sup>-</sup> (Machado & Serralheiro, 2017). In addition, it manifests as an oxidative stress at the subcellular level, mediated by reactive oxygen species (Hernández, 2019).

In this case, the use of these waters is conditional on the tolerance of crops to salinity, and the management practices such as irrigation and fertilization (Freitas, Figueirêdo, Porto, Costa, & Cunha, 2014). Thus, fertilization with phosphorus is extremely important in plants grown under salt stress conditions. This is related to the functions that phosphorus performs in plant metabolism, especially in terms of its capacity to store energy (F. R. A. Oliveira, Oliveira, Medeiros, Sousa, & Freire, 2010), being essential in a number of cellular processes, including the maintenance of membrane synthesis of biomolecules, structures. and formation of high-energy molecules. It also helps in cell division, enzyme activation/inactivation, and carbohydrate metabolism (Razaq, Zhang, Shen, & Salahuddin, 2017).

Considering the socioeconomic importance of watermelon, especially mini-watermelon, it is important to conduct studies investigating phosphate fertilization as a way to mitigate the effects of salt stress on this crop, under the conditions of the Brazilian semi-arid region. In this context, the objective of this study was to evaluate the gas exchange, growth, and production of miniwatermelon cv. Sugar Baby cultivated with saline water and phosphorus fertilization.

# **Materials and Methods**

The experiment was carried out in a protected environment (greenhouse) at the Center for Sciences and Agri-Food Technology (CCTA) of the Federal University of Campina Grande (UFCG), located in the municipality of Pombal, PB, Brazil, at the geographic coordinates of 6°47'20" S latitude and 37°48'01" W longitude, and an altitude of 194 m.

Five levels of irrigation water electrical conductivity—ECw (0.3, 1.3, 2.3, 3.3, and 4.3 dS m<sup>-1</sup>) and four doses of phosphorus (60, 80, 100, and

120% of the recommended levels of Novais, Neves e Barros (1991) for experiments in pots) were evaluated in a randomized block design, arranged in a 5x4 factorial scheme with three replicates, with each plot consisting of one plant. The dose relative to 100% corresponded to 300 mg of  $P_2O_5$  kg<sup>-1</sup> of soil.

Watermelon (*Citrullus lanatus*), cultivar Sugar Baby, was used in the experiment. This cultivar stands out for its early cycle, and the fruits are ready for harvesting 75 days after planting. It is a versatile plant with vigorous foliage and is tolerant to high temperatures. It produces round fruits with a dark green rind, weighing around 2 to 4 kg. It has a soft pulp with high sugar content and an intense red color (S. S. da Silva et al., 2019).

Plants were cultivated in 20-L plastic pots adapted as lysimeters. Two holes were made at the base of the pots and connected to transparent 4-mm-diameter drains. The end of the drain inside the lysimeter was connected to a nonwoven geotextile (Bidim OP 30) to prevent clogging by soil material. A container was placed below each drain to collect the drained water and estimate water consumption by plants. The pots were filled with a 0.5-kg layer of crushed stone, followed by 23.5 kg of a Neossolo Regolítico (Psamment) with a sandy clay loam texture, from the rural area of the municipality of São Domingos, PB, whose chemical and physical characteristics (Table 1) were obtained according to the methodology proposed by Teixeira, Donagemma, Fontana e Teixeira (2017).

Four seeds of the watermelon cv. Sugar Baby were equidistantly distributed in each lysimeter, at 2 cm depth. After the emergence of seedlings, thinning was performed in two stages, when the plants had two and three pairs of true leaves, respectively, leaving one plant per container in the last thinning operation.

Chemical characteristics										
pH H <sub>2</sub> O)	OM	Р	$K^+$	$Na^+$	$Ca^{2+}$	$Mg^{2+}$	Al <sup>3+</sup>	$\mathrm{H}^{+}$		
(1:2,5)	g kg-1	(mg kg <sup>-1</sup> )	cmol kg <sup>-1</sup>							
5.58	2.93	39.2	0.23	1.64	9.07	2.78	0.00	8.61		
	Chemical characteristics				Physical characteristics					
EC <sub>se</sub>	CEC	SAR	ESP	Size fraction (g kg <sup>-1</sup> )		Water content (dag kg <sup>-1</sup> )				
(dS m <sup>-1</sup> )	cmol <sub>c</sub> kg <sup>-1</sup>	(mmol L <sup>-1</sup> ) <sup>0.5</sup>	%	Sand	Silt	Clay	33.42 kPa <sup>1</sup>	1519.5 kPa <sup>2</sup>		
2.15	22.33	0.67	7.34	572.70	100.70	326.60	25.91	12.96		

