

NPK combinations mitigate the deleterious effects of salt stress on the morphophysiology of West Indian Cherry

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Abstract

Under salt stress, plant growth and development are negatively affected due to physiological changes, requiring strategies such as fertilization management to minimize these effects. In this scenario, this study aimed to evaluate the effect of combinations of nitrogen, phosphorus, and potassium on the growth, leaf water status, electrolyte leakage, and gas exchange of West Indian Cherry grown under water stress in the second year of production. The experiment was conducted in a protected environment in Campina Grande - PB. The treatments were distributed in a randomized block design with a 2 × 10 factorial arrangement with three replications corresponding to two electrical conductivity levels of irrigation water– ECw (0.6 and 4.0 dS m⁻¹) and ten combinations of fertilization with nitrogen, phosphorus, and potassium (80-100-100; 100-100-100; 120-100-100; 140-100-100; 100-80-100; 100-120-100; 100-140-100; 100-100-80; 100-100-120, and 100-100-140% of the recommendation in the second year of production). Irrigation with the ECw of 4.0 dS m⁻¹ negatively affected plant growth, the leaf water status, electrolyte leakage, and the leaf gas exchange of West Indian Cherry. However, the 40% increase (C4 -140-100-100% of the recommended N-P₂O₅-K₂O level) in the nitrogen level mitigated the deleterious effects of salt stress on the relative water content, internal CO₂ concentration, and the CO₂ assimilation rate of West Indian Cherry plants in the second year of production.

Keywords: *Malpighia emarginata*, mineral nutrition, salinity

Introduction

The semi-arid region of Brazil shows low rainfall and high evaporation rates, which favors salt accumulation in water sources used for irrigation and prevents their use in agricultural production (Dantas et al., 2022). Adding to this scenario, the estimates indicate that climate changes will reduce the incidence of rainfall by about 15% and cause temperature increases from 1 to 4 °C in semi-arid areas, making the situation even worse (Marengo et al., 2009). From this perspective, salt stress reduces the soil osmotic potential, hindering water and nutrient uptake by plants. Furthermore, the excess absorption of ions such as sodium (Na⁺) and chloride (Cl⁻) causes toxicity and nutrient imbalance (Dutra et al., 2022), directly affecting plant growth and physiology.

Thus, due to the limitations in the use of saline water for irrigation, the development of fruit farming in the Brazilian semi-arid region can be compromised, affecting

the country's visibility as one of the largest fruit producers in the world in a current scenario, only surpassed by China and India (FAO, 2021).

From this perspective, West Indian Cherry (*Malpighia emarginata*) is a fruit of tropical climate with a wide economic and nutritional potential, especially due to the high vitamin C content present in its fruits (Mezadri et al., 2008). In 2017, the Brazilian production of this fruit amounted to approximately 61 thousand tons produced in about 7,000 hectares, with the Northeast region being responsible for 78.1% (47,607 tons) of national production, mainly in the States of Pernambuco, Ceará, Sergipe, and Paraíba (IBGE, 2017), putting Brazil in the leadership of worldwide West Indian Cherry production (FAO, 2021).

The use of saline water in irrigated fruit farming can be made possible by adequate fertilization management, contributing to agricultural sustainability in the semi-arid region of Brazil. Therefore, properly

managing fertilization with nitrogen, phosphorus, and potassium can mitigate the deleterious effects caused by salinity due to the reduction in the uptake of Na^+ and Cl^- ions through competitive inhibition (Silva et al., 2022). In this scenario, it is understood that combinations of nitrogen, phosphorus, and potassium can significantly contribute to the morphophysiology of West Indian Cherry since similar previous studies have already shown promising results, e.g., the mitigation of salt stress and the consequent increase in sugar apple production (Sá et al., 2021), significant improvements in the growth, physiology, and production of the West Indian Cherry cv. "BRS Jaburu" (Sá et al., 2019), and reductions in the effects of salt stress on the anthocyanin and ascorbic acid contents of West Indian Cherry fruits (Lacerda et al., 2021). Therefore, the present study aimed to evaluate the effect of combined fertilization with nitrogen, phosphorus, and potassium on the growth, leaf water status, electrolyte leakage, and gas exchange of West Indian Cherry grown under salt stress in the second year of production.

Material And Methods

The experiment was developed in a protected environment at the Academic Unit of Agricultural Engineering– UAEA of the Federal University of Campina Grande – UFCG, in the municipality of Campina Grande, Paraíba (7°15'18" S, 35°52'28" W, and at a mean elevation of 550 m a.s.l.). The plant nursery used in the study was bow-shaped, measuring 30 m in length, 21 m in width, and with a height of 3.0 m. The structure was covered by low-density polyethylene (150 microns) with an infrared treatment. The data on temperature (maximum and minimum) and relative air humidity in the experimental location observed in the period are shown in (Figure 1).

The experiment began in March 2020 with the acquisition of grafted West Indian Cherry seedlings. The rootstock and scion corresponded to the cultivars Junco

and Flor Branca, respectively, and the cleft grafting technique was used. The seedlings were provided by a commercial orchard registered with the National Registry of Seeds and Seedlings, located in the São Gonçalo District, Sousa – PB.

The study was conducted in 200-L pots adapted as drainage lysimeters filled with a 1.0 kg layer of gravel and 230 kg of soil classified as Entisol (USA, 2014). The soil used in the containers came from the 0-20 cm depth layer and was collected in the municipality of Riachão do Bacamarte – PB, and its physicochemical characteristics (Table 1) were determined according to (Teixeira et al., 2017).

