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Original Article

Salt stress and organic fertilization on the growth and biochemical metabolism of *Hylocereus costaricensis* (red pitaya) seedlings

Estresse salino e fertilização orgânica sobre o crescimento e metabolismo bioquímico de mudas de *Hylocereus costaricensis* (pitaia vermelha)

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Abstract

Red pitaya (*Hylocereus costaricensis*) is a promising species, with high cultivation potential due to the organoleptic and functional qualities of its fruits. However, irrigation water salinity can affect the crop yield. Therefore, materials rich in organic substances can minimize the damage caused by excess salts in soil and/or water. Thus, the objective of this study was to evaluate the influence of organic matter sources as attenuators of salt stress on the production and biochemical responses of red pitaya seedlings. A completely randomized design in 4 × 5 factorial scheme, with five sources of organic matter (humus, sheep manure, biofertilizer, organic compost and sand + soil) and four salinities (0.6, 2.6, 4.6 and 6.6 dS m^{-1}) with four replicates and two plants per plot was used. The shoot length, root length, cladode diameter, number of cladodes, number of sprotus, root volume, shoot dry mass, root dry mass and total dry mass, root and shoot dry mass ratio, chlorophyll a, b and total, amino acids and soluble sugars were evaluated at 120 days after the treatments began to be applied. Red pitaya is moderately tolerant to salinity (ECw from 4.0 to 6.0 dS m^{-1}). Organic compost and sheep manure attenuate the harmful effects of salinity on red pitaya seedlings. Under salt stress conditions, red pitaya plants increase their levels of proline, amino acids and total sugars.

Keywords: cactaceae, initial growth, organic matter, salinity.

Resumo

A pitaia vermelha (*Hylocereus costaricensis*) é uma promissora espécie, com elevado potencial de cultivo devido às qualidades organolépticas e funcionais de seus frutos. Entretanto, a salinidade da água de irrigação pode afetar o rendimento produtivo da cultura. Diante disso, materiais ricos em substâncias orgânicas podem minimizar os danos provocados pelo excesso de sais no solo e/ou na água. Assim, o objetivo deste trabalho foi avaliar a influência de fontes de matéria orgânica como atenuante do estresse salino na produção e respostas bioquímicas de mudas de pitaia vermelha. O delineamento inteiramente casualizado em esquema fatorial 4 × 5, com cinco fontes de matéria orgânica (húmus, esterco ovino, biofertilizante, composto orgânico e areia + solo) e quatro salinidade (0.6, 2.6, 4.6 e 6.6 dS m^{-1}), com quatro repetições e duas plantas por vaso foi utilizado. O comprimento da parte aérea, comprimento da raiz, diâmetro do cladódio, número de cladódios, número de brotos, volume da raiz, massa seca da parte aérea, massa seca da raiz e massa seca total, razão da massa seca da raiz e da parte aérea, clorofila a, b e total, aminoácidos e os açúcares solúveis foram avaliados aos 120 dias após o início da aplicação dos tratamentos. A pitaia vermelha é moderadamente tolerante a salinidade. O composto orgânico e estrume ovino atenuam os efeitos nocivos da salinidade nas mudas de pitaia vermelha. Sob condições de estresse salino, as plantas aumentam os níveis de aminoácidos e açúcares totais.

Palavras-chave: cactáceas, crescimento inicial, matéria orgânica, salinidade.

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1. Introduction

Red pitaya (*Hylocereus costaricensis*) is a climbing plant with cladode-type stem of triangular shape, juicy and spiny. Adventitious roots originate from its stem and assist the plant in fixing its structure in the different types of soils, as well as in the assimilation of nutrients.

The market exploited by this crop is diversified due to the growing niche in fruit commercialization. The fruit can be eaten fresh or processed as ice cream, salads and wines. The fruit can also be used by the industry for food and pharmaceutical purposes. The presence of phenolic compounds and antioxidants, red pigments called "betalains", in the fruit of pitaya aids in digestion processes and prevents cancer and other problems associated with heart attacks (Moreno-Ley et al., 2021). Vietnam, Malaysia, Colombia, Mexico, Costa Rica and Nicaragua are currently the world's leading producers of pitaya (FAO, 2019).

In Brazil, the main producing states are São Paulo, Minas Gerais, Bahia, Paraná and Goiás. However, some factors indicate a high potential for increase in the planted area and production of pitaya in northeastern Brazil. First, this crop has crassulacean acid metabolism (CAM); consequently, this plant grows and develops under extremely dry conditions under high temperatures (Ortiz-Hernández and Carrillo-Salazar, 2012), a common scenario for most states in northeastern Brazil. In addition, pitaya has a high added value, allowing a high economic return for small producers in the Brazilian semi-arid region (Nunes et al., 2014).

Despite the adaptability of *H. costaricensis* to conditions with low water availability, another limiting factor in semi-arid regions is the high salinity of the water used in irrigation. Rapid evaporation, high nutrient leaching, and poor management of irrigation and fertilization in agricultural areas accelerate the salinization of underground reservoirs, the main source of water for the semi-arid region, and soil (Shazma et al., 2011). In many situations, salinity reaches levels that make cultivation unfeasible or reduce the yields of many plant species (Sousa et al., 2012). When exposed to salinity of water and soil, sensitive plants have their growth and yield hampered due to disorders of the osmotic process. This imbalance affects the absorption and translocation of minerals in plant cells, besides hindering water absorption by the roots (Silva et al., 2013). In addition to nutrient acquisition disorders, high concentrations of Na^+ and Cl^- in cells can cause toxicity and production of reactive oxygen species (ROS), degrading the cell membrane of plants (Isayenkov and Maathuis, 2019).

