Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga
ISSN 1983-2125 (online)

http://dx.doi.org/10.1590/1983-21252024v3712048rc

Scientific Article

Proline on the induction of tolerance of sour passion fruit seedlings to salt stress

Prolina na indução de tolerância de mudas de maracujazeiro-azedo ao estresse salino

Larissa F. S. Santos[1]*, Geovani S. de Lima[1], Vera L. A. de Lima[1], André A. R. da Silva[1], Reynaldo T. de Fátima[1], Thiago F. de L. Arruda[1], Lauriane A. dos A. Soares[2], Jessica D. Capitulino[1]

[1]Post Graduate Program in Agricultural Engineering, Universidade Federal de Campina Grande, Campina Grande, PB, Brazil. [2]Academic Unit of Agricultural Sciences, Center of Agrifood Science and Technology, Universidade Federal de Campina Grande, Pombal, PB, Brazil.

ABSTRACT - Water sources in the Brazilian semi-arid region commonly contain high levels of dissolved salts in their composition, standing out as one of the abiotic stresses that limit the expansion of irrigated fruit growing, especially salt stress-sensitive crops such as sour passion fruit. Thus, the use of elicitors, such as proline, can be an effective alternative to mitigate salt stress in plants. In this context, the objective of this study was to evaluate the effects of foliar application of proline on chlorophyll fluorescence, growth, quality and tolerance of sour passion fruit irrigated with saline water during the seedling formation phase. The experiment was conducted from July to October 2022, under greenhouse conditions in Campina Grande, PB, Brazil, using a completely randomized design, in a 5 × 4 factorial scheme, with five levels of electrical conductivity of irrigation water - ECw (0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}) and four concentrations of proline (0, 5, 10 and 15 mM), with four replicates and two plants per plot. Water salinity from 0.6 dS m^{-1} reduces the maximum fluorescence, variable fluorescence, quantum yield of photosystem II and growth of 'BRS GA1' sour passion fruit seedlings. Foliar application of proline at concentrations ranging from 6 to 8.05 mM increases the growth in plant height, stem diameter and leaf area of sour passion fruit seedlings. The sour passion fruit genotype 'BRS GA1' is sensitive to water salinity, with a salinity threshold level of 0.6 dS m^{-1} and a reduction per unit increase in electrical conductivity of 10.49%.

Keywords: *Passiflora edulis* Sims.. Salinity. Osmolyte synthesis.

RESUMO - As fontes hídricas do semiárido brasileiro comumente apresentam altos teores de sais dissolvidos em sua composição, destacando-se como um dos estresses abióticos que limita a expansão da fruticultura irrigada, principalmente de culturas sensíveis ao estresse salino como o maracujazeiro-azedo. Assim, a utilização de substâncias elicitoras, como prolina, pode ser uma alternativa eficaz para mitigar o estresse salino nas plantas. Nesse contexto, objetivou-se com este trabalho avaliar os efeitos da aplicação foliar de prolina na fluorescência da clorofila, crescimento, qualidade e tolerância de maracujazeiro-azedo irrigados com águas salinas durante a fase de formação de mudas. A pesquisa foi conduzida no período de julho a outubro de 2022, em casa de vegetação em Campina Grande – PB, utilizando-se o delineamento inteiramente casualizado, em esquema fatorial 5 × 4, sendo cinco níveis de condutividade elétrica da água de irrigação CEa - (0,6; 1,2; 1,8; 2,4 e 3,0 dS m^{-1}) e quatro concentrações de prolina (0, 5, 10 e 15 mM) com quatro repetições e duas plantas por parcela. A salinidade da água a partir de 0,6 dS m^{-1} reduz a fluorescência máxima, variável, eficiência quântica do fotossistema II e o crescimento de mudas maracujazeiro-azedo 'BRS GA1'. A aplicação foliar de prolina em concentração variando de 6 a 8,05 mM aumenta o crescimento em altura de plantas, diâmetro de caule e área foliar de mudas de maracujazeiro-azedo. O genótipo de maracujazeiro-azedo 'BRS GA1' é sensível a salinidade da água, sendo o nível de salinidade limiar de 0,6 dS m^{-1} e a redução por aumento unitário da condutividade elétrica de 10,49%.