The treatments consisted of two levels of electrical conductivity of irrigation water - ECw (0.6 and 4.0 dS m^{-1}) and 10 fertilization combinations (C) with nitrogen, phosphorus, and potassium – NPK ($C_1 = 80\text{-}100\text{-}100$; $C_2 = 100\text{-}100\text{-}100$; $C_3 = 120\text{-}100\text{-}100$; $C_4 = 140\text{-}100\text{-}100$; $C_5 = 100\text{-}80\text{-}100$; $C_6 = 100\text{-}120\text{-}100$; $C_7 = 100\text{-}140\text{-}100$; $C_8 = 100\text{-}100\text{-}80$; $C_9 = 100\text{-}100\text{-}120$; and $C_{10} = 100\text{-}100\text{-}140\%$ of the recommendation of Cavalcante (2008) for $\text{N-P}_2\text{O}_5\text{-K}_2\text{O}$), under a 2×10 factorial arrangement distributed in randomized block design with three replications, totaling 60 experimental units.

The combination of 100-100-100% corresponded to the application of 100, 60, and 60 g of N, P_2O_5 , and K_2O per plant, respectively, in the first year of cultivation. The salinity levels were established according to studies conducted by (Silva et al., 2020).

The irrigation water with the electrical conductivity levels of 0.6 and 4.0 dS m^{-1} was prepared by dissolving NaCl, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ at the equivalent proportion of 7:2:1, respectively, in local tap water ($\text{ECw} = 0.38 \text{ dS m}^{-1}$). The preparation of irrigation water considered the relationship between the ECw and the concentration of salts (Richards, 1954), according to Equation 1. After the irrigation water was prepared, the electrical conductivity was determined and adjusted before its use.

$$Q \approx 10 \times \text{ECw} \quad (1)$$

Where: Q – sum of cations ($\text{mmol}_e \text{ L}^{-1}$); ECw – water electrical conductivity (dS m^{-1}).

Thirty days after the seedlings were transplanted to the lysimeters, irrigation with saline water began by adopting a two-day irrigation schedule. Water was applied to each lysimeter according to the respective treatments to maintain soil moisture close to field capacity, and the volume applied was determined according to the water requirement of the plants, estimated based on the soil water balance using Equation 2:

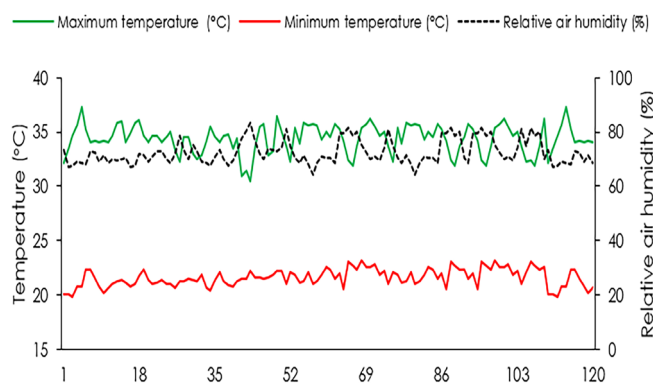


Figure 1. Daily maximum and minimum temperatures and relative air humidity observed inside the plant nursery during the experimental period.

Table 1. Chemical and physical characteristics of the soil (0-20 cm) used in the experiment before the application of treatments

| Chemical characteristics | | | | | | | | | | |
|--------------------------------------|------------------------------------|--|---|------------------------------------|------------------|--------------------------------------|-----------------------------------|-------|-------------------------|-------------------------|
| pH H ₂ O | O.M. | P | K ⁺ | Na ⁺ | Ca ²⁺ | Mg ²⁺ | Al ³⁺ + H ⁺ | | | |
| 1:2.5 | g dm ⁻³ | mg dm ⁻³ |cmol _c kg ⁻¹ | | | | | | | |
| 6.5 | 8.1 | 79 | 0.24 | 0.51 | 14.90 | 5.40 | 0.90 | | | |
| Chemical characteristics | | | | | | Physical characteristics | | | | |
| EC _{se} | CEC | SAR _{se} | ESP | SB | V | Particle fraction | | | Moisture content | |
| dS m ⁻¹ | cmol _c kg ⁻¹ | (mmol L ⁻¹) ^{0.5} | % | cmol _c kg ⁻¹ | % | (g kg ⁻¹) | | | (dag kg ⁻¹) | |
| 2.15 | 21.95 | 0.16 | 2.3 | 21.05 | 95.89 | Sand | Silt | Clay | 33.42 kPa ¹ | 1519.5 kPa ² |
| | | | | | | 572.7 | 100.7 | 326.6 | 25.91 | 12.96 |

pH – potential of hydrogen; O.M. – organic matter; Walkley-Black Wet digestion; Ca²⁺ and Mg²⁺ - extracted with 1 M KCl at pH 7.0; Na⁺ and K⁺ - extracted with NH₄OAc 1 M at pH 7.0; Al³⁺ + H⁺ - extracted with CaOAc 0.5 M at pH 7.0; EC_{se} – Electrical conductivity of the saturation extract; CEC – Cation exchange capacity; SAR_{se} – Sodium adsorption ratio of the saturation extract; ESP – Percentage of exchangeable sodium; SB – Sum of bases (K⁺ + Ca²⁺ + Mg²⁺ + Na⁺); V – Base saturation ([SB/CEC] × 100); ¹⁻² – Referring to field capacity and the permanent wilting point, respectively.

$$VI = \frac{(Va - Vd)}{(1-FL)} \tag{2}$$

Where: VI – water volume to be used at the irrigation event (mL); Va – water volume applied at the previous irrigation event anterior (mL); Vd – water volume drained after the previous irrigation event (mL); LF – 0.10 leaching fraction, applied every 90 days to prevent excess salt accumulation.