Some studies have evaluated the susceptibility of other pitaya species to salt stress. Nong et al. (2019), who evaluated a set of RNA sequences and transcriptome analysis in roots of red pitaya (*Hylocereus polyrhizus*) under salt stress and observed that 261 genes were up-regulated and 61 down-regulated at all three time points. Glycolysis to gluconeogenesis was one of the most significantly modulated pathways. Santos et al. (2020) evaluated the response of red pitaya (*Hylocereus costaricensis*) to different salinity levels and salt stress attenuators (H_2O_2) and, according to the authors, salinity caused a negative influence, because it reduced the resistance of pitaya seedlings, since the plants had difficulties in adequately developing their root system and shoots, which resulted in smaller biomass production by the crop. In the species *Stenocereus thurberi*, the effects of salinity mainly interfered in plant germination (Orozco et al., 2017). However, few studies have sought to quantify tolerance levels, understand tolerance mechanisms and identify strategies to mitigate the damage caused by salt stress to the species *H. costaricensis*.

An alternative to overcome the problems arising from salt stress is the adoption of agricultural practices aimed at mitigating the salinity effect on the plant. These practices include the application of attenuators capable of causing a positive physiological response in the plant when exposed to salinity conditions. The use of organic matter can be a viable tool. Sources of organic matter, such as manure, organic compost and biofertilizer, when applied to the soil, increase cation exchange capacity (CEC) and anion exchange capacity (AEC) (Silva et al., 2013; Silva Júnior et al., 2017). Using these organic sources can minimize the salinity effect, whether from the soil or irrigation water, since the soil increases its capacity to adsorb salts (Ma et al., 2020; Ran et al., 2021). This greater retention of salts by the soil + organic compost system could reduce the intense absorption by plants, mitigating salt stress on pitaya.

The exploitation of exotic fruit crops with high market potential is an economic opportunity for many regions. For producers in the semi-arid region, *H. costaricensis* is an excellent option due to its adaptation to dry and hot conditions. However, the problem of salinity in semi-arid regions should also be overcome for the development of cultivation areas. Strategic planning for semi-arid regions will only be possible after the generation of information about the salinity effect on *H. costaricensis*. To obtain such information, the following hypotheses were raised: *H. costaricensis* can tolerate low salinity levels, and organic sources can minimize salinity effects during the initial period of orchard implementation. The objectives to answer these hypotheses were 1) to evaluate the tolerance of *H. costaricensis* seedlings to different salt concentrations in soil and 2) to identify sources of organic matter capable of attenuating salt stress in *H. costaricensis* seedlings.

2. Materials and Methods

2.1. Plant material

The cladodes used to produce the seedlings were removed from pathogen-free plants from germplasm bank of the fruit growing sector of the Universidade Federal Rural Semi-Árido (UFERSA). Cladodes were collected at the top of the plants and standardized with length of 15 cm (Marques et al., 2011). Then, the upper parts were immersed in Bordeaux mixture solution (100:50 v/v).

2.2. Experimental conditions and design

The experiment was carried out in a greenhouse at the Universidade Federal Rural do Semi-Árido (UFERSA), Mossoró, Rio Grande do Norte, Brazil. The climate of the region is considered hot and dry, with an average annual temperature of 27.5 °C, relative humidity of 68.9% and average precipitation of 673.9 mm (Alvares et al., 2013).

The cladodes were grown in plastic pots with capacity for 5 dm^3. Irrigation was carried out through a drip system, using microtube emitters, and water was supplied by means of a reservoir (100 L fiber box). Each microtube had length of 0.50 m and average flow rate of 1.7 L $hour^{-1}$. To manufacture the substrates, sand, soil and four organic sources were used (organic compost, sheep manure, humus and biofertilizer. The mixture constituted the substrates used in the following proportions: Sand+soil (1:1), sand+soil+organic compost (1:1:3), sand+soil+sheep manure (1:1:3), sand+soil +humus (1:1:3), sand+soil+biofertilizer (1:1:3) and their chemical analyzes are contained in Table 1.

Organic compost, cattle manure and humus were added to the substrate at the time of planting the cladodes. The biofertilizer was added in three applications, at the time of planting the cladodes and 15 days after planting. 533,33 ml were applied in each application, totaling 1.600 liters. The application of saline water treatments began 10 days after planting, beginning of root development.

The water with the lowest electrical conductivity - ECw (0.6 dS m^{-1}) came from the UFERSA supply system. The other concentrations were obtained by mixing NaCl salts, dissolved in the local supply water, according to the equation of Richards (1954) and checked using a properly calibrated digital conductivity meter (Tec-4MP-Piracicaba - Brazil).

The experimental design was completely randomized, in a 5 × 4 factorial scheme, with four replicates and two plants per replicate, totaling 20 combinations (treatments).

Table 1. Analysis of the substrate at the beginning and end of the experiment.