 Table 1

 Chemical and physical characteristics of the soil used in the experiment, before the application of the treatments

pH – Hydrogen potential, OM – Organic matter: Walkley-Black Wet Digestion;  $Ca^{2+}$  and  $Mg^{2+}$  extracted with 1 M KCl at pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted with 1 M NH<sub>4</sub>OAc at pH 7.0; Al<sup>3+</sup>+H<sup>+</sup> extracted with 0.5 M CaOAc at pH 7.0; ECse – Electrical conductivity of the saturation extract; CEC – Cation exchange capacity; SAR – Sodium adsorption ratio of the saturation extract; ESP – Exchangeable sodium percentage; <sup>1,2</sup> referring to the limits of field capacity and permanent wilting point, respectively.

The waters were prepared in such a way as to have an equivalent proportion of 7:2:1 of Na<sup>+</sup>:Ca<sup>2+</sup>:Mg<sup>2+</sup>, respectively, with the salts NaCl, CaCl<sub>2</sub>.2H<sub>2</sub>O, and MgCl<sub>2</sub>.6H<sub>2</sub>O. This is the predominant ratio in sources of water used for irrigation in small farms in the Northeast region, considering the relationship between ECw and concentration of salts, according to Richards (1954), Eq. 1:

Q (mmolc 
$$L^{-1}$$
) = 10 x ECw (dS m<sup>-1</sup>) .....(1)

Where:

Q = Quantity of salts to be applied  $(mmol_{c} L^{-1})$ ;

ECw = Electrical conductivity of water (dS m<sup>-1</sup>)

Irrigation was performed daily at 17:00 h, applying in each container the volume corresponding to that obtained by the water balance, determined by Eq. 2:

$$VI = \frac{(Va - Vd)}{(1 - LF)}$$
.....(2)

Where: VI = Volume of water to be applied (mL);Va = volume applied in the previous irrigation event (mL); Vd = volume drained (mL), and LF = leaching fraction of 0.2. Fertilization with nitrogen and potassium was performed as recommended for pot experiments (Novais et al., 1991), applying via fertigation, at 15day intervals distributed throughout the crop cycle, 100 and 150 mg kg<sup>-1</sup> soil of N and K<sub>2</sub>O, respectively. Urea (45% N) and potassium chloride (60% K<sub>2</sub>O) were used as sources of nitrogen and potassium, respectively.

P supply was performed according to preestablished treatments, using monoammonium phosphate (48%  $P_2O_5$ ), applying one third of the recommended dose as a basal level and the other two thirds in three equal applications, at 10-day intervals, with the first application at 15 days after sowing. Fertilization with micronutrients was performed weekly, and was applied through the foliar application using 1.0 g L<sup>-1</sup> of Ubyfol [(N (15%),  $P_2O_5$  (15%),  $K_2O$  (15%), Ca (1%), Mg (1.4%), S (2.7%), Zn (0.5%), B (0.05%), Fe (0.5%), Mn (0.05%), Cu (0.5%), Mo (0.02%)].

The effects of treatments on the crop were determined at 60 days after transplanting, through measuring gas exchange (CO<sub>2</sub> assimilation rate (*A*), transpiration (*E*), stomatal conductance (*gs*) and intercellular CO<sub>2</sub> concentration (*Ci*)) on the third leaf counted from the apex. Based on these

data, the intrinsic water use efficiency (*WUEi*) (*A/gs*) [( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) (mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>)<sup>-1</sup>] and the instantaneous carboxylation efficiency (*A/Ci*) [( $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) ( $\mu$ mol mol<sup>-1</sup>]<sup>-1</sup> were quantified using the portable photosynthesis meter "LCPro+" from ADC BioScientific Ltd.

Growth was evaluated based on main stem length (MSL), stem diameter (SD), and number of leaves (NL). Production components were measured at 70 days after sowing (DAS) by determining fruit equatorial diameter (FED), fruit polar diameter (FPD), and fresh fruit weight (FFW). MSL was measured as the distance between the plant collar and the insertion of the apical meristem, while SD was measured at 5 cm from the plant collar, while NL was counted considering leaves that were fully expanded, with a minimum length of 3 cm and with at least 50% of their area photosynthetically active. Fruit fresh weight was analyzed using a digital scale, and the results were expressed in g per plant.

The collected data were subjected to an analysis of variance by F test at a probability level of 0.05 and, when significant, linear and quadratic polynomial regression analysis was performed for the factors salinity level and phosphorus dose, using the statistical program SISVAR ESAL (Ferreira, 2019).

