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Original Article

Morphophysiology of cowpea under salt stress and application of carbon-based nanobiostimulant in the vegetative stage[1]

Morfofisiologia de feijão-caupi sob estresse salino e aplicação de nanobioestimulante de carbono na fase vegetativa

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HIGHLIGHTS:

Carbon-based nanobiostimulant stimulates the physiology of cowpea at concentrations of up to 320 mg L⁻¹.
240 mg L⁻¹ of the nanobiostimulant improves the growth of cowpea under salt stress.
Growth and gas exchange of cowpea are reduced by the electrical conductivity of irrigation water of 4.0 dS m⁻¹.

ABSTRACT: Several technologies have been implemented to improve plant performance in irrigated crops, and one of them is the use of nanobiostimulants. Therefore, the aim of the present study was to explore the effects of applying different concentrations of a carbon-based biostimulant on the morphophysiology of cowpea plants subjected to varying electrical conductivities of irrigation water. The experiment was performed in a completely randomized design, in a 2 × 6 factorial scheme, with two electrical conductivities of irrigation water (ECw: 0.35 and 4.0 dS m^{-1}) and six concentrations of nanobiostimulant applied through the leaves (0, 80, 160, 240, 320, and 400 mg L^{-1}), with four replications. Growth and gas exchange variables were evaluated 31 days after sowing (V9 stage). Concentrations between 240 and 320 mg L^{-1} of carbon-based nanobiostimulant promoted greater increases in growth and physiological variables, but they caused decreases in non-photochemical quenching. Water electrical conductivity of 4.0 dS m^{-1} reduced the number of leaves (17.1%), stem diameter (10.0%), SPAD index (10.3%), net photosynthesis (10.9%), stomatal conductance (46.4%), transpiration (34.5%), instantaneous carboxylation efficiency (22.4%), and photochemical quenching (4.5%); in contrast, it increased the ratio between internal and ambient CO_2 concentration (18.0%), leaf temperature (2.9%), water use efficiency (32.5%), and non-photochemical quenching (12.4%). Concentrations of 240 to 320 mg L^{-1} of the nanobiostimulant enhance the development and gas exchange of cowpea plants under non-stress conditions, whereas the concentration of 240 mg L^{-1} promotes the maximum increase in plant height under salinity.

Key words: carbon dots, growth, gas exchange, salinity, *Vigna unguiculata* L.

RESUMO: Diversas tecnologias vêm sendo implementadas para melhorar o desempenho das plantas em cultivos irrigados e uma delas é o uso de nanobioestimulantes. Assim, o objetivo deste estudo foi investigar os efeitos da aplicação de diferentes concentrações de bioestimulante à base de carbono na morfofisiologia de plantas de feijão-caupi submetidas a diferentes condutividades elétricas da água de irrigação. O experimento foi realizado em delineamento inteiramente casualizado, em arranjo fatorial 2 × 6, com duas condutividades elétricas da água de irrigação (CEa: 0,35 e 4,0 dS m^{-1}) e seis concentrações do nanobioestimulante aplicadas via foliar (0, 80, 160, 240, 320 e 400 mg L^{-1}), com quatro repetições. Variáveis de crescimento e de trocas gasosas foram avaliadas 31 dias após a semeadura (estádio V9). Concentrações entre 240 e 320 mg L^{-1} do nanobioestimulante de carbono proporcionaram maiores incrementos no crescimento e nas variáveis fisiológicas, no entanto, causaram decréscimos no quenching não-fotoquímico. A condutividade elétrica da água de 4,0 dS m^{-1} reduziu número de folhas (17,1%), diâmetro do caule (10,0%), índice SPAD (10,3%), fotossíntese líquida (10,9%), condutância estomática (46,4%), transpiração (34,5%), eficiência instantânea de carboxilação (22,4%), quenching fotoquímico (4,5%); em contraste, aumentou razão entre concentração interna e ambiente de CO_2 (18,0%), temperatura foliar (2,9%), eficiência do uso da água (32,5%) e quenching não-fotoquímico (12,4%). As concentrações de 240 a 320 mg L^{-1} do nanobioestimulante potencializam o desenvolvimento e as trocas gasosas do feijão-caupi em condições sem estresse, enquanto que a concentração de 240 mg L^{-1} promove aumento máximo de altura de plantas sob salinidade.

Palavras-chave: carbon dots, crescimento, trocas gasosas, salinidade, *Vigna unguiculata* L.

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Introduction

The use of carbon dots (CDs) nanotechnology in the development of plant biostimulants emerges as a promising scientific approach for agriculture, given their high water solubility, chemical stability, low toxicity, biocompatibility, and significant electron donation and reception capacity (Li et al., 2020). In addition, CDs promote more efficient absorption by leaves due to their reduced size and are able to promote the activation of reactions inherent to plant metabolism, stimulating growth, aiding in uptake of nutrients and increasing the photosynthetic rate and tolerance of plants to biotic and abiotic stresses (Maswada et al., 2020).

Studies indicate various effects of CDs on the growth and physiology of crops. Tan et al. (2021), with foliar application of CDs at a concentration of 150 mg L^{-1}, observed increases in stomatal conductance and photosynthetic rate, leading to improved development in rice and corn plants. Under abiotic stresses, for instance, Ji et al. (2023) found significant enhancements in photosynthesis and nitrogen metabolism in soybean plants.

The quest for novel management strategies to alleviate the impact of abiotic stresses, such as salinity, has driven an increased adoption of biostimulants. The use of CDs emerges as a potential alternative to support the utilization of saline water in agriculture. This approach aims to minimize the adverse effects associated with soil osmotic potential reduction, nutritional disturbances, and specific ion-induced impacts, which lead to plant toxicity, decreased photosynthesis, and pigment degradation (Wang et al., 2022).

As a leguminous crop of significant socio-economic and nutritional importance, cowpea (*Vigna unguiculata* L.) stands out among crops whose development and gas exchange processes are impacted by salinity (Costa et al., 2020). In this study, the aim was to explore the effects of applying different concentrations of a carbon dots-based biostimulant on the morphophysiology of cowpea plants subjected to varying electrical conductivities of irrigation water.