Treat	pH	EC	O.M.	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	SB	TCE	ESP
		d Sm^{-1}	g kg^{-1}	mg dm^{-3}			cmolc dm^{-3}			%	
Initial substrate											
Test	7.8	2.98	21.1	436.85	257.39	1402.6	2.1	1.1	9.96	9.96	61.26
BIO	8.14	3.41	23.58	100.66	759.34	1757.5	2.3	0.9	12.79	12.79	59.79
CO	7.68	6.5	24.2	214.55	164.28	1483.7	3.4	3.3	13.57	13.57	47.54
EO	8.04	7.5	20.79	189.88	141.19	1422.8	3.2	2	11.75	11.75	52.67
HU	7.7	9.63	28.76	258.21	397.94	1341.7	3.4	2.2	12.45	12.45	46.86
Final substrate											
SS + 0.6	8.26	0.57	24.72	313.47	483.81	1283.1	1	0.7	8.52	8.52	65.52
BIO + 0.6	8.27	1.72	26.55	206.33	247.35	4832.5	2.8	1.3	25.75	25.75	81.62
CO + 0.6	7.88	8.5	29.17	496.96	789.46	1270.7	4.4	2	13.95	13.95	39.63
EO + 0.6	8.11	5.22	26.83	243.03	418.01	1179.5	9	3.1	18.3	18.3	28.04
HU + 0.6	7.87	6.38	28.72	256.32	538.48	1372.1	4.3	3.16	14.81	14.81	40.31
SS + 2.6	8.14	6.58	22.96	373.58	136.92	1585.1	4	0.76	12	12	57.43
BIO + 2.6	8.65	6.56	33.62	200	418.01	1635.8	0.9	1.95	11.03	11.03	64.48
CO + 2.6	7.96	9.53	26.83	300.61	177.07	1595.2	3.6	1.5	12.49	12.49	55.55
EO + 2.6	8.4	9.74	31.96	277.19	458.17	1757.5	2.9	1.7	13.42	13.42	56.98
HU + 2.6	7.77	7.54	28.45	186.71	699.11	1605.4	3	1.7	13.47	13.47	51.84
SS + 4.6	9.33	3.5	10.14	364.09	383.42	1635.8	1.7	1.25	11.05	11.05	64.42
BIO + 4.6	8.84	5.2	30.93	117.74	333.22	1635.8	1.7	1.22	10.89	10.89	65.35
CO + 4.6	8.11	6.3	23.59	150.65	478.25	1757.5	6.7	6.8	22.37	22.37	34.18
EO + 4.6	8.34	7.4	23.58	215.19	347.74	1798.1	12.7	6.59	28	28	27.93
HU + 4.6	7.85	8.53	25.55	163.91	638.87	1848.8	13.5	4.2	27.38	27.38	29.38
SS + 6.6	7.74	8.5	24.93	186.23	232.83	1585.1	1.1	2	10.59	10.59	65.1
BIO + 6.6	7.6	9.5	28.52	306.35	182.63	1645.9	11.6	7.49	26.72	26.72	26.8
CO + 6.6	7.89	7.8	27.45	278.46	548.52	1767.6	4.1	4.85	18.04	18.04	42.62
EO + 6.6	8.04	6.3	29.89	208.86	157	1666.2	3.5	3.55	14.7	14.7	49.31
HU + 6.6	8.95	6.2	24.37	206.45	147.01	1543.1	5.3	2.8	12.3	12.03	48.44

pH = substrate hydrogenic potential; EC = Electrical conductivity; O.M. = Organic Matter; SB = Sum of Bases; TCE = Total Cation Exchange Capacity; ESP = Exchangeable Sodium Percentage.

The treatments corresponded to five sources of organic matter (OS): humus (HU), sheep manure (SM), biofertilizer (BIO), organic compost (OC) and the control (sand + soil (S+S)) and four salinities (S): 0.6, 2.6, 4.6 and 6.6 dS m^{-1}. The analyzes of the substrates after the experiment are described in Table 1.

2.3. Variables analyzed

The evaluations were performed at 120 days after the application of the treatments, this period being considered the production of seedlings. full development of the root system was observed. The variables were measured: shoot length (SL), root length (RL), cladode diameter (CD), number of cladodes (NC), number of sprotus (NS), root volume (RV), shoot dry mass (SDM), root dry mass (RDM) and total dry mass (TDM), root and shoot dry mass ratio (RDM/SDM).

The tolerance of red pitaya to salinity was evaluated according to (Oliveira et al., 2018): RP = [(PTWoS - PTWiS)/PTWoS]*100, where: RP = Reduction of total dry biomass production (%); PTWoS = Total dry biomass production in the treatment without salinity; PTWiS = Total dry biomass production in the treatments with salinity.

To determine chlorophyll contents, 0.002 g of fresh massfrom cladodes was weighed and homogenized in 3 mL of 80% acetone. After macerated, the material was centrifuged at 10,000 RPM for 7 minutes. The supernatant was collected, placed in a glass cuvette and analyzed at wavelengths of 645 nm, 663 nm, 652 nm and 470 nm to determine chlorophyll a, b, total, with the aid of an Agilent Cary 60 mass spectrophotometer. The results were expressed in mg chlorophyll per gram of fresh weight according to Whitham et al. (1971) and Arnon (1949): $CHLa = [(12.7^*A_{663}) - (2.69^*A_{645})] * (V/(1000^*W))$; $CHLb = [(22.9^*A_{645}) - (4.68^*A_{666})] * (V/(1000^*W))$ and $CHLtotal = [(20.2^*A_{663}) -(2.69^*A_{645})] * (V/(1000^*W))$, where: A = is the absorbance reading at the indicated wavelength; V = is the final volume of the extract used; W = is the weight of the fresh material used.

In obtaining the crude extract, after chlorophyll extraction, the precipitate was agitated in vortex containing monobasic potassium phosphate buffer solution at 0.1 M. After shaken, the material was centrifuged at 10,000 RPM for 5 minutes. The supernatant was collected and stored in a freezer at 20 °C to determine soluble sugars.

Soluble sugars were quantified using the method of Yemm and Willis (1954). A 50-µL aliquot of the crude extract was collected and completed with 950 µL of distilled water. The stock solution used to obtain the curve was prepared using glycine at concentrations of 0 µmol up to 0.1 µmol. After completing the reaction time, quantification of the samples was performed in an Agilent Cary 60 mass spectrophotometer at wavelength of 570 nm.

Amino acids were determined by the Ninhydrin test method (Yemm et al., 1955), using 5% Ninhydrin in Methyl Cellosolve and sodium citrate as buffer at 0.2 M, pH = 5.0. After adding the amino acid standard and reagents, the mixture was stirred and kept in a water bath at 100 ºC for 20 minutes to develop the color. Subsequently, the mixture was supplemented with 60% ethanol and stirred again. After cooling, reading was performed at λ = 570 nm in the U.V. spectrophotometer.