Fertilization with nitrogen, phosphorus, and potassium was performed via topdressing and split into 24 plots at 15-day intervals. Calcium nitrate, monoammonium phosphate, and potassium sulfate were used as sources of nitrogen, phosphorus, and potassium, respectively. Every 15 days, 1.0 g L⁻¹ of Dripsol® (Mg: 1.1%; Zn: 4.2%; B: 0.85%; Fe: 3.4%; Mn: 3.2%; Cu: 0.5%; Mo: 0.05%) was applied on the adaxial and abaxial leaf surfaces to supply the plants with micronutrients. Furthermore, crop management practices such as cleaning pruning, manual hoeing, soil scarification, and phytosanitary control were performed during the experiment.

At the end of the first year of cultivation, the plants were subjected to 15 days of water stress followed by a cleaning pruning, after which the second production year began. For the second year, the water electrical conductivity levels and the combinations of NPK fertilization used in the first year were maintained. However, the NPK combinations were adapted for the second production year of West Indian Cherry, following the suggestion of (Cavalcante, 2008).

The application of treatments in the second year of production began 15 days after pruning (DAP). The management of fertilization, irrigation, and phytosanitary control used in the first year of cultivation were also kept for the second year.

The following variables were evaluated 120 days after pruning (DAP): relative water content, percentage of electrolyte leakage, and the leaf gas exchange variables of stomatal conductance (gs), transpiration (E), CO₂ assimilation rate (A), and internal CO₂ concentration

(Ci). With the leaf gas exchange results, the instantaneous water-use efficiency (WUEi) (A/E) and the instantaneous carboxylation efficiency (CEi) (A/Ci) were quantified. The gas exchange parameters were measured in the third leaf pair located to the east of the plant, counted from the apex of the main branch. An irradiation of 1200 μmol photons m⁻² s⁻¹ and an airflow of 200 mL min⁻¹ were applied using a portable photosynthesis measurer model LCPro+ of ADC BioScientific Ltda.

At the same time (120 DAP), the branch growth of West Indian Cherry was evaluated using a metric tape by dividing the plant into quadrants, with quadrant 1 corresponding to the northern branches (1 - North), quadrant 2 to the eastern branches (2 - East), quadrant 3 to the southern branches (3 - South), and quadrant 4 to the western branches (4 - West).

The relative water content (RWC) was determined by removing six leaves from the main branch to obtain six leaf disks 12 mm wide from each leaf. Immediately after collection, the branches were weighed to prevent loss of moisture, thus obtaining their fresh mass (FM); next, these samples were placed in a beaker, immersed in 50 mL of distilled water, and stored for 24 h. After this period, the excess water from the disks was removed with a paper towel, and the turgid mass (TM) of the samples was obtained. Next, the samples were oven-dried to constant weight at 65 ± 3 °C to obtain the dry mass (DM) of the samples. The RWC was determined according to the methodology described by (Lima et al., 2015) using Equation 3:

$$RWC = \frac{FM - DM}{TM - DM} \times 100 \tag{3}$$

Where: RWC – relative water content (%), FM - fresh leaf mass (g), TM – turgid mass (g), and DM – dry mass (g).

The percentage of electrolyte leakage (% IEL) was determined using a copper perforator to obtain five leaf disks 12 mm wide per experimental unit, which were washed and placed in Erlenmeyer® containers containing

50 mL of distilled water. After closed in aluminum paper, the Erlenmeyer® containers were stored at 25 °C for 24 h, after which the initial conductivity of the medium (Xi) was measured using a benchtop conductivity meter (MB11, MS Techonopon®). Next, the Erlenmeyer® containers were subjected to the temperature of 90 °C for 120 minutes in a drying oven (SL100/336, SOLAB®), after which the material was cooled and the final conductivity was measured (Xf). The percentage of electrolyte leakage in the leaf blade was expressed as the percentage of initial electrical conductivity in relation to the electrical conductivity after the treatment for 120 minutes at 90° C, according to the methodology proposed by (Scotti-Campos et al., 2013), considering Equation 4:

$$\% \text{ IEL} = \frac{X_i}{X_f} \times 100 \tag{4}$$

Where: % IEL - percentage of electrolyte leakage, Xi - initial electrical conductivity, and Xf - final electrical conductivity.

The data were tested for the normality of distribution (Shapiro-Wilk test). Next, the analysis of variance (F-test) was performed for the electrical conductivity levels of irrigation water. The means of the fertilization combinations (NPK) were compared using the Scott-Knott clustering test, and both analyses were performed using the software Sisvar (Ferreira, 2019).

Results And Discussion

The interaction between the electrical conductivity levels of irrigation water and the fertilization combinations influenced ($p \leq 0.01$) the relative water content (RWC) (Table 2). In isolation, the salinity levels affected ($p \leq 0.01$) the RWC and the percentage of electrolyte leakage (% IEL), and the fertilization combinations interfered with the RWC.

