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Growth and physiology of ‘Sunrise’ papaya seedlings in response to salinity and humic acid¹

Crescimento e fisiologia de mudas de mamoeiro ‘Sunrise’ em resposta à salinidade e ácido húmico

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HIGHLIGHTS:

Humic acid treatment at 3.50 mL L⁻¹ alleviated the deleterious effects of salt stress on the growth of papaya seedlings.

Humic acid increased stomatal conductance, net photosynthesis, and transpiration in papaya seedlings.

Application of humic acid at >2 mL L⁻¹ increased the chlorophyll content of papaya.

ABSTRACT: Salinity is one of the major obstacles to agriculture in semi-arid regions, as it affects the physiological processes, growth, and yield of numerous crops. Hence, the application of salt stress attenuators is of paramount importance, as it enables the use of saline water for agricultural purposes. Among these, humic acid promotes the growth as well as water and nutrient uptake of plants. To this end, the present study evaluated the effects of humic acid on the growth and physiology of *Carica papaya* seedlings irrigated with saline water. The experiment followed the randomized block design with five levels of electrical conductivity (0.5, 1.15, 2.75, 4.35, and 5.0 dS m⁻¹) and five concentrations of humic acid (0.5, 0.94, 2.0, 3.06, and 3.5 mL L⁻¹). Growth, gas exchange, chlorophyll content, and chlorophyll a fluorescence were evaluated. Irrigation with 4.9 dS m⁻¹ water and application of 3.05 mL L⁻¹ humic acid promoted the growth of papaya seedlings. Irrigation with high-salinity water (4.96 and 3.09 dS m⁻¹) coupled with the application of 3.05 and 2.21 mL L⁻¹ humic acid increased internal CO₂ concentration, transpiration, instantaneous water use efficiency, carboxylation efficiency, and chlorophyll b content. Overall, humic acid (3.5 mL L⁻¹) attenuated the deleterious effects of salt stress, promoting the growth and improving the performance of papaya seedlings under moderate salinity (4 dS m⁻¹).

Key words: *Carica papaya* L., moderate salinity, soil conditioner, saline stress, humic substances

RESUMO: A salinidade é um dos grandes entraves na agricultura em regiões semiáridas, pois afeta os processos fisiológicos, crescimento e produção de diversas culturas. Assim, o uso de atenuadores do estresse salino é de extrema importância, pois permite o uso de águas salinas para fins agrícolas. Dentre estes, destacam-se os ácidos húmicos que promovem o crescimento e a absorção de água e nutrientes. Assim, o objetivo deste trabalho foi avaliar o efeito do ácido húmico sob o crescimento e a fisiologia de mudas de mamão irrigadas com água salina. O delineamento experimental utilizado foi em blocos casualizados com cinco níveis de condutividade elétrica (0,5; 1,15; 2,75; 4,35 e 5,0 dS m⁻¹), e cinco concentrações de ácido húmico (0,5; 0,94; 2,0; 3,06 e 3,5 mL L⁻¹). Foram avaliados o crescimento, trocas gasosas, índice de clorofilas e fluorescência da clorofila a. A irrigação com água de 4,9 dS m⁻¹ e aplicação de 3,05 mL L⁻¹ de ácido húmico proporcionou maior crescimento das mudas de mamoeiro. A irrigação de mudas de mamoeiro com alta salinidade (4,96 e 3,09 dS m⁻¹) aliada à aplicação de 3,05 e 2,21 mL L⁻¹ de ácido húmico proporcionou aumento da concentração interna de CO₂, taxa de transpiração, eficiência instantânea do uso da água, eficiência de carboxilação e teor de clorofila b. O ácido húmico (3,5 mL L⁻¹) atenua os efeitos deletérios da salinidade no mamoeiro, sendo capaz de aumentar o crescimento e melhorar o desempenho das mudas de mamão em salinidade moderada (4 dS m⁻¹).

Palavras-chave: *Carica papaya* L., condicionador de solo, estresse salino, salinidade moderada, substâncias húmicas

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INTRODUCTION

Papaya (*Carica papaya* L.) is of great economic importance for social development in Brazil. Papaya fruit production reached 1,256,706 tons in 2021, with the Northeast Region accounting for 56% of the total yield. In particular, Paraíba state is the fourth-largest producer of papaya seedlings (IBGE, 2021). However, unavailability of natural resources owing to rainfall variability coupled with high evaporative demand necessitates the use of saline water for irrigation (Silva et al., 2020).