2.4. Statistical analysis

The data were subjected to the Shapiro-Wilk normality test and Bartlett homogeneity test, being within the normality and homogeneity standards, so they were subjected to analysis of variance. The data were subjected to analysis of variance by F test ($p \leq 0.05$); qualitative effects were subjected to Tukey test, while quantitative effects were subjected to regression analysis. The relationship between the evaluated parameters of the different jelly formulations was estimated considering Pearson's correlation coefficient. The variables were subjected to multivariate analysis. The multivariate analysis of the data was performed by principal component analysis (PC), in order to better show the distribution of different substrates and saline water concentrations with growth and biochemical characteristics. The analyses were performed in the statistical program R (R Core Team, 2020).

3. Results

3.1. Growth of red pitaya under salt stress and organic matter sources

The interaction between the evaluated factors, salinity (S) × organic matter sources (OS), was significant for diameter of cladode (DC), number of cladode (NC), number of sprouts (NS), shoots dry mass (SDM), root dry mass (RDM), total dry mass (TDM). Salinity as a single factor affected shoot length (SL), root volume (RV) and shoot/root ratio (S/R), respectively. The OS affected shoot length. There was no difference between the number of cladodes for any treatment tested.

The presence of organic sources increased cladode diameter at the concentration equivalent to 0.6 dS m^{-1}. However, the S+S and OC treatments increased the DC to salinities of 3.8 and 3.1 dS m^{-1} (Figure 1a). The NC was higher in the organic source HU and salinity of 6.6 dS m^{-1} (Figure 1b). At the concentration of 0.6 dS m^{-1}, the presence of HU and OC increased the number of shoots compared to the control (S+S), but the increase in salt concentration reduced the number of shoots produced (Figure 1C).

The increase in salinity did not alter SDM, RDM and TDM in the S+S treatment, which had mean values of 19.10, 1.29, and 20.39, respectively (Figure 2a, 2b and 2c). The addition of OC and SM increased SDM by 97.2 and 98.1%, respectively, compared to the control S+S (Figure 2a). This behavior was also observed for TDM in substrates with these organic sources, with increments of 95.8 and 96.4% for OC and SM, respectively (Figure 2c). In addition, the increase in salt concentration up to 6.6 dS m^{-1} stimulated the linear accumulation of SDM and TDM at approximate rates of 3.1 and 3.2 g/dSm^{-1} (both variables) for OC and SM, respectively (Figure 2a and 2c).

The addition of BIO and HU did not alter the SDM and TDM of pitaya plants compared to S+S under salinity of 0.6 dS m^{-1} (Figure 2a and 2c). However, under saline

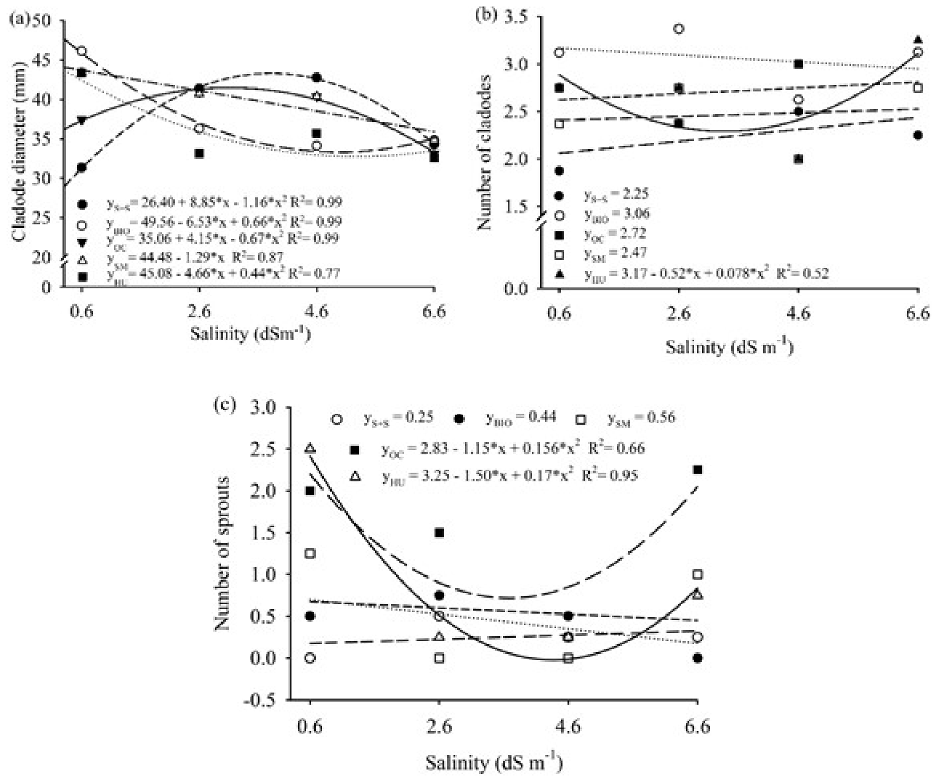

Figure 1. Cladode diameter (a), number of cladodes (b) and number of shoots (c) of red pitaya (*H. costaricensis*) under salt stress and organic matter sources.

