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Original Article

Effect of calcium-enriched microalgae extract on mitigating saline stress in papaya seedlings

Efeito do extrato de microalgas enriquecido com cálcio na mitigação do estresse salino em mudas de mamoeiro

I. R. S. do Nascimento[a]*, L. D. R. Silva[b], E. N. S. Rodrigues[a], J. M. F. L. Cruz[c], D. B. Araújo[a], C. D. S. Pereira[b], L. M. F. Silva[d], W. E. Pereira[e], M. C. Silva[f], J. P. O. Santos[g] and O. R. Farias[h]

[a]Universidade Federal da Paraíba – UFPB, Departamento de Fitotecnia e Ciências Ambientais, Programa de Pós-graduação em Agronomia, Areia, PB, Brasil

[b]Universidade Federal de Lavras – UFLA, Departamento de Agronomia, Programa de Pós-graduação em Agronomia, Lavras, MG, Brasil

[c]Universidade Federal de Lavras - UFLA, Departamento de Fitopatologia, Programa de Pós-Graduação em Fitopatologia, Lavras, MG, Brasil

[d]Universidade Federal da Paraíba – UFPB, Departamento de Solos e Engenharia Rural, Areia, PB, Brasil

[e]Universidade Federal da Paraíba – UFPB, Departamento de Ciências Fundamentais e Sociais, Areia, PB, Brasil

[f]Universidade Federal da Paraíba – UFPB, Departamento de Biociência, Programa de Pós-graduação em Biodiversidade, Areia, PB, Brasil

[g]Instituto Federal do Tocantis – IFTO, Campus Avançado Lagoa da Confusão, TO, Brasil

[h]Universidade Federal da Paraíba – UFPB, Programa de Pós-doutorado em Agronomia, Areia, PB, Brasil

Abstract

The papaya (*Carica papaya* L.) is among the mainly fruit species produced in tropical and subtropical climate. The salinity of water in agricultural areas is considered a limiting factor for the expansion of papaya. This study aimed to evaluate calcium-enriched microalgae extract (EMa-Ca) as an attenuator of saline stress in irrigation water on the growth and physiology of Formosa papaya seedlings, hybrid Tainung. The experiment was conducted in a protected environment, with treatments distributed in a 5 × 2 factorial scheme, comprising five electrical conductivities of irrigation water (0.50; 1.10; 2.50; 3.90 and 4.50 dSm^{-1}) with the presence and absence of EMa-Ca in the substrate. Evaluated characteristics were: plant height, number of leaves, stem diameter, leaf area, dry masses weight of roots, aboveground parts and total. Gas exchanges and chlorophyll indices (*a*, *b* and *total*) were also evaluated. The application of EMa-Ca resulted in an increase of 6.05% in height and 6.33% in trunk diameter. The number of leaves decreased with an increase in electrical conductivity, and the leaf area was reduced by 33%. All seedling dry masses showed greater declines in the absence of EM-Ca. The EMa-Ca increased net photosynthesis, CO_2 concentration, transpiration and stomatal conductance by 39.13%, 30.43%, 38.88% and 42.85%, respectively. For chlorophyll without the use of EMa-Ca, a decrease rate of 1.21%, 0.41% and 1.62% was observed for *Chla*, *Chlb* and *Chlt*, respectively. Therefore, the EMa-Ca application (1.0 ml/L) significantly enhance the vegetative development, gas exchanges, and chlorophyll indices of papaya seedlings under saline stress conditions.

Keywords: abiotic stress, biostimulant, *Carica*, microorganisms, nutrition.

Resumo

O mamão (*Carica papaya* L.) está entre as principais espécies frutíferas produzidas em clima tropical e subtropical. A condição de salinidade da água nas áreas agrícolas pode ser considerada um fator limitante para a expansão do mamão. Este estudo teve como objetivo avaliar o extrato de microalga enriquecido com cálcio (EMa-Ca) como atenuador do estresse salino em água de irrigação no crescimento e na fisiologia de mudas de mamão Formosa, híbrido Tainung. O experimento foi conduzido em ambiente protegido, com tratamentos distribuídos em esquema fatorial 5 × 2, compreendendo cinco condutividades elétricas da água de irrigação (0,50; 1,10; 2,50; 3,90 e 4,50 dSm^{-1}) com a presença e ausência no substrato de EMa-Ca. As características avaliadas foram: altura da planta, número de folhas, diâmetro do caule, área foliar, peso de massa seca das raízes, partes aérea e total. Também foram avaliadas as trocas gasosas e os índices de clorofila (a, b e total). A aplicação de EMa-Ca resultou em aumento de 6,05% na altura e 6,33% no diâmetro do tronco. O número de folhas diminuiu com aumento da condutividade elétrica e a área foliar reduziu 33%. Todas as massas secas das mudas apresentaram maiores declínios na ausência do EM-Ca. O EMa-Ca aumentou a fotossíntese líquida, concentração de CO_2, transpiração e condutância estomática em 39,13%, 30,43%, 38,88% e 42,85%, respectivamente. Para a clorofila sem a utilização do EMa-Ca observa-se uma taxa de diminuição de 1,21%, 0,41% e 1,62% para *Chla*, *Chlb* e *Chlt*, respectivamente. Portanto, a aplicação de EMa-Ca (1,0 ml/L) melhora significativamente o desenvolvimento vegetativo, as trocas gasosas e os índices de clorofila de mudas de mamoeiro sob condições de estresse salino.

Palavras-chave: estresse abiótico, bioestimulante, *Carica*, microrganismos, nutrição.

*e-mail: izaias.agronomia@gmail.com
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1. Introduction

Papaya (*Carica papaya* L.) is among the mainly fruit species produced in tropical and subtropical climate. Its socioeconomics importance is tied to the agricultural development and income generation in producing regions, making a significant contribution to strengthening the national economy through both fresh and processed products. It originates from southern Mexico and regions of Central America, being cultivated in countries in Asia, Africa, Central America and the Caribbean, South America and Oceania, being the fourth most exported tropical fruit, after pineapple, avocado and mango (FAO, 2024).