The relative water content (Figure 2) of the West Indian Cherry plants irrigated with the ECw of 0.6 dS m⁻¹ differed ($p \leq 0.01$) from those irrigated with the ECw of 4.0 dS m⁻¹ under all fertilization combinations. The plants

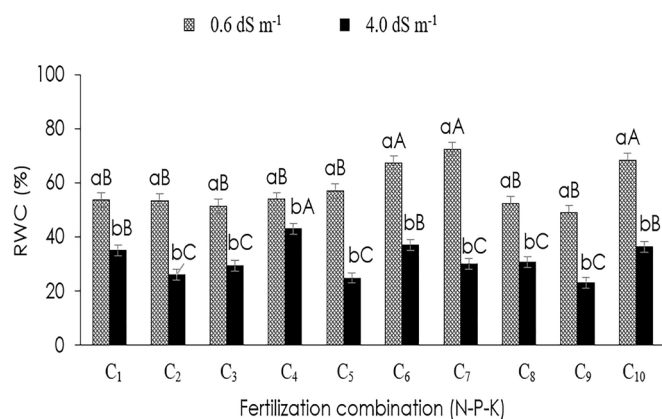


Figure 2. Relative water content – RWC in West Indian Cherry leaves as a function of the interaction between irrigation water salinity– ECw and the combinations of NPK fertilization 120 days after pruning in the second year of production. C₁ = 80-100-100; C₂ = 100-100-100; C₃ = 120-100-100; C₄ = 140-100-100; C₅ = 100-80-100; C₆ = 100-120-100; C₇ = 100-140-100, C₈ = 100-100-80, C₉ = 100-100-120, and C₁₀ = 100-100-140% of the recommended N-P₂O₅-K₂O level; Means with the same uppercase letters indicate no significant difference between the N-P₂O₅-K₂O fertilization combinations for the same water by the Scott-Knott test at 0.05 of probability, and equal lowercase letters in the same fertilization combination indicate no significant difference between salinity levels (Fisher test, $p \leq 0.05$).

irrigated with the ECw of 0.6 dS m⁻¹ and fertilized with combinations C₆, C₇, and C₁₀ recorded the highest RWC values, differing ($p \leq 0.01$) from the other combinations. Also, the plants irrigated with the ECw of 0.6 dS m⁻¹ and fertilized with combinations C₆, C₇, and C₁₀ had a RWC on average 15.9% higher compared to those under 100-100-100% N-P₂O₅-K₂O (C₂) and irrigated with the same salinity level. Although the mean RWC values are considered low, especially for plants under stress, studies with West Indian Cherry (Malpighia emarginata D.C.) and soursop (Annona muricata L.) showed similar results. In West Indian Cherry, a RWC of 40.07 was observed when using 125% K₂O regardless of the salinity condition (Pinheiro et al., 2019). For soursop, irrigation with the water electrical conductivity of 4.0 dS m⁻¹ resulted in a RWC of 70% and 80% compared to irrigation with 0.8 dS m⁻¹ (Silva et al., 2021). Another important factor to be considered is that, at the moment of the evaluations, the soil moisture content was 13.21% and 18.52% when irrigation was performed with 0.6 and 4.0 dS m⁻¹, respectively, which can justify a possible increase in the soil osmotic potential and thus reduce water availability to plants, resulting in a lower relative water content in the leaves.

It is known that the relative water content (RWC), for representing the maximum water volume that the cell vacuoles can receive under total turgidity, is an important indicator of the water status of plants under abiotic stresses (Khatami et al., 2022). Therefore, the increase in the RWC as a function of the higher offer of K₂O and

Table 2. Summary of the analysis of variance for the relative water content (RWC) and percentage of electrolyte leakage (% IEL) of West Indian Cherry irrigated with saline water and subjected to different fertilization combinations with NPK 120 days after pruning in the second year of production

| Source of variation | GL | Mean square | |
|------------------------------------|----|----------------------|--------------------|
| | | RWC | % IEL |
| Water electrical conductivity –ECw | 1 | 10138.44** | 81.77** |
| Fertilization combinations – NPK | 9 | 277.94** | 1.72 ^{ns} |
| Interaction (ECw × NPK) | 9 | 322.11** | 1.51 ^{ns} |
| Blocks | 2 | 102.60 ^{ns} | 0.06 ^{ns} |
| Residual | 38 | 35.11 | 0.99 |
| CV (%) | | 13.00 | 10.01 |

^{ns, **} respectively not significant and significant at $p \leq 0.05$ and $p \leq 0.01$.

P_2O_5 , observed in the present study, may have occurred because potassium plays important roles in physiological processes. (Lima et al., 2018) add that the balanced potassium fertilization in West Indian Cherry improved the osmotic regulation and the maintenance of ionic homeostasis under salt stress conditions. Furthermore, the increase in K_2O and P_2O_5 can reduce the uptake of Na^+ and Cl^- by plants due to the competition between these nutrients in absorption sites (Meneghette et al., 2017), which can have effects in the reduction of the deleterious effects of salt stress.

It should be noted that, despite the RWC reductions in the plants irrigated with the ECw of 4.0 dS m^{-1} (Figure 2), the 40% increase in the nitrogen level ($C_4 = 140-100-100\%$ of the recommended N- P_2O_5 - K_2O) resulted in the highest RWC value (42.91%), differing ($p \leq 0.01$) from the other combinations. For (Ashraf et al., 2018), adequate nitrogen concentrations can contribute to a higher synthesis of low molecular weight compounds such as glycine, betaine, and proline, which act as membrane osmoprotectants and macromolecules, assisting in plant osmotic adjustment to salinity.