#### **Results and Discussion**

According to the summary of the analysis of variance (Table 2), there was a significant effect of the interaction between factors (SL x PD) on the CO<sub>2</sub> assimilation rate (A) and instantaneous carboxylation efficiency (*CEi*) of mini-watermelon plants. Except for *CEi*, the water salinity levels significantly influenced all the variables evaluated. Phosphorus doses had a significant effect only on intercellular CO<sub>2</sub> concentration (*Ci*) and the instantaneous carboxylation efficiency of mini-watermelon plants, at 70 days after sowing.

Table 2

Summary of the analysis of variance for stomatal conductance (gs), intercellular CO<sub>2</sub> concentration (Ci), transpiration (E), CO<sub>2</sub> assimilation rate (A), intrinsic water use efficiency (*WUEi*) and instantaneous carboxylation efficiency (*CEi*) of mini-watermelon plants cv. Sugar Baby cultivated with saline water and phosphorus doses, at 60 days after sowing

Source of variation	DF	Mean squares						
Source of variation		gs	Ci	Ε	A	WUEi	CEi	
Saline levels (SL)	4	0.010**	769.67*	1.795*	68.81**	1339.16**	0.0003 <sup>ns</sup>	
Linear regression	1	0.032**	2731.30*	6.491**	272.37**	5068.57**	0.0015 <sup>ns</sup>	
Quadratic regression	1	0.003 <sup>ns</sup>	57.75 <sup>ns</sup>	0.563 <sup>ns</sup>	1.20 <sup>ns</sup>	270.68 <sup>ns</sup>	$0.00001^{ns}$	
Phosphorus doses (PD)	3	0.001 <sup>ns</sup>	4742.41*	0.511 <sup>ns</sup>	15.64 <sup>ns</sup>	40.22 <sup>ns</sup>	0.0019**	
Linear regression	1	0.001 <sup>ns</sup>	8780.43**	0.109 <sup>ns</sup>	6.10 <sup>ns</sup>	11.57 <sup>ns</sup>	$0.001^{*}$	
Quadratic regression	1	0.0008 <sup>ns</sup>	2444.81*	0.526 <sup>ns</sup>	13.18 <sup>ns</sup>	48.02 <sup>ns</sup>	$0.001^{*}$	
Interaction (SL x PD)	12	0.002 <sup>ns</sup>	2054.53 <sup>ns</sup>	0.249 <sup>ns</sup>	24.81*	98.76 <sup>ns</sup>	$0.0008^{*}$	
Blocks	2	0.0006 <sup>ns</sup>	2330.51*	0.283 <sup>ns</sup>	1.77 <sup>ns</sup>	24.00 <sup>ns</sup>	0.0011 <sup>ns</sup>	
Residual	38	0.001	604.99	0.238	6.88	75.85	0.0001	
CV(%)		17.54	11.44	14.26	16.84	12.41	19.01	

DF - degrees of freedom; CV (%) - coefficient of variation; \*significant at 0.05 probability level; \*\* significant at 0.01 probability level; ns not significant.

The stomatal conductance of watermelon plants decreased linearly with the increase in water salinity levels. Based on the regression equation (Figure 1A), there was a decrease in gs of 6.76% per unit increase in ECw. Comparatively, plants irrigated using water with an ECw of 4.3 dS m<sup>-1</sup> had a reduction in gs of 27.60% (0.065 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) in comparison to those irrigated with the lowest level of water salinity (0.3 dS m<sup>-1</sup>). The reduction in stomatal conductance may be related to the decrease in the turgor pressure of guard cells, due to the decrease in water absorption caused by the reduction in the osmotic potential of the soil solution. Stomatal closure is a protective strategy against salt stress, making it possible for the plant to save water and improve efficiency in its use. However, the lower gs leads to a reduction in CO<sub>2</sub> diffusion through the leaf mesophyll. In addition, the supply of CO<sub>2</sub> to RuBisCO (ribulose-1,5bisphosphate carboxylase oxygenase) is hampered, which predisposes the photosynthetic apparatus to increased energy dissipation and negative regulation of photosynthesis when plants are subjected to high light and temperature (Chaves, Flexas, & Pinheiro, 2009). A decrease in stomatal conductance was also observed by Sá et al. (2016) in a study with bell pepper cv. All Big subjected to water salinity (ECw of 0.6 and 3.0 dS m<sup>-1</sup>).