Salinity affects the germination and growth of papaya seedlings, compromising orchards in the future (Lima-Neto et al., 2016). This is a result of water absorption restriction (osmotic effect) and changes in metabolism, nutrient absorption, and ionic balance (ionic effect) (Zhang et al., 2019), which promote the generation of reactive oxygen species, thereby damaging the cellular components and photosynthetic pigments (Ibrahim et al., 2018; Dias et al., 2020).

Application of humic substances can serve as an alternative in areas where only brackish water is available for irrigation (Guo et al., 2019; Pham et al., 2021). As such, humic compounds enhance microbial activity, promote nutrient absorption, increase soil permeability, and stimulate plant growth (Liu et al., 2019). Furthermore, humic acid can improve the photosynthetic efficiency of plants by regulating various metabolic, hormonal, biochemical, molecular, and physiological activities (Shah et al., 2018). Dias et al. (2020) obtained promising results with humic application for the production of 'Hawaii' papaya; however, further studies are warranted on other cultivars and under various soil and climatic conditions. To this end, the present study evaluated the effects of humic acid on the growth and physiology of papaya seedlings irrigated with saline water.

MATERIAL AND METHODS

The experiment was conducted during November-December 2021 in an uncontrolled greenhouse with a transparent plastic cover at the Center for Human, Social and Agrarian Sciences, Federal University of Paraíba, Bananeiras, State of Paraíba, Brazil (6° 45' 27.78" S 35° 38' 46.40" W; 617 m a.s.l.). According to Köppen's classification, the local climate is of type "As," with a dry and hot summer and rainy winter (Alvares et al., 2013).

The treatments were arranged in randomized blocks using the matrix of central composite box (Tekindal et al., 2014), combining different electrical conductivities of irrigation water (ECw) with concentrations of humic acid (HA). The minimum (- α) and maximum (α) values of ECw and HA ranged respectively from 0.5 to 5.0 dS m⁻¹ and 0.5 to 3.5 mL L⁻¹, totaling nine treatments (Table 1); four replicates were set per treatment, divided into three blocks, with one plant per plot. ECw and HA values were based on the study by Dias et al. (2020) on 'Hawaii' papaya.

Diamond Grow™ liquid, containing 14% humic acid, was applied manually at the indicated concentrations using a 10 mL graduated pipette, divided into three applications (10, 20, and 30 days after the start of saline water irrigation); the concentration of humic acid present in the product was calculated in mL L⁻¹ for application corresponding each treatment. All humic acid

Table 1. Combinations of treatments generated through the matrix of central composite box

Treatment	Level		Concentration	
	ECw	HA	ECw	HA
T ₁	-1	-1	1.15	0.94
T ₂	-1	1	1.15	3.06
T ₃	1	-1	4.35	0.94
T ₄	1	1	4.35	3.06
T ₅	- α	0	0.50	2.00
T ₆	α	0	5.00	2.00
T ₇	0	α	2.75	3.50
T ₈	0	- α	2.75	0.50
T ₉	0	0	2.75	2.00

ECw - electrical conductivity of irrigation water; HA - humic acid

treatments were applied in the late afternoon. No fertilization other than humic acid was applied.

As the substrate, red-yellow Latosol (Oxisol - American Classification of Soil Taxonomy), tanned bovine manure, and washed sand were mixed at the ratio of 3:1:1 and placed in 5 dm³ polyethylene pots. Soil chemical analysis revealed the following characteristics: pH = 5.52; organic matter = 28.72 g kg⁻¹; P = 18.72 mg kg⁻³; Ca²⁺ = 4.80 cmol_c dm⁻³; Mg²⁺ = 1.20 cmol_c dm⁻³; Na⁺ = 0.04 cmol_c dm⁻³; base saturation = 6.13 cmol_c dm⁻³; H⁺ + Al³⁺ = 1.16 cmol_c dm⁻³; Al³⁺ = 0.0 cmol_c dm⁻³; cation exchange capacity = 7.29 cmol_c dm⁻³; V = 84.15%; K⁺ = 0.09 cmol_c dm⁻³; organic carbon = 16.6 g kg⁻¹; fulvic acid fraction = 2.85 g kg⁻¹; humic acid fraction = 3.31 g kg⁻¹; humin fraction = 8.33 g kg⁻¹; and EC from the saturation extract (dS m⁻¹) = 0.41.