Material and Methods

The experiment was conducted between October and November 2022, in a greenhouse of the Department of Plant Science of the Universidade Federal do Ceará - UFC, Pici Campus, Fortaleza, Ceará, Brazil (3° 44' 25" S and 38° 34' 32" W, with altitude of 12 m). During the experimental period, the maximum and minimum temperatures and the relative humidity of air inside the greenhouse were monitored daily with a digital thermo-hygrometer, ranging from 34.1 to 42.2 °C, 23.5 to 26.2 °C and 49.5 to 73%, respectively (Figure 1).

The experimental design was completely randomized in a 2 × 6 factorial arrangement, referring to two electrical conductivities of irrigation water (ECw: 0.35 and 4.0 dS m^{-1}) and six concentrations of biostimulant based on carbon dots applied through the leaves (0, 80, 160, 240, 320, and 400 mg L^{-1}) with four replicates. Each plot consisted of two plants (one plant per pot), totaling 96 pots. The electrical conductivity of the irrigation water (ECw) was set at 4.0 dS m^{-1}, taking into account the crop's moderate tolerance to salinity. The concentrations of the biostimulant were determined based on the manufacturer's recommendations, following preliminary tests of the product on leguminous plants, namely common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*).

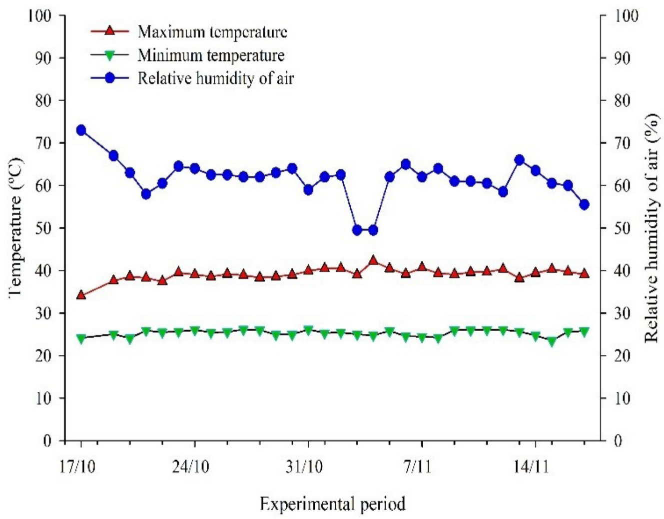


Figure 1. Daily means of maximum and minimum temperatures and relative humidity of air during the experimental period

For this experiment, seeds of the cowpea cultivar 'Pingo de Ouro 1-2' (SisGen CE3D84C-temporary), provided by the UFC Cowpea Germplasm Bank (BAG), were used. Sowing was carried out manually (3 seeds) in 18 L polyethylene pots, filled with a 2 cm layer of crushed stone number 1 at the bottom to facilitate drainage, and 15 kg of soil. Seven days after sowing (DAS), manual thinning was performed, leaving only one plant in each pot.

The soil classified as Neossolo Flúvico (Entisol-Fluvent) used in this study was collected at a depth of 0-20 cm in the non-cultivated area of the Vale do Curu Experimental Farm, belonging to UFC and located in Pentecoste, Ceará. The sample was sent to the Laboratory of Chemical Analysis and Soil Physics of the Department of Soil of UFC to determine the main chemical attributes (Table 1).

Chemical fertilization was performed following the method outlined by Melo et al. (2018), applying 0.34 g pot^{-1} of potassium chloride and 0.38 g pot^{-1} of single superphosphate as basal dose. 0.5 g pot^{-1} of urea was applied 15 days after the emergence of the seedlings.

The biostimulant was developed and provided by the Brazilian company Krilltech, where it is marketed in its

Table 1. Chemical attributes of the soil used in the experiment

C	OM	Ava. P	K	Mg	Ca	Na	H+Al	S	T	V	ESP	pH	EC	Fe	Cu	Zn	Mn
(g kg^{-1})		(mg kg^{-1})	(cmol$_c$ dm^{-3})							(%)		H_2O	(dS m^{-1})	(mg kg^{-1})			
3.3	5.7	57	0.2	1.2	2.7	0.2	0.7	4.3	4.9	87	4	7	0.3	57.1	0.6	1.8	33.2

C – Carbon; OM - Organic matter; Ava. P- Available phosphorus; K - Potassium; Mg - Magnesium; Ca - Calcium; H + Al - Potential acidity at pH 7.0; SB - Sum of exchangeable bases; T - Cation exchange capacity of soil at pH 7.0; V - Percentage of base saturation; ESP - Exchangeable sodium percentage; pH - Hydrogen potential; EC - Electrical conductivity; Fe - Iron; Cu – Copper; Zn – Zinc; Mn - Manganese concentrated formula with 400 g L^{-1} of arboline (carbon dots). Arboline is a spheroidal-shaped particle with a size of 3.1 nm, elemental composition represented by carbon (C, 61%), nitrogen (N, 21.3%), oxygen (O, 11.4%), and hydrogen (H, 6.3%), and surface composition with C (67.4%), O (21%), and N (11.6%).

To prepare the solutions and obtain the application concentrations (0, 80, 160, 240, 320, and 400 mg L^{-1}), the dilution stage was necessary, fixing the solution to a volume of 70 mL for each treatment with nanobiostimulant, with each plant receiving by foliar spray 10 mL of the solution with carbon dots, whose concentration varied according to the treatments. For treatment without nanobiostimulant (0 mg L^{-1}), 10 mL of distilled water was sprayed on each plant. The application of the nanobiostimulant was performed only once at 23 days after sowing (DAS) and comprised the V5 phenological stage of cowpea, when the third trifoliate leaf had leaflets separated and fully expanded. The application was carried out using a sprayer with a capacity of 100 mL.