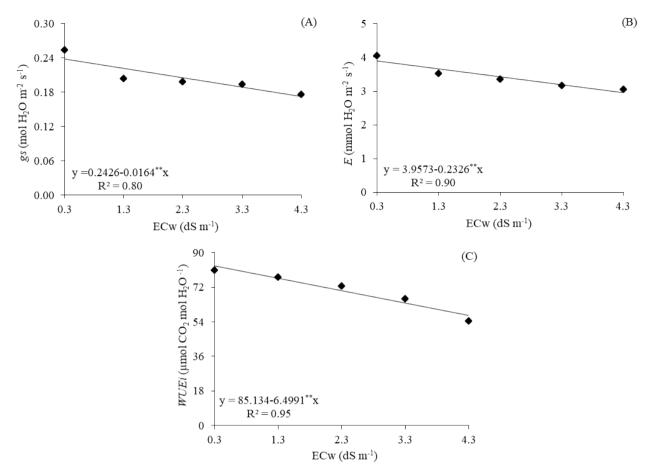
In mini-watermelon plants, leaf transpiration also reduced linearly with an increase in water salinity levels and, according to the regression equation (Figure 1B), there was a reduction of 5.87% per unit increase in ECw. When subjected to water salinity from 4.3 dS m<sup>-1</sup>, the reduction in Ewas 23.93% (0.930 mmol  $H_2O m^{-2} s^{-1}$ ) compared to those grown under ECw of 0.3 dS m<sup>-1</sup>. Reduction in the transpiration of mini-watermelon plants resulted from a decrease in stomatal conductance (Figure 1A) imposed by salt stress. It is likely that the reduction in leaf transpiration may, at least in part, be associated with the closure of stomata and with non-stomatal causes related to the osmotic and toxic effects of excess salts (Lúcio et al., 2013). However, stomatal closure contributes to a decrease in the flow

of toxic ions (Na<sup>+</sup> and Cl<sup>-</sup>) within the transpiration flow in plants (Acosta-Motos et al., 2017). Furtado, Pereira, Andrade, Pereira e Silva (2012), in a study evaluating the effects of salt stress (ECw of 0.3 and 5.0 dS m<sup>-1</sup>) on the gas exchange of the watermelon cv. Crimson Sweet, concluded that the highest rate of leaf transpiration was observed in plants irrigated with 0.3 dS m<sup>-1</sup> water.

Regarding the intrinsic water use efficiency of mini-watermelon plants (Figure 1C), it was verified that, as water salinity increased, there was a reduction in WUEi of 7.63% per unit increase in ECw. In plants grown under the highest level of ECw (4.3 dS m<sup>-1</sup>), WUEi was reduced by 31.25% (25.99  $\mu$ mol CO<sub>2</sub> mol H<sub>2</sub>O<sup>-1</sup>) compared to those subjected to water salinity of 0.3 dS m<sup>-1</sup>. As the intrinsic water use efficiency is obtained through the relationship between the CO<sub>2</sub> assimilation rate and stomatal conductance, in which the observed values relate to the amount of carbon fixed by the plant for a given stomatal conductance, it is likely that the plants that have the capacity to maintain reduced water use efficiency under saline conditions are those that are sensitive to salt stress (Flowers & Flowers, 2005).

Water salinity also promoted a linear reduction in the intercellular CO<sub>2</sub> concentration of miniwatermelon plants (Figure 2A), equal to 2.21% per unit increase in ECw. There was a reduction of 20.00 µmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> (8.90%) between plants irrigated using water with ECw of 4.3 dS m<sup>-1</sup> as compared to those subjected to the lowest salinity level (0.3 dS  $m^{-1}$ ). It is likely that the reduction in Ci is related to the lower CO<sub>2</sub> diffusion in the intercellular space of the leaf mesophyll, due to the stomatal closure caused by salt stress (W. J. D. Oliveira, Souza, Cunha, Silva, & Veloso, 2017). With the closure of the stomata, initially there is a reduction in the intercellular CO<sub>2</sub> concentration in the substomatal chamber and then, when the stress becomes severe, there is an increase in Ci (Romero, Alarcón, Valbuena, & Galeano, 2017). Corroborating the results obtained in this study, S. S. da Silva et al. (2019) evaluated the gas exchange of watermelon cv. Sugar Baby under strategies of irrigation with saline waters (ECw of 0.8 and 3.2 dS m<sup>-1</sup> applied at different phenological stages) and

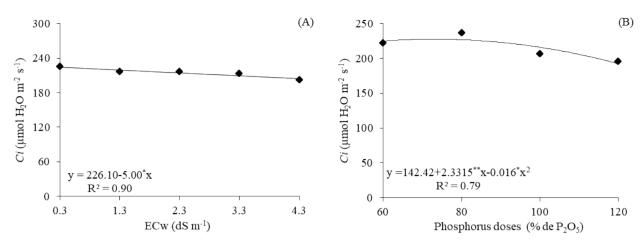
found that irrigation using water with ECw of 3.2 dS  $m^{-1}$  during the fruit maturation stage resulted in a reduction in the intercellular CO<sub>2</sub> concentration.