Palavras-chave: *Passiflora edulis Sims.*. Salinidade. Síntese de osmólitos.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

Received for publication in: July 3, 2023.
Accepted in: March 7, 2024.

***Corresponding author:**
<englarissafss@gmail.com>

INTRODUCTION

Belonging to the Passifloraceae family, sour passion fruit is a fruit crop appreciated and cultivated in several regions of Brazil, due to the favorable edaphoclimatic conditions established for the use of its fruit in the fruit pulp industry, as well as for fresh consumption (PIRES et al., 2008). Passion fruit is widely used in traditional medicine for being rich in vitamins A and C, folic acid, and nutrients such as calcium, iron, and potassium (CORRÊA et al., 2016).

The semi-arid region of Northeast Brazil has an irregular distribution of rainfall, high evaporation rates in most months of the soil, intensifying water scarcity events, and salinization of water sources and soil (LIMA et al., 2021). In this region, it is common to find surface water and groundwater sources with high levels of salts and with varying cationic and anionic compositions depending on climatic conditions, climatic zone, and time of year (PINHEIRO et al., 2022), standing out as one of the abiotic stresses that limit the expansion of irrigated fruit growing in the region.

The excess of salts present in the water sources causes a reduction in the osmotic potential of the soil solution and restricts the absorption of water and nutrients by plants, leading to a nutritional imbalance, in addition to causing toxicity by specific ions, interfering with physiological, enzymatic, and metabolic processes (ANDRADE et al., 2022). Salt stress also induces oxidative stress, due to the imbalance between the production of reactive oxygen species and their detoxification by enzymatic and non-enzymatic reactions, consequently leading to photooxidative damage, membrane lipid peroxidation, protein denaturation, and, finally, cell death (MOHAMED et al., 2020).

Application of elicitors should be considered as an alternative to mitigate salt stress in plants. Among these, foliar application of proline stands out. Proline is a compound with osmoprotective characteristics, with the capacity to eliminate reactive oxygen species and capacity for cellular defense, when the plant is subjected to salt stress, inducing tolerance to salts and reducing oxidative damage (WANG et al., 2017). Under conditions of salt stress, proline is a key signaling molecule capable of triggering multiple responses that are part of the acclimatization process through the stabilization of membranes and proteins, promoting the scavenging of free radicals, and acting on cell signaling and oxidation balance (GHARSALLAH et al., 2016).

In view of the above, the objective of this study was to evaluate the effects of foliar application of proline on chlorophyll fluorescence, growth, quality and tolerance of sour passion fruit irrigated with saline waters during the seedling formation phase.

MATERIAL AND METHODS

The experiment was conducted from July to October 2022 under greenhouse conditions, at the Academic Unit of Agricultural Engineering (UAEA) of the Federal University of Campina Grande (UFCG), Campina Grande Campus, PB, Brazil, located by the geographic coordinates: 7º 15’18” S, 35º 52’ 28” W and altitude of 550 m. The data collected on temperature (maximum and minimum) and relative humidity of the air inside the greenhouse are presented in Figure 1.