The irrigation water of lower electrical conductivity (0.35 dS m^{-1}) came from the public supply system of the municipality of Fortaleza, Ceará. The treatment with the higher electrical conductivity (4.0 dS m^{-1}) was prepared every week in reservoirs with a capacity of 100 L, by dissolving NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ salts in the supply water in equivalent proportion of 7:2:1, respectively, following the relationship between the electrical conductivity of water (ECw) and its concentration ($mmol_c$ $L^{-1} \approx EC \times 10$), as outlined by Richards (1954). These salts were chosen as they represent the primary salts found in brackish waters of the Northeast region, predominantly in this proportion (Medeiros et al., 2003). The ECw was measured through daily readings with a portable conductivity meter to ensure the control of the salinity levels.

All pots were irrigated daily with low-salinity water (0.35 dS m^{-1}) until the imposition of salt stress, which was initiated at phenological stage V3 (12 DAS). From that moment on, the pots of the treatments with high salinity started to be irrigated with water of 4.0 dS m^{-1} until phenological stage V9 (31 DAS), totaling 19 days under salt stress, maintaining the same daily watering schedule. The volume of water was applied based on the water balance obtained according to the drainage lysimeter methodology (Silva et al., 2021), leaving the soil at field capacity and applying, once a week, a leaching fraction of 15%, in order to avoid excessive accumulation of salts in the soil (Ayers & Westcot, 1999).

This application was performed locally to avoid contact with the leaves of the plants, and the volume was determined by Eq.1:

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

where:

VI - volume of water to be used in the irrigation (mL);

Vw and Vd - volume of water applied and drained in the previous irrigation, respectively; LF - leaching fraction.

At 31 DAS, when the plants reached the V9 phenological stage, plant height was determined using a graduated tape, measuring from the plant's base to the last leaf insertion. Stem diameter was measured using a digital caliper at a height of 3 cm from the plant base. The number of leaves was obtained by direct counting of the trifoliate leaves. To check for possible increases in plant growth with the application of the biostimulant, the relative growth rate was calculated with plant height data at 21 DAS (before application) and 31 DAS (after application), according to Benincasa (2003).

Also at 31 DAS, measurements were made of the net photosynthetic rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}), internal CO_2 concentration (Ci, μmol CO_2 mol^{-1} air) and leaf temperature, in the third fully expanded trifoliate leaf from the apex, between 8:00 a.m. and 12:00 a.m., using an infrared gas analyzer (IRGA, LI-6400XT, LI-COR Biosciences Inc., Lincoln, Nebraska, USA), under saturating radiation (1300 μmol m^{-2} s^{-1}), controlled CO_2 concentration (400 μmol mol^{-1}) and under ambient temperature conditions. With these data, it was possible to obtain the Ci/Ca ratio, the instantaneous carboxylation efficiency (iCE, A/Ci, [μmol m^{-2} s^{-1}(μmol mol^{-1})$^{-1}$]), and the instantaneous water use efficiency (WUE, A/E, [μmol CO_2 m^{-2} s^{-1}(μmol H_2O m^{-2} s^{-1})$^{-1}$]).

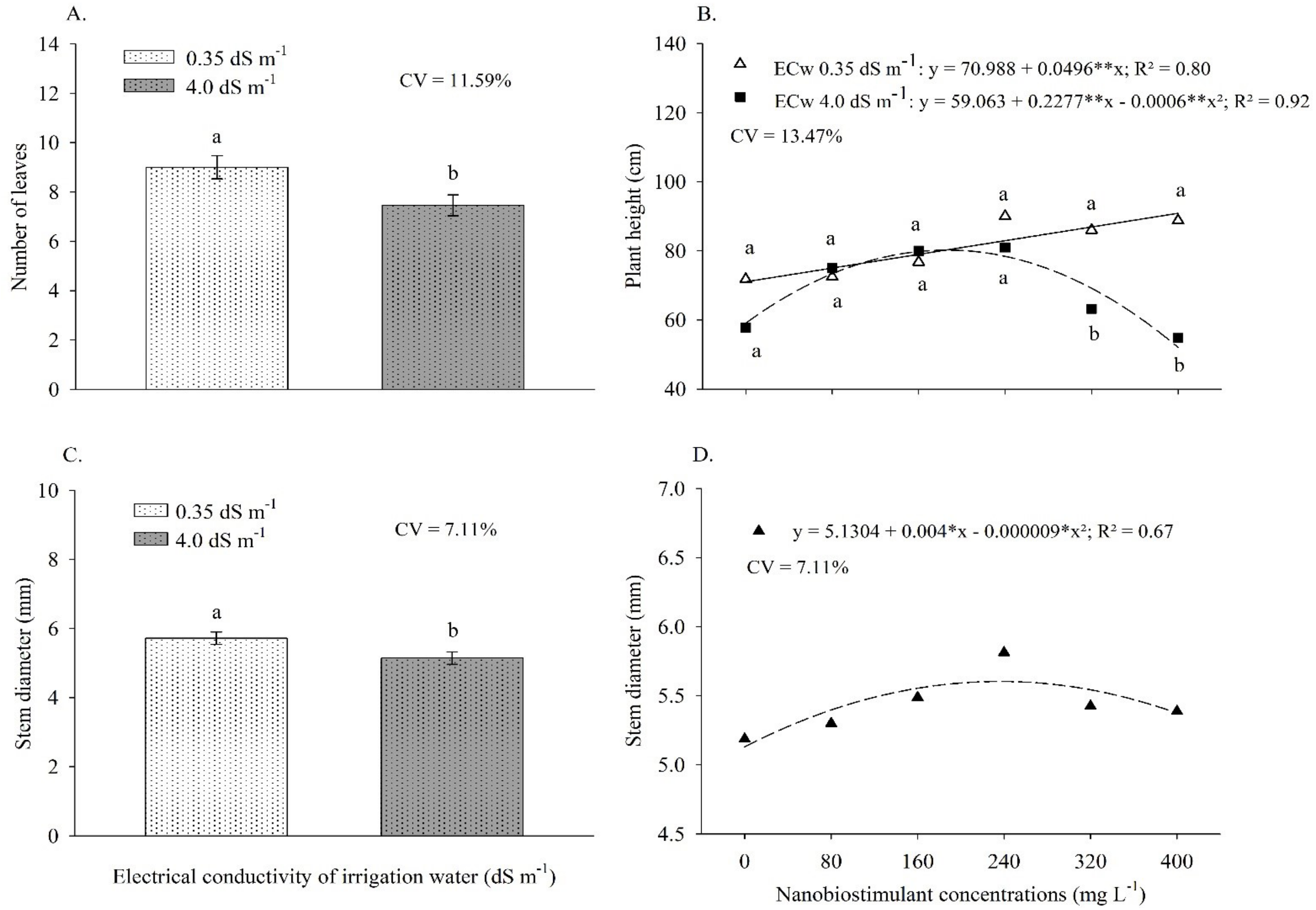
At the time of determining gas exchange, parameters related to chlorophyll *a* fluorescence in the light were also quantified by means of an IRGA-coupled fluorometer: effective quantum yield of PSII (ϕPSII), electron transport rate (ETR), photochemical quenching (qP), and non-photochemical quenching (qN). In addition, SPAD index readings were also performed, that is, the green intensity of the leaves analyzed by estimating the relative chlorophyll index, using a chlorophyll meter (Minolta SPAD-502), with readings in the third fully expanded trifoliate leaf from the apex of the cowpea plant.