Papaya seeds (*Carica papaya* L. 'Sunrise'; Feltrin®) were used. Seedlings were produced by sowing two seeds in a container with a volumetric capacity of 150 mL and depth of approximately 3 cm. Thinning was performed 10 days after sowing (DAS), retaining one (most vigorous) seedling per container. At 14 DAS, seedlings were selected and standardized, and those approximately 10 cm tall were transplanted into polyethylene bags with a volumetric capacity of 3.5 dm³. The bags were arranged with a spacing of 0.3 × 0.3 m.

Saline water of the required electrical conductivity was prepared by adding sodium chloride to the supply water (ECw = 0.5 dS m⁻¹); ECw values were confirmed using a portable conductivity meter (CD-860 microprocessor; Instrutherm®). Irrigation with saline water was initiated at 15 DAS, performed manually according to the water requirement of the plants; soil moisture was maintained close to 100% of the field capacity, as ascertained using drainage lysimetry (Bernardo et al., 2019).

Plant height, stem diameter, leaf number, and leaf area were evaluated at 30 DAS. Plant height (expressed in cm) was obtained by measuring the distance between the soil surface and the highest part of the plant using a graduated ruler. Stem diameter (expressed in mm) was measured using a digital caliper. Leaf number was determined by counting all leaves. Leaf area was determined according to Eq. 1 (Campostrini & Yamanishi, 2001):

$$LA = L \times W \times N \times f \quad (1)$$

where:

- LA - leaf area (cm²);
- L - length of the third leaf from the apex (cm);
- W - width of the third leaf from the apex (cm);

N - number of leaves per plant; and,
f - correction factor for papaya (0.89).

Gas exchange, chlorophyll a, chlorophyll b, chlorophyll total, and chlorophyll a fluorescence were evaluated at 31 DAS. Gas exchange was ascertained using an infrared gas analyzer (IRGA; LCpro-SD Portable Photosynthesis System, ADC BioScientific, Hoddesdon, England). Measurements were performed between 8:00 and 11:00 a.m. using artificial light ($1,200 \mu\text{mol m}^{-2} \text{s}^{-1}$) at the reference ambient CO_2 concentration of $385 \mu\text{mol CO}_2 \text{mol}^{-1}$ of air and ambient temperature. In addition, stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), net photosynthesis (A , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$), internal carbon concentration (C_i , $\mu\text{mol CO}_2 \text{mol air}^{-1}$), water use efficiency (WUE , A/E), and intrinsic carboxylation efficiency (iCE , A/C_i) were evaluated. Chlorophyll a, chlorophyll b, and chlorophyll total contents were measured on the third leaf from the apex of each plant using a digital chlorophyll meter (CFL 1030; ClorofiLOG®, Falker, Porto Alegre, Brazil).