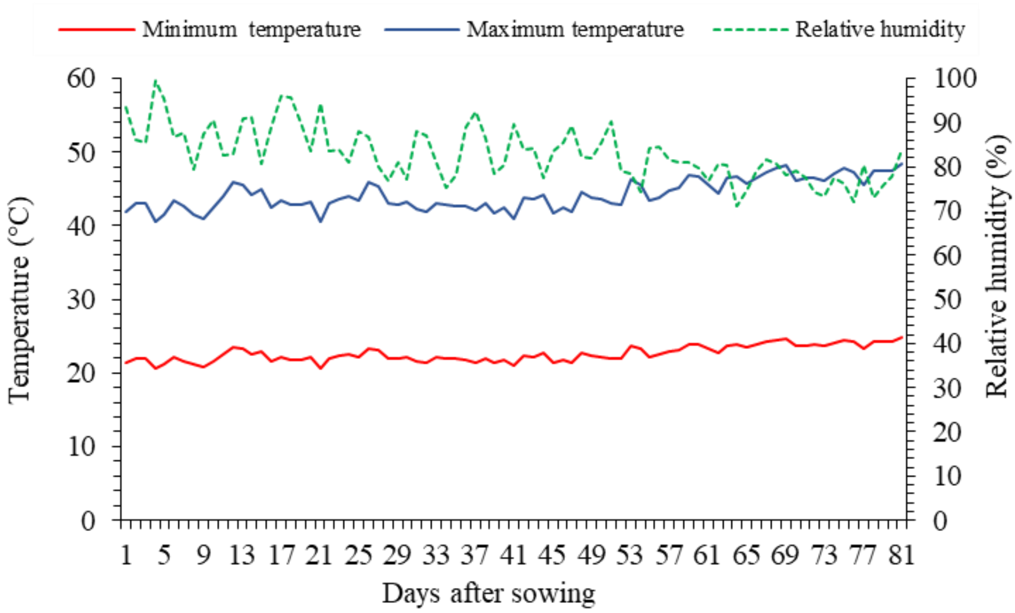


Figure1. Data of maximum and minimum temperature and relative humidity during the experimental period.

The treatments consisted of five levels of electrical conductivity of irrigation water (0.6, 1.2, 1.8, 2.4 and 3.0 dS m^{-1}) and four proline concentrations (0, 5, 10 and 15 mM), in a 5 × 4 factorial arrangement, distributed in a completely randomized design, with four replicates, two plants per plot, totaling 160 experimental units. Salinity levels were defined based on a study conducted by Ramos et al. (2022) using sour passion fruit, while proline concentrations were established according to studies conducted by Veloso et al. (2018) using guava crop.

The cultivar ‘BRS Gigante Amarelo’, currently named ‘BRS GA1’, was obtained from seeds and used in the present study. Sowing was carried out using 3 seeds per polyethylene bag with dimensions of 10 × 25 cm and capacity of 3 kg, equidistantly at 1 cm depth. The substrate used was composed of a mixture of sandy loam soil, sand and earthworm humus, at ratio of 2:1:1, respectively, on a volume basis. At 37 days after sowing (DAS), thinning was carried out, leaving one plant per bag, the one that visually showed greatest morphological vigor. The soil was collected at 0-20 cm depth in an area of the municipality of Lagoa Seca, PB. Physical and chemical attributes of the soil are presented in Table 1 and were determined according to the methodology proposed by Teixeira et al. (2017).

Table 1. Chemical and physical characteristics of the soil used before the application of the treatments.

Chemical characteristics								
pH H_2O)	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H^+
(1:2.5)	dag kg^{-1}	(mg kg^{-1})	------- $cmol_c\ kg^{-1}$ -------					
6.7	12.75	5.96	89.51	0.09	3.72	0.95	0.00	0.91
------- Chemical characteristics -------				------- Physical characteristics -------				
EC_{se}	CEC	SAR	ESP	Particle-size fraction (g kg^{-1})			Moisture (dag kg^{-1})	
(dS m^{-1})	$cmol_c\ kg^{-1}$	(mmol L^{-1})$^{0.5}$	%	Sand	Silt	Clay	33.42 kPa[1]	1519.5 kPa[2]
0.50	5.89	0.70	1.52	727	211	62	Sandy loam	

pH – hydrogen potential, OM – organic matter: Walkley-Black Wet Digestion; Ca^{2+} and Mg^{2+} extracted with 1 M KCl at pH 7.0; Na^+ and K^+ extracted using 1 M NH_4OAc at pH 7.0; $Al^{3+}+H^+$ extracted using 0.5 M CaOAc at pH 7.0; EC_{se} - electrical conductivity of the saturation extract; CEC – cation exchange capacity; SAR - Sodium adsorption ratio of the saturation extract; ESP - Exchangeable sodium percentage; [1,2] corresponds to field capacity and permanent wilting point, respectively.

