

## Water status, cell damage and gas exchanges in West Indian cherry (*Malpighia emarginata*) under salt stress and nitrogen fertilization

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### Abstract

This study was conducted to evaluate water status, cell damage and gas exchanges of West Indian cherry grown under saline water irrigation and nitrogen (N) fertilization in the post-grafting stage. The experiment was carried out in drainage lysimeters under greenhouse conditions in Regolithic Neosol with sandy loam texture. Treatments consisted of two levels of electrical conductivity of water (ECw) (0.8 and 4.5 dS m<sup>-1</sup>) and four N doses (70; 85; 100 and 115% of the N recommendation), arranged in randomized blocks, with three replicates. The dose relative to 100% corresponded to 200 g of N per plant per year. Irrigation with 4.5 dS m<sup>-1</sup> electrical conductivity water resulted in a reduction in stomatal conductance, transpiration, CO<sub>2</sub> assimilation rate and instantaneous carboxylation efficiency but increased cell damage percentage and internal CO<sub>2</sub> concentration in West Indian cherry plants. Inhibition of CO<sub>2</sub> assimilation rate in West Indian cherry plants is related to non-stomatal effects. Irrigation with 4.5 dS m<sup>-1</sup> water and fertilization with 115% of N recommendation intensified leaf water saturation deficit in the West Indian cherry crop. The BRS Jaburu West Indian cherry was sensitive to 4.5 dS m<sup>-1</sup> water salinity.

**Keywords:** *Malpighia emarginata*, salinity, nitrogen.

**Abbreviations:** gs\_stomatal conductance; E\_transpiration; A\_CO<sub>2</sub> assimilation rate, Ci\_internal CO<sub>2</sub> concentration; ECI\_instantaneous carboxylation efficiency; WUE\_instantaneous water use efficiency; ECw\_electrical conductivity of the irrigation water; water saturation deficit\_WSD, percentage of cell membrane damage\_%D),

### Introduction

Belonging to the Malpighiaceae family, West Indian cherry (*Malpighia emarginata*) is a fruit crop cultivated in Brazil, especially in the semi-arid region of the Northeast, due to the versatility of its production, standing out for its high content of ascorbic acid. In addition, it contains important bioactive compounds, such as anthocyanins, carotenoids, phenolic compounds, natural dyes, compounds with known action in the prevention of degenerative diseases, biological activity and health maintenance (Dembitsky et al., 2011). It has become an important option to diversify the economy for both enabling farmworkers to work, where they live and generating jobs for fruit growers.

Although the semi-arid region of Northeast Brazil has favorable edaphoclimatic conditions for West Indian cherry cultivation, the rainfall scarcity combined with high evaporative demand

lead to water deficit during most of the year, which makes irrigation an essential practice to guarantee the agricultural production (Oliveira et al., 2010). Thus, an alternative to increase water availability to crops can be the use of saline waters and the electrical conductivity values commonly found in the region with higher than 1.5 dS m<sup>-1</sup> (Neves et al., 2010). Excess of salts in the water reduces the osmotic potential of the soil solution, affecting water availability and causing toxicity by specific ions, nutritional imbalance and other damages related to indirect effects through physical and chemical changes in the soil (Neves et al., 2009).

In addition, presence of excessive salts in the root zone can negatively affect cell membrane integrity (Silva et al., 2010) and cause reduction in the photosynthetic capacity, leading to reduction in transpiration and increase in cell mechanical

resistance. It will happen due to the mechanism of stomatal closure, decreasing water losses by transpiration and CO<sub>2</sub> supply to the leaves (Amorim et al., 2010). Nonetheless, crop sensitivity to salt stress depends on other factors such as species, cultivar, types of salts, stress intensity and duration, crop and irrigation managements, edaphoclimatic conditions and fertilization (Deuner et al., 2011).

However, the deleterious effects caused by salinity on crops have been overcome due to the adoption of adequate fertilization management practices. In this context, mineral nutrition stands out among the main strategies employed to increase crop yield and profitability. Nitrogen (N) is one of the main macronutrients responsible for such increase (Chaves et al., 2011) due to the functions of this element in plant metabolism. It performs structural function, participating in the synthesis of amino acids, proteins, coenzymes, nucleic acids, vitamins and chlorophyll, which are among the organic compounds essential to plant survival (Cantarella, 2007).