The percentage of electrolyte leakage (% IEL) (Figure 3) of the plants irrigated with 0.6 dS m^{-1} differed ($p \leq 0.01$) from those grown under the ECw of 4.0 dS m^{-1} . When comparing the mean values between treatments, the plants under the ECw of 4.0 dS m^{-1} show a higher % IEL value (23.24%) compared to those irrigated with the lowest salinity (0.6 dS m^{-1}), which recorded a % IEL of 12.25%. Therefore, the % IEL increase observed in the plants irrigated with the ECw of 4.0 dS m^{-1} may be related to the cell membrane damage induced by reactive oxygen species (ROS). For (Astaneh et al., 2022), plants under salt stress conditions show an expressive increase in the

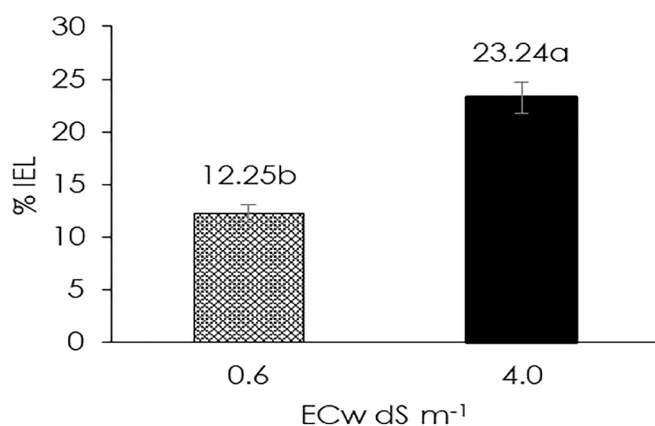


Figure 3. Mean percentage of electrolyte leakage - % IEL in the leaf blade of West Indian Cherry as a function of the electrical conductivity of irrigation water under different NPK concentrations 120 days after pruning in the second year of production.

production of ROS, which disrupts the cell membrane and causes greater electrolyte leakage. Furthermore, the salt excess in the irrigation water promotes the accumulation of toxic ions, which induce lipid peroxidation and destabilize the production of lipids, proteins, and membrane nucleic acids, limiting the maintenance of cell turgor (Sharma et al., 2020). This observation corroborates the hypothesis of the possible consequences caused by the reduction in the relative leaf water content of West Indian Cherry in the present study (Figure 2).

A similar trend was observed by (Silva et al., 2021) in a study with the West Indian Cherry cv. 'BRS 336 Jaburu' under ECw levels ranging from 0.3 to 4.3 dS m^{-1} . In their study, the researchers found a higher IEL (26.64%) in plants grown under the ECw of 4.3 dS m^{-1} and a lower IEL (12.44%) at the ECw of 0.3 dS m^{-1} .

There was an effect of the interaction ($p \leq 0.01$) between the salinity levels and fertilization combinations on the internal CO_2 concentration (C_i) and the CO_2 assimilation rate (A) (Table 3). All gas exchange variables were significantly affected by the salinity levels of irrigation water when analyzed in isolation, contrasting with the fertilization combinations, which did not affect any variable.

The C_i (Figure 4) of the plants irrigated with the electrical conductivity of 4.0 dS m^{-1} differed ($p \leq 0.01$) from those grown under the ECw of 0.6 dS m^{-1} . There was an increase in the C_i of the plants irrigated with the highest salinity level (4.0 dS m^{-1}) under all fertilization combinations

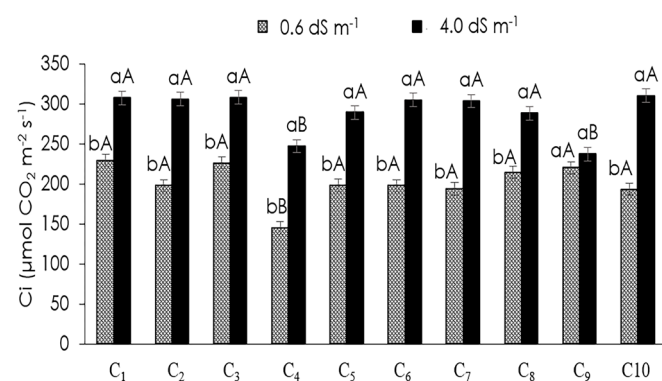


Figure 4. Internal CO_2 concentration - C_i of West Indian Cherry as a function of the interaction between irrigation water salinity - ECw and NPK fertilization combinations 120 days after pruning in the second production year.

Table 3. Summary of the analysis of variance for the internal CO₂ concentration (Ci), stomatal conductance (gs), transpiration (E), CO₂ assimilation rate (A), instantaneous carboxylation efficiency (CEi), and instantaneous water-use efficiency (WUEi) of West Indian Cherry plants irrigated with saline water and subjected to different combinations of NPK fertilization 120 days after pruning in the second year of production

| Source of variation | GL | Mean squares | | | | | |
|------------------------------------|----|-----------------------|----------------------|---------------------|--------------------|--------------------|------------------------|
| | | Ci | gs | E | A | WUEi | CEi |
| Water electrical conductivity -ECw | 1 | 43201.7** | 0.0008* | 0.081* | 16.01** | 33.6** | 4.9×10 ^{-4**} |
| Fertilization combinations - NPK | 9 | 2728.2 ^{ns} | 0.0002 ^{ns} | 0.021 ^{ns} | 0.59 ^{ns} | 2.08 ^{ns} | 2.4×10 ^{-5ns} |
| Interaction (ECw × NPK) | 9 | 8723.2** | 0.0001 ^{ns} | 0.025 ^{ns} | 1.62** | 7.61 ^{ns} | 2.7×10 ^{-5ns} |
| Blocks | 2 | 20003.5 ^{ns} | 0.0011 ^{ns} | 0.167 ^{ns} | 2.11 ^{ns} | 2.96 ^{ns} | 4×10 ^{-ns} |
| Residual | 38 | 1593.13 | 0.0001 | 0.017 | 0.43 | 4.09 | 1.2×10 ⁻⁵ |
| CV (%) | | 16.3 | 27.9 | 20.3 | 21.4 | 20.2 | 26.8 |

^{ns, *} respectively not significant and significant at $p \leq 0.05$ and $p \leq 0.01$.

except C₉ (100-100-120% of the recommended N-P₂O₅-K₂O level). The increase in the internal CO₂ concentration could be related to the increase in Rubisco oxygenase to the detriment of carboxylase, thus resulting in higher photorespiration under stress (Prywes et al., 2022).