**Figure 1.** Stomatal conductance -gs (A), transpiration -E (B) and intrinsic water use efficiency -WUEi (C) of miniwatermelon plants cv. Sugar Baby as a function of water salinity -ECw, at 60 days after sowing.

Regarding the effects of phosphorus doses on the intercellular CO<sub>2</sub> concentration of mini-watermelon plants (Figure 2B), the equation shows that a maximum estimated value of 227.35  $\mu$ mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> was obtained when plants were fertilized with an estimated dose of 73% of the P<sub>2</sub>O<sub>5</sub> recommendation. There was a reduction of 32.91  $\mu$ mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in *Ci* between plants grown at a dose of 120% and those fertilized with 60% of the P<sub>2</sub>O<sub>5</sub> recommendation. Being a structural component of macromolecules, phosphorus plays a fundamental role in plant metabolism. It participates in energy-

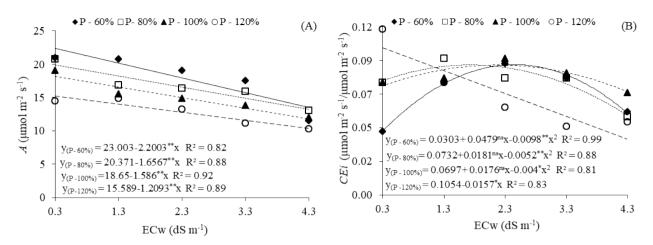
rich compounds, such as adenosine triphosphate (ATP), responsible for storing energy and donating electrons to maintain the biochemical phase of photosynthesis. It is also required for esterification reactions with sugars and other compounds involved in photosynthesis and respiration (Ceconi, Poletto, Lovato, & Muniz, 2007), being a key element in various metabolic pathways and biochemical reactions, such as numerous stages of the  $C_3$  and  $C_4$  photosynthetic pathways and glycolysis (Kuwahara & Souza, 2009).



**Figure 2.** Intercellular  $CO_2$  concentration – *Ci* of mini-watermelon plants cv. Sugar Baby as a function of water salinity - ECw (A) and phosphorus doses (B) at 60 days after sowing.

The interaction between factors (SL x PD) significantly influenced the CO<sub>2</sub> assimilation rate of mini-watermelon plants. According to the regression equations (Figure 3A), there were linear reductions in the A of plants fertilized with 60, 80, 100, and 120% of the  $P_2O_5$  recommendation, which were respectively equal to 9.56, 8.13, 8.50, and 7.75% per unit increase in ECw. Thus, plants irrigated with water from 4.3 dS  $m^{-1}$  reduced A by 8.80, 6.62, 6.34, and 4.83 µmol m<sup>-2</sup> s<sup>-1</sup> compared to those under the lowest salinity level (0.3 dS  $m^{-1}$ ). Therefore, it becomes evident that the decrease in CO<sub>2</sub> assimilation rate in mini-watermelon depends on the dose of P<sub>2</sub>O<sub>5</sub>. The observed decrease in CO<sub>2</sub> assimilation rate (Figure 3A) may be related to stomatal closure, as evidenced by stomatal conductance (Figure 1A) and by the reduction in the intercellular CO<sub>2</sub> concentration (Figure 2A). The high concentration of ions such as Na<sup>+</sup> and Cl<sup>-</sup> in the leaves can also be considered as a factor that contributes to the reduction of A, due to damage to enzymes and structures of the membrane (Coelho, Simões, Salviano, Mesquita, & Alberto, 2018), indicating that the decrease in CO<sub>2</sub> assimilation rate is related to factors of stomatal and non-stomatal origin.