Brazil ranks third in the world as the largest papaya exporter, with Mexico and Guatemala leading the list (Lucena et al., 2021). However, in Brazilian territory, the states with the highest production (Espírito Santo and Bahia) are showing a reduction in productivity along with other traditional areas and this is driving the expansion of the crop to new agricultural areas (Lucena et al., 2021). The salinity of water in agricultural areas can be considerate a limiting factor for papaya expansion, particularly in semi-arid regions, commonly characterized by limited freshwater resources (Álvarez-Méndez et al., 2022). The importance of research is highlighted to enhance the papaya production under restricted soil and water conditions, such as water salinity and low annual precipitation.

Salinity has negative effects on the growth of papaya plants at different stages of development (Nascimento Neto et al., 2020). It induces water stress and cytotoxicity caused by the excessive absorption of ions, such as chloride and sodium (NaCl) (Sá et al., 2013a). Additionally, salinity triggers oxidative stress by generating reactive oxygen species, which damage cellular compounds and photosynthetic pigments (Dias et al., 2019; Hasanuzzaman et al., 2021). The increased salinity in the root and leaf zone reduces growth and biomass accumulation in the vegetative parts of papaya, in agreement with previous reports on the salt stress tolerance of papaya hybrids (Elder et al., 2000; Sá et al., 2013a; Nascimento-Neto et al., 2020).

The use of microalgae in agriculture serve as an important source of biologically active compounds, contributing phenolic compounds, polysaccharides, hormone-like substances and, proteins known for their benefits as antioxidants agents, plant growth promoters and their ability to enhance plant resilience to abiotic and biotic stress (Kusvuran, 2021; Ronga et al., 2019). Moreover, the microalgae extract present in biostimulants have the capacity to diminish stress in crops, for example, salinity and drought (González-Pérez et al., 2022). The calcium ion (Ca^{2+}) plays a crucial role by signaling in the plant's physiological and biochemical pathways to development resistance to saline stress. This generates an antagonist effects on sodium (Na^{+}) and chloride (Cl^{-}) that causes cytotoxity effects in plants (Seifikalhor et al., 2019). Therefore, microalgae extract enriched with calcium (EMa-Ca) can be an alternative to mitigate the effects of water salinity on papaya seedlings.

Technical and scientific information involving the studied factors (salinity × biostimulant) in *C. papaya* are limited. Sá et al. (2013b) stated that the exogenous application of gibberellin in *C. papaya* plants shows significant potential in mitigating saline stress when compared to auxins and cytokines. Therefore, new research is necessary to ensure the use and occupation of areas considerate limited to agriculture due to factors such as water salinity, especially when facing climate changes and the intense use of chemical inputs to attend the nutritional demand of the world's population.

Therefore, the presence of microalgae and calcium can minimize the adverse effects caused by water salinity on the morphological and physiological characteristics of papaya seedlings. This study aimed to evaluated calcium-enriched microalgae extract (EMa-Ca) as an attenuator of saline stress in irrigation water on the growth and physiology of Formosa papaya seedlings, hybrid Tainung.

2. Material and Methods

2.1. Location and experimental design

The experiment was conducted in a protected environment from September to October of 2021 (42 days) at the Federal University of Paraíba, in Areia, Paraíba (PB) (latitude 6º 57' 26'' S and longitude 35º 45' 31'' W, altitude 574.62 m a.s.l.). The region climate is characterized as type "As", according to the Köppen classification with average annual precipitation of 1200 mm (Francisco and Santos, 2017). Meteorological conditions were collected throughout the experimental period (Figure 1).

The design used in the experiment was randomized blocks with three replicates. Treatments were arranged in a 5 × 2 factorial scheme, consisting of five electrical conductivities of irrigation water (0.50, 1.10, 2.50, 3.90 and 4.50 dSm^{-1}) in the presence and absence of microalgae extract enriched with calcium (EMa-Ca). The substrate (MecPlant®) used has classification of type I (pH (H_2O); $_{1:25}$ = 5.0; P = 233,27 mg kg^{-1}; Na^{+} = 14.69 $cmol_c\ kg^{-1}$; $H^{+} + Al^{+}$ = 20.62 $cmol_c\ kg^{-1}$; Al^{3+} = 0.0 $cmol_c\ kg^{-1}$; Ca^{2+} = 8.9 $cmol_c\ kg^{-1}$; Mg^{2+} = 8.9 $cmol_c\ kg^{-1}$; K^{+} = 97.59 $cmol_c\ kg^{-1}$; Total organic carbon = 35.25 g kg^{-1}), specific for seedling production, and the containers employed were plastic bags of 1 dm^3.

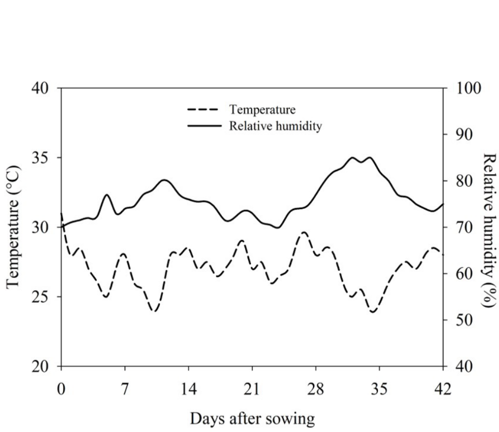


Figure 1. Daily average temperature and relative humidity of the air inside the protected environment during the experimental period.

The sowing was conducted with three seeds per container at 2 centimeters depth. After 10 days of emergence (DAE), thinning was performed, maintaining one plant per container. Seeds used were from Formosa, hybrid Tainung.

2.2. Experimental conduction and evaluated characteristics

Soil applications of calcium-enriched microalgae extract (EMa-Ca) were applied fertigation at a dose of 1 ml/L, resulting in a solution of 100 ml/plant supplied in the same quantity six times over an interval of seven days, resulting in 0.6 ml/plant of EMa-Ca. The initial application at plant emergence, on the fifth day after sowing (DAS). The microalgae extract enriched with calcium used was the commercial product Ferticell Cálcio 880 Plus®, which has the following guarantees: 25% microalgae extract (*Chlorella* spp.) and 26% of calcium ($CaCO_3$).