Given its importance as constituent of various compounds, N is involved in several biochemical reactions, which are necessary for plant metabolism, standing out as activator of various enzymatic systems, many of which participate in the processes of photosynthesis and respiration, resulting in stimulus to growth (Epstein and Bloom, 2006). In this context, various studies have found that the accumulation of these organic solutes increased the osmotic adjustment capacity of plants to salinity (Silva et al., 2008; Barhoumi et al., 2010).

Based on this premise, this study aimed to evaluate the water status, cell damage and gas exchanges in West Indian cherry irrigated with saline water and N fertilization, in the post-grafting stage.

## Results and discussion

### *Effect of saline stress and nitrogen doses on water status and cell damage in West Indian cherry*

According to the summary analysis of variance (Table 1), water salinity levels had significant influence on water saturation deficit (WSD), percentage of cell membrane damage (%D), stomatal conductance (*g<sub>s</sub>*), internal CO<sub>2</sub> concentration (*C<sub>i</sub>*), leaf transpiration (*E*), CO<sub>2</sub> assimilation rate (*A*) and instantaneous carboxylation efficiency (*E<sub>ci</sub>*) in the West Indian cherry plants. Nitrogen doses (ND) did not cause significant difference in any of the variables analysed. However, the interaction between factors (SL x ND) significantly affected leaf water saturation deficit in West Indian cherry plants during the fruiting stage.

Leaf water saturation deficit was significantly affected by the interaction between factors (SL x ND). According to the regression equations (Fig 1A), the data of plants subjected to irrigation with low-salinity water (0.8 dS m<sup>-1</sup>) fitted to a quadratic model, whose minimum estimated value (15.15%) was obtained in West Indian cherry plants, which received the highest N dose. As N dose was increased, there was an accentuated reduction in WSD. However, the highest leaf water saturation deficit was occurred when West Indian cherry plants were subjected to the highest EC<sub>w</sub> level (4.5 dSm<sup>-1</sup>). The increase was 8.81% for every 15% increase in N dose. The

regression equations (Fig 1A) show that with 4.5 dS m<sup>-1</sup> water and dose of 115% of N recommendation, the WSD was increased by 3.83%, compared with plants subjected to EC<sub>w</sub> of 0.8 dS m<sup>-1</sup>. Increments of leaf water saturation deficit in West Indian cherry can be attributed to the high salt concentration in the irrigation water (EC<sub>w</sub>=4.5 dS m<sup>-1</sup>). The increasing N doses is associated with changes in the concentration of cell sap (Fig 1B), resulting from the reduction in the osmotic potential of the soil solution, which contributed to the decrease in leaf water potential. It negatively influences the water status needed to maintain physiological processes that are essential to plant survival (Benzarti et al., 2014).

According to the means comparison test for the percentage of cell membrane damage (%D) (Fig 1B), plants irrigated with low-salinity water (0.8 dS m<sup>-1</sup>) significantly differed from those subjected to EC<sub>w</sub> of 4.5 dS m<sup>-1</sup>. Comparing the means between treatments, it is noted that plants irrigated with lowest water salinity level had the lowest %D in the leaf tissue (10.66%), evidencing that cell membrane integrity was maintained in the West Indian cherry leaf tissues. Conversely, plants under high-salinity water showed the highest %D (14.08%). Thus, it is evident that high concentrations of salts caused disorganization in cell membrane, leading to reduction in homeostasis, a situation that reflects the extension of the lipid peroxidation caused by reactive oxygen species (ROS). The superoxide radical ( $\bullet\text{O}_2^-$ ), besides other highly reactive chemical species such as singlet oxygen ( $^1\text{O}_2$ ), the hydroxyl radical ( $\bullet\text{OH}^-$ ) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) can initiate lipid peroxidation (Mendes et al., 2011). Evaluating the behavior of three citrus scion/rootstock combinations under greenhouse conditions and different levels of irrigation water salinity (0.6 to 3.0 dS m<sup>-1</sup>), Sousa et al. (2017) also found that irrigation with water salinity leads to increment in the damage to cell membrane.

