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Original Article

Photosynthetic pigments and quantum yield of West Indian cherry under salt stress and NPK combinations

Pigmentos fotossintéticos e rendimento quântico de aceroleira sob estresse salino e combinações de NPK

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Abstract

West Indian cherry cultivation has proved to be an important economic activity in northeastern Brazil. However, irrigation with brackish waters limits cultivation, requiring new strategies to minimize the effect of salt stress. In this context, the present study aimed to evaluate the effect of nitrogen (N), phosphorus (P), and potassium (K) combinations on the photosynthetic pigments and quantum yield of West Indian cherry cultivated under salt stress, in the second year of production. The assay was conducted in a protected environment by adopting an experimental design in randomized blocks, with treatments distributed in a 2×10 factorial arrangement referring to two electrical conductivity levels of irrigation water - ECw (0.6 and 4.0 dS m^{-1}) and 10 NPK fertilization combinations - FC (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), with three replications, each consisting of one plant. Irrigation with the electrical conductivity of 4.0 dS m^{-1} negatively affected the synthesis of photosynthetic pigments and the photochemical efficiency of the West Indian cherry cv. Flor Branca. The NPK combinations did not attenuate the effects of salt stress on the analyzed variables. However, the combinations referring to 120-100-100%, 140-100-100%, and 100-120-100% of NPK recommendation improved the quantum yield of photosystem II by reducing the initial fluorescence and increasing the maximum fluorescence of the West Indian cherry cv. Flor Branca.

Keywords: *Malpighia emarginata,* mineral nutrition, salinity.

Resumo

Na região Nordeste do Brasil, o cultivo da acerola tem se mostrado uma importante atividade econômica; entretanto, a irrigação com águas salobras limita o cultivo, sendo necessário a busca por estratégias que minimizem o efeito do estresse salino. Neste contexto, objetivou-se avaliar combinações de adubação com nitrogênio (N), fósforo (P) e potássio (K) sobre os teores de pigmentos fotossintéticos e o rendimento quântico do fotossistema II da aceroleira cv. Flor Branca cultivada sob estresse salino, no segundo ano de produção. O ensaio foi conduzido em ambiente protegido sob delineamento experimental em blocos casualizados, com os tratamentos distribuídos em esquema fatorial 2×10, referentes a dois níveis de condutividade elétrica da água de irrigação - CEa (0,6 e 4,0 dS m^{-1}) e 10 combinações de adubação com NPK - CA (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 e 100-100-140% da recomendação referente ao segundo ano de produção), com três repetições cada uma constituída por uma planta. A irrigação com água de 4,0 dS m^{-1} de condutividade elétrica reduziu os teores de pigmentos fotossintéticos e o rendimento quântico do fotossistema II da aceroleira. As combinações de NPK não atenuaram os efeitos do estresse salino sobre as variáveis analisadas. Entretanto, as combinações de 120-100-100, 140-100-100 e 100-120-100% da recomendação de NPK melhoraram o rendimento quântico do fotossistema II, uma vez que reduziram a fluorescência inicial e aumentaram a fluorescência máxima da aceroleira cv. Flor Branca.

Palavras-chave: *Malpighia emarginata,* nutrição mineral, salinidade.

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1. Introduction

West Indian cherry (*Malpighia emarginata*) is a tropical fruit species with a wide economic and nutritional potential, mainly due to the high vitamin C content present in the fruits (Mezadri et al., 2008). Brazil is the largest producer of this species worldwide (FAO, 2021), with its Northeast region concentrating 78% (47.607 tons) of the national production of West Indian cherry, mainly in the States of Pernambuco, Ceará, Sergipe, and Paraíba (IBGE, 2017). However, problems related to water resources are still a limiting in factor to increasing productivity.

Abiotic salt stress is one of the main restrictions to productivity and agricultural sustainability (Wei et al., 2020; Gökçe et al., 2023), and global climate changes have increased atmospheric temperatures and drought events, favoring water scarcity and salinity (McFarlane et al., 2020; Silva et al., 2020). Approximately 20% of agricultural lands in the world are affected by salt stress, mostly in arid and semiarid regions (Abdelraheem et al., 2019; Hou et al., 2023).

Excess of salts in water and/or soil causes the osmotic effect, which hinders the uptake of nutrients and water by plants (Souza et al., 2023) and causes nutrient imbalance. Sanity damages the thylakoid membrane, inhibiting the biosynthesis of photosynthetic pigments, which reduces the quantum yield of photosystem II, consequently affecting the photosynthetic efficiency (Sharma et al., 2020; Arruda et al., 2023). In this scenario, chlorophyll fluorescence is an important process that serves as an indicator of the physiological responses of plants and has been used to study the inhibition of photosystem II activity under stress conditions (Dubey et al., 2021; Khatri and Rathore, 2022).

Plants must use strategies to reduce the uptake of Na^+ and maintain ionic homeostasis, increasing the accumulation of osmolytes and activating the antioxidant defense system (Siddiqui et al., 2017). The adequate management of fertilization could be an efficient strategy to reduce the adverse effects of salinity (Machado and Serralheiro, 2020). Nitrogen acts directly in the synthesis of photosynthetic pigments and participates in the formation of important proteins and amino acids in plant metabolism (Siddiqui et al., 2019). Phosphorus, in turn, integrates structural compositions and participates in respiration and photosynthesis, in addition to being involved in energy release processes for metabolic reactions, which favors nitrogen uptake and assimilation (Simão et al., 2018). Potassium favors carbohydrate formation and translocation and acts in the regulation of stomatal movement and water uptake by plants (Araújo et al., 2012; Silva et al., 2022).

From this perspective, this study is based on the hypothesis that the adequate combination of nitrogen, phosphorus and potassium can mitigate the deleterious effects caused by irrigation with saline water on the synthesis of photosynthetic pigments and the quantum yield of West Indian cherry. Therefore, this study aimed to evaluate effect of different combinations of nitrogen, phosphorus and potassium on the photosynthetic pigments and quantum yield of the photosystem II of the West Indian cherry cv. Flor Branca grown under salt stress in the second year of production.