Irrigation was conducted daily with water conductivity following the treatment levels. During the first five days, the containers were kept close to field capacity, after this period, the plants received daily water slides based on evaporation from a mini evaporation tank prepared according to Pereira et al. (2007), with a diameter of 60 cm and a height of 25 cm. To obtain the irrigation depth, the equation was used: Lam = Ev, where: Lam = Irrigation depth to be applied (mm); Ev = evaporation in the mini tank (mm). However, until the seedlings emerged all treatments were irrigated with non-saline water. The electrical conductivity values of the irrigation water were obtained from saline water (CEa = 15.7 dSm $^{-1}$) originating from the Jacaré reservoir, located in the municipality of Remígio, PB (latitude 6° 53' 30'' S and longitude 35° 49' 51'' W, 535 m). This water was diluted in non-saline water (CEa = 0.48 dSm^{-1}) supplied by the public system of the municipality of Areia, PB. The analysis of the water after dilution was in accordance with the methods established by Richards (1947) (Table 1).

At 45 days after the emerge (DAE) the seedlings were assessed for their growth, measuring: plant height (cm) using a millimeter ruler; stem diameter (mm) using digital calipers; number of leaves by tallying fully development leaves; leaf area (cm^2) using a scanner printer (HP Deskjet F4480 Inject Multifunction Printer, Chongqing, China) for subsequent determination using the program ImageJ®. Dry root, aboveground and total masses (g) were obtained by weighing on an analytical balance, after the material was placed in a forced air circulation oven at 65º C until reaching constant mass over 72 hours, both equipment manufactured in the United States of America (USA).

Gas exchange and chlorophyll indices (*a*, *b* and *total*) evaluation were conducted at the first leaf from the top of the seedling, at the same time at 45 DAE, between 9 a.m. and 11 a.m. using an infrared gas analyzer – IRGA (LI-6400XT, LI-COR®, Lincoln Nebrasca, USA) with an attached light source of 1.200 µmol m^{-2} s^{-1}. The analyzed gas exchange variables included net CO_2 assimilation rate (A - µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s - mol m^{-2} s^{-1}), internal CO_2 concentration (C_i - µmol CO_2 mol^{-1}), and transpiration (E - mmol H_2O m^{-2} s^{-1}). The determination of leaf chlorophyll indices *a* (*Chla*) and *b* (*Chlb*) were carried out on the mid-third of the first leaf using the portable clorofiLOG FALKER® (manufactured Rio Grande do Sul, Brasil) and the readings are expressed in relative units referred as Falker chlorophyll index (ICF, dimensionless). From those readings, the total chlorophyll index (*Chlt* = *Chla* + *Chlb*) was calculated.

2.3. Statistical analysis

The data underwent two-way analysis of variance using the F test ($p<0,05$). The means of electrical conductivity were subjected to a polynomial regression analyses, while means of the application of the microalgae extract were compared using the F test. The statistical analyses were performed using the R statistical software, with the packages 'emmeans' (Lenth, 2024) and 'stats' (R Core Team, 2024).

3. Results

3.1. Height and diameter of stem

The height and stem diameter of papaya seedlings responded independently to irrigation with saline water (p_{height} = 5.177e-09 and $p_{diameter}$ = 7.501e-10) and EMa-Ca (p = 0.002222 and $p_{diameter}$ = 0.001057). The increase in salinity, decreased the height of papaya seedlings by 1.29 cm for each unit increase in electrical conductivity

Table 1. Chemical characterization of saline waters (SA) used in the experiment with papaya seedlings in the presence and absence of calcium-enriched microalgae extract.

Water	C.E.	Ca^{2+}	Mg^{2+}	Na^+	K^+	Cl^-	CO_3^{2-}	HCO_3^-	SO_4^{2-}	RAS	Class.
H_2O	dS m^{-1}	................................mmol$_c$ L^{-1}..................................								mmol L^{-1}	
SA	0.50	1.03	1.26	2.41	2.41	3.90	0.0	1.70	0.53	2.26	C2S1
SA	1.10	1.62	3.64	4.68	0.38	9.90	0.0	1.80	0.73	2.89	C3S1
SA	2.50	3.07	9.39	11.36	0.44	29.90	0.0	2.05	1.61	4.55	C4S2
SA	3.90	5.50	15.71	44.74	0.53	49.90	0.0	2.30	2.19	13.74	C4S4
SA	4.50	5.90	15.86	48.61	0.53	54.90	0.0	2.05	2.41	14.74	C4S4

C.E. = Eletrical conductivity at 25ºC; RAS = Sodium adsorption ratio – RAS = =$Na^+/[(Ca^{2+}+Mg^{2+})/2]$; Class. = Classification; C1 and C4 = low and high risk of soil salinization; S1 and S4 = low and high risk of soil sodification.

(Figure 2A). Additionally, with the application of EMa-Ca, there was an increase in height and steam diameter of papaya seedlings by 6.05% and 6.33%, respectively (Figure 2C, D). The diameter of the stem responded to the electrical conductivity levels of the water. The highest stem diameter (7.99 mm) was observed at a conductivity of 0.73 dS m^{-1}, while the maximum conductivity (4.5 dS m^{-1}) caused a 26.28% reduction in steam diameter (Figure 2A, B). When EMa-Ca was used, there was an increase of 0.44 mm in stem diameter (Figure 2D).

3.2. Number of leaves and leaf area

The number of leaves and leaf area also responded independently to both saline water irrigation ($p_{n.leaves}$ = 0.0001402 and $p_{l.area}$ = 3.994e-06) and to EMa-Ca presence ($p_{n.leaves}$ = 0.0004139 and $p_{l.area}$ = 0.0001797). The number of leaves decreased from 7.26 to 6.38 leaves with an increase in electrical conductivity from 0.5 to 4.5 dS m^{-1} (Figure 3A). In presence of EMa-Ca, the number of leaves changed from 6.52 to 7.11 (Figure 3C). Saline water for irrigation led to a 33% reduction in leaf area (142.87 to 95.33 cm^2) when compared at conductivity values of 0.5 against 4.5 dS m^{-1} (Figure 3B). On the other hand, EMa-Ca raised leaf area in 19.12% (108.71 to 129.49 cm^2) (Figure 3D).