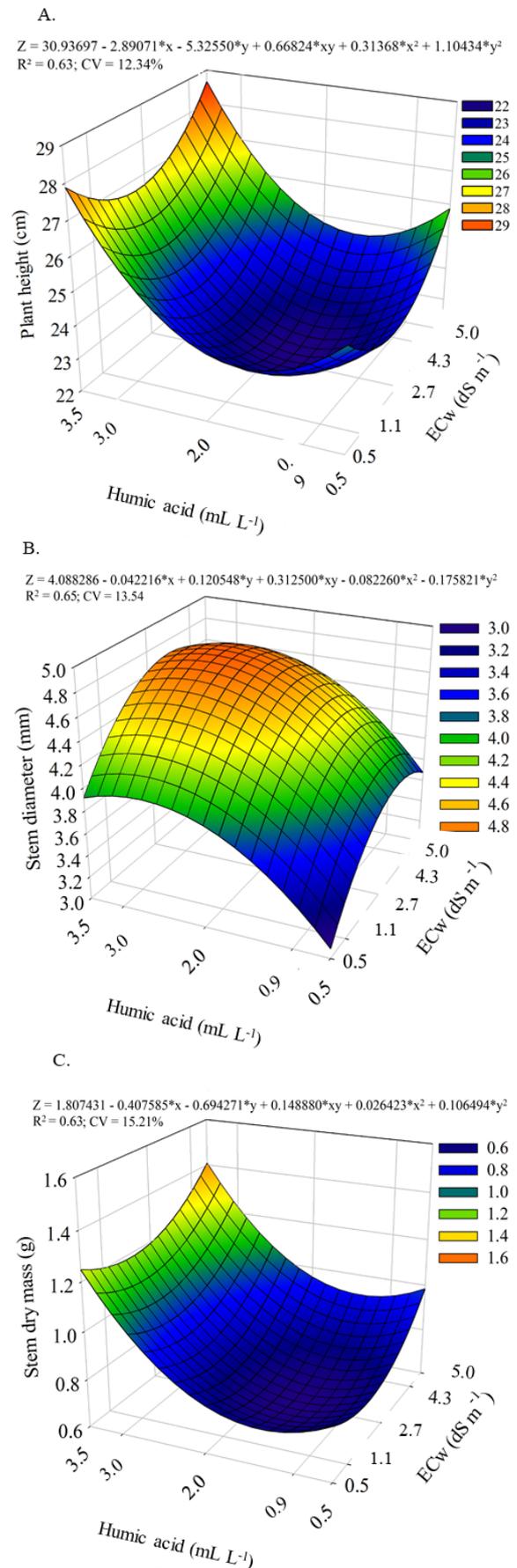
At the end of the experiment (at 32 DAS), leaves and stems were harvested, and fresh mass was ascertained using a balance with 0.001 g resolution. The plant parts were then deposited in Kraft paper bags and placed in an oven with forced air circulation at $65 \text{ }^\circ\text{C}$ until a constant weight was obtained. Plant dry mass was measured using a balance (0.001 g).

The obtained data were submitted to analysis of variance with F-test ($p < 0.01 \leq 0.05$). When significant results were obtained, regression analysis was performed on the quantitative factors (humic acid concentration and electrical conductivity level). All analyses were performed using R version 4.1.2. (R Core Team, 2021).

RESULTS AND DISCUSSION

Application of humic acid mitigated the harmful effects of salinity on the growth of *C. papaya* seedlings (Figure 1), with significant increments attaining the height of 28.58 cm under ECw of 4.99 dS m^{-1} and HA concentration of 3.05 mL L^{-1} (Figure 1A). Despite significant effect of the interaction of factors, adequate adjustment for leaf number was not possible ($z = 7.905653 - 0.411836x - 0.675634y + 0.257960xy - 0.009364x^2 + 0.015094y^2$; $R^2 = 0.53$; $\text{CV} = 18.04\%$). Likewise, the highest values of stem diameter and stem dry mass were 4.7 mm and 1.57 g, respectively, obtained at the ECw of 4.99 and 4.89 dS m^{-1} and HA concentrations of 3.03 and 3.05 mL L^{-1} (Figures 1B and C).

Salinity stimulated the shoot growth of papaya plants. In general, saline conditions reduce growth, gas exchange, and biomass in papaya plants (Diniz et al., 2018; Sousa et al., 2019; Dias et al., 2020). This reduction can be attributed to difficulty in absorbing water from the soil with reduced water potential owing to high salt concentration, which leads to the closure of stomata and suppression of photosynthesis (Oliveira et al., 2017). In addition, salinity increases the absorption of Na^+ and Cl^- and reduces the absorption of K^+ , Ca^{2+} , and NO_3^- , leading to nutritional imbalance and plant growth suppression due to nutrient deficiency (Sá et al., 2013; Coelho et al., 2015; Oliveira et al., 2015; Silva et al., 2017). In the present study, however, salinity



* $p < 0.05$ or ** $p < 0.01$, according to F-test

Figure 1. Plant height (A), stem diameter (B), and stem dry mass (C) of *Carica papaya* seedlings subjected to different levels of irrigation water salinity (ECw) and concentrations of humic acid (32 days after sowing)

of up to 5 dS m⁻¹ was not deleterious to seedling growth, due perhaps to the moderate tolerance of papaya seedlings to salinity (Diniz et al., 2018) or the short period of exposure to salt stress.