The normality of the data was verified using the Shapiro-Wilk test and, then, the data of the analyzed variables were subjected to analysis of variance, evaluating the significance of the treatments by the F test ($p \leq 0.05$). When significant, the means of ECw were compared using Tukey's test, while the concentrations of the nanobiostimulant were evaluated through polynomial regression ($p \leq 0.05$). For statistical analysis and graphing, the statistical package R v.4.0.2 (R Core Team, 2020) and Sigmaplot (version 11.0) were used, respectively.

Results and Discussion

Plants subjected to irrigation water electrical conductivity of 4.0 dS m^{-1} (salt stress) had reductions of 17.1% in the number of leaves (Figure 2A) when compared to plants exposed to the lowest electrical conductivity (0.35 dS m^{-1} – no stress). The interaction between the factors indicated that plants exposed to salt stress exhibited lower average heights compared to those subjected to the non-stress treatment at concentrations of 320 and 400 mg L^{-1} of the nanobiostimulant, with reductions of 18.8 and 44.8%, respectively (Figure 2B).

At both ECw levels, it is possible to verify that the application of the nanobiostimulant increased plant height at all doses tested, when compared to 0 mg L^{-1}. Non-stressed plants showed a 27.9% increase in height when comparing the dose of 400 mg L^{-1} with that of 0 mg L^{-1} of the biostimulant. In



Means followed by the same letter do not differ at $p \leq 0.05$ by Tukey's test. *, ** - Significant at $p \leq 0.05$ and $p \leq 0.01$ by F test, respectively. Vertical bars indicate standard error (n=96)

Figure 2. Number of leaves (A), plant height (B), and stem diameter (C and D) of cowpea (*Vigna unguiculata*) as a function of the electrical conductivity of irrigation water and/or of the nanobiostimulant concentration, at 31 days after sowing

contrast, stressed plants reached their maximum height (80.7 cm) when subjected to a concentration of 190 mg L^{-1} of the nanobiostimulant, surpassing plants exposed to the 0 mg L^{-1} dose by 36.6% (Figure 2B).

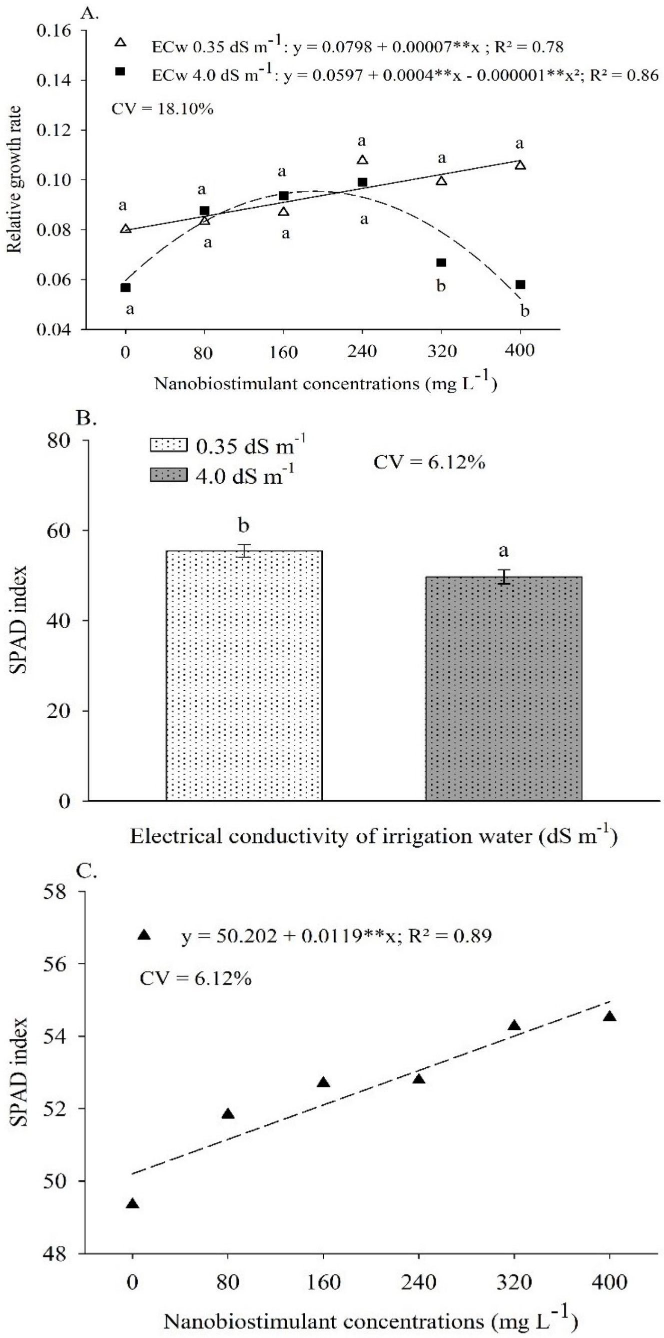
Regarding the isolated effects on stem diameter, plants subjected to salt stress had reductions of 10% in this variable, when compared to plants exposed to the lowest electrical conductivity (Figure 2C). It was observed that, with the increase in the concentrations of the nanobiostimulant, there were increases in the stem diameter of the plants, and the concentration of 222 mg L^{-1} was the one that promoted the maximum gain in this variable, being 8.7% higher than that obtained with the 0 mg L^{-1} dose (Figure 2D).