Various studies have reported increases in the internal CO₂ concentration as a function of the increase in the electrical conductivity of irrigation water, e.g., the study of (Dias et al., 2018) with West Indian Cherry, in which the authors observed that plants grown under the highest ECw (3.8 dS m⁻¹) showed a Ci of 278.58 μmol CO₂ m⁻² s⁻¹, corresponding to an increase of 38.03% (76.75 μmol CO₂ m⁻² s⁻¹) in relation to plants irrigated with the ECw of 0.3 dS m⁻¹. In another study, (Lima et al., 2019) observed a higher Ci (330.83 μmol CO₂ m⁻² s⁻¹) in plants irrigated with the ECw of 4.5 dS m⁻¹, with a 93.57% increase (159.92 μmol CO₂ m⁻² s⁻¹) in relation to plants irrigated with the ECw of 0.8 dS m⁻¹.

Despite the Ci increase observed in plants irrigated with the ECw of 4.0 dS m⁻¹, those grown under combinations C₄ (140-100-100% of the recommended N-P₂O₅-K₂O level) and C₉ (100-100-120% of the recommended N-P₂O₅-K₂O level) differed ($p \leq 0.01$) from the other combinations, showing Ci reductions (Figure 4) of 19.28 and 22.55% in combinations C₄ and C₉, respectively, compared to the plants of the control treatment (C₂) irrigated with the same salinity level. According to (Ahanger et al., 2017), potassium is involved in the translocation and maintenance of water balance and participates in various biochemical and physiological functions, e.g., osmoregulation and the reduction in excessive uptake of ions such as Na⁺. This scenario may have contributed to the better efficiency of Rubisco carboxylase and, consequently, Ci reduction (Figure 4). Furthermore, the 40% increase in the N level may have favored the synthesis of amino acids and soluble sugars, which contribute to the osmotic adjustment of plants to salinity (Ashraf et al., 2018).

Stomatal conductance (Figure 5A) in plants irrigated with the ECw of 0.6 dS m⁻¹ differed significantly

in relation to plants grown under the ECw of 4.0 dS m⁻¹. When comparing the means of the treatment, the plants under the ECw of 0.6 dS m⁻¹ showed the highest gs (0.028 mmol H₂O m⁻² s⁻¹), whereas those grown under the highest salinity (4.0 dS m⁻¹) recorded a gs of 0.020 mmol H₂O m⁻² s⁻¹, i.e., a reduction of 28.6%. According to (Silva et al., 2021), plants tend to close their stomata under salt stress in response to the reduction in the relative water content in the leaves (Figure 2). However, despite the reduction in stomatal conductance in plants irrigated with the highest salinity (4.0 dS m⁻¹), there was no restriction in the internal CO₂ concentration (Figure 4) due to the Ci increase in the plants subjected to the highest salinity.

Lima et al., (2020) also observed reductions in the gs when evaluating the gas exchange in West Indian Cherry plants under salt stress (ECw of 0.8 and 4.5 dS m⁻¹) 90 days after formation pruning in the second production year. The authors observed the highest gs value (0.177 mmol H₂O m⁻² s⁻¹) in plants irrigated with the ECw of 0.8 dS m⁻¹, whereas plants subjected to the highest ECw (4.5 dS m⁻¹) showed a gs of 0.074 mmol H₂O m⁻² s⁻¹, i.e., a reduction of 58.2% (0.103 mmol H₂O m⁻² s⁻¹) compared to the plants irrigated with the ECw of 0.8 dS m⁻¹.

Transpiration (Figure 5B) also differed significantly as a function of the electrical conductivity of irrigation

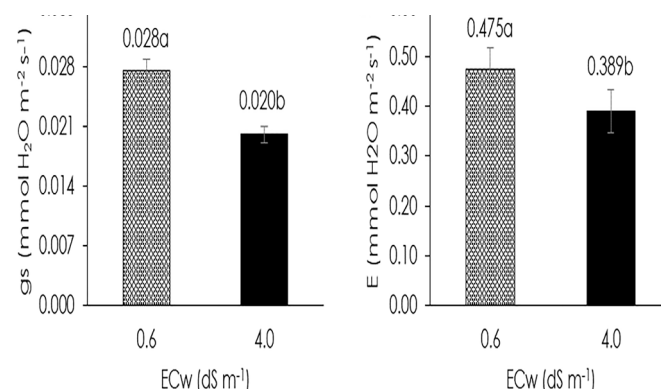


Figure 5. Stomatal conductance - gs (A) and transpiration - E (B) of West Indian Cherry as a function of irrigation water salinity-ECw (A) under different combinations of NPK fertilization 120 days after pruning in the second year of production.

water, achieving its highest value ($0.475 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) in plants irrigated with the lowest ECw (0.6 dS m^{-1}). However, the West Indian Cherry plants irrigated with the ECw of 4.0 dS m^{-1} showed an E of $0.389 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, i.e., a reduction of 18.1% compared to those grown under the ECw of 0.6 dS m^{-1} . Thus, the partial stomatal closure (Figure 5A) associated with the osmotic effects of salinity decreased leaf transpiration (Figure 5B).

The reduction in transpiration is associated with water restriction events caused by salt accumulation in the soil, decreasing the osmotic potential close to the roots and reducing the water uptake. The reduction in transpiration is a way to avoid water loss to the environment by maintaining the water potential in the leaves and preventing the dehydration of guard cells (Lima et al., 2020).