2. Material and Methods

2.1. Location of the experiment

The experiment was conducted in drainage lysimeters in a greenhouse of the Agricultural Engineering Academic Unit of the Federal University of Campina Grande, in the municipality of Campina Grande, Paraíba, Brazil (coordinates: 7º 15’18” S, 35º 52’28” W, at an elevation of 550 m a.s.l.). The temperature (maximum and minimum) and mean relative humidity of air data during the experimental period are shown in Figure 1.

2.2. Treatments and experimental design

The treatment consisted of two electrical conductivity levels of irrigation water - ECw (0.6 and 4.0 dS m^{-1}) and ten fertilization combinations (FC) with nitrogen, phosphorus and potassium – NPK (FC1 = 80-100-100; FC2 = 100-100-100; FC3 = 120-100-100; FC4 = 140-100-100; FC5 = 100-80-100; FC6 = 100-120-100; FC7 = 100-140-100; FC8 = 100-100-80; FC9 = 100-100-120; and FC10 = 100-100-140% of the NPK recommendation for the species, according to Cavalcante, 2008), in a 2×10 factorial arrangement distributed randomly and three replications, totaling 60 experimental replications (Figure 2).

The fertilization combination control – FC2 (100-100-100%) corresponded to the annual application of 100, 60, and 60 g of N, P_2O_5, and K_2O per plant, using as a reference the recommendation for the second year of cultivation, whereas the salinity treatments used in the present assay were based on the study conducted by Silva et al. (2020).

2.3. Setting up and execution of the experiment

In March 2020, grafted West Indian cherry seedlings (having as rootstock and scion the cultivars Junco and Flor Branca, respectively) were purchased from a commercial nursery registered with the National Registry of Seeds and Seedlings, located in the São Gonçalo District, municipality of Sousa, Paraíba, Brazil.

The grafted seedlings (30 cm high, four-months-old) were transplanted to 200-L drainage lysimeters whose inside was covered with a geotextile fabric and filled with 1.0 kg of gravel (type 1: from 9.5 to 19 mm) and 230 kg of soil (Figure 3).

The soil used to carry out the study was classified as an *Neossolo Regolítico* in the Brazilian soil classification system (EMBRAPA, 2018), which corresponds to a Entisol (United States, 2014), collected in the municipality of Riachão do Bacamarte – PB (7° 15’ 34” S and 35° 40’ 1” W),

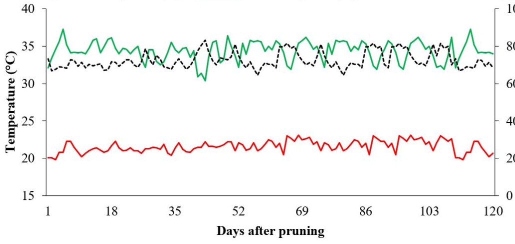

Figure 1. Daily maximum and minimum temperatures and relative air humidity inside the greenhouse during the experimental period.

from the 0-20 cm layer. The physicochemical characteristics were determined according to Teixeira et al. (2017), and the results are shown in Table 1.

The irrigation water with the electrical conductivity levels of 0.6 and 4.0 dS m^{-1} was prepared by dissolving NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ in the equivalent proportion of 7:2:1, respectively, in local tap water (ECw = 0.38 dS m^{-1}), according to Medeiros et al. (2003). The irrigation water was prepared by considering the relationship between the ECw and the concentration of salts (Richards, 1954), according to Equation 1:

$$Q \cong 10 \times ECw \tag{1}$$

where: Q – sum of cations ($mmol_c$ L^{-1}); and ECw – water electrical conductivity (dS m^{-1}).

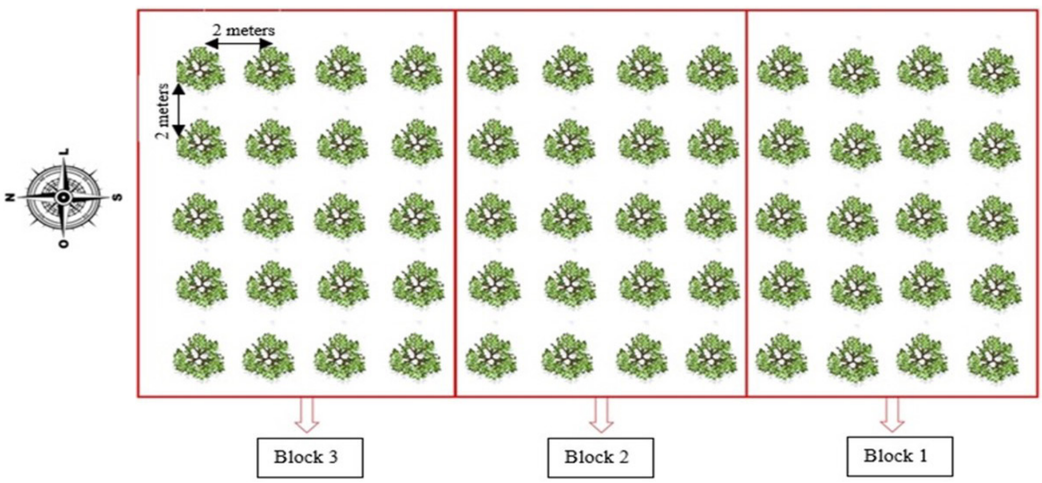

Figure 2. Layout of the experimental area.