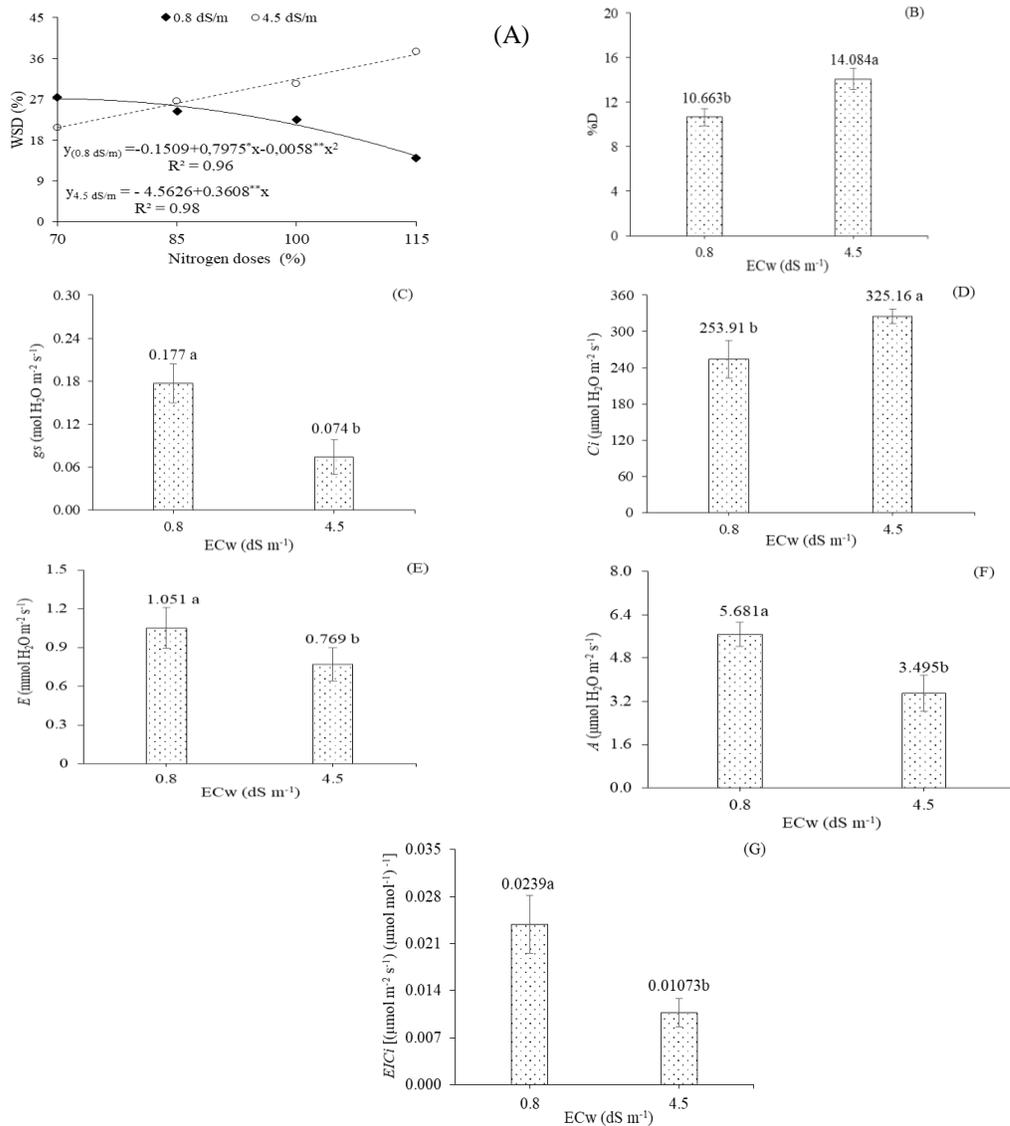
### *Effect of saline stress and nitrogen doses on gas exchanges in West Indian cherry*

Stomatal conductance in West Indian cherry was significantly affected by the irrigation with different salinity levels and, according to the means comparison test (Fig 1C), in plants subjected to the highest EC<sub>w</sub> level (4.5 dS m<sup>-1</sup>), *g<sub>s</sub>* decreased by 58.19% (0.103 mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) in comparison to those irrigated with the lowest salinity level (0.8 dS m<sup>-1</sup>). The stomatal closure observed in plants irrigated with high-salinity water (Fig 1C) possibly occurred due to the osmotic effect of the dissolved salts. This situation has deleterious effect on the gas exchanges due to reduction in osmotic potential of the solution, which limits the water flux from the soil to plants. Consequently, it reduces leaf water potential, characterizing the water stress (Lima et al., 2015). However, reduction in stomatal opening can be considered as a strategy to decrease water losses to the atmosphere and, consequently, maintain high turgor of guard cells, directly affecting the formation of carbohydrates in the photosynthesis and the production. Sá et al. (2017), evaluated the effects of saline water irrigation (EC<sub>w</sub> from 0.6 to 3.8 dS m<sup>-1</sup>) on the same cultivar (BRS 366 Jaburu),