With regard to the instantaneous carboxylation efficiency (CEi) of watermelon (Figure 3B), it can be noted that, for plants fertilized with P<sub>2</sub>O<sub>5</sub> doses of 60, 80, and 100% of the recommendation, the results were best described by a quadratic model, with maximum estimated value of 0.089 µmol m<sup>-2</sup> s<sup>-1</sup>/µmol m<sup>-2</sup> s<sup>-1</sup> when plants were subjected to water salinity of 2.6, 2.0, and 2.5 dS m<sup>-1</sup>. However, under dose equivalent to 120% of the  $P_{2}O_{5}$  recommendation, there was a linear and decreasing effect with a reduction of 14.89% per unit increase in ECw. When comparing the CEi of plants fertilized with the highest  $P_2O_5$  dose (120%) and subjected to ECw of 4.3 dS m<sup>-1</sup>, it was possible to observe a reduction of 62.36% (0.0628 µmol m<sup>-2</sup>  $s^{-1}/\mu$ mol m<sup>-2</sup> s<sup>-1</sup>) in comparison to those that were irrigated using water of the lowest salinity level (0.3 dS m<sup>-1</sup>). The reduction in *CEi* is an indication that factors of non-stomatal origin also influenced the photosynthetic activity of the plants, such as low activity of the enzyme Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), probably due to low substrate availability (ATP and NADPH) for enzyme activation and regeneration (Hussain, Luro, Costantino, Ollitrault, & Morillon, 2012).



**Figure 3.**  $CO_2$  assimilation rate - A (A) and instantaneous carboxylation efficiency – CEi (B) of mini-watermelon plants cv. Sugar Baby as a function of the interaction between water salinity levels – ECw and phosphorus doses at 60 days after sowing.

The summary of the analysis of variance (Table 3) shows a significant effect of the interaction between factors (SL x PD) on the main stem length of mini-watermelon plants. Water salinity levels

significantly influenced all variables analyzed (MSL, SD, NL, FFW, FED, and FPD). Phosphate fertilization promoted a significant effect only on MSL and SD at 70 DAS.

Table 3

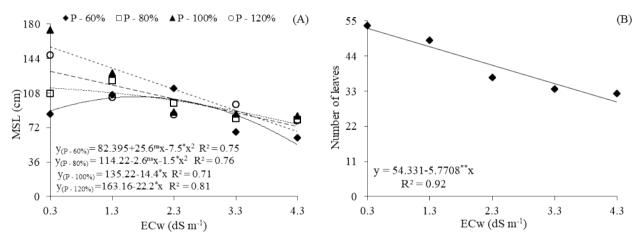
Summary of the analysis of variance for main stem length (MSL), stem diameter (SD), number of leaves (NL), fresh fruit weight (FFW), fruit equatorial diameter (FED) and fruit polar diameter (FPD) of mini-watermelon fruits cv. Sugar Baby cultivated with saline waters and phosphorus doses at 70 days after sowing

Source of variation	DF	Mean squares							
Source of variation		MSL	SD	NL	FFW	FED	FPD		
Saline levels (SL)	4	6331.53**	7.19**	1088.01**	251046.98**	19.08**	17.53**		
Linear regression	1	24196.80**	28.37**	3996.30**	940976.57**	71.54**	65.71**		
Quadratic regression	1	1080.21*	$0.008^{\text{ns}}$	175.07 <sup>ns</sup>	34935.91*	1.50 <sup>ns</sup>	0.58 <sup>ns</sup>		
Phosphorus doses (PD)	3	1740.95**	1.93*	75.24 <sup>ns</sup>	9977.34 <sup>ns</sup>	2.22 <sup>ns</sup>	0.68 <sup>ns</sup>		
Linear regression	1	2610.75**	0.04 <sup>ns</sup>	14.74 <sup>ns</sup>	69.88 <sup>ns</sup>	2.85 <sup>ns</sup>	0.41 <sup>ns</sup>		
Quadratic regression	1	1.35 <sup>ns</sup>	3.16*	97.53 <sup>ns</sup>	10984.46 <sup>ns</sup>	3.10 <sup>ns</sup>	0.61 <sup>ns</sup>		
Interaction (SL x PD)	12	956.38**	0.30 <sup>ns</sup>	60.28 <sup>ns</sup>	10442.59 <sup>ns</sup>	1.00 <sup>ns</sup>	1.30 <sup>ns</sup>		
Blocks	2	561.80 <sup>ns</sup>	$0.001^{ns}$	11.57 <sup>ns</sup>	9985.94 <sup>ns</sup>	2.57 <sup>ns</sup>	1.11 <sup>ns</sup>		
Residual	38	116.01	0.27	54.12	8269.16	0.86	0.76		
CV(%)		10.81	7.48	17.92	24.69	9.64	9.06		

DF - degrees of freedom; CV (%) - coefficient of variation; \*significant at 0.05 probability level; \*\* significant at 0.01 probability level; ns not significant.