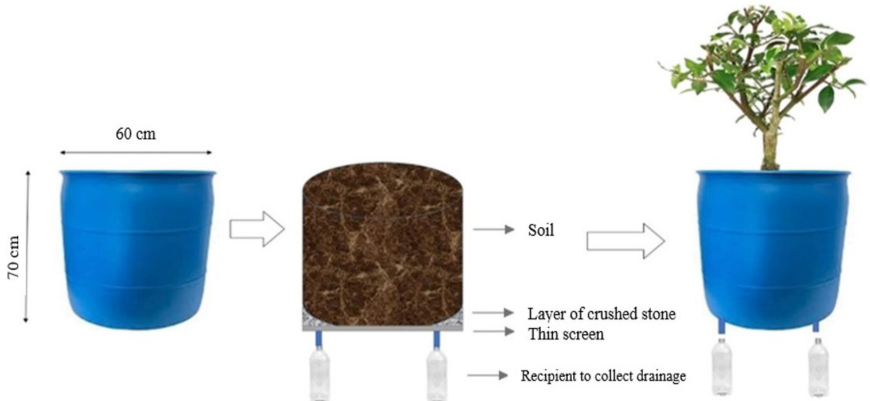

Figure 3. Illustration of the filling of drainage lysimeters.

Table 1. Chemical and physical attributes of the soil (0-20 cm depth) used in the experiment.

Chemical attributes										
pH H_2O	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 attributes						Physical attributes				
EC_{se}	CEC	SAR_{se}	ESP	SB	V	Particle fraction g kg^{-1}			Moisture content dag kg^{-1}	
dS m^{-1}	$cmol_c$ kg^{-1}	(mmol $L^{-1})^{0.5}$	%	$cmol_c$ kg^{-1}	%	Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
2.15	21.95	0.16	2.3	21.05	95.9	572.7	100.7	326.6	25.91	12.96

pH – potential of hydrogen; O.M. – organic matter: Walkley-Black Wet digestion; Ca^{2+} and Mg^{2+} - extracted with 1 M KCl at pH 7.0; Na^+ and K^+ - extracted with NH_4OAC 1 M at pH 7.0; $Al^{3+} + H^+$ - 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 – Exchangeable sodium percentage; SB – Sum of bases ($K^+ + Ca^{2+} + Mg^{2+} + Na^+$); V – Base saturation ([SB/CEC] × 100). [1–2] – Referring to field capacity and the permanent wilting point, respectively.

Further details regarding the execution and management of the assay adopted in the first year of cultivation can be found in Souza et al. (2023). At the end of the first year of experimental cultivation, the West Indian cherry plants were subjected to water stress for 15 days, after which the first cycle pruning was performed, thus starting the second year of production. The electrical conductivity of water and the NPK fertilization combinations used in the second year of production were the same as in the first year.

Fifteen days after pruning, irrigation with saline water was performed by adopting a two-day irrigation schedule. Water was applied to each lysimeter according to the treatments in order to maintain soil moisture close to field capacity, the volume applied being determined according to the plants water requirements, estimated by the water balance in the soil using Equation 2:

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

where: VI – water volume to be used in the irrigation event (mL); Va – water volume applied in the previous irrigation event (mL); Vd - volume drained after the previous irrigation event (mL); andLF – leaching fraction - 0.10 applied every 30 days to prevent excess salt accumulation.

Fertilization with nitrogen, phosphorus and potassium was applied as topdressing and split into 24 applications at 15-day intervals. Calcium nitrate, monoammonium phosphate and potassium sulfate were used as sources of nitrogen, phosphorus + nitrogen, and potassium, respectively. Every 15 days, a Dripsol® micro solution (with the following composition: Mg -1.1%; Zn - 4.2%; B - 0.85%; Fe - 3.4%; Mn - 3.2%; Cu - 0.5%; and Mo - 0.05%), at the concentration of 1.0 g L^{-1} was applied via foliar application on the adaxial and abaxial surfaces, using a backpack sprayer (model Jacto XP, with a capacity of 12 L), with a JD 12P nozzle, a maximum working pressure of 88 psi, and applying a mean volume of 400 mL. Crop management practices such as weed removal, hoeing, soil scarification, and phytosanitary controls were performed as per necessity during the experiment.

2.4. Traits analyzed

The following variables were evaluated 120 days after pruning (DAP): chlorophyll fluorescence: initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv = Fm - F_0), and quantum efficiency of photosystem II (Fv/Fm), and the photosynthetic pigments: chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), total chlorophyll (Chl *t*), and carotenoids (Car).

The chlorophyll fluorescence was evaluated in the third leaf counted from the apex of the plant's main branch between 8:00 and 10:00 a.m., using an OS5p Pulse Modulated Chlorophyll Fluorometer made by Opti Science and adopting the Fv/Fm protocol. This protocol was performed after the leaves were adapted to the dark for at least 30 min using a clip from the equipment to ensure that all acceptors were oxidized, i.e., the reaction centers were opened (Sá et al., 2015).

The photosynthetic pigments (chlorophyll *a*, *b*, chlorophyll total, and carotenoids) were quantified in the third leaf (the same leaf used for chlorophyll fluorescence analysis), according to Arnon (1949), with plant extracts obtained using disk samples of leaf (diameter of 12 mm and a mean mass of approximately 34.2 mg) from the blade of the third fully expanded leaf counting from the apex of previously identified branches. Each sample used 6.0 mL of acetone P.A. diluted 80% in deionized water. Through these extracts, it was possible to determine the chlorophyll and carotenoid concentrations in the solutions using a spectrophotometer at the absorbance wavelengths (ABS) of 470, 647, and 663 nm through Equations 3, 4, 5, and 6:

$$\text{Chl } a = (12.25 \times \text{ABS663}) - (2.79 \times \text{ABS647}) \quad (3)$$

$$\text{Chl } b = (21.5 \times \text{ABS647}) - (5.10 \times \text{ABS647}) \quad (4)$$

$$\text{Chl } t = (7.15 \times \text{ABS663}) + (18.71 \times \text{ABS647}) \quad (5)$$

$$\text{Car} = \frac{\left|(1000 \times \text{ABS470}) - (1.82 \times \text{Cl } a) - (85.02 \times \text{Cl } b)\right|}{198} \quad (6)$$

where: Chl *a* - chlorophyll *a*;Chl *b* - chlorophyll *b*; Chl *t* - chlorophyll total; and Car - carotenoids;

The values obtained for the contents of chlorophyll *a*, *b*, chlorophyll total, and carotenoids in the leaves were expressed as µg mL^{-1}.