3.3. Dry mass part aerial, root and total

The dry mass of papaya seedlings responded to the interaction of saline water irrigation and EMa-Ca (p_{aerial} = 0.004787, p_{root} = 0.0002649 and p_{total} = 0.0003106). The increase in salinity in water irrigation reduced the plant dry mass of the aboveground part (MSPA), root (MSR) and total (MST) with less intensity in those that received the enhriched-calcium microalgae extract. When comparing MSPA in the presence and absence of EMa-Ca at maximum salinity (4.5 dS m^{-1}), there was an increase of 26.22% in MSPA with the application of EMa-Ca (Figure 4A). For MSR (B) and MST (C) there was better development in the presence of EMa-Ca up to salinity concentrations, respectively, from 2.5 and 3.9 dS m^{-1} (Figure 4B, C).

3.4. Net photosynthesis, internal CO_2 concentration, transpiration, stomatal conductance

The gas exchanges of the papaya seedlings were affected individually by saline water irrigation (p_A = 7.741e-08, p_{Ci} = 0.004936, p_E = 1.6e-09 and p_{gs} = 4.648e-08) and EMa-Ca application (p_A = 9.601e-05, p_{Ci} = 1.733e-07, p_E = 0.0001725 and p_{gs} = 0.001544) (Figure 5 and 6). The net photosynthesis, leaf transpiration, internal concentration of CO_2 and stomatal conductance were reduced at 40.82%, 89.3%, 48.72% and 34.16%, respectively, when comparing to the values of water with 0.5 and 4.5 dSm^{-1}. For net photosynthesis (Figure 5A) the reduction was 0.57 (µmol CO2 m^{-2} s^{-1}) for each 1.0 dS m^{-1} increase in conductivity. For the concentration of CO_2 (Figure 5B), transpiration (Figure 5C) and stomatal conductance (Figure 5D), the conductivities of

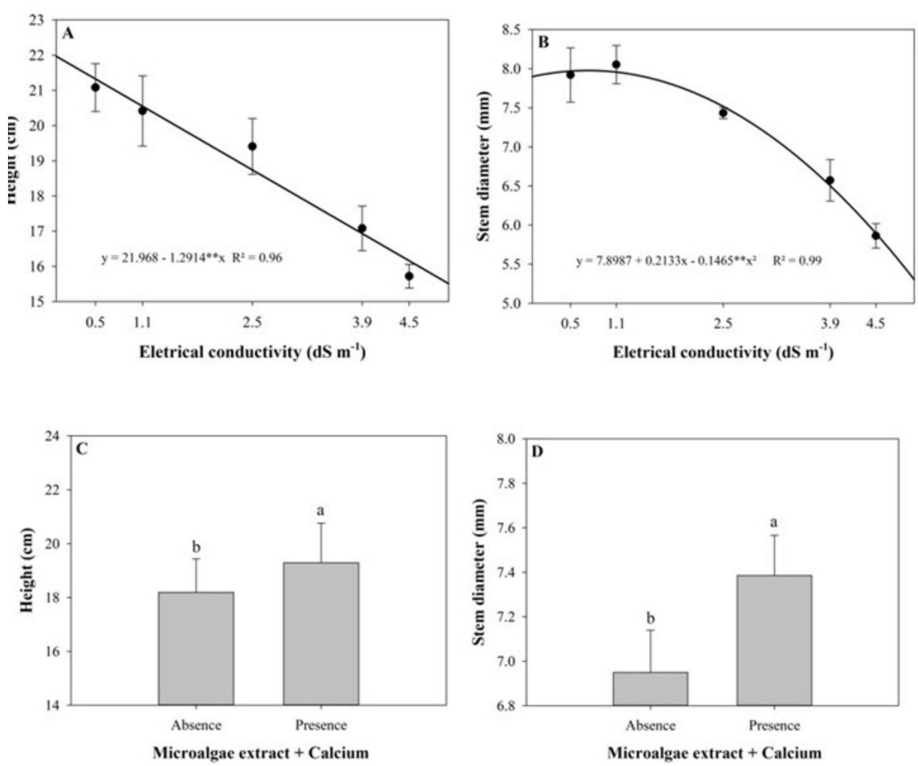
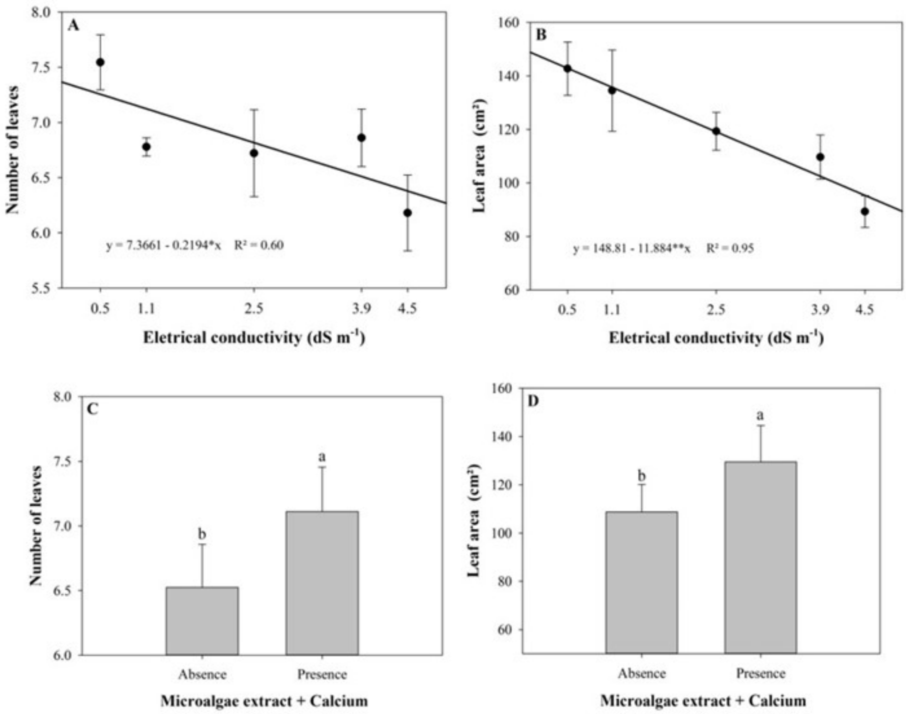


Figure 2. Height and diameter of the stem of papaya seedlings as a function of electrical conductivity of irrigation water (A and B) and use of microalgae extract enriched with calcium (C and D).