The irrigation waters were prepared from the dissolution of NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$, at ratio of 7:2:1, the predominant ratio in the sources of water used for irrigation in the Northeast region, taking as a basis the water from the local supply system of Campina Grande, PB. Irrigation water was prepared considering the relationship between ECw and the concentration of salts (RICHARDS, 1954), according to Equation 1.

$$Q = 640 \times ECw \quad (1)$$

where:
Q - quantity of salts to be applied (mg L^{-1});
ECw - electrical conductivity of water (dS m^{-1}).

Prior to sowing, the soil moisture content was raised to the level corresponding to field capacity with water of lowest salinity level (0.6 dS m^{-1}). Subsequently, irrigation was carried out daily, at 5 p.m., applying to each bag the amount of water necessary to keep soil moisture close to field capacity. At 27 DAS, irrigation with water from the different treatments began, and the volume of water was applied according to the water needs of the plants, determined by the water balance, according to Equation 2.

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

where:
VI = volume of water to be used in the next irrigation event (mL);
Va = volume applied in the previous irrigation event (mL);
Vd = volume drained (mL); and
LF = leaching fraction of 0.20 (to reduce the gradual accumulation of salts in the soil).

Fertilization with nitrogen, potassium and phosphorus was carried out as topdressing according to the recommendation of Novais, Neves and Barros (1991), at doses of 100, 150 and 300 mg kg^{-1} of N, K and P, respectively, in the form of urea, potassium chloride and monoammonium phosphate. Fertilization with nitrogen, phosphorus and potassium was split into 8 portions and applied at 24, 31, 38, 45, 52, 59, 66 and 73 DAS, via fertigation. As source of micronutrients, Dripsol Micro® (1.2% magnesium, 0.85% boron, 3.4% iron, 4.2% zinc, 3.2% manganese, 0.5% copper and 0.06% molybdenum) was foliar applied at a concentration of 1 g L^{-1}. Foliar fertilization was carried out at 7-day intervals.

Proline concentrations were obtained in each application event by dilution of proline in distilled water. The applications were carried out weekly using a manual sprayer with a 1-cm adjustable conical metal nozzle with service pressure of 300 Psi and flow rate of 1.1 L min^{-1}, in addition to an adjuvant to improve the application efficiency. A cardboard box was used to avoid drift between plots. Plants subjected to the concentration of 0 mM were sprayed only with distilled water. The applications were carried out from 5 p.m. due to the lower temperature. The adjuvant solution was added to make better use of the product, and the average volume applied per plant was 200 mL.

Pest control was performed using a chemical insecticide classified as synthetic pyrethroid, with Deltamethrin as the active ingredient. Invasive plants were controlled by manual uprooting along the experimental period, so as to avoid interspecific competition for water and nutrients, favoring the full development of the crop.

At 66 DAS, chlorophyll fluorescence was analyzed through the variables initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv), and quantum yield of photosystem II (Fv/Fm) in leaves (third fully expanded leaf counted from the apical bud) pre-adapted to the dark using leaf clips for 30 min, between 7 and 10 a.m., to ensure that all first acceptors were oxidized, i.e., with open reaction centers, using Opti Science's OS5p pulse-modulated fluorometer.

Growth variables, namely stem diameter (SD), plant height (PH) and leaf area (LA), as well as biomass accumulation were analyzed at 80 DAS. Plant height (cm) was measured as the distance from the collar to the insertion of the apical meristem, and SD (mm) was measured at 2 cm from the plant collar. LA was determined according to

Cavalcante et al. (2002), using Equation 3.

$$LA = 0.78 \times L \times W \quad (3)$$

where:
LA - leaf area (cm^2);
L - leaf length (cm);
W - leaf width (cm);
0.78 - mathematical constant.

Biomass accumulation was also evaluated at 80 DAS, through stem dry mass (StDM), leaf dry mass (LDM) and root dry mass (RDM). To determine these variables, the plants were cut close to the soil surface and separated into leaves, stem and roots. To obtain the dry mass, each part of the plant was placed in properly identified paper bags and dried in a forced circulation oven at 65 ºC until reaching constant weight. Subsequently, this material was weighed on a 0.001 g precision scale to obtain LDM (g per plant), StDM (g per plant) and RDM (g per plant), which were summed to obtain the total dry mass (TDM). StDM + LDM data were then used to calculate ShDM (g per plant). R/S ratio ($g\ g^{-1}$) was obtained through the ratio between RDM and ShDM.