The West Indian cherry production data referring to this study, previously presented by Silva Filho et al. (2023a), were used to discuss the correlation between the production variables and the variables referring to chlorophyll fluorescence and photosynthetic pigments to explore and analyze the impact of various factors on the yield of West Indian cherry irrigated with saline water and fertilized with different combinations of nitrogen, phosphorus, and potassium.

2.5. Statistical analysis

The data were tested for the normality of distribution (Shapiro-Wilk test) at the probability level of 0.05. Then, the analysis of variance was performed by Fisher's test (F) for the electrical conductivity levels of irrigation water. The means of the fertilization combinations (NPK) were compared by the Scott-Knott clustering test. Both analyses were performed using the software SISVAR, version 5.6 (Ferreira, 2019).

A multivariate analysis of principal components (PCs) was performed to synthesize the amount of relevant information obtained from the sets of original data into fewer dimensions, resulting from linear combinations of the original variables generated based on eigenvalues higher than one ($\lambda \geq 1.0$) in the correlation matrix, explaining percentages higher than 10% in the total variance (Kaiser, 1960; Govaerts et al., 2007). Based on the reduction of dimensions, the original data of the variables of each component were subjected to a multivariate analysis of variance (MANOVA) by the Hotelling test at $p \leq 0.05$. For PC4, the analysis of variance (ANOVA) was only performed to project the graphs. The variables with a correlation coefficient equal to or higher than 0.5 were maintained in each principal component (PC), according to Hair et al. (2009). The software Statistica v. 7.0 was used in the statistical analyses (StatSoft Inc, 2004).

3. Results and Discussion

According to the analysis of variance (Table 2), there was no significant effect of fertilization combinations and interaction ($p > 0.05$) between the electrical conductivity levels of irrigation water and fertilization combinations on the contents of photosynthetic pigments (Chl *a*, Chl *b*, Chl *t*, and Car) of West Indian cherry, 120 days after pruning. In isolation, the salinity levels affected ($p \leq 0.01$) the contents of Chl *a*, Chl *b*, Chl *t*, and Car at $p \leq 0.05$.

When comparing the means of the ECw levels, the plants under the ECw of 4.0 dS m^{-1} showed the lowest Chl *a* (456.02 µg mL^{-1}), whereas those grown under the lowest water salinity level (0.6 dS m^{-1}) showed the highest Chl *a* value (572.81 µg mL^{-1}) (Figure 4A). A similar effect was also observed with chlorophyll *b*, i.e., the lowest Chl *b* value (131.82 µg mL^{-1}) was obtained in plants irrigated with the ECw of 4.0 dS m^{-1}, corresponding to a reduction of 16.37% (25.80 µg mL^{-1}) compared to those grown under the ECw of 0.6 dS m^{-1} (Figure 4B).

Similar results were obtained in the research developed by Silva et al. (2021), when they evaluated the photosynthetic pigments of West Indian cherry under salt stress (ECw ranging from 0.3 to 4.3 dS m^{-1}). The authors observed a reduction of 35.68% in plants irrigated with the ECw of 4.3 dS m^{-1} in comparison to those grown under the ECw of 0.3 dS m^{-1}.

Photosynthetic pigments are determinants for plant growth and development (Sarkar and Kalita, 2023). Excess of salt in the irrigation water inhibits the activity of 5-aminolevulinic acid, which is a chlorophyll precursor, in addition to increasing the activity of the enzyme chlorophyllase, which acts by degrading photosynthetic pigment molecules, damaging the chloroplasts and limiting the activity of pigmentation proteins (Cavalcante et al., 2011; Ferraz et al., 2015; Nigam et al., 2022). Reductions in the contents of chlorophyll *a* and chlorophyll *b* in West Indian cherry plants as a function of irrigation with brackish water were also verified by other researchers (Lima et al., 2018; Dantas et al., 2021; Dias et al., 2021).

The total chlorophyll content (Figure 5A) of West Indian cherry plants irrigated with water of low electrical conductivity (0.6 dS m^{-1}) was 730.43 µg mL^{-1}, on average 24.3% higher than the value observed in plants grown under irrigation with the ECw of 4.0 dS m^{-1}. The reduction in chlorophyll biosynthesis could be a form of adaptation to the stress condition faced by the crop, aiming to save energy and capture less light energy to prevent oxidative stress, possibly due to the photooxidation of pigments, damaging plants through the oxidation of membrane lipids, proteins, and nucleic acids (Shoukat et al., 2023). Furthermore, the reduction in the contents of photosynthetic pigments could be due to the degradation of the photosynthetic apparatus as a result of the increase in the activity of the enzyme chlorophyllase (Bhatt et al., 2022).

Table 2. Summary of the analyses of variance referring to the contents of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), chlorophyll total (Chl *t*), and carotenoids (Car) of West Indian cherry irrigated with saline water and subjected to different fertilization combinations (FC) with NPK, 120 days after pruning, in the second year of cultivation.

Source of variation	GL	Mean square			
		Chl *a*	**Chl *b***	**Chl *t***	**Car**
Water electrical conductivity – ECw	1	204,617.12**	9,982.15**	304,987.80**	6,218.02*
Fertilization combinations – FC	9	10,380.78^{ns}	4,190.15^{ns}	19,446.74^{ns}	217.29^{ns}
Interaction (ECw × FC)	9	25,346.94^{ns}	1,744.61^{ns}	34,772.75^{ns}	1,279.02^{ns}
Blocks	2	33,255.73^{ns}	3,585.84^{ns}	57,675.46^{ns}	308.14^{ns}
Residuals	38	2,7963.97	2,351.14	35,261.29	1,128.62
CV (%)		22.51	23.50	18.49	20.83

$^{ns, *, and **}$: non-significant significant at a $p \leq 0.05$ and 0.01, respectively. GL: degree of freedom; CV: coefficient of variation.