In recent years, studies have shown that the application of carbon dots can enhance plant growth in agricultural crops (Maswada et al., 2020). This action capacity of nanobiostimulants is due to the size of their particles, the controlled and specific delivery at the site of highest metabolic rate of the plant (leaves), and also their nano formulation, which allows the supply of one or more macro or micronutrients to the plant (Juárez-Maldonado et al., 2019; Li et al., 2020). In the present study, the nanobiostimulant used, in addition to carbon dots, also has nitrogen added to its surface, and the contribution of this nutrient tends to favor metabolic reactions that lead to plant growth.

On the other hand, it was evident that the salinity of the irrigation water affected the growth of cowpea plants and that the application of the nanobiostimulant was not enough to minimize the harmful effects of salt stress. This reduced growth may be attributed to limitations in water absorption by the roots due to the accumulation of salts in the soil solution, affecting processes such as cell expansion. It could also be related to a reduction in stomatal opening, photosynthetic rate, and the supply of carbohydrates and/or growth hormones to meristematic regions. Additionally, nutritional imbalances caused by the high concentration of toxic ions in the protoplasm could contribute to the observed effects (Khare et al., 2020). Andrade et al. (2019) also found that the increased salinity of irrigation water decreased the growth of cowpea plants.

The trend of effect of salinity and nanobiostimulant concentrations was also repeated for relative growth rate of the plant height (RGR, Figure 3A), indicating that plants exposed to salt stress had lower RGR than those exposed to the treatment without stress at concentrations of 320 and 400 mg L^{-1}, respectively, 16.5 and 44.6%.

In plants irrigated with water of 0.35 dS m^{-1}, the RGR increased linearly with increasing concentrations of the nanobiostimulant, with an increase of 35.1% when comparing the dose of 400 mg L^{-1} with that of 0 mg L^{-1}. For plants irrigated with water of 4.0 dS m^{-1}, a quadratic behavior was



Means followed by the same letter do not differ at $p \leq 0.05$ by Tukey's test. ** - Significant at $p \leq 0.01$ by F test. Vertical bars indicate standard error (n=96)

Figure 3. Relative growth rate of the plant height (A) and SPAD index (B and C) of cowpea (*Vigna unguiculata*) as a function of the electrical conductivity of irrigation water and/or of the nanobiostimulant concentration, at 31 days after sowing

observed, with maximum RGR (0.10) when subjected to the concentration of 200 mg L^{-1} of the nanobiostimulant, surpassing the concentration of 0 mg L^{-1} by 67% (Figure 3A).

The positive outcome of the nanobiostimulant may be associated not only with the direct and indirect supply of nutrients, but also with the provision of molecules that enhance the plant's metabolic efficiency and activate essential reactions for cellular division, expansion, and differentiation processes. As a result, the promotion of growth is observed (Dell'Aversana et al., 2021).

For the SPAD index, plants subjected to salt stress showed a reduction of 10.3% when compared to non-stressed plants (Figure 3B). Decreases in this variable with the increase in the salinity of irrigation water were also verified by Thuc et al. (2023), when studying the effects of drought and salinity on the growth, production, and nutritional content of cowpea. This reduction in the SPAD index in plants under stress can be explained both by the negative effects of the excessive amount of sodium ions present in cells, which can end up blocking electron transport, and by oxidative damage on chlorophylls caused by excess of reactive oxygen species (Zhang et al., 2019).