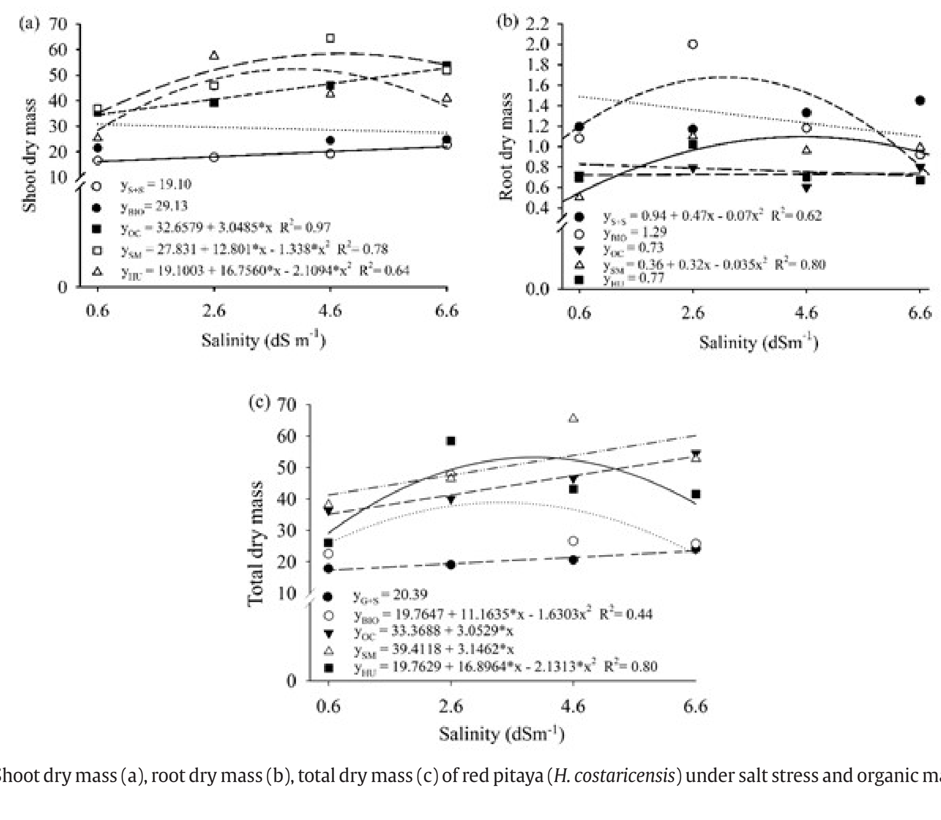

Figure 2. Shoot dry mass (a), root dry mass (b), total dry mass (c) of red pitaya (*H. costaricensis*) under salt stress and organic matter sources.

conditions equivalent to 2.6 dS m^{-1}, the presence of HU increased the SDM (101.8%) and TDM (123.5%) compared to the salt concentration of 0.6 dS m^{-1}. Such increase in SDM and TDM in the presence of HU was reduced when plants were subjected to salt concentrations of 4.6 and 6.6 dS m^{-1} (Figure 2a and 2c).

The organic source OC caused linear increment in shoot dry mass as a function of increasing salinity, with increment of 53.1% in the ECw of 6.6 dS m^{-1} compared to the control (Figure 2a). The organic sources SM and HU favored the SDM up to salinities of 4.8 and 3.9 dS m^{-1} (Figure 2a). Root dry mass was positively affected by the organic sources S+S and SM up to ECw of 3.3 and 4.5 dS m^{-1} (Figure 2b). SM and OC promoted linear increments in the total dry mass, whereas HU was beneficial up to the ECw of 3.9 dS m^{-1} (Figure 2c).

Salinity affected the behavior of SL and RV similarly for all organic sources and the control, resulting in lack of interaction between the factors salinity and organic sources ($P > 0.05$). SL was reduced from the ECw of 1.8 dS m^{-1}, with a decrease of 25.1% at the highest ECw (6.6 dS m^{-1}) (Figure 3a). The increase in salinity negatively affected RV, which decreased by 37.7% at the ECw of 6.6 dS m^{-1} compared to the control (Figure 3b). The root and shoot ratio was increasing up to the salinity of 4.9 dS m^{-1} (Figure 3c).

The organic sources OC, SM and HU promoted higher SL compared to the S+S and BIO. BIO promoted higher SL compared to S+S (Table 2).

3.2. Biochemical responses of red pitaya under salt stress and organic matter sources

The interaction between the evaluated factors, salinity (S) and organic matter sources (OS), was significant for amino acids, chlorophyll *a*, chlorophyll *b* and total chlorophyll ($p<0.01$). Salinity as a single factor affected the contents of total sugars (Figure 4).

Chlorophyll *a* content was higher under the treatments S+S and HU at 6.6 dS m^{-1}, respectively. For the BIO, there was a linear reduction as salinity increased (Figure 4a). Chlorophyll *b* content was higher under the organic source OC and the S+S control at salt concentrations of

Table 2. Mean values for shoot length (SL) of red pitaya (*H. costaricensis*) subjected to different organic sources.

Treatments	Shoot length
sand + soil (S+S)	26.70 c
biofertilizer (BIO)	46.94 b
organic compost (OC)	86.52 a
sheep manure (SM)	87.00 a
humus (HU)	81.47 a
CV (%)	24.74

Means followed by the same letter do not differ statistically by Tukey test ($p < 0.05$). CV=Coefficient of variation