**Table 1.** Summary of analysis of variance for leaf water saturation deficit (WSD), percentage of cell membrane damage (%D), stomatal conductance (*gs*), internal CO<sub>2</sub> concentration (*Ci*), leaf transpiration (*E*), CO<sub>2</sub> assimilation rate (*A*), instantaneous water use efficiency (*WUEi*) and instantaneous carboxylation efficiency (*EICi*) in West Indian cherry plants grown under different levels of water salinity and nitrogen doses, during the fruiting stage, in the second year of cultivation.

Source of variation	DF	Mean squares								
		WSD	%D	<i>gs</i>	<i>Ci</i>	<i>E</i>	<i>A</i>	<i>WUEi</i>	<i>EICi</i>	
Saline levels (SL)	1	267.60**	70.21**	0.0640**	30459.37*	0.47*	28.66**	7.98 <sup>ns</sup>	0.0010*	
N doses (ND)	3	17.81 <sup>ns</sup>	1.21 <sup>ns</sup>	0.0009 <sup>ns</sup>	457.37 <sup>ns</sup>	0.07 <sup>ns</sup>	1.33 <sup>ns</sup>	3.21 <sup>ns</sup>	0.00004 <sup>ns</sup>	
Linear regression	1	10.93 <sup>ns</sup>	2.94 <sup>ns</sup>	0.0014 <sup>ns</sup>	357.07 <sup>ns</sup>	0.001 <sup>ns</sup>	0.60 <sup>ns</sup>	5.06 <sup>ns</sup>	0.000001 <sup>ns</sup>	
Quadratic regression	1	6.44 <sup>ns</sup>	0.33 <sup>ns</sup>	0.0013 <sup>ns</sup>	0.04 <sup>ns</sup>	0.16 <sup>ns</sup>	0.05 <sup>ns</sup>	3.65 <sup>ns</sup>	0.000001 <sup>ns</sup>	
Interaction (SL x ND)	3	721.48**	7.64 <sup>ns</sup>	0.0032 <sup>ns</sup>	1150.81 <sup>ns</sup>	0.05 <sup>ns</sup>	2.05 <sup>ns</sup>	4.47 <sup>ns</sup>	0.00002 <sup>ns</sup>	
Blocks	2	16.29 <sup>ns</sup>	0.11 <sup>ns</sup>	0.0048 <sup>ns</sup>	8631.54 <sup>ns</sup>	0.21 <sup>ns</sup>	0.20 <sup>ns</sup>	1.99 <sup>ns</sup>	0.00004 <sup>ns</sup>	
Residual	14	282.01	2.68	0.0025	1879.20	0.07	1.23	3.96	0.00005	
CV (%)		17.62	13.25	10.25	14.97	15.29	24.23	17.79	13.41	

<sup>ns</sup>, \*\*, \* respectively, not significant, significant at  $p < 0.01$  and  $p < 0.05$ .



**Fig 1.** Leaf water saturation deficit – WSD (A) as a function of the interaction between levels of irrigation water salinity – ECw and nitrogen doses, and percentage of cell membrane damage - %D (B), stomatal conductance – *gs* (C), internal CO<sub>2</sub> concentration – *Ci* (D), leaf transpiration – *E* (E), CO<sub>2</sub> assimilation rate – *A* (F) and instantaneous carboxylation efficiency – *EICi* (G) in West Indian cherry plants irrigated using solutions with different ECw levels, during the fruiting stage. Bars represent the standard error of the mean (n=3). Mean with different letter indicates that treatments differ by Tukey test,  $p < 0.05$ .