The deleterious effects of salt stress were also observed on the CO_2 assimilation rate (Figure 6) through reductions in the A parameter under all fertilization except C_4 , even without restrictions to CO_2 entry into the substomatal chamber (Figure 4). It is also noted that the highest A value ($3.86 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was recorded in the plants irrigated with the ECw of 0.6 dS m^{-1} and grown under fertilization combination C_4 (140-100-100 % of the recommended N-P₂O₅-K₂O level), differing ($p \leq 0.01$) from the other combinations. It should also be noted that the C_4 combination also provided the highest A value ($2.48 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in the plants irrigated with the ECw of 4.0 dS m^{-1} . This increase could be related to the fact that supplementary N application may have increased the uptake of NO_3^- to the detriment of Cl^- , reducing the Cl^-/N ratio in the leaves, which can reestablish ionic homeostasis and reduce the effects of salt stress in plants (Ibrahim et al., 2018).

According to the means comparison test for the instantaneous carboxylation efficiency (Figure 7A) and the instantaneous water-use efficiency (Figure 7B), the plants irrigated with the ECw of 4.0 dS m^{-1} obtained the lowest CEi ($0.014 \text{ [}(\mu\text{mol m}^{-2} \text{ s}^{-1}) (\mu\text{mol mol}^{-1})^{-1}\text{]}$) and WUEi values ($4.21 \text{ [}(\mu\text{mol m}^{-2} \text{ s}^{-1}) (\mu\text{mol mol}^{-1})^{-1}\text{]}$). When comparing the CEi and WUEi of West Indian Cherry plants irrigated with the ECw of 4.0 dS m^{-1} in relation to those grown under the ECw of 0.6 dS m^{-1} , there was a 36.4% reduction in the CEi and a 28.5% reduction in the WUEi. The CEi is a variable used to identify the action of non-stomatal factors that interfere with the CO_2 assimilation rate (Lima et al., 2022). Therefore, the reduction in the CEi of plants can be related to the increase in photorespiration and the reduction in the activity of carboxylase due to the low substrate availability (ATP and NADPH) for enzyme activation

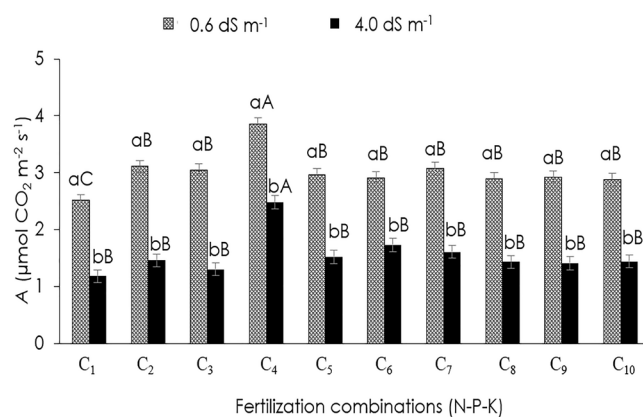


Figure 6. CO_2 assimilation rate - Ci of West Indian Cherry as a function of the interaction between irrigation water salinity - ECw and the combinations of NPK fertilization 120 days after pruning in the second year of production. $C_1 = 80-100-100$; $C_2 = 100-100-100$; $C_3 = 120-100-100$; $C_4 = 140-100-100$; $C_5 = 100-80-100$; $C_6 = 100-120-100$; $C_7 = 100-140-100$; $C_8 = 100-100-80$; $C_9 = 100-100-120$, and $C_{10} = 100-100-140\%$ of the recommended N-P₂O₅-K₂O level; Means with the same uppercase letters indicate no significant difference between the combinations of N-P₂O₅-K₂O fertilization by the Scott-Knott test at 0.05 of probability, and equal lowercase letters in the same fertilization combination indicate no significant difference between salinity levels (Fisher test, $p < 0.05$).

Figure 6. CO_2 assimilation rate - Ci of West Indian Cherry as a function of the interaction between irrigation water salinity - ECw and the combinations of NPK fertilization 120 days after pruning in the second year of production.

and regeneration resulting from the accumulation of salts in leaf tissues, especially Na^+ and Cl^- (Prywes et al., 2022), and the action of other environmental factors favoring to oxygenation of RuBisCO and the increase in the photorespiratory pathway, significantly reducing the carbon compounds (Voss et al., 2013).

Dias et al., (2021) evaluated the effect of salt stress (ECw ranging from 0.6 to 3.8 dS m^{-1}) on the gas exchange parameters of West Indian Cherry and observed that the highest CEi values ($0.024 \text{ [}(\mu\text{mol m}^{-2} \text{ s}^{-1}) (\mu\text{mol mol}^{-1})^{-1}\text{]}$) were obtained in plants irrigated with the ECw of 0.6 dS m^{-1} . On the other hand, the lowest CEi values ($0.0144 \text{ [}(\mu\text{mol m}^{-2} \text{ s}^{-1}) (\mu\text{mol mol}^{-1})^{-1}\text{]}$) were observed in the plants grown under the ECw of 3.8 dS m^{-1} , corresponding to a reduction of

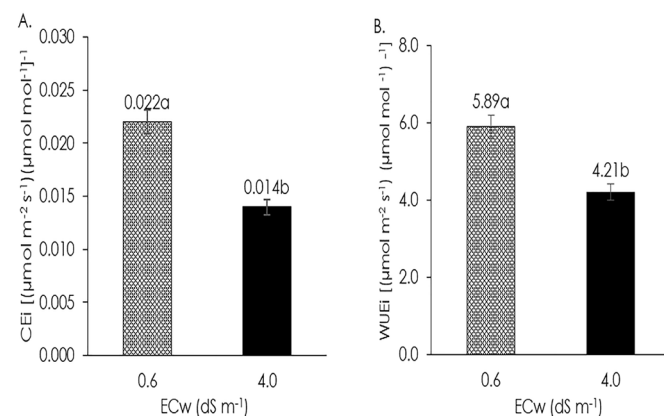


Figure 7. Instantaneous carboxylation efficiency - CEi (A) and instantaneous water-use efficiency - WUEi (B) of West Indian Cherry as a function of irrigation water salinity- ECw (A) 120 days after pruning in the second year of production.