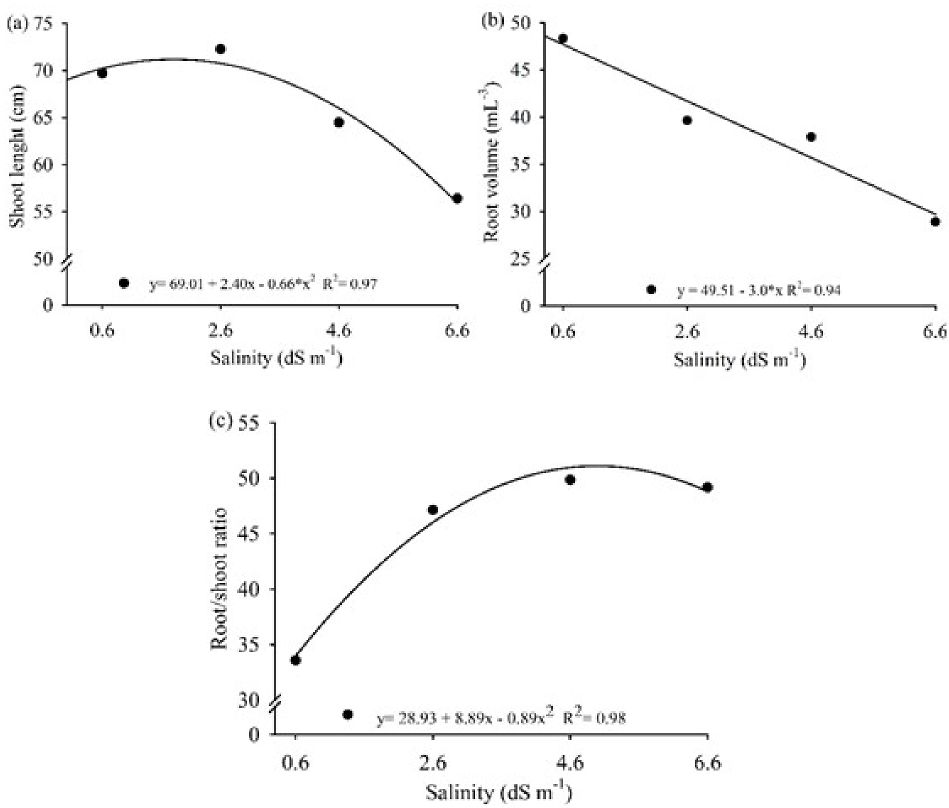

Figure 3. Shoot length (a), root volume (b) and root and shoot relationship (c) of red pitaya (*H. costaricensis*) under salt stress.

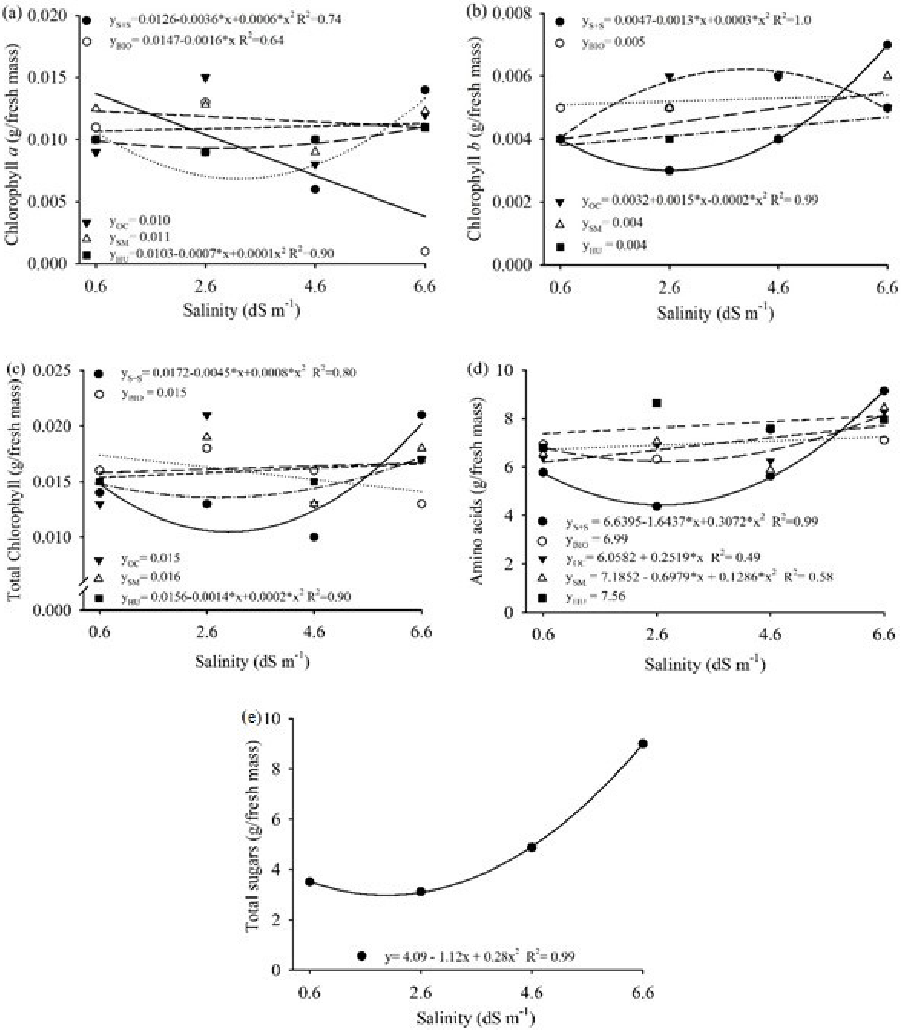

Figure 4. Chlorophyll *a* (a), chlorophyll *b* (b), total chlorophyll (c), amino acids (d) and and total sugars (e) of red pitaya (*H. costaricensis*) under salt stress and organic matter sources.

3.7 and 6.6 dS m^{-1} (Figure 4b). The highest total chlorophyll contents were obtained with the organic sources S+S and HU at salinity of 6.6 dS m^{-1} (Figure 4c). OC and SM and the control also promoted the highest amount of amino acids at the highest electrical conductivity (6.6 dS m^{-1}). For the other organic sources (BIO and HU), there were no statistical differences due to the increase in salinity (Figure 4d). Salinity levels above 2.0 dS m^{-1} increased the amount of total sugars (Figure 4e).

3.3. Multivariate analysis

The multivariate analysis of principal components (PC) explained 59.88% of the accumulated variability of the data, corresponding to 33.25% and 26.64% of variance for PC1 and PC2, respectively (Figure 5). Treatments T11 (4.6 - S+S), T1 (0.6 - S+S) and T6 (2.6 - S+S) were the ones that presented the greatest contributions to PC1, with 20.80%, 13.45% and 12.66% respectively. In PC2, treatments T16 (6.6 - S+S) presented the greatest contributions, with 42.12% (Figure 5). The variables that showed the greatest positive corrections in PC1 ($p<0.001$) were RSR ($r^2=0.90$), SDM ($r^2=0.88$), SL ($r^2=0.88$), TDM, ($r^2=0.87$) and CD ($r^2= 0.75$). In PC2, the variables ChlorB ($r^2=0.86$), TChlor ($r^2=0.82$), ChlorA ($r^2=0.77$), Amino ($r^2=0.68$) were the ones that presented correlations (Figure 5).