Application of humic acid, particularly at high concentrations, stimulated plant growth. The effect of humic acid as a growth promoter has been demonstrated in papaya due to increased water content in the root zone and the ion exchangeable ability of the soil solution (Guo et al., 2019; Al-Abadi & Al-hayany, 2021). This effect may be related to changes in the expression of genes linked to the assimilation of nutrients or the synthesis and transport of phytohormones, which affects the photosynthetic processes and stimulates plant growth (Barone et al., 2019). In addition, humic acid can improve cell membrane stability, reduce Na⁺ accumulation, and increase K⁺ accumulation, thereby mitigating salinity-induced lipid peroxidation and leaf necrosis (Saidimoradi et al., 2019).

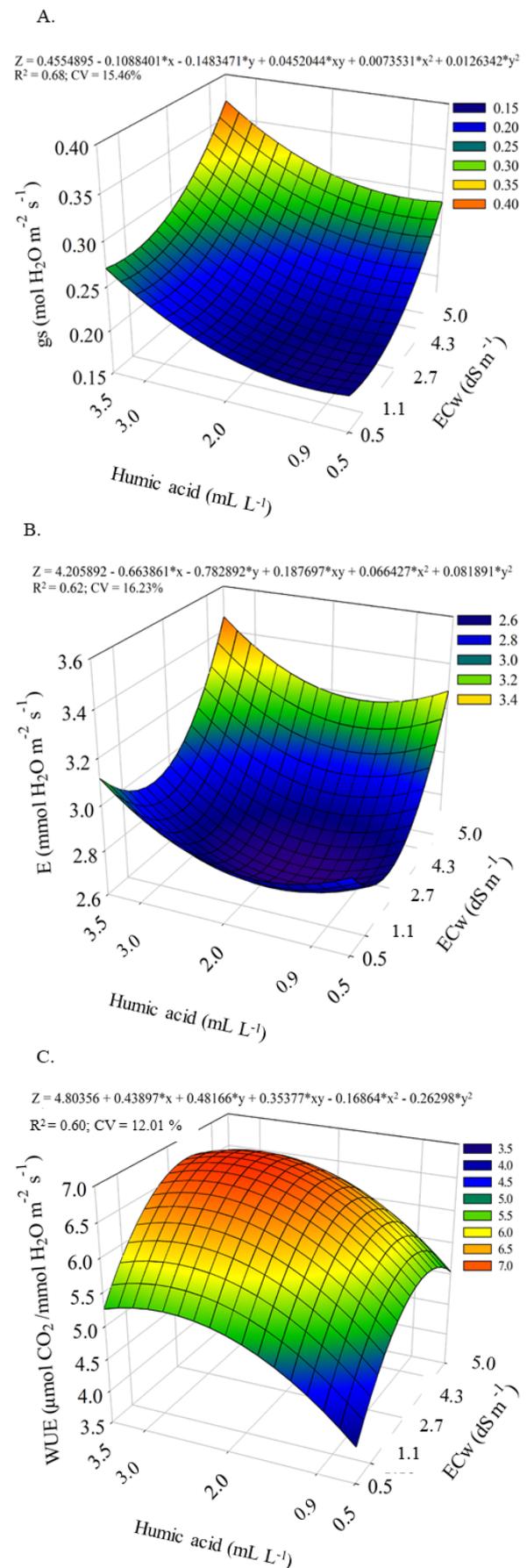
Humic acid increased *g_s* and *E*. At the EC_w of 4.96 and 5.0 dS m⁻¹ and HA concentration of 3.05 and 3.5 mL L⁻¹, the estimated values of *g_s* and *E* were 0.44 and 3.5 mmol H₂O m⁻² s⁻¹, respectively (Figures 2A and B).

Furthermore, the different concentrations of humic acid and levels of electrical conductivity affected *A* and *C_i*; however, the data could not be adjusted satisfactorily (*A*: $z = 11.804062 + 1.414767x + 2.254550y + 0.078616xy - 0.256850x^2 - 0.563171y^2$; $R^2 = 0.58$; $CV = 16.71\%$; *C_i*: $z = 276.48987 - 18.01648 - 17.77253y + 5.11006xy + 1.46737x^2 + 0.53046y^2$; $R^2 = 0.56$; $CV = 13.31\%$).

Similarly, WUE was higher in plants treated with humic acid and exposed to salt stress, reaching high values [(7.24 μmol CO₂ (mmol H₂O m⁻² s⁻¹)⁻¹] at the EC_w of 4.50 dS m⁻¹ and HA concentration of 3.05 mL L⁻¹ (Figures 2C).