**Table 2.** Physical and chemical characteristics of the soil used in the experiment.

Chemical characteristics								
pH (H <sub>2</sub> O) (1:2.5)	OM g kg <sup>-1</sup>	P mg kg <sup>-1</sup>	K <sup>+</sup> .....cmol <sub>c</sub> kg <sup>-1</sup> .....	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Al <sup>3+</sup>	H <sup>+</sup>
5.58	2.93	39.2	0.23	1.64	9.07	2.78	0.00	8.61
..... Chemical characteristics.....			..... Physical characteristics.....					
CEC cmol <sub>c</sub> kg <sup>-1</sup>	ESP (%)	ECse dS m <sup>-1</sup>	SAR (mmol L <sup>-1</sup> ) <sup>0.5</sup>	Size fraction (g kg <sup>-1</sup> )			Water content - dag kg <sup>-1</sup>	
22.33	7.34	2.15	0.67	Sand	Silt	Clay	33.42 kPa <sup>1</sup>	1519.5 kPa
				572.7	100.7	326.6	25.91	12.96

pH – hydrogen potential, OM – Organic matter: Walkley-Black Wet Digestion; Ca<sup>2+</sup> and Mg<sup>2+</sup> extracted with 1 M KCl at pH 7.0; Na<sup>+</sup> and K<sup>+</sup> extracted using 1 M NH<sub>4</sub>OAc 1M at pH 7.0; Al<sup>3+</sup> + H<sup>+</sup> extracted using 0,5 M CaOAc pH 7.0; ECse – electrical conductivity of the saturation extract; CEC - Cation exchange capacity; SAR – Sodium adsorption ratio - (mmol L<sup>-1</sup>)<sup>0.5</sup>; ESP – Exchangeable sodium percentage.

also found reduction in *gs*, which was equal to 14% per unit increase in irrigation water salinity.

Unlike the trend observed for stomatal conductance, the internal CO<sub>2</sub> concentration of West Indian cherry plants irrigated with 4.5 dS m<sup>-1</sup> water was statistically higher than that of plants subjected to ECw of 0.8 dS m<sup>-1</sup> (Fig 1D). There was an increase of 71.25 μmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in the *Ci* of plants subjected to irrigation with ECw of 4.5 dS m<sup>-1</sup> compared with those subjected to 0.8 dS m<sup>-1</sup>. Based on the data of internal CO<sub>2</sub> concentration (Fig 1D), it becomes evident that its lower value observed in plants subjected to ECw of 0.8 dSm<sup>-1</sup> is associated with the higher CO<sub>2</sub> assimilation rate (Fig 1F). However, the increase in internal CO<sub>2</sub> concentration in plants cultivated under high salinity (4.5 dS m<sup>-1</sup>) suggests that the carbon entering the leaf mesophyll cell was not being metabolized by the photosynthetic apparatus due to the low activity of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), as reported by Habermann et al. (2003). In addition, such increase in internal CO<sub>2</sub> concentration is an indication that there was no restriction in CO<sub>2</sub> acquisition by the crop. However, when it reached the mesophyll cells, the fixation process during the carboxylation phase was compromised (Freire et al., 2014), which can be related to the degradation of the photosynthetic apparatus in response to the senescence of leaf tissues, resulting from the stress caused by the excess of salts (Silva et al., 2013).

Leaf transpiration (*E*) also significantly differed as a function of the different salinity levels (Fig 1E), and its highest value (1.05 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) was obtained when low-salinity water (0.8 dS m<sup>-1</sup>) was used in irrigation. Nonetheless, West Indian cherry plants irrigated using water with ECw of 4.5 dS m<sup>-1</sup> showed *E* of 0.76 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, i.e., reduction of 26.83% in comparison to those subjected to ECw of 0.8 dS m<sup>-1</sup>. Thus, the partial closure of the stomata (Fig 1C) associated with the osmotic effects of salinity led to reduction in leaf transpiration (Fig 1E). This may have affected the capacity of water absorption by the root system as a strategy of this species to tolerate the salt stress, aiming to reduce the absorption of toxic ions dissolved, especially Na<sup>+</sup> and Cl<sup>-</sup>, decreasing the toxicity by specific ions (Syvertsen and Garcia-Sanchez, 2014).

Carbon dioxide assimilation rate (*A*) is directly influenced by stomatal conductance and based on the means comparison test (Fig 1F), West Indian cherry plants subjected to ECw of 0.8 dSm<sup>-1</sup> were statistically different from those irrigated with 4.5