The high salinity concentrations (6.6 dsm) in the EO (T19) and HU (T20) substrates showed high levels of chlorophyll. As observed, the sugar contents were high in these substrates, showing positive correlations with ClorB (r=0.49) and Amino (r=0.55). On the other hand, it was observed that RL and RV reduced with high salinity concentrations (>4.6 dsm-1). The substrates (T2, T3, T5, T10, T13 and T14) showed the greatest attenuating effect on applied salinity concentrations, providing better means of RV, RL, CD, SL, TDM, SDM, RSM.

The Bio substrate proved to be efficient in attenuating the effects of salinity, providing high levels of root dry matter (RDM). The higher RDM caused lower plant shoot

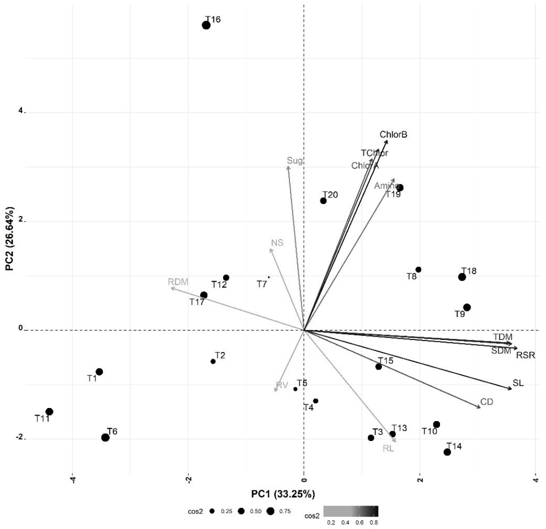

Figure 5. Principal component analysis (PC) performed on different substrates and saline water concentrations with growth and biochemical characteristics (n = 200). T1 (0.6 - S+S), T2 (0.6 - BIO), T3 (0.6 -OC), T4 (0.6 - SM), T5 (0.6 - HU), T6 (2.6 - S+S), T7 (2.6 - BIO), T8 (2.6 -OC), T9 (2.6 - SM), T10 (2.6 - HU), T11 (4.6 - S+ S), T12 (4.6 - BIO), T13 (4.6 - OC), T14 (4.6 - SM), T15 (4.6 - HU), T16 (6.6 - S+S), T17 (6.6 - BIO), T18 (6.6 - OC), T19 (6.6 - SM), T20 (6.6 - HU).

growth (SL) (r= -0.56) and root/shoot ratio (RSR) (r = -0.73). This reduction in RV was influenced by the high salinity, and this is observed with the negative correlation between Sugars and RV (r = -0.52).

4. Discussion

The greater supply of nutrients due to the addition of OC, SM, BIO and HU enabled greater growth in diameter for pitaya plants in the absence of salt stress. The presence of organic sources can provide nutrients that are essential to the initial growth of pitaya plants, especially N, an important mineral for the vegetative development of the crop (Gonçalves et al., 2013).

Increasing the salt concentration to 6.6 dS m^{-1} prevented the absorption of nutrients provided by the organic sources, eliminating the beneficial effect of the addition of SM, BIO and HU on cladode diameter. Plants tend to absorb lower amounts of nutrients from the soil due to osmotic deregulation in root cells (Roy et al., 2014). Similar results were obtained by Silva et al. (2018), who evaluated sources of organic fertilization and salinity levels in the initial growth of passion fruit (*Passiflora edulis* Sims) and found that the sources of organic matter used (bovine, goat and poultry manure) may favor the stem diameter of the seedlings, but the increase in salinity levels cause negative effects, leading to changes in plant growth and diameter.

As a mechanism of tolerance, the plant tends to reduce the number of shoots produced when subjected to abiotic stress conditions. In environments with high concentrations of soluble salts, plants adopt tolerance mechanisms in order to minimize damage to their structure. The osmotic effect present in saline environments may also explain this behavior observed in the plants, because the high salinity levels negatively reduced the length of roots. By reducing the rate of shoot production, plants consequently reduce their transpiration rate, decreasing water loss, which can ensure a higher survival rate (Fariduddin et al., 2012). With this, the plant ends up also reducing its photosynthetic capacity, since its area of light absorption is compromised, leading to decrease in size as well, as noted with *Passiflora edulis* (Mesquita et al., 2012), *Capsicum annuum* (Santos et al., 2019) and *Solanum melongena* (Sousa et al., 2022).

Some plants can expand their survival mechanisms and have become tolerant to salt stress conditions through alternative processes (Kashyap et al., 2020). In the case of pitaya, its ability to survive in saline environments can be explained by its physiological structure. Like the other Cactus species, pitaya has CAM (Crassulacean Acid Metabolism) photosynthetic metabolism, which assists in the concentration of carbon dioxide.

This results in greater efficiency in the use of water for other metabolic activities. The ability to store water and solutes in their tissues guarantees the growth and development of these plants under salt stress conditions. Salinity tolerant plants can selectively accumulate ions and thus maintain the osmotic adjustment, ensuring water absorption (Llanes et al., 2021). When evaluating the salinity tolerance of cactus, Schuch and Kelly (2008) observed that saguaro cactus and golden barrel cactus were tolerant to all salinity levels without altering the total dry mass content; these cactus species relied on osmotic adjustment based on increased osmotic potential and higher concentrations of sodium and chloride in the stem tissue. However, this behavior may vary according to genus.

In saline environments the availability of nutrients is limited, since high concentrations of Na^{+} and Cl^{-} result in antagonistic effect for other nutrients, causing the plant to be unable to absorb. The increase of these salts can extract nutrients (K^{+}, Ca^{+2}, NH_4^{+}, P and NO_3^{-}) bound to organic matter; for this reason, it was possible to observe in the present study that the growth of pitaya was favored, because there was an increase in the amount of nutrients present in the substrate. Another factor of extreme importance is the activity of microorganisms present in the organic matter; when decomposing it, these microorganisms produce substances (organic acids) that promote the release of nutrients and improve the physicochemical properties of the soil, thus facilitating water absorption and promoting greater availability of nutrients, as observed in *Arabidopsis thaliana* (Kaddour et al., 2012) *and Citrullus lanatus* (Silva Júnior et al., 2017).