Seedling quality was evaluated by the Dickson Quality Index (DICKSON; LEAF; HOSNER, 1960) using the data of plant height (PH), stem diameter (SD), total dry mass (TDM), shoot dry mass (ShDM) and root dry mass (RDM), according to Equation 4.

$$DQI = \frac{(TDM)}{(PH/SD) + (ShDM/RDM)} \quad (4)$$

where:
DQI - Dickson quality index;
PH - height of plants (cm);
SD - stem diameter (mm);
TDM - total dry mass (g per plant);
ShDM - shoot dry mass (g per plant); and
RDM - root dry mass (g per plant).

The tolerance of sour passion fruit to salt stress in the seedling formation phase was determined based on the relative accumulation of total dry mass per plant, using the plateau followed by linear decrease model of Maas and Hoffman (1977). Model parameters were fitted by minimizing the square of errors with the Microsoft Excel solver tool.

The data obtained were subjected to the distribution normality test (Shapiro-Wilk test) at 0.05 probability level. After that, the data were subjected to analysis of variance by the F test at 0.05 and 0.01 probability levels, and when significant, linear and quadratic polynomial regression analysis was performed using statistical software SISVAR Version 5.6. When there was a significant effect of the interaction, response surface graphs were constructed using SigmaPlot software, version 12.5.

RESULTS AND DISCUSSION

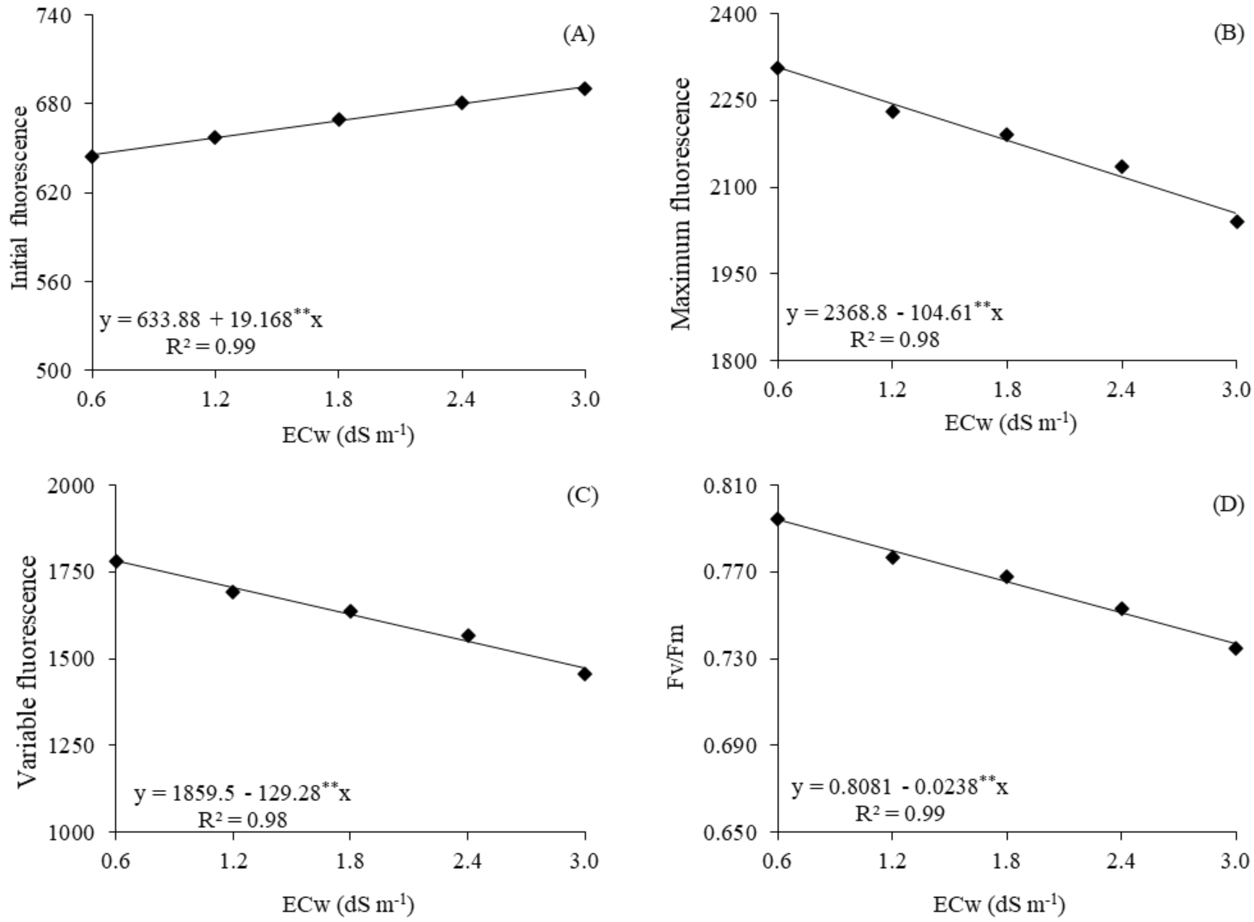
There was a significant effect of water salinity levels on all fluorescence variables (Table 2). Proline concentrations and the interaction between the factors (SL × PRO) did not significantly affect any of the variables measured in sour passion fruit at 66 DAS.