dS m<sup>-1</sup> water. There was a reduction of 38.48% in *A* (2.18 μmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) between plants subjected to the highest (4.5 dS m<sup>-1</sup>) and lowest (0.8 dS m<sup>-1</sup>) salinity levels. The reduction in CO<sub>2</sub> assimilation rate in plants irrigated with high-salinity water can be attributed to the stomatal closure. This evidenced in the present study by the stomatal conductance (Fig 1C), by which the consequent reduction in the normal CO<sub>2</sub> flux towards the carboxylation site was one of the main factors responsible for the reduction in photosynthesis (Bosco et al., 2009). In addition, the reduction in cell turgor associated with the toxic effects, particularly of Na<sup>+</sup> and Cl<sup>-</sup>, also contributed to the reduction in *A*. Likewise, Sousa et al. (2016), in a study evaluating gas exchanges of citrus (scion/rootstock combinations) as a function of irrigation water salinity (ECw from 0.6 to 3.0 dS m<sup>-1</sup>), also observed reduction in CO<sub>2</sub> assimilation rate, equal to 20.33% per unit increase in ECw. According to the means comparison test (Fig 1G), the instantaneous carboxylation efficiency (*EICi*) was significantly reduced in West Indian cherry plants irrigated with the highest ECw level (4.5 dS m<sup>-1</sup>), compared with those subjected to 0.8 dS m<sup>-1</sup>. The *EICi* of plants irrigated with 4.5 dS m<sup>-1</sup> water was decreased by 55.10% [0.0131 (μmol m<sup>-2</sup> s<sup>-1</sup>) (μmol mol<sup>-1</sup>)<sup>-1</sup>], compared to plants under the lowest salinity level (0.8 dS m<sup>-1</sup>). The expressive reduction in the *EICi* of West Indian cherry plants subjected to 4.5 dS m<sup>-1</sup> could be resulted from the low CO<sub>2</sub> assimilation rate (Fig 1F), which is attributed to the CO<sub>2</sub> found in the substomatal chamber (Fig 1C). It is evident that if the internal CO<sub>2</sub> concentration increases, a reduction in CO<sub>2</sub> consumption in the chloroplasts may occur due to reduction in photosynthesis. The *EICi* ratio will also decrease. Lúcio et al. (2013) added that the osmotic and toxic effects of excess salts associated with stomatal and non-stomatal causes are also responsible for the reduction in the *EICi* ratio.

## Materials and methods

### Localization, experimental procedure, treatments and plant material

The experiment was carried out in pots equipped with drainage lysimeters under greenhouse conditions, at the Center of Technology and Natural Resources of the Federal University of Campina Grande (CTRN/UFCG), located in the municipality of Campina Grande, PB, Brazil, at the local

geographic coordinates 7° 15' 18" S and 35° 52' 28" W, at 550 m of altitude.

The experimental design was randomized blocks with three replicates in a 2 x 4 factorial arrangement. The treatments consisted of two levels of electrical conductivity of the irrigation water – ECw (0.8 and 4.5 dS m<sup>-1</sup>) and four N doses [70; 85; 100 and 115% of the dose recommended by Cavalcante et al. (2008)]. The dose relative to 100% corresponded to 200 g of N per plant per year.

Irrigation solutions with the respective ECw levels were prepared by dissolving the salts NaCl, CaCl<sub>2</sub>.2H<sub>2</sub>O and MgCl<sub>2</sub>.6H<sub>2</sub>O, at equivalent proportion of 7:2:1, respectively, in water (ECw = 0.6 dS m<sup>-1</sup>) from the public supply system of Campina Grande, PB, based on the relationship between ECw and the concentration of salts (10\*mmol<sub>c</sub> L<sup>-1</sup> = ECw dS m<sup>-1</sup>), according to Richards (1954).

### Establishment and management of the experiment

The lysimeters were filled with a 1-kg layer of crushed stone nº 0, followed by 250 kg of eutrophic Regolithic Neosol with sandy loam texture, properly pounded to break up clods, from the rural area of the municipality of Esperança, PB. Its chemical and physical characteristics (Table 2) were obtained according to the methodologies proposed by Donagema et al. (2011).

A 4-mm-diameter drain was installed at the bottom of each lysimeter to collect drained water for evaluation and to determine plant water consumption. The tip of the drain inside the pot was wrapped with a nonwoven geotextile (Bidim OP 30) to avoid clogging by soil material.

The rootstocks used in the experiment were 'Crioula' West Indian cherry seedlings from EMBRAPA Tropical Agroindustry, located in Pacajus-CE. At transplantation, the seedlings were 240 days old. During the acclimation period in the greenhouse, West Indian cherry plants were irrigated with low-salinity water (0.8 dS m<sup>-1</sup>). BRS 366 Jaburu was used as scion variety. This cultivar stands out for its high yield (57 t ha<sup>-1</sup>), which favors the production of vitamin C (2,648 mg 100g<sup>-1</sup>), with height of about 1.87 cm and crown diameter of 2.18 m. Its fruits are shiny, with mean weights of 4 to 5 g when unripe, adequate to obtain vitamin C, and 6 to 7 g after ripening (EMBRAPA, 2012).