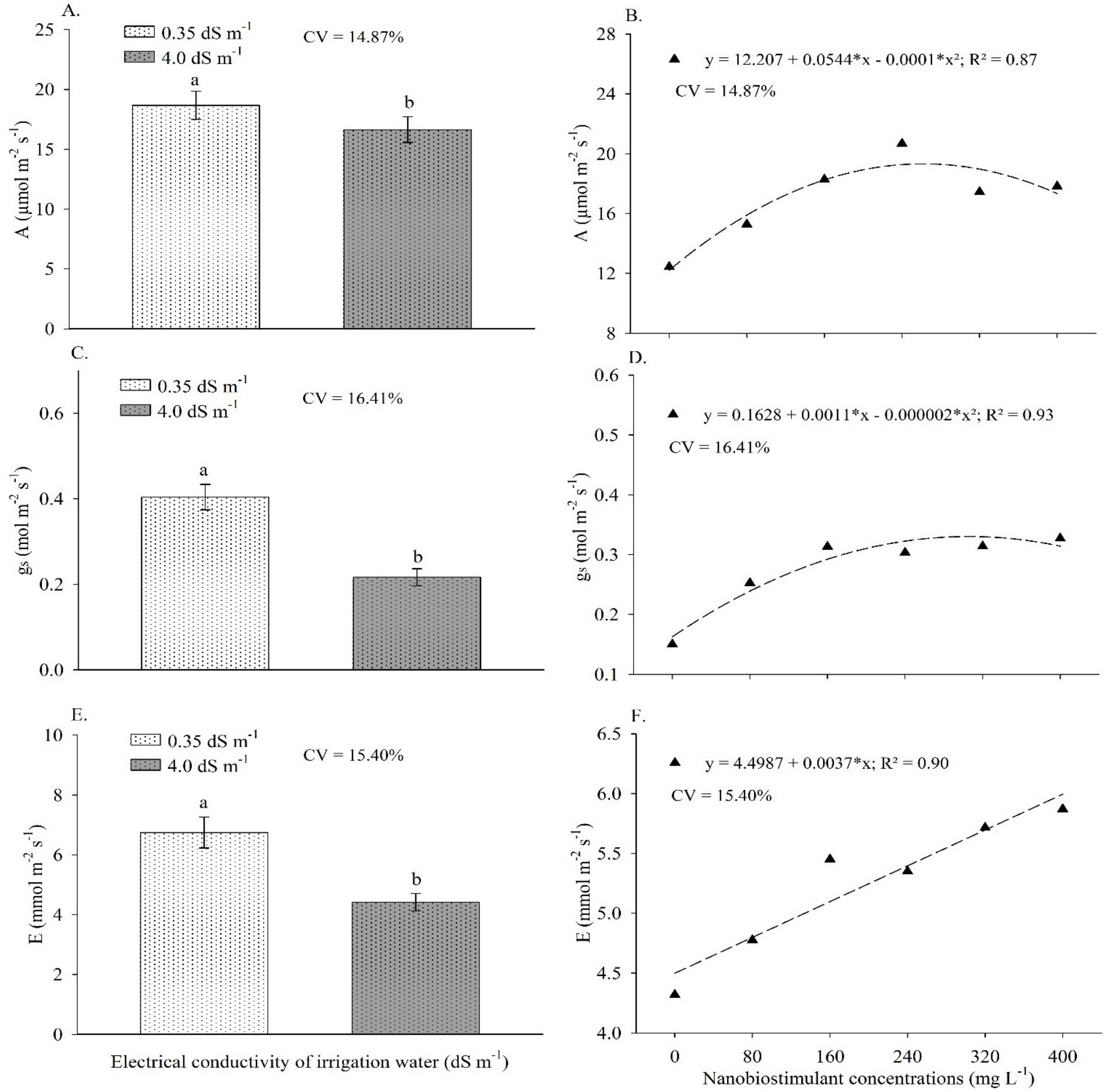
The increase in carbon-based nanobiostimulant concentrations promoted linear increments in the SPAD index, increasing it by 9.5% when comparing the highest and lowest concentrations applied (Figure 3C). The carbon dots (CDs) studied here contain surface functional groups that are abundant in nitrogen, which may have contributed to an increase in chlorophyll synthesis. Additionally, CDs, for being effective electron donors and receptors and possessing free radical scavenging properties, may have contributed to reduced oxidation and increased accumulation of this pigment (Li et al., 2020). The beneficial effect of carbon dots (e.g., multi-walled carbon nanotubes - MWCNTs-COOH) on photosynthetic pigments and SPAD index was also found by Gohari et al. (2020) in basil (*Ocimum basilicum* L.).

The net photosynthetic rate (A, Figure 4A) and stomatal conductance (gs, Figure 4C) of plants subjected to salt stress had reductions of 10.9 and 46.4%, respectively, when compared to non-stressed plants. As for the effect of the nanobiostimulant on these variables, it was found that the concentration of 272 mg L^{-1} was the one that promoted maximum photosynthesis, which reached a rate of 19.6 μmol CO_2 m^{-2} s^{-1} (Figure 4B). On the other hand, the concentration of 275 mg L^{-1} was the one that promoted the highest stomatal conductance, 0.31 mol CO_2 m^{-2} s^{-1} (Figure 4D). Both concentrations surpassed that of 0 mg L^{-1} by 60.6 and 92.9%, respectively.

Regarding transpiration, plants subjected to salt stress had a reduction of 34.5% when compared to plants exposed to the lower electrical conductivity (Figure 4E). As for the effect of the nanobiostimulant, it was found that, with increasing concentrations, the plants showed a 32.9% increase when comparing transpiration at the higher and lowest applied concentrations (Figure 4F).

A typical response of plants under salt stress is observed, where there is a reduction in stomatal conductance as a protection mechanism against the effects of osmotic stress (Arif et al., 2020). As a result, there are consequent drops in transpiration, affecting the absorption and transport of nutrients, and also in the photosynthetic rate, since it limits the diffusion of CO_2 in leaf mesophyll cells, potentially increasing the chances of photochemical damage due to the absorption of excessive energy in photosystem II combined with the lower availability of CO_2 (Zörb et al., 2019).

Although carbon dots have several benefits in vegetables grown under water deficit conditions (Xiong et al., 2018), no significant effects were found for cowpea plants subjected to salt stress. Even with the reduction of transpiration, the fact that these nanoparticles activate transport proteins on the membrane may have led to the facilitation of sodium ion entry, thus culminating in lower photosynthesis efficiency added to lower stomatal conductance (Juárez-Maldonado et al., 2019).



Means followed by the same letter do not differ at p ≤ 0.05 by Tukey's test. * - Significant at p ≤ 0.05 by F test. Vertical bars indicate standard error (n=96)

Figure 4. Net photosynthesis (A- A and B), stomatal conductance (gs- C and D), and transpiration (E- D and E) of cowpea (*Vigna unguiculata*) as a function of the electrical conductivity of irrigation water (A, C, E) and of the nanobiostimulant concentration (B, D, F), at 31 days after sowing

There is still much discussion about the effect of nanoparticles and their influence on gas exchange. Nanomaterials induce better photosynthesis and improves stomatal conductance, transpiration rate, and chlorophyll content (Siddiqui et al., 2015), as occurred in the present study with carbon dots.

Salt stress also caused changes in the Ci/Ca ratio (Figure 5A) and leaf temperature (Figure 5B), increasing them by 18.0 and 2.9% compared to non-stressed plants. This higher Ci/Ca ratio suggests greater accumulation of carbon in the substomatal chamber; i.e., the carbon present is not being used in the photosynthesis process, and this indicates a limitation that is no longer stomatal but biochemical linked to the functioning of the photosynthetic machinery (Mukherjee et al., 2021). The increase in leaf temperature, on the other hand, is closely linked to the high temperature of the cultivation environment and the reduction of transpiration, an important process for the regulation and cooling of the leaf surface (Huang et al., 2022).