The main stem length of mini-watermelon plants was significantly affected by the interaction between factors (SL x PD). From the regression equations (Figure 4A), it is verified that the MSL data of plants fertilized with 60 and 80% of the  $P_2O_5$  recommendation were described by a quadratic model, with maximum estimated values of 103.96 and 113.30 cm under ECw of 1.9 and 0.3 dS m<sup>-1</sup>, respectively. For plants that received 100 and 120% of  $P_2O_5$ , linear decreases were observed as the ECw levels increased, corresponding to 10.64 and 13.60% per unit increase in ECw, i.e. equivalent reductions of 44.0 and 56.7% in the MSL of plants irrigated with ECw of 4.3 dS m<sup>-1</sup> compared to those under

water salinity of 0.3 dS m<sup>-1</sup>. The reduction in growth in plants under salinity can be attributed to osmotic stress caused by the reduction in the external water potential and to the ionic effect caused by the accumulation of ions in plant tissues (Lima, Nobre, Gheyi, Soares, & Silva, 2014). Another factor that possibly contributed to the reduction in MSL was the competitive mechanism: Na<sup>+</sup> occuping the sites of absorption of K<sup>+</sup> and Mg<sup>2+</sup>, and the ion Cl<sup>-</sup> acting on N and P absorption sites, inhibiting their absorption (Lucena, Siqueira, Martinez, & Cecon, 2012) and, thereby, contributing to the reduction in plant growth.



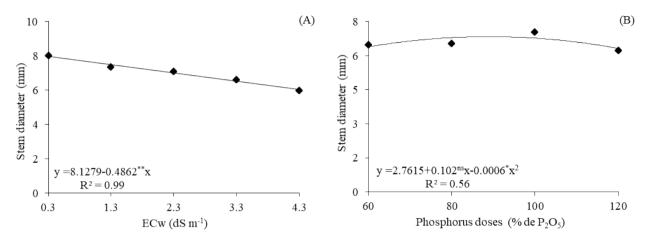
**Figure 4.** Main stem length – MSL of mini-watermelon plants cv. Sugar Baby as a function of the interaction between water salinity levels – ECw and phosphorus doses (A) and number of leaves as a function of ECw levels, 70 days after sowing.

The number of leaves of mini-watermelon plants decreased significantly with the increase in irrigation water salinity (Figure 4B), with a 10.62% reduction per unit increase in ECw. By comparing the NL of plants subjected to ECw of 4.3 dS m<sup>-1</sup> to that of plants under irrigation with the lowest salinity level (0.3 dS m<sup>-1</sup>), it was possible to note a reduction of 23.08 (43.88%) leaves. The lower formation of leaves in plants grown under stress which occurred in the present study can be considered a morphological or anatomical alteration to maintain the absorption of water and nutrients

under saline conditions and reduce transpiration and maintain a higher water content (Bezerra et al., 2018). Ribeiro, Sales, Eloi, Moreira e Sales (2012), in a study conducted to evaluate the effects of irrigation water salinity (ECw ranging from 0.17 to 5.5 dS m<sup>-1</sup>) on the initial growth of watermelon, also found a reduction in the number of leaves, equal to 9.87% per unit increase in ECw.

Water salinity also inhibited the stem diameter growth of mini-watermelon plants. According to the regression equation (Figure 5A), the SD decreased by 5.98% per unit increase in ECw. When plants were subjected to a salinity of 4.3 dS m<sup>-1</sup>, their SD decreased by 24.36% (1.94 mm) compared to those cultivated under the lowest salinity level (0.3 dS m<sup>-1</sup>). The decrease in the growth in stem diameter observed in plants under salt stress is possibly associated with energy expenditure due to various metabolic alterations such as lipid peroxidation, a reduction in chlorophyll content, an increased

synthesis of reactive oxygen species, and enzymatic antioxidant activity (Queiroz, Sodek, & Haddad, 2012). A reduction in the stem diameter of plants cultivated under salt stress has also been observed by Ribeiro et al. (2012) in watermelon cv. Crimson Sweet, by Araújo et al. (2016) in melon, and by Bezerra et al. (2018) in guava plants cv. Paluma.

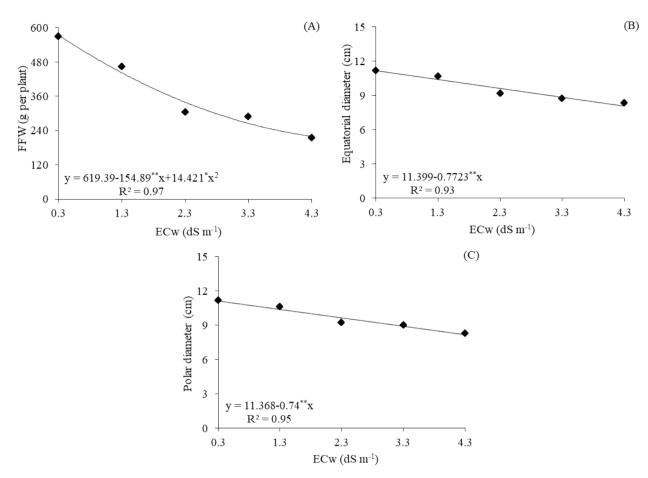


**Figure 5.** Stem diameter of mini-watermelon plants cv. Sugar Baby as a function of water salinity – ECw (A) and phosphorus doses (B) at 70 days after sowing.