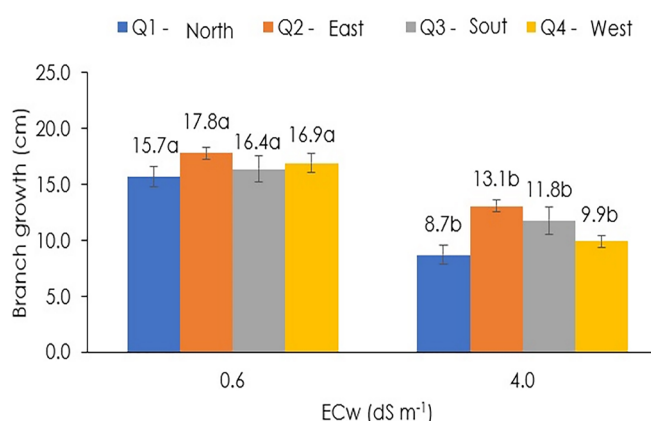
40.0% in relation to those irrigated with the lowest salinity (0.6 dS m⁻¹). Plants try to overcome osmotic stress by reducing the uptake of toxic ions, stomatal conductance (Figure 5A), and transpiration (Figure 5B) to increase the water-use efficiency and the relative water content in their leaves. However, as observed in the present study, this mechanism was not sufficient to increase the instantaneous water-use efficiency in West Indian Cherry plants under salt stress conditions. The WUEi reduction as a function of the increase in the electrical conductivity of irrigation water can be attributed to the change in the soil osmotic potential caused by salt excess, resulting in the reduction of water availability to plants (Pinheiro et al., 2022).

When evaluating branch growth in the different quadrants, there was an influence (p ≤ 0.01) of the electrical conductivity of irrigation water (Table 4) 120 days after pruning.

Irrigation with the ECw of 4.0 dS m⁻¹ negatively affected branch growth in West Indian Cherry in all quadrants analyzed (Figure 8) when comparing the plants irrigated with the highest salinity (4.0 dS m⁻¹) with those irrigated with the lowest salinity (0.6 dS m⁻¹), with reductions of 44.6, 26.4, 28.0, and 41.4% in this variable in the North, East, South, and West quadrants, respectively. The branches of the North and West quadrants were the most damaged. In turn, the branches of the East quadrant, irrigated with the 0.6 dS m⁻¹, obtained the highest growth (17.8 cm).

Plant growth is influenced by light and the biosynthesis of substances, phototropism, and photostimulation. Auxin, produced by the apical meristem, is a plant hormone whose functions include cell growth and elongation (Larcher, 2003). Light causes the migration of auxin to the darkest or shaded side of the plant, where the auxin promotes greater cell elongation and plant growth, which may have influenced the higher branch growth in the eastern quadrant.

Salt stress is one of the main factors that reduce plant growth and plant yield. In this study, branch growth and leaf gas exchanges were compromised in plants



Means with equal lowercase letters in the same quadrant indicate no significant difference between salinity levels (Fisher test, p < 0.05).

Figure 8. Branch growth of West Indian Cherry in the different quadrants as a function of irrigation water salinity 120 days after sowing in the second year of production.

exposed to the higher electrical conductivity of irrigation water. At high concentrations, water and/or soil salinity affects water and nutrient uptake, compromising growth and all plant physiological processes (Silva et al., 2021).

Conclusions

1. Irrigation with the ECw of 4.0 dS m⁻¹ negatively affects plant growth, the leaf water status, electrolyte leakage in the leaf blade, and leaf gas exchange in West Indian Cherry plants 120 days after pruning.
2. The 40% increase in the nitrogen level (C₄-140-100-100% of the recommended N-P₂O₅-K₂O level) mitigates the deleterious effects of salt stress on the relative water content, the internal CO₂ concentration, and the CO₂ assimilation rate of West Indian Cherry plants in the second year of production.
3. The proper management of fertilization is recommended for West Indian Cherry producers in the semi-arid region of Brazil to mitigate the deleterious effects of salt stress caused by irrigation with saline water.

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Table 4. Summary of the analysis of variance for the branch growth of West Indian Cherry in different quadrants (Q1 - North, Q2 - East, Q3 - South, and Q4 - West) 120 days after pruning (DAP) in the second year of production

| Sources of variation | GL | Mean Squares | | | |
|-------------------------------------|----|---------------------|--------------------|--------------------|---------------------|
| | | Q1 - North | Q2 - East | Q3 - South | Q4 - West |
| Water electrical conductivity - ECw | 1 | 723.8** | 335.6* | 318.8** | 715.5** |
| Fertilization combinations - NPK | 9 | 50.1 ^{ns} | 59.2 ^{ns} | 56.5 ^{ns} | 35.8 ^{ns} |
| Interaction (ECw × NPK) | 9 | 36.8 ^{ns} | 40.6 ^{ns} | 95.0 ^{ns} | 69.9 ^{ns} |
| Blocks | 2 | 213.9 ^{ns} | 8.8 ^{ns} | 13.8 ^{ns} | 200.4 ^{ns} |
| Residual | 38 | 30.1 | 51.9 | 43.9 | 51.1 |
| CV (%) | | 24.1 | 24.2 | 24.6 | 29.1 |

^{ns}, * respectively not significant and significant at p ≤ 0.05 and p ≤ 0.01.

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