Figures 3. Number of leaves and leaf area of papaya seedlings as a function of irrigation with saline water (A and B) and application of microalgae extract enriched with calcium (C and D).

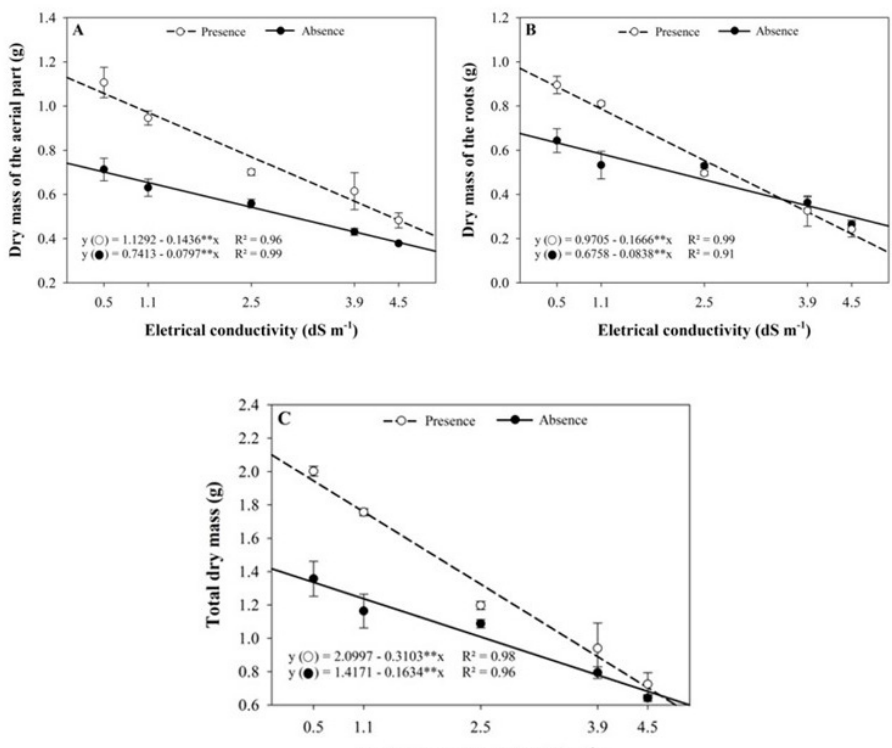


Figure 4. Dry mass of the aerial part (A) roots (B) and total (C) of papaya seedlings irrigated with saline water, without and with calcium-enriched microalgae extract.

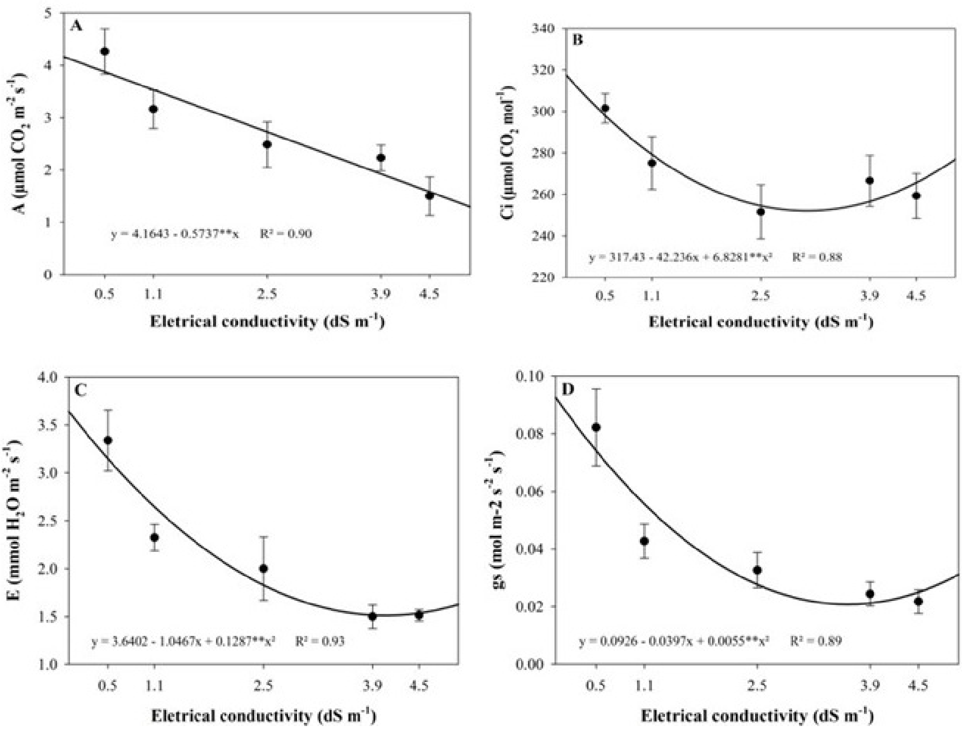


Figure 5. A – Net photosynthesis (A), Ci – internal CO_2 concentration (B); E – transpiration (C) and gs- stomatal conductance (D) of papaya seedlings irrigated with saline water.