In saline environments, high electrical conductivity can compromise the absorption of nutrients and cause problems for the development of plants, hampering their growth due to reduced root growth. The absorption of nutrients by plants is reduced by the increase in the osmotic potential of the soil, as it negatively affects the absorption of water as well, since it decreases the external water potential, which compromises the availability of water within the plant, resulting in negative effects on the capacity to translocate nutrients within the plants and causing an increase in solute levels (Ibrahimova et al., 2021).

The increase in salinity levels results in high concentrations of sodium carbonate, so the pH of the substrate is influenced and may reach very high values. Consequently, the availability of some nutrients (zinc, iron, copper and phosphorus) is limited, resulting in the deficiency of these nutrients in plants and impairing their growth.

To ensure their survival under adverse conditions, plants develop mechanisms that help in their adaptation and development in these environments; in this context, some species show reduction in their growth as a way to minimize stress. The excess of salts in these environments directly restricts the absorption of water and nutrients by the plant due to increased osmotic pressure in the soil (Ma et al., 2020).

With a lower water absorption, plant cells and structures undergo a reduction in moisture content, becoming less hydrated, so there is a reduction in the CO_2 permeability inside the plants, reducing their photosynthetic capacity and consequently their cell expansion (Morais et al., 2012). The number of stomata also decreases, reducing the transpiration rate and leading to a reduction in water loss, which is satisfactory for plants, because the availability of water inside cells increases ensuring a higher water use efficiency in physiological and biochemical activities (Ma et al., 2020; Ran et al., 2021)

The physical structures of the substrate are compromised by the high electrical conductivity of the water, because the accumulation salts leads to dispersion of the clay fraction, creating a dense layer that prevents proper root development, generating indirect consequences on plant growth, since it hinders CO_2 fixation (Khataar et al., 2018; Ran et al., 2021). In the present study, it was possible to observe a reduction in root volume, but there was no decrease in root dry mass, which implies a modification in the structures of the root system of pitaya plants, ensuring a better distribution in the environment, which can be considered as an adaptive capacity of the plant. According to Zobel et al. (2007), changes in root structures enable the plant to tolerate adverse conditions; in the case of salt stress, this allows a greater absorption of water and nutrients.

The root morphology and architecture are directly related the absorption of nutrients. In the case of ions such as NaCl, some plants through modification in their structures can limit the absorption and consequently the translocation of this element to the shoots, which results in decreased toxicity inside the cells, ensuring higher survival rate and better vegetative growth (Acosta-Motos et al., 2017). Results similar to those of the present study were observed in loquat plants, for which the authors found that salinity resulted in the redistribution of root system dry mass, favoring the development of the roots, which can be considered as an adaptive response to maintain the stem/root balance when the absorption capacity of the roots (García-Legaz et al., 2008).

The length of the root system indicates the ability of a plant to absorb water from the deeper layers of the soil, therefore, as the salinity level increased, the length of the roots drastically reduced, causing a decrease in the absorption of water and nutrients from the layers further away from the zone of absorption. Segundo (Lanza and Reis, 2021), this behavior is considered an adaptive capacity for survival of the species, since longer roots result in greater exploration of the deeper layers of the soil, enabling plants to absorb water and nutrients that are not available in the surface layers. This behavior occurs under saline stress conditions due to excess ions result in a lower soil water potential than that of the plant, consequently the absorption of water and nutrients by the roots becomes difficult (Ma et al., 2020).

The greater amount of chlorophyll *a* under the organic sources OC and SM is associated with higher supply of nutrients and water availability, favoring the synthesis of this pigment. However, under severe stress conditions there is a reduction in the content of this pigment due to the higher activity of the chlorophyllase enzyme, degrading chlorophyll molecules (Houimli et al., 2010). The increase in chlorophyll *b* content occurs due to the reduction in the chlorophyll *a* content of pitaya plants, a mechanism already observed in other plants to optimize the use of light energy under stress conditions (Brito et al., 2016). In other plant species, such as *Opuntia ficus-indica*, the chlorophyll content in cladodes was not affected by the increase in salinity (Salazar and Véliz, 2008).

The increase in the amount of amino acids, even under saline conditions, occurred in the presence of organic sources, indicating a possible role of organic matter in providing soluble amino acids that could be used by *H. costaricensis* (Cha-Um and Kirdmanee, 2011).

The increments up to total soluble sugars up to 6.6 dS m^{-1} were adaptive changes of *H. costaricensis* plants to osmotically adjust the cell water status. These biochemical mechanisms have already been observed for several plant species (Álvarez et al., 2018). The accumulation of macromolecules with chemiosmotic potential, such soluble sugars, is a mechanism that allows the plant to reduce cell water potential and facilitates the absorption of nutrients under saline conditions (Chen and Jiang, 2010). In addition, the higher concentration of soluble sugars in *H. costaricensis* plants may have reduced the absorption of Na^+ and Cl^-, alleviating the oxidative stress caused by salinity. This behavior for the total sugars was observed in all plants of *H. costaricensis*, regardless of the presence or absence of organic sources, suggesting that this mechanism of protection against salinity is inherent to the species.

5. Conclusion

Red pitaya is moderately tolerant (ECw from 4.0 to 6.0 dS m^{-1}) salt concentrations in the soil since the presence of salts did not affect the average growth attributes of this species.

Organic sources increase the red pitaya's tolerance for growth. The species showed mechanisms biochemical mechanisms of protection against salt stress, mainly by increasing the total soluble sugars and amino acids. These mechanisms are independent of the application or not of organic sources. Red pitaya is a fruit crop with high agricultural potential for regions with higher soil and water salinity. In addition, the application of organic fertilizer should be recommended to improve the performance of this fruit crop in these saline regions.

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