Table 2. Summary of the analysis of variance for initial fluorescence (F_0), maximum fluorescence (Fm), variable fluorescence (Fv) and quantum yield of photosystem II (Fv/Fm) in sour passion fruit plants cultivated under water salinity and proline concentrations, at 66 days after sowing (DAS).

Sources of variation	DF	Mean squares			
		F_0	Fm	Fv	Fv/Fm
Salinity levels (SL)	4	5308.47**	160747.30**	243919.42**	0.0082**
Linear regression	1	21160.69**	630276.09**	962653.01**	0.0324**
Quadratic regression	1	73.11ns	3922.35ns	3291.35ns	0.0001ns
Proline concentrations (PRO)	3	105.16ns	8867.49ns	9116.02ns	0.0002ns
Linear regression	1	29.16ns	14941.27ns	17973.42ns	5.24e-4ns
Quadratic regression	1	168.22ns	7808.95ns	6408.20ns	1.0e-7ns
Interaction (SL × PRO)	12	76.45ns	5017.71ns	6002.38ns	0.0002ns
Residual	12	123.81	24746.36	27300.58	0.00046
CV (%)		1.66	7.21	10.16	2.82

DF - degrees of freedom; CV (%) - coefficient of variation; (*) significant at $p \leq 0.05$; (**) significant at $p \leq 0.01$; (ns) not significant.

Initial fluorescence (F_0) increased with the increase in irrigation water salinity (Figure 2A), by 3.02% per unit increment of ECw. An increase of 7.12% was observed when comparing the F_0 of plants cultivated under ECw of 3.0 $dS\ m^{-1}$ with the value of those irrigated with 0.6 $dS\ m^{-1}$ water. Such increase in F_0 is a sign of salt stress effects, since it results in lower utilization of photochemical energy in the reaction centers of photosystem II, which is the starting point for the functioning of the photosystem (KALAJI et al., 2018). In contrast to the results obtained in the present study, Diniz et al. (2021) studied the effect of irrigation with saline waters of up to 3.1 $dS\ m^{-1}$ on ‘Gigante Amarelo’ passion fruit (*Passiflora edulis* Sims) and observed reduction in F_0 from ECw of 1.1 $dS\ m^{-1}$.



** - Significant at $p \leq 0.01$ by the F test.

Figure 2. Initial fluorescence – F_0 (A), maximum fluorescence – Fm (B), variable fluorescence – Fv (C) and quantum yield of photosystem II – Fv/Fm (D) of sour passion fruit plants, as a function of water salinity - ECw, at 66 days after