According to Figure 5C, it is observed that salt stress caused a decrease in the instantaneous carboxylation efficiency (iCE) by 22.4% when compared to plants under non-stressed treatment, which can be noticed by analyzing the accumulation of Ci in the substomatal chamber and the reduction of the photosynthetic rate, previously identified (Mukherjee et al., 2021). For the effect of the nanobiostimulant on this variable, it was found that the concentration of 250 mg L^{-1} was the one that led to the maximum iCE, promoting an increase of 22.2%

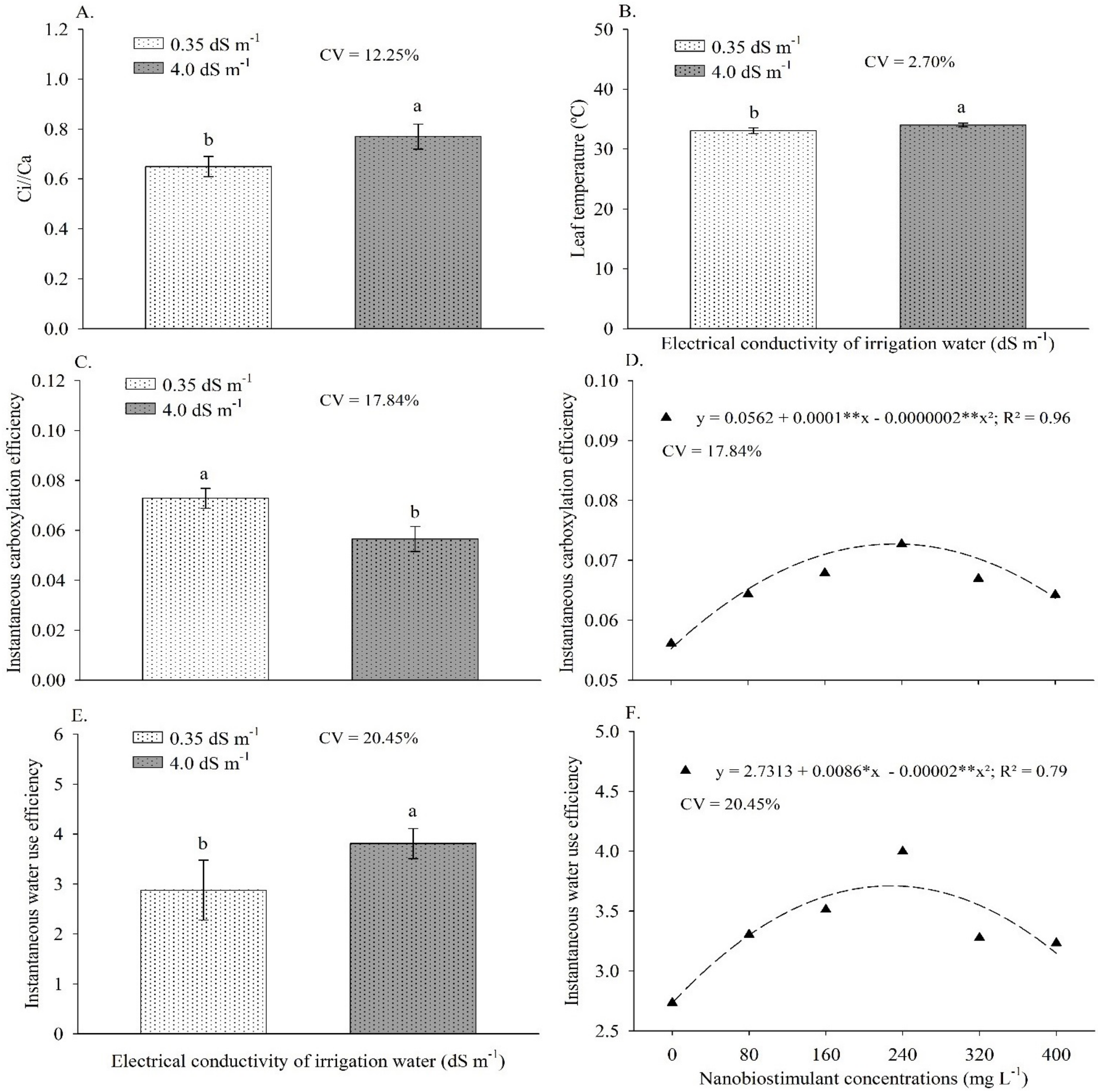


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Figure 5. Ci/Ca (A), leaf temperature (B), instantaneous carboxylation efficiency [μmol m^{-2} s^{-1}(μmol mol^{-1})$^{-1}$] (C and D) and instantaneous water use efficiency [μmol CO_2 m^{-2} s^{-1}(μmol H_2O m^{-2} s^{-1})$^{-1}$] (E and F) of cowpea (*Vigna unguiculata*) as a function of the electrical conductivity of irrigation water (A, B, C, D) and of the nanobiostimulant concentration (E, F), at 31 days after sowing