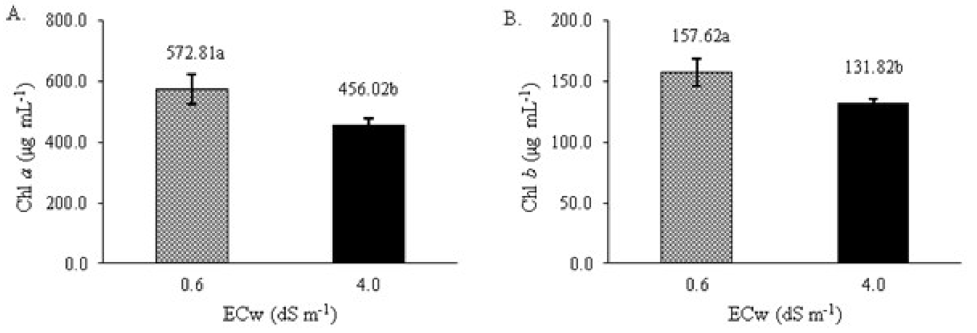

Figure 4. Chlorophyll *a* - Chl *a* (A) and chlorophyll *b* - Chl *b* (B) of the West Indian cherry cv. Flor Branca as a function of the electrical conductivity levels of irrigation water - ECw, 120 days after pruning, in the second year of cultivation. Means with different letters indicate a significant difference by Fisher's F-test for the water electrical conductivity (ECw). Vertical lines represent the standard error of the mean (n=3).

For the carotenoid content (Figure 5B), the plants irrigated with the ECw of 4.0 dS m^{-1} showed reductions of 22.02% (20.36 µg mL^{-1}) in relation to the 72.09 µg mL^{-1} of Car quantified in plants that received low-salinity water (0.6 dS m^{-1}). Thus, it can be inferred that the West Indian cherry plants irrigated with the lowest electrical conductivity (0.6 dS m^{-1}) showed normal functioning against photooxidation, whereas plants irrigated with the higher electrical conductivity (4.0 dS m^{-1}) showed metabolism changes and, consequently, greater damage to the photosynthetic membranes. These results corroborate the studies conducted by Dias et al. (2021), in which the authors observed a linear reduction in the carotenoid content as the electrical conductivity of irrigation water increased from 0.6 to 3.8 dS m^{-1} in West Indian cherry fertilized with phosphorus-potassium combinations in the second year of cultivation.

Therefore, the results obtained in this study can be justified due the salt stress can reduce the production of photosynthetic pigments, probably to induce the degradation of β-carotene, reducing the content of carotenoids, which are integrated components of the thylakoid membranes, acting in the capture and transference of light to the chlorophyll.

The interaction between the electrical conductivity irrigation water and fertilization combinations had no significant influence ($p > 0.05$) on the fluorescence variables of chlorophyll *a* in West Indian cherry 120 days after pruning (Table 3). In isolation, the electrical conductivity levels of irrigation water affected ($p \leq 0.01$) the initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv), and quantum efficiency of photosystem II (Fv/Fm), whereas the fertilization combinations had a significant effect on the F_0 and Fm.

When comparing the means between the electrical conductivity levels, the plants under the ECw of 0.6 dS m^{-1} showed the lowest F_0 (165.91), whereas the plants irrigated with the higher salinity (4.0 dS m^{-1}) showed an F_0 of 276.52, i.e., an increase of 66.67% (Figure 6A). The F_0 increase results in lower utilization of available energy, which highlights the damage caused by salt stress in the capture of light energy by the photosynthetic pigments (Martins et al., 2019; Lotfi et al., 2020).

The initial fluorescence of West Indian cherry plants (Figure 6B) fertilized with 120-100-100% (FC3), 140-100-100% (FC4), and 100-120-100% (FC6) of the NPK recommendation was statistically lower, on average 25.96% (68.82) below the values of plants that received the other combinations, showing no differences between each other.

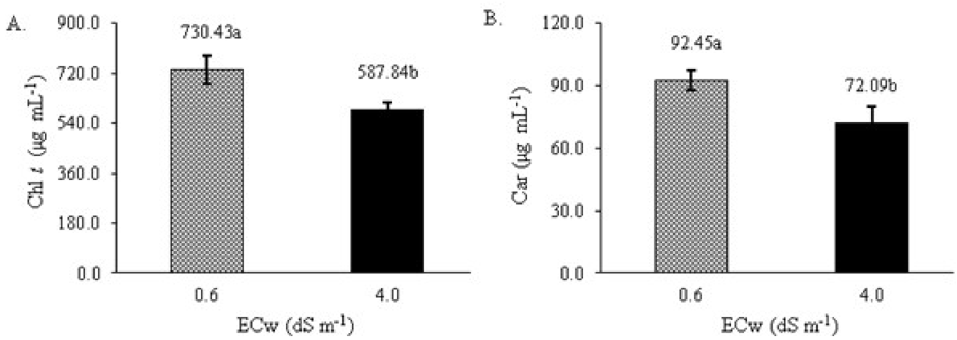

Figure 5. Chlorophyll total - Chl *t* (A) and carotenoids - Car (B) of the West Indian cherry cv. Flor Branca as a function of the electrical conductivity of irrigation water, 120 days after pruning, in the second year of cultivation. Means with different letters indicate a significant difference by Fisher's F-test for the water electrical conductivity (ECw). Vertical lines represent the standard error of the mean (n=3).

Table 3. Summary of the analyses of variance referring to initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv), and quantum efficiency of photosystem II (Fv/Fm) of West Indian cherry irrigated with saline water and subjected to different fertilization combinations (FC) with NPK, 120 days after pruning, in the second year of cultivation.

Source of variation	GL	Mean square			
		F_0	Fm	Fv	Fv/Fm
Water electrical conductivity - ECw	1	183,511.94**	35,225.57**	249,746.29**	0.054**
Fertilization combinations - FC	9	464.53**	1,440.29*	1158.44ns	0.001ns
Interaction (ECw × FC)	9	29.03ns	327.18ns	262.42ns	0.001ns
Blocks	2	187.10ns	1,263.75ns	483.19ns	0.0002ns
Residuals	38	72.44	528.34	726.25	0.0005
CV (%)		3.85	5.38	5.71	2.95

ns, *, **: not significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively. GL: degree of freedom; CV: coefficient of variation.

Overall, NPK combinations FC3, FC4, and FC6 improved the utilization of available energy, highlighting the importance of these nutrients through adequate combinations for physiological activities, as reported by Silva et al. (2021).

The maximum fluorescence (Figure 7A) also differed significantly as a function of the electrical conductivity of irrigation water, with the highest value (621.62) corresponding to 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 Fm of 573.16, i.e., a reduction of 7.80% compared to plants under the ECw of 0.6 dS m^{-1}. The maximum fluorescence is the point at which the plant's fluorescence reaches its maximum capacity and virtually all the quinone is reduced (Veloso et al., 2023). According to Manaa et al. (2019), the Fm reduction is a reflection of the slowdown in photosynthetic activity, reducing energy capture to prevent an excessive excitation of electrons, which are precursors in the formation of reactive oxygen species. In a study conducted by Dias et al. (2018) with the West Indian cherry cv. BRS 366-Jaburu under salt stress (ECw from 0.8 to 3.8 dS m^{-1}), the authors also observed an Fm reduction (15.48%) as a function of irrigation with the highest salinity level (3.8 dS m^{-1}).

The maximum fluorescence of West Indian cherry showed significant differences as a function of the NPK combinations (Figure 7B), with the highest Fm being achieved by plants that received the NPK combination corresponding to 140-100-100% (FC4) of the recommendation, with no significant differences in relation to the 120-100-100% (FC3) and 100-120-100% (FC6) combinations and differing significantly from the other combinations studied, which did not differ among them.

The plants under combinations FC3, FC4, and FC6 possibly maintained the leaf turgor due to the osmotic adjustment capacity promoted by the synthesis of compatible osmolytes, e.g., amino acids, stimulated by the increase in N and P, thus reducing the imbalance between transpiration and water uptake by the plant, favoring osmotic homeostasis (Sá et al., 2015) and, as a result, increasing the maximum fluorescence of West Indian cherry.

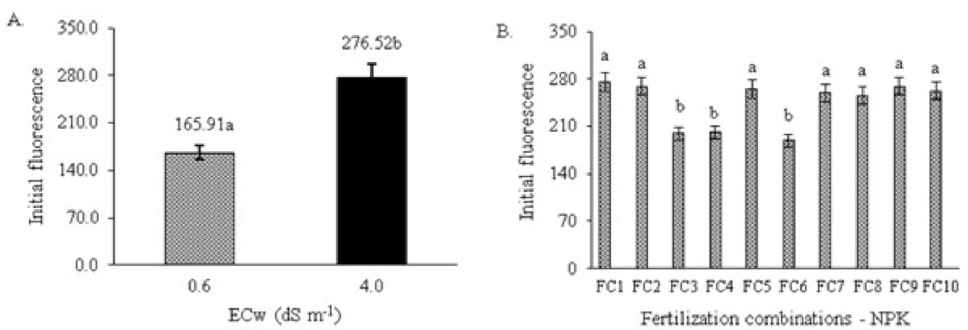

Figure 6. Initial fluorescence - F_0 of West Indian cherry cv. Flor Branca as a function of the electrical conductivity levels of irrigation water (A) and fertilization combinations (B) 120 days after pruning in the second year of cultivation. FC1 = 80-100-100; FC2 = 100-100-100; FC3 = 120-100-100; FC4 = 140-100-100; FC5 = 100-80-100; FC6 = 100-120-100; FC7 = 100-140-100, FC8 = 100-100-80, FC9 = 100-100-120, and FC10 = 100-100-140% of the recommended N-P_2O_5-K_2O level; means with same letters indicate that there are no significant differences between fertilization combinations N-P_2O_5-K_2O at a 0.05 of probability by the Scott-Knott test. Means with different letters indicate a significant difference by F-test for the electrical conductivity of water (ECw). Vertical lines represent the standard error of the mean (n=3).

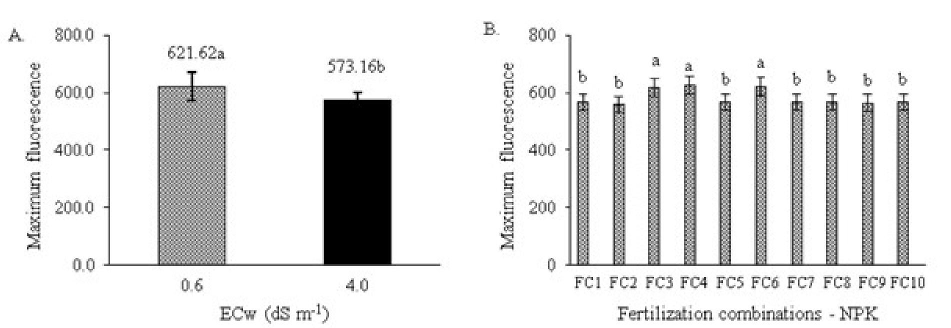

Figure 7. Maximum fluorescence – F_m of the West Indian cherry cv. Flor Branca as a function of the electrical conductivity levels of irrigation water (A) and fertilization combinations (B), 120 days after pruning, in the second year of cultivation. FC1 = 80-100-100; FC2 = 100-100-100; FC3 = 120-100-100; FC4 = 140-100-100; FC5 = 100-80-100; FC6 = 100-120-100; FC7 = 100-140-100, FC8 = 100-100-80, FC9 = 100-100-120 e FC10 = 100-100-140% of the recommended N-P_2O_5-K_2O level; means with same letters indicate that there are no significant differences between fertilization combinations with N-P_2O_5-K_2O at a 0.05 of probability by the Scott-Knott test. Means with different letters indicate a significant difference by F-test for the electrical conductivity of water (ECw). Vertical lines represent the standard error of the mean (n=3).

Plants under the ECw of 0.6 dS m^{-1} showed a higher Fv (536.28) compared to those under the ECw of 4.0 dS m^{-1} (407.25) (Figure 8A). Since it refers to the active potential energy in the photosystem, the Fv reduction (24.06%) demonstrates limitations in the activation of the electron transport chain, which is responsible for producing adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NDPH) in the Calvin cycle, reducing the plant's photosynthetic capacity (Taiz et al., 2017; Lotfi et al., 2020).

The quantum efficiency of photosystem II (Figure 8B) of West Indian cherry plants under irrigation with water of low salinity (0.6 dS m^{-1}) was 0.77, on average 11.6% higher than the value observed (0.69) in plants irrigated with the ECw = 4.0 dS m^{-1}. This response demonstrates the lower activity of the enzyme chlorophyllase, which reduces the chlorophyll contents (Figure 4) and, consequently, affects energy capture and transport between reaction centers and the free plastoquinone (Çiçek et al., 2018).

The multidimensional space of the original variables was reduced to four principal components (PC1, PC2, PC3, and PC4). The eigenvalues and the percentage of variation explained by each component jointly represented 89.47% of the total variation, with PC1 amounting to 52.62% of the variance, followed by PC2 with 13.36%, PC3 with 12.90%, and PC4 with 10.58% of the total variance (Table 4).

In PC1, salt stress (ECw=4 dS m^{-1}) reduced the quantum efficiency of PSII, which is related to the reduction in the content of photosynthetic pigments (Chl *a*, Chl *b*, Car, and Chl *t*) and chlorophyll fluorescence (Fo, Fm, Fv, and Fv/Fm), a process that triggered lower values of production variables (PFD, TFW, and MFW) compared to non-stressed plants (ECw=0.6 dS m^{-1}). However, under fertilization combinations FC4, FC8, and FC9, the stressed plants showed an increase in the pigment contents, the quantum efficiency of PSII, and production. In PC2, the reduction in fruit size (PFD and EFD) is related to the increase in the total number of fruits (TFN) (Figures 9A and B), whereas, in PC3, the increase in the number of fruits is converted into a higher total fruit weight, which is due to the supply of phosphorus in the FC9 combination (Figures 9C and D).

These effects actually occurred because potassium acts in stomatal opening and closure, reducing stomatal conductance (*gs*) and the CO_2 assimilation rate (*A*) and increasing the internal CO_2 concentration (*Ci*) (Silva Filho et al., 2023b).

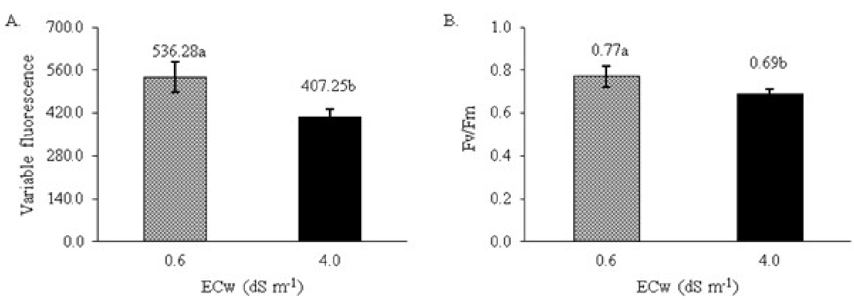

Figure 8. Variable fluorescence - Fv (A) and quantum efficiency of photosystem II - Fv/Fm (B) of the West Indian cherry cv. Flor Branca as a function of the electrical conductivity levels of irrigation water, 120 days after pruning, in the second year of cultivation. Means with different letters indicate a significant difference by F-test for the electrical conductivity of water (ECw). Vertical lines represent the standard error of the mean (n=3).

Table 4. Eigenvalues, percentage of the total variance explained, multivariate analysis of variance (MANOVA), and correlation coefficients (r) between original variables and principal components.

	Principal components			
	PC1	**PC2**	**PC3**	**PC4***
Eigenvalues (λ)	6.84	1.73	1.67	1.37
Percentage of the total variance explained (s^2%)	52.62	13.37	12.90	10.58
Hotelling test (T^2) for the water electrical conductivity (ECw)	≤0.01	≤0.01	≤0.01	0.04
Hotelling test (T^2) for fertilization combination (FC)	≤0.01	≤0.01	≤0.01	0.11
Hotelling test (T^2) for the interaction (ECw× FC)	≤0.01	≤0.01	≤0.01	0.68

PCs	Correlation coefficient (r)												
	F_0	Fm	Fv	Fv/Fm	Chl *a*	Chl *b*	Chl *t*	Car	PFD	EFD	TNF	TFW	MFW
PC1	-0.91	-0.79	-0.91	-0.90	-0.77	-0.62	-0.80	-0.73	-0.35	-0.58	-0.40	-0.61	-0.79
PC2	-0.02	0.38	0.10	0.02	-0.36	-0.21	-0.35	-0.18	0.60	0.61	-0.52	-0.34	0.36
PC3	-0.13	-0.30	-0.26	-0.18	-0.33	0.24	-0.20	-0.18	0.42	0.40	0.68	0.66	0.12
PC4	0.33	0.30	0.28	0.26	-0.32	-0.52*	-0.41	-0.31	-0.45	-0.15	0.30	0.25	0.08

PCs - principal components; PC1 - principal components1; PC2 - principal components 2; PC3 - principal components 3; PC4 - principal components 4; F_0 - initial fluorescence; Fm - maximum fluorescence; Fv - variable fluorescence; Fv/Fm - quantum efficiency of photosystem II; Chl *a* - chlorophyll *a*; Chl *b* - chlorophyll *b*; Chl *t* – chlorophyll toral; Car - carotenoids; PFD - polar fruit diameter; EFD - equatorial fruit diameter; TNF - total number of fruits; TFW - total fruit weight; and MFW - mean fruit weight. *Variable subjected to ANOVA.

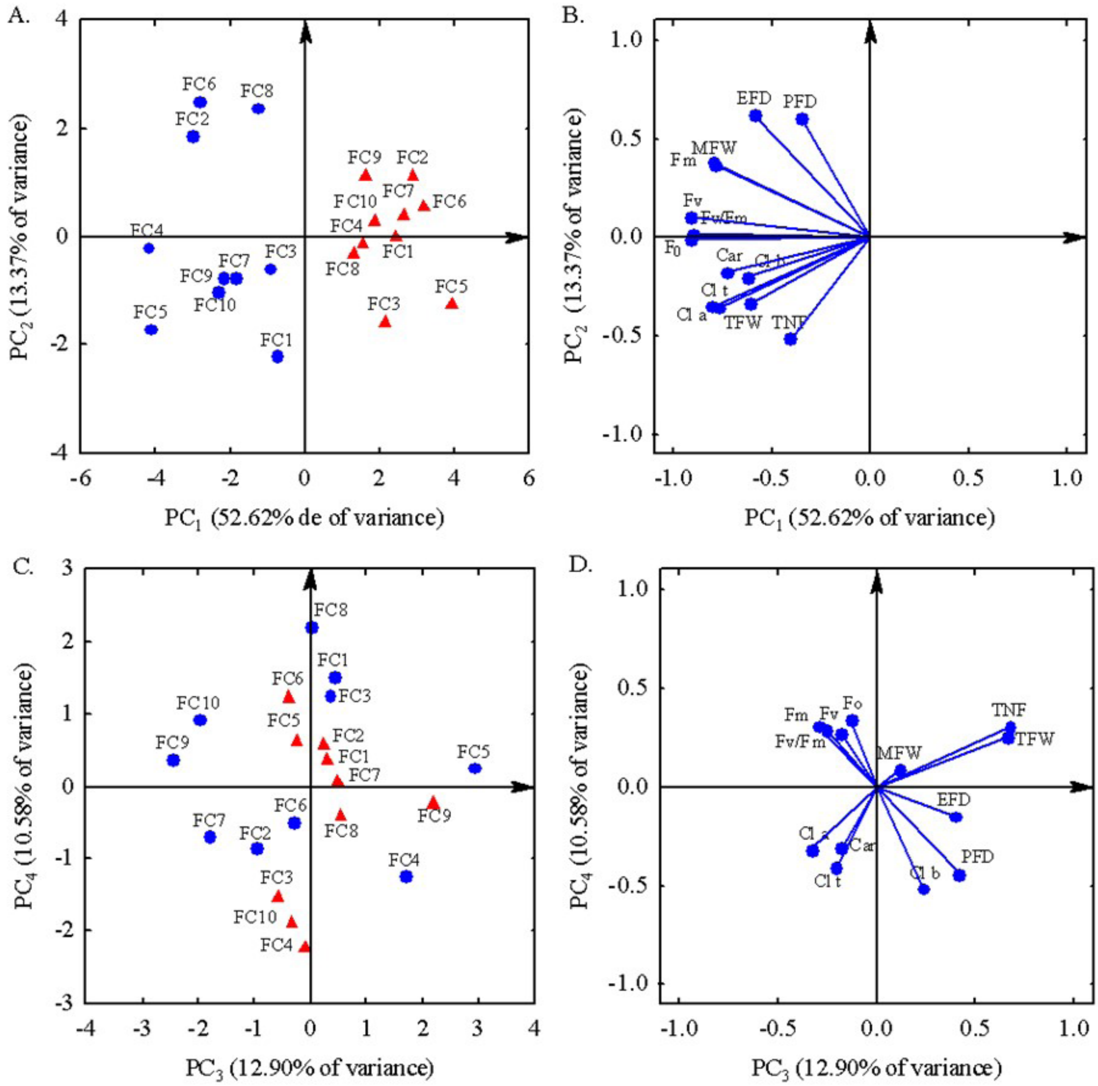

Figure 9. Two-dimensional scores of the principal components (PC) for the electrical conductivity of irrigation water and NPK (A and C) and the correlation coefficients of the variables analyzed (B and D) with the first four principal components (PC1, PC2, PC3, and PC4). PCs - principal components; PC1 - principal components1; PC2 - principal components 2; PC3 - principal components 3; PC4 - principal components 4. Fertilization combination (FC) with nitrogen, phosphorus and potassium (NPK), being: FC1 = 80-100-100%; FC2 (control) = 100-100-100%; FC3 = 120-100-100%; FC4 = 140-100-100%; FC5 = 100-80-100%; FC6 = 100-120-100%; FC7 = 100-140-100%; FC8 = 100-100-80%; FC9 = 100-100-120% and FC10 = 100-100-140%. Chl *a* – chlorophyll *a*; Chl *b*– chlorophyll *b*; Car– carotenoids; Chl *t* – chlorophyll *total*; F_{0} - initial fluorescence; Fv - variable fluorescence; Fm - maximum fluorescence and Fv/Fm quantum efficiency of photosystem II; total number of fruits (TNF), total fruit weight (TFW), mean fruit weight (MFW), polar (PFD), and equatorial fruit diameter (EFD). ● electrical conductivity of irrigation water (ECw) = 0.6 dS m^{-1} and ▲ ECw = 4.0 dS m^{-1}.

4. Conclusions

Irrigation with the electrical conductivity of water of 4.0 dS m^{-1} promotes negative effects on the photosynthetic pigments and the quantum yield of photosystem II of the West Indian cherry cv. Flor Branca. The NPK combinations did not mitigate the effects of salt stress on the photosynthetic pigments and the quantum yield of photosystem II. However, the 120-100-100%, 140-100-100%, and 100-120-100% combinations of the NPK recommendations improved the utilization of available energy by reducing the initial fluorescence and increasing the maximum fluorescence of the West Indian cherry cv. Flor Branca.

Multivariate analysis indicated that irrigation with saline water (ECw=4 dS m^{-1}) reduced the quantum efficiency of PSII, consequently decreasing the contents of photosynthetic pigments and chlorophyll fluorescence, resulting in lower production. Plants fertilized with the combinations 140-100-100%, 100-100-80%, and 100-100-120% of the NPK recommendation for the second production year increased the pigment contents, the quantum efficiency of PSII, and production.

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