Phosphorus doses significantly influenced the stem diameter of mini-watermelon plants. The regression equation (Figure 5B) shows that the maximum estimated value of SD (7.09 mm) was obtained in plants fertilized with 88% of the  $P_2O_5$ recommendation and, from this dose, there was a downward trend. By comparing plants fertilized with 120% of P<sub>2</sub>O<sub>5</sub> with those that received 60% of the recommendation, it was possible to observe a decrease of 0.36 mm in SD. Under salt stress conditions, the reduction in plant growth occurs due to the restriction in nutrient absorption caused by osmotic and ionic stresses, resulting from the high concentration of salts in the soil solution, in particular, the ions Na<sup>+</sup> and Cl<sup>-</sup>, the disorganization of the membrane system, and the production of reactive oxygen species (Lucena et al., 2012).

Regarding the fresh weight of mini-watermelon fruits (Figure 6A), the values decreased quadratically

with increases in ECw levels, and the maximum estimated value (574.22 g per plant) was obtained in plants grown under the lowest water salinity level (0.3 dS m<sup>-1</sup>), decreasing sharply from that ECw level and reaching a minimum value (222.00 g per plant) at the highest ECw level (4.3 dS m<sup>-1</sup>). By comparing the FFW of plants irrigated with an ECw of 4.3 dS m<sup>-1</sup> with that of plants subjected to water salinity of 0.3 dS m<sup>-1</sup>, it was possible to note a reduction of 354.22 (61.68%) g per plant. The reduction in FFW may be related to difficulties in the absorption of water and nutrients by plants, arising due to the decrease in the osmotic potential of the soil solution caused by the excess of salts. This situation resulted in stomatal closure, observed in this study through the reduction in stomatal conductance (Figure 1A) and, consequently, had a negative effect on the  $CO_2$  assimilation rate (Figure 3A) resulting in the production of fruits with reduced weight. Costa et al. (2013), in an experiment conducted to evaluate the production and quality of three watermelon cultivars subjected to different levels of irrigation water salinity (ranging from 0.57 to 4.91 dS m<sup>-1</sup>), found that the number of fruits, yield, and weight reduced linearly with increasing water salinity.



**Figure 6.** Fresh fruit weight - FFW (A), fruit equatorial diameter (B) and fruit polar diameter (C) of mini-watermelon plants cv. Sugar Baby as a function of water salinity - ECw, 70 days after sowing.

The equatorial (Figure 6B) and polar (Figure 6C) diameters of mini-watermelon fruits decreased linearly as the ECw levels increased, by 6.77 and 6.50% per unit increase in ECw, respectively. In plants grown under ECw of 4.3 dS m<sup>-1</sup>, FED and FPD were reduced by 3.08 and 2.96 cm compared to those irrigated with 0.3 dS m<sup>-1</sup> water. The production of fruits with a smaller diameter under salt stress conditions is also related to the diversion of energy for the maintenance of metabolic activities, as previously explained by Queiroz et al. (2012). S. S. da Silva et al. (2019) also observed the formation

of fruits with a smaller diameter in plants irrigated using water with ECw of 3.2 dS m<sup>-1</sup> in the vegetative and vegetative/flowering stages. According to these authors, this decrease in fruit size is a consequence of the reduction in water potential caused by excess salts in the soil.

#### Conclusions

Watermelon plants cv. Sugar Baby are sensitive to water salinity from 0.3 dS m<sup>-1</sup>, showing reductions in gas exchange, growth, and production. The reduction in the  $CO_2$  assimilation rate in watermelon plants cv. Sugar Baby is associated with factors of stomatal and non-stomatal origin.

Phosphorous doses equivalent to 73 and 88% of the recommendation promote an increase in the intercellular  $CO_2$  concentration and stem diameter, respectively, in mini-watermelon plants.

Fertilization with  $P_2O_5$  doses ranging from 60 to 120% of the recommendation does not mitigate the effects of salt stress on the cultivation of watermelon cv. Sugar Baby.

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