Humic acid improved soil fertility by increasing porosity and amphipathic particles that favor water and nutrient retention (Guo et al., 2019). In fact, we observed an increase in the *g_s*, *E*, and WUE of plants that were treated with high concentrations of humic acid, suggesting that humic acid increased the water-holding capacity of soil. Interestingly, this effect was potentiated by the combination of high concentration of humic acid and salinity of the irrigation water, suggesting that although salinity increased transpiration, the water content of soil was sufficient to maintain the stomata open without dehydration. Similar results have been observed in a previous study on potato plants under water deficit, in which the application of humic acid increased *g_s* and net CO₂ assimilation, even in dry soils (Man-Hong et al., 2020). As such, the net assimilation of CO₂ was higher when the plants were exposed to the combination of moderate salinity and humic acid. These results suggest that although water relations did not act as a limiting factor for photosynthesis, salinity may have induced metabolic damage owing to ionic toxicity and oxidative stress, ultimately suppressing the net photosynthesis. Meanwhile, the application of humic acid likely mitigated the ionic toxicity caused by salinity. This speculation is supported by the fact that the application of humic acid is related to the enhancement of antioxidant capacity as well as increase in the retention of toxic ions (Na⁺ and Cl⁻) in the soil but decrease in their absorption (Guo et al., 2019; Saidimoradi et al., 2019).

The chlorophyll indices of papaya seedlings were affected by the factors studied separately. As such, the application of humic