Before transplanting the seedlings, the soil was brought to field capacity using the solutions of the respective treatments. After transplanting, irrigation was daily performed, by applying a water volume to maintain soil moisture close to field capacity. The volume was applied according to the crop water requirement, estimated by water balance: volume applied minus volume drained in the previous irrigation, plus a leaching fraction of 0.10.

Fertilization with potassium and phosphorus were carried out as recommended by Cavalcante (2008), by applying equivalent to 333.3 and 230.7 g per plant, respectively, of potassium chloride and monoammonium phosphate. Nitrogen and potassium were split into 12 equal parts, applied monthly. Phosphorus was supplied in 3 equal applications, at 20-day intervals. To meet probable deficiencies of micronutrients, plants were weekly sprayed with a solution of Ubyfol containing 1.5 g L<sup>-1</sup> [(N (15%); P<sub>2</sub>O<sub>5</sub> (15%); K<sub>2</sub>O (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%)].

### Traits measured

At 90 days after pruning of formation, in the second year of cultivation, i.e., during the stage of full flowering of the West Indian cherry crop, the following parameters were evaluated: water saturation deficit (WSD), percentage of cell membrane damage (%D) and gas exchanges, through stomatal conductance (*gs*), transpiration (*E*), CO<sub>2</sub> assimilation rate (*A*), internal CO<sub>2</sub> concentration (*Ci*), instantaneous water use efficiency (*WUEi*) and instantaneous carboxylation efficiency (*E/Ci*). Stomatal conductance (*gs* - mol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), CO<sub>2</sub> assimilation rate (*A*) (μmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>), transpiration (*E*) (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) and internal CO<sub>2</sub> concentration (*Ci*) (μmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>) were measured at the height of the middle third of the crown, using a portable infrared gas analyser (IRGA - LCPro+ Portable Photosynthesis System®). After data collection, instantaneous water use efficiency (*WUEi*) (*A/E*) [(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>] and instantaneous carboxylation efficiency (*E/Ci*) (*A/Ci*) [(μmol m<sup>-2</sup> s<sup>-1</sup>) (μmol mol<sup>-1</sup>)<sup>-1</sup>] were quantified. At the same time, the percentage of cell membrane damage was determined following the methodology of Scotti-Campos *et al.* (2013), according to Eq. 1:

$$\%D = \frac{ECi}{ECf} \times 100 \dots \dots \dots (1)$$

Where: %D = percentage of cell membrane damage (%); ECi= initial electrical conductivity (dS m<sup>-1</sup>); ECf= final electrical conductivity (dS m<sup>-1</sup>);

Leaf water saturation deficit was determined following the methodology described by Taiz & Zeiger (2013), according to Eq. 2:

$$WSD = \frac{TW-FW}{TW-DW} \times 100 \quad (2)$$

Where: WSD = water saturation deficit (%); FW= fresh weight of leaf (g); TW= turgid weight of leaf (g); DW = dry weight of leaf (g).

### Statistical analysis

The data obtained in the experiment were subjected to analysis of variance by F test and when significant, means comparison test (Tukey at 0.05 probability level) was applied to the water salinity levels and regression analysis was applied to the N doses. When the interaction between factors (SL X ND) was significant, a follow-up analysis was carried out for saline levels with respect to N doses, using the statistical program SISVAR-ESAL (Ferreira, 2011).

### Conclusions

Irrigation with 4.5 dS m<sup>-1</sup> electrical conductivity water resulted in a reduction in stomatal conductance, transpiration, CO<sub>2</sub> assimilation rate and instantaneous carboxylation efficiency, but increased cell damage percentage and internal CO<sub>2</sub> concentration in West Indian cherry plants. Inhibition of CO<sub>2</sub> assimilation rate in West Indian cherry plants is related to non-stomatal effects. Irrigation with 4.5 dS m<sup>-1</sup> water associated with the highest N dose intensifies leaf water saturation deficit in West Indian cherry. Nitrogen doses did not mitigate the

effects of the salt stress on water saturation deficit, cell damage and gas exchanges in West Indian cherry plants in the post-grafting stage. BRS Jaburu West Indian cherry is sensitive to 4.5 dS m<sup>-1</sup> water salinity.

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