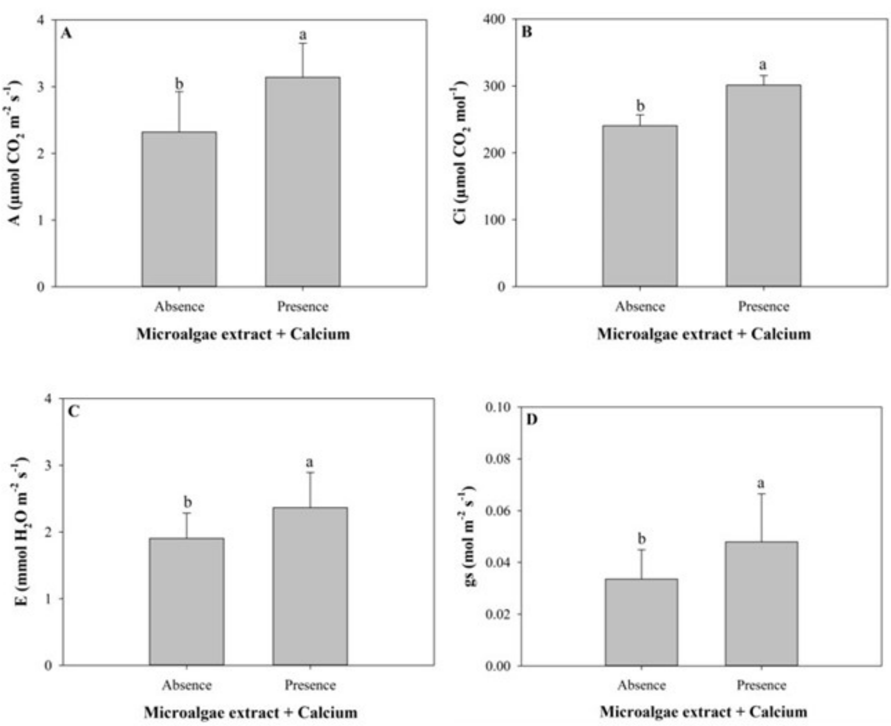


Figure 6. A – Net photosynthesis (A), Ci – internal concentration of CO_2 (B), E – perspiration (C) and gs – stomatal conductance (D) of papaya seedlings irrigated with saline water, with and without microalgae extract enriched with calcium.

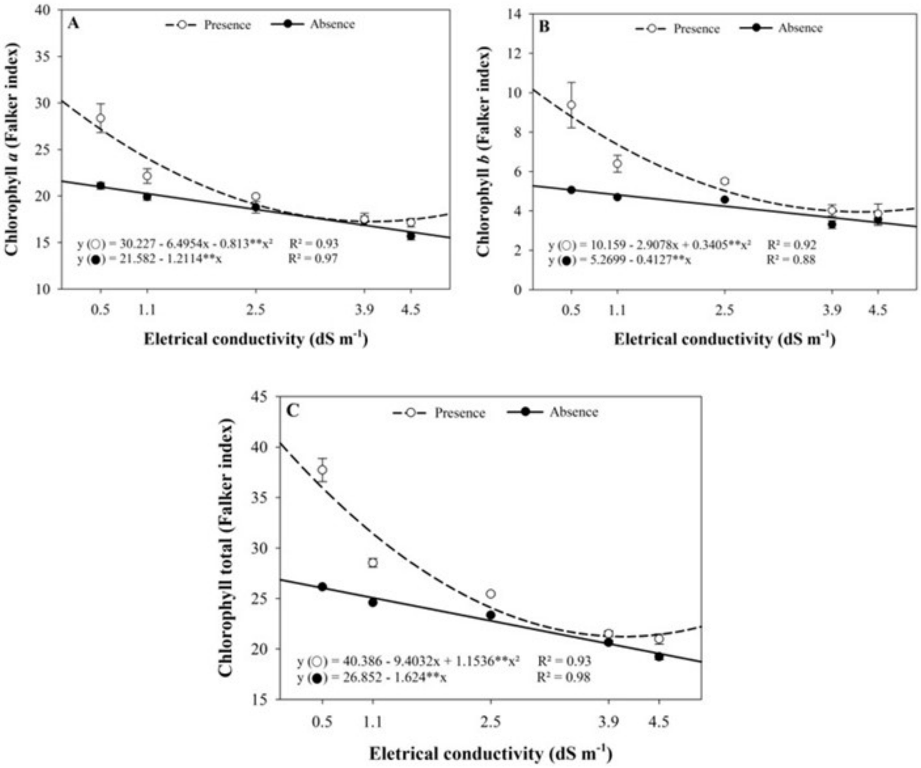


Figure 7. Chlorophyll index *Chla* (A), *Chlb* (B) and *Chlt* (C) of papaya seedlings irrigated with saline water, with and without microalgae extract enriched with calcium.

3.09 dS m^{-1}, 4.07 dS m^{-1} and 3.61 dS m^{-1} had the lowest results, respectively, 252.12 µmol CO_2 mol^{-1}, 1.51 mmol H_2O m^{-2} s^{-1} and 0.0209 mol m^{-2} s^{-1} respectively.

The application of EMa-Ca in the subtract of papaya seedlings increased the gas exchange variables. The microalgae extract increased the net photosynthesis, internal concentration of CO_2, transpiration and stomatal conductance in 35.44%, 25.31%, 24.31% and 42.99%, respectively, due to the benefits of the calcium-enriched biostimulant (Figura 6A, B, C, D). The assimilation net CO_2 from 2.32 to 3.14 µmol CO_2 m^{-2} s^{-1} (Figure 6A). For intern CO_2 concentration (Figure 6B), transpiration (Figure 6C) and stomatal conductance (Figure 6D), the best results were 301.21 µmol CO_2 mol^{-1}, 2.37 mmol H_2O m^{-2} s^{-1} and 0.048 mol m^{-2} s^{-1}, respectively.

3.5. Chlorophyll index a, b and total

The chlorophyll index a, b and total responded to the interaction between both water salinity irrigation and EMa-Ca (p_{ChlA} = 0.0004085, p_{ChlB} = 0.001549 and p_{ChlT} = 0.0004519). For all chlorophyll (*Chla*, *Chlb* and *Chlt*), without the use of the product, there was a reduction with increasing salinity. The decrease rate was 1.21%, 0.41% and 1.62%, respectively, for *Chla* (A), *Chlb* (B) e *Chlt* (C) as conductivity increased in 1 dS m^{-1} (Figure 7). When EMa-Ca was used, there was a reduction in conductivity values of 3.99, 4.26 and 4.08 dS m^{-1} for *Chla*, *Chlb* and *Chlt*, respectively (Figure 7A, B).