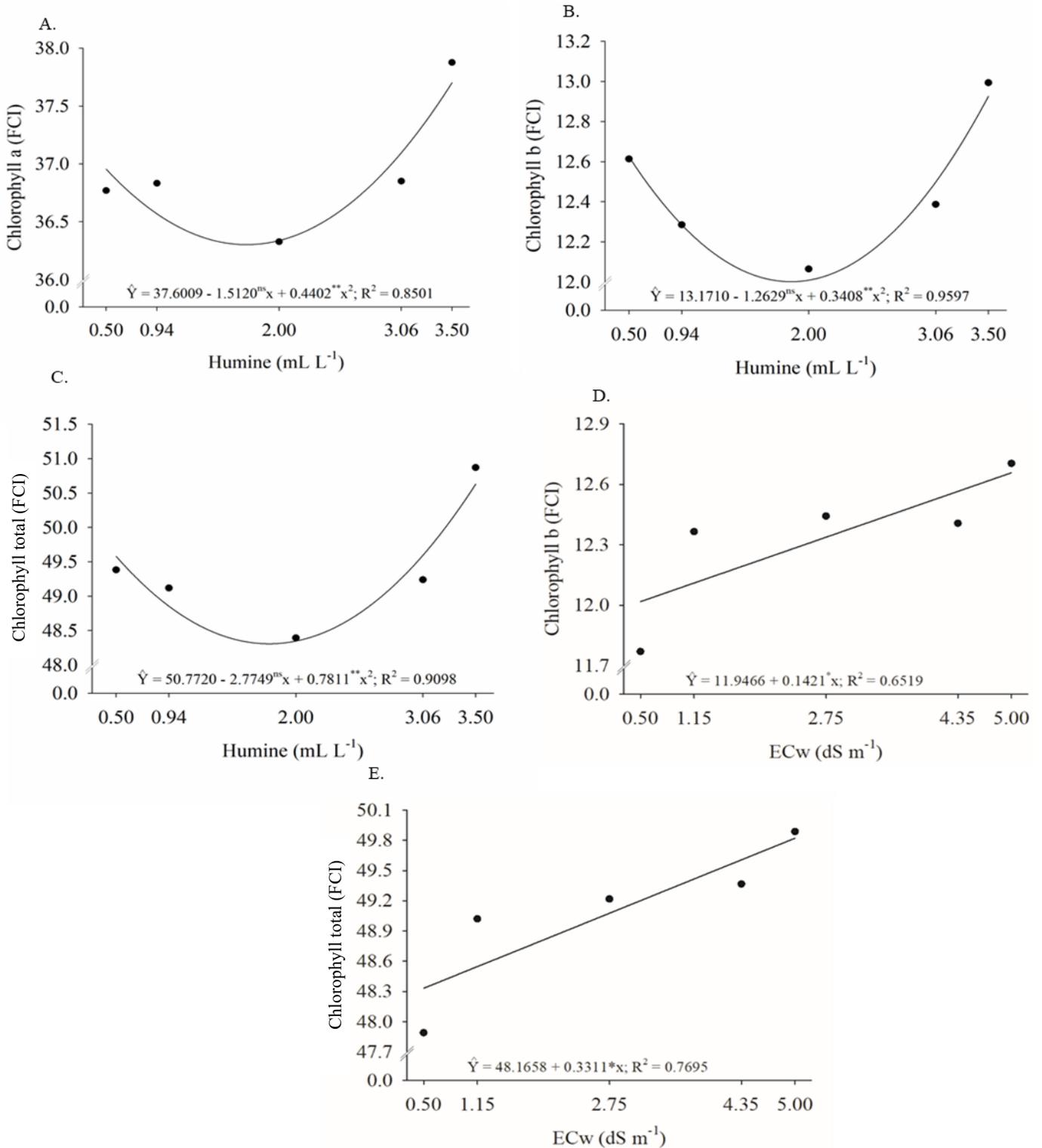
* $p < 0.05$ according to F-test

Figure 2. (A) Stomatal conductance (*g_s*), (B) transpiration, and (C) water use efficiency (WUE) of *Carica papaya* seedlings subjected to different levels of irrigation water salinity (EC_w) and concentrations of humic acid (32 days after sowing)

acid reduced chlorophyll a, chlorophyll b, and chlorophyll total contents at concentrations up to 2 mL L⁻¹, followed by successive increment; the maximum values (37.7, 12.92, and 50.6 Falker Chlorophyll Index [FCI], respectively) were recorded at the HA concentration of 3.5 mL L⁻¹ (Figures 3A, B, and C). This small reduction and successive increment can be explained by the fact that at concentrations below 2 mL L⁻¹, humic acid induced chlorophyll degradation, while higher concentrations did not produce this deleterious effect. Humic substances derived

from lignin decomposition have been reported to increase chlorophyll content, although the beneficial effect depends on the application dose (Saidimoradi et al., 2019), which explains the trends observed in the present study.

Furthermore, the salinity of irrigation water significantly affected the contents of chlorophyll b and chlorophyll total, showing linear increment as a function of salt addition to irrigation water; specifically, between the lowest and highest EC_w, increases of respectively 5.32% (Figure 3D) and 3.08%



* p < 0.05 or ** p < 0.01 according to F-test

Figure 3. Chlorophyll a (A), chlorophyll b (B and D), and chlorophyll total (C and E) content of *Carica papaya* seedlings subjected to different levels of irrigation water salinity (EC_w) and concentrations of humic acid (32 days after sowing)

(Figure 3E) were noted. No significant effect was noted on chlorophyll a fluorescence.

Salinity promotes the activity of chlorophyllase, the enzyme responsible for chlorophyll degradation, due perhaps to the greater translocation of chloride instead of nitrate mediated by the high concentration of salts in the plant, ultimately reducing the chlorophyll content (Ibrahim et al., 2018). Nevertheless, our results demonstrated higher chlorophyll b and chlorophyll total contents at the highest salt concentrations tested. This may be related to the intrinsic tolerance of papaya plants to salinity, since chlorophyll b is an accessory pigment present in the antenna complexes that is accumulated under stress to protect the photosystems (Lokstein et al., 2021). The increase in chlorophyll content with the application of humic acid, in turn, may be connected to its action of stimulating the synthesis and attenuating the degradation of chlorophyll, thereby contributing to the dissipation of excess excitation energy in photosystem II and protection of the photosystems under saline conditions (Ozfidan-Konakci et al., 2018).

In summary, the application of humic acid appears to be a viable alternative for the production of papaya seedlings using brackish water for irrigation, provided that the increases in growth and gas exchange are directly linked to plant productivity (Ramos et al., 2021; Kumar et al., 2022). These findings contribute to broadening our knowledge of the physiological aspects of *C. papaya* growth with saline water irrigation as well as the practical application of humic acid to improve papaya seedling production under salt stress.

CONCLUSIONS

The application of humic acid (3.5 mL L⁻¹) coupled with moderate salinity of irrigation water (4 dS m⁻¹) stimulates the growth and gas exchange of *Carica papaya* seedlings. Humic acid not only attenuates saline stress but also promotes the development of papaya seedlings irrigated with saline water.

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