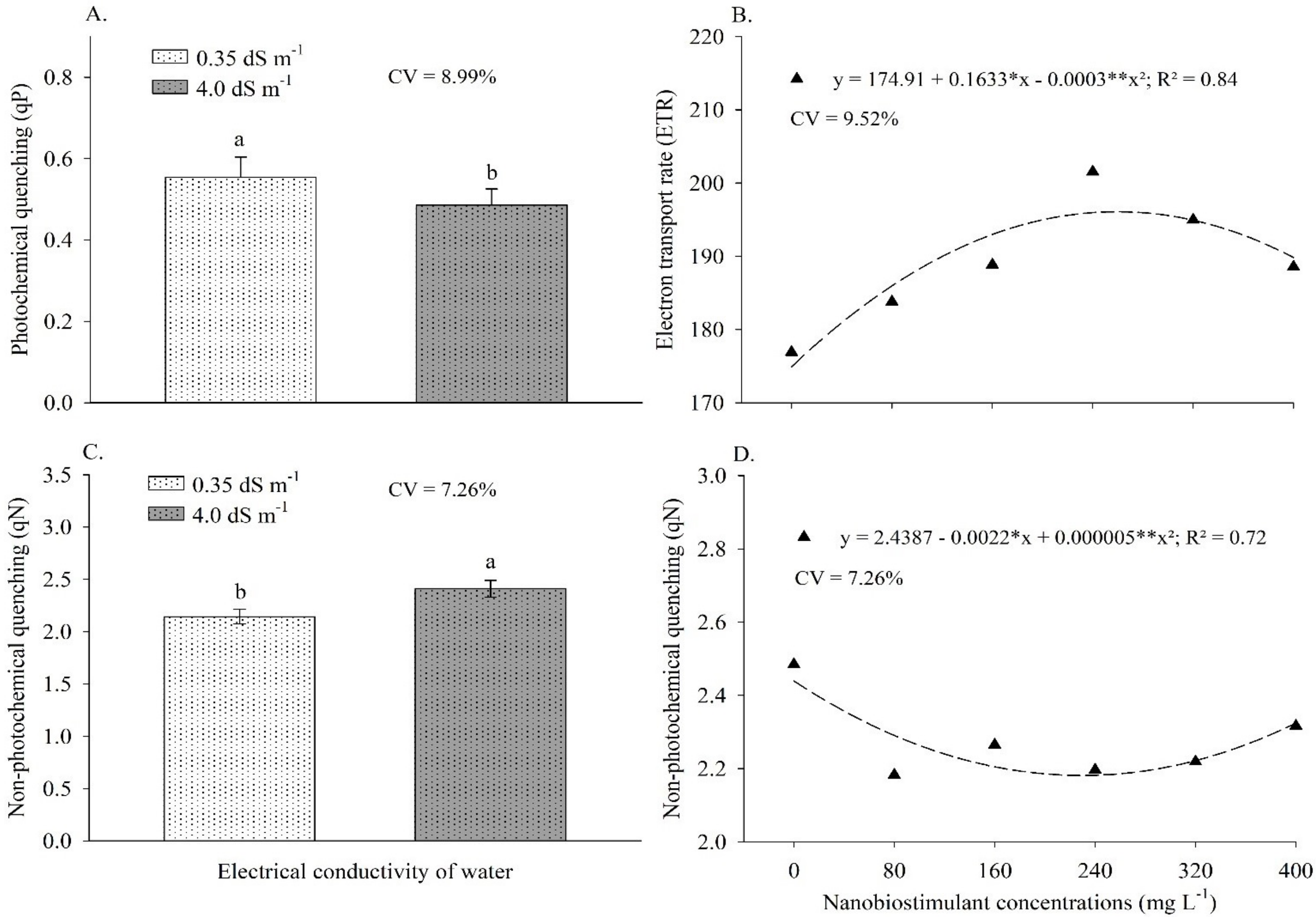
compared to the concentration of 0 mg L^{-1} (Figure 5D).

For instantaneous water use efficiency (WUE), it was observed that plants subjected to the highest salinity showed increases of 32.5% in the WUE compared to plants that were not exposed to stress (Figure 5E), which may demonstrate a tolerance response of this cowpea plant to salt stress with regard to stomatal regulation. For the effect of the nanobiostimulant on this variable, it was found that the concentration of 215 mg L^{-1} was the one that led to the maximum WUE, promoting an increase of 33.9% compared to the concentration of 0 mg L^{-1} (Figure 5F).

Studies show a relationship between carbon dots and the expression of genes that encode aquaporins, which allow the transport of water, gases, nutrients, and small molecules, and with the regulation of stomatal opening (Kou et al., 2021).

In Figure 6A and 6C, respectively, it is possible to observe a 4.5% reduction in photochemical quenching (qP) and a 12.4% increase in non-photochemical quenching (qN) in plants subjected to the higher salinity, compared to plants not exposed to stress.

This reduction in qP may be related to both the lower chlorophyll content and the negative effects of the excessive amount of sodium ions present in cells, ultimately causing damage to the photosystems (Zhang et al., 2019). On the other hand, the increase in qN constitutes a defense mechanism for plants to protect themselves from photoinhibition, in which



Means followed by the same letter do not differ at $p \leq 0.05$ by Tukey's test. *, ** - Significant at $p \leq 0.05$ and $p \leq 0.01$ by F test, respectively. Vertical bars indicate standard error (n=96)

Figure 6. Photochemical quenching (A), electron transport rate (B) and non-photochemical quenching (C and D) of cowpea (*Vigna unguiculata*) as a function of the electrical conductivity of irrigation water and of the nanobiostimulant concentration, at 31 days after sowing

they begin to dissipate the excess energy of the PSII reaction center in a non-photochemical way (Guedes et al., 2023).

Regarding the impact of the nanobiostimulant, it was observed that the concentration of 272 mg L^{-1} resulted in the highest electron transport rate (Figure 6B) and 220 mg L^{-1} was the one that led to the lowest non-photochemical quenching (Figure 6D), promoting an increase of 12.7% and a decrease of 9.9% compared to the concentration of 0 mg L^{-1}, respectively. Thus, the effectiveness of the nanobiostimulant in increasing photosynthesis is observed, with much of the energy investment concentrated in processes that lead to increased photosystem yield (Guedes et al., 2023).

The fact that carbon dots are good electron donors and receivers makes them act as intermediates in the electron transfer process. Chandra et al. (2014) found that some amine-functionalized carbon dots can strongly conjugate on the surface of chloroplasts and transfer electrons to them, generating positive photosynthetic responses.

Conclusions

1. Under non-stressful conditions (0.35 dS m^{-1}), concentrations between 240 and 320 mg L^{-1} of the carbon-based nanobiostimulant provide greater stimulation to the growth and gas exchange of cowpea plants during the vegetative stage.

2. The concentration of 240 mg L^{-1} of the nanobiostimulant promotes the maximum increase in height and relative growth rate of cowpea plants under conditions of salt stress (4.0 dS m^{-1}).

3. Irrigation with saline water (4.0 dS m^{-1}) diminishes the growth and physiological variables of cowpea, especially in plants that did not receive the biostimulant.

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