4. Discussion

4.1. Height and diameter of the stem of papaya seedlings

Irrigation with water contemning high concentration of soluble salts, contributes to the substrate salinization, promoting negative effects on plant growth. Either through the reduction of water uptake (osmotic effects) or due to the phytotoxicity of sodium and chloride ions on plants tissue. These effects can cause a reduction in cell division and elongation, resulting in decreased height and stem diameter (Sá et al., 2013a, b).

The highest height and diameter values of the papaya stem were observed with the application of microalgae extract enriched with calcium, justified by the biostimulant properties inherent to microalgae. These organisms contain growth phytohormones, amino acids, micronutrients and various active compounds that promotes hormonal and nutritional balance, enhancing plant development (Ronga et al., 2019; Al Dayel and El Sherif, 2021). Moreover, the presence of calcium in the formula likely aids in reducing the toxicity of Na^+ and Cl^-, enhancing the efficiency of the antioxidant enzymatic system and the reactive oxygen species detoxification system (ROS) (Roy et al., 2019).

Reductions in stem diameter and height with increasing water salt concentration were also recorded by Sa et al. (2013a) and Sa et al. (2013b) for papaya seedlings (Sunrise Solo and Tainung) in initial growth. Nascimento-Neto et al.

(2020), observed a reduction in the height and diameter of the stem in 'Sunrise Solo' papaya seedlings with an increase in the conductivity of the water supplied daily than on alternate days. Cavalcante et al. (2010), studying salt sources in 'Havaí' papaya seedlings, also observed that the increase in the electrical conductivity of the water harms the growth in height and diameter of the plant stem.

The use of EMa-Ca and irrigation with less saline water ensure seedlings of high agronomic standers for establishing a productive and long-lasting orchard, given that the stem diameter is directly correlated to a highest fruit production in papaya plants. The average height of the papaya seedlings (15-20 cm) remains consistent for the species even under saline stress (Dantas et al., 2013). This fact can be related to the cultivar genetics and the presence of EMa-Ca that favored the plant metabolism, once that height increase is observed on the extract application.

4.2. Number of leaves and leaf area

With salinity increase, the reduction in number of leaves and leaf area can be a consequence to the damage provoked by salt accumulation in vegetal tissue due to saline stress. The saline environment generally hinders the leaf development of plants, and as a strategy to survive, they reduce their leaf area, aiming to minimize light interception as well as water loss through transpiration in search of homeostasis (Arif et al., 2020). However, the reduction in leaf area decreases photosynthesis, causing seedlings to have energy deficiencies and less biomass to withstand field conditions. The results obtained in this study are consistent with those found by Parés et al. (2008), showing that when CE is superior to 2 dSm^{-1}, the leaf area decreases in 36% in papaya.

These reductions in number of leaves and leaf area with the increase of salinity were also observed by Sá et al. (2013a) when evaluating the growth of papaya cultivars irrigated with saline water; they described a reduction in leaf area due to stress. Sá et al. (2013b) while studying the initial growth and dry mass accumulation of papaya cultivars in saline water in hydroponic system, reported a reduction in leaf area. Thus, it is emphasized that this is a plant strategy to neutralize the adverse effects caused by salinity.

The salinity accumulation around root zone can reduce water absorption, causing inhibition of meristematic activity, cellular elongation. It can also cause oxidative damage due to the accumulation of reactive oxygen species that harm cells (Hasanuzzaman et al., 2021). In papaya seedlings, Rocha et al. (2017) observed that the use of microalgae-based biostimulant (*Spirulina platensis*) gradually increased the number of leaves with the age of the seedlings, which also ensured a stem diameter increase. Dias et al. (2019) working with seedlings of *Passiflora edulis*, found that a concentration of 1% *Spirulina platensis* in the substrate favored a higher number of leaves.

It is evident that the microalgae extract composed of *Chlorella* spp. increases supplementation with metabolites and hormones that often act as antioxidants, blocking oxidative reactions induced by abiotic stress and increasing the plant's antioxidant potential (González-Pérez et al., 2022), and this may justify the better leaf development of papaya seedlings, regardless of the salinity level of the water, in the presence of EMa-Ca.

Calcium in intracellular processes possibly acted as a signaling molecule in the physiological and biochemical pathways of seedlings to develop resistance to salt stress (Larbi et al., 2020). Thus, the presence of calcium possibly promoted a significant reduction in the concentration of sodium in both leaves and roots and an increase in the concentration of potassium and calcium in different plant tissues, as it triggers sodium exclusion mechanisms (Larbi et al., 2020).

4.3. Dry mass part aerial, root and total

EMa-Ca favored a reduction in the detrimental effects of salinity on the accumulation of the dry matter in seedlings. Al Dayel and El Sherif (2021), evaluating the effects of microalgae extracts (*Chlorella vulgaris* and *Nannochloropsis salina*), observed a decrease in the salinity effects on the dry mass of roots, leaves and stem in *Moringa oleifera*. These reductions in salinity stress are attributed to the activity of microalgae in boosting the activity of antioxidant enzymes (catalase and superoxide dismutase), proportioning the reduction of oxygen reactive species, favoring vegetative growth due to accumulation and translocation of photoassimilates (Taiz et al., 2017).

The calcium present in the used extract possibly contributed to the reduction of saline stress through its role as secondary messenger in plant metabolism, providing plants with the ability to adjust to environments with high salt content. This happens by activating a signal transduction system composed of different sensors, such as calmodulin and calcineurin B-like proteins (Seifikalhor et al., 2019). Bezerra et al. (2020), studying the effect of calcium application to mitigate the effects of water salinity in *Passiflora edulis*, described an increase in the number of productive branches. Therefore, it can be inferred that calcium acted in the stress signalization systems, balancing the absorption of essential nutrients for the plant to alleviate the salinity effects on the aboveground part, consequently improving photosynthesis and the total biomass accumulation of the plants.

4.4. Net photosynthesis, internal CO_2 concentration, transpiration, stomatal conductance

A reduction in stomatal opening, influenced by the increase of abscisic acid (ABA) concentration, is a defense mechanism to reduce the water loss by transpiration and maintain osmotic balance, especially under saline stress conditions (Taiz et al., 2017). Similarly, the decrease in leaf growth is the earliest response of glycophytes exposed to saline stress, followed by a decline in gas exchange (Abobatta, 2020).

In this study it was observed a decrease in all evaluated gas exchange parameters in papaya seedlings. This can be related to the lower water potential of the root system and the accumulation of Cl^- and Na^+ in vegetative parts. In this context, the decline in leaf gas exchange in response to salinity is associated with both an increase in Na^+ and Cl^- concentration in the leaves (Cavalcante et al., 2010;

Larbi et al., 2020). Orsini et al. (2012), evaluating the functional connection between morphologic/physiologic characteristics and stress tolerance of strawberry cultivars, described that the accumulation of NaCl in leaves under saline stress was related to reductions in net photosynthesis and stomatal conductance. However, it was emphasized that a lower transpiration rate delays the accumulation of toxic ions in leaves, and low stomatal density is important for adaption to saline stress.

Analyzing only the EM-Ca application, it is possible to observe that the metabolic process of plants can be intensified in the presence of microalgae such as *Chlorella* sp., due to their contribution to the supplementation of both primary metabolites (carbohydrates, proteins and lipids), amino acids such as proline, arginine and tryptophan, as well as various hormones (auxins, gibberellins, and cytokines) (Ronga et al., 2019). This improved the gas exchange characteristics in papaya seedlings under saline conditions.

The stomatal resistance induced by saline stress must be reduced to increase transpiration and CO2 assimilation (Rouphael et al., 2017). In this context, the contribution of the used microalgae (*Chlorella* sp.) to gas exchange can be linked to its reinforcement of plant metabolism, mainly with amino acids such as proline, which, when associated with gibberellin, is of primary importance for inducing stress tolerance through its metabolism in the plant (Ali et al., 2019). Rouphael et al. (2017), evaluating the influence of a biostimulant (*Eclonia maxima*) x salinity in *Cucurbita pepo*, described an isolated effect and an increase in the net photosynthesis rate and transpiration rate with the use of the biostimulant.

The biostimulant composed of Ca^{+} and *Chlorella* sp. extract, possibly acting in conjunction on the metabolism of papaya seedlings, may justify the significant changes in gas exchanges. Larbi et al. (2020) described that the supplementation with Ca^{2+} can inhibit the translocation of Na^{+} from the roots to the aboveground parts, improving the Na^{+} exclusion mechanism at the root level.

4.5. Chlorophyll index a, b and total

The increase of electrical conductivity of irrigation water decreased the chlorophyll indices of papaya seedlings leaves with lower intensity in plants in the substrate with biostimulant. For the *Chlt* index values of seedlings irrigated with water of 1.1 and 4.5 dS m^{-1}, they were higher, respectively, 21.3% and 10% in the presence of EMa-Ca, when compared to seedlings in the absence of EMa-Ca and same dose of saline water. The linear reduction in leaf chlorophyll content is related to the rising of chlorophyllase activity induced by NaCl, causing adverse effects on membrane stability and the protein-pigment-lipid complex (Taiz et al., 2017). Similarly, a possible excessive chloride absorption promoted antagonism to absorption and assimilation of nitrate ions (NO_3^-), the main nitrogen form absorbed by plant, necessary for the composition of the chlorophyll molecule, consequently reducing plant photosynthetic activity (Lucas et al., 2024).

As mentioned before, calcium acts reducing the deleterious effects of salinity and can favor the NO_3^- absorption by plants to ensure metabolic processes including formation of chlorophyll molecules. Thorough Ca^{2+} sensors present in the plasma membrane causing specific phosphorylation and domain distribution patterns during distinct phases of responses to low and high NO_3^- (Chu et al., 2021). Fertilization with microalgae of the genus *Chlorella* spp. improves plants tolerance to stress due to a greater nutritional balance (N, P, K^+, Ca^{2+}, Mg^{2+}), increasing secondary metabolites and antioxidant enzymes content (superoxide dismutases, catalases, ascorbate peroxidases and phenol peroxidases) (Kusvuran, 2021). Al Dayel and El Sherif (2021) observed that in *Moringa oleifera* the microalgae extract of *Chlorella vulgaris* besides ensuring higher photosynthetic pigments rates in non-saline condition, also effected the survival and maintenance of these pigments under saline water at 1.2 dS m^{-1}. Therefore, the formulation of EMa-Ca mitigated the action of saline on chlorophyll indices up to a conductivity of 2.5 dS m^{-1}.

Although the literature cites papaya as a crop that is moderately tolerant to salinity levels ranging from 3 to 6 dS m^{-1} (Ayers and Westcot, 1999). Elder et al. (2000), observed that papaya seedlings from the Formosa group (hybrid Tainung) showed a considerable decline in growth due to the increase in the electrical conductivity of irrigation water (1400–4000 S/cm). There was also a reduction in photosynthetic activity and chlorophyll levels. Sa et al. (2013a), concluded with the same hybrid (Tainung) used in this study that it presents greater sensitivity to salinity when compared to cultivars from the 'Solo' group.

Based on the results presented, the application of calcium-enriched microalgae extract is an alternative to optimize the quality of papaya seedlings. It not only boost growth and physiologic characteristics, but it also can mitigate the deleterious effects of salinity. Therefore, researches with biostimulant should be further developed to determine the best method of application and dose, ensuring the sustainability of the agricultural production when facing saline stress.

5. Conclusion

The growth, dry mass accumulation, gas exchange and chlorophyll indices of Tainung papaya seedlings were reduzidos by the increase of electric conductivity of irrigation water. The application of (1.0 ml/L) calcium-enriched microalgae extract improves significantly the vegetative growth, gas exchange and chlorophyll index of papaya seedlings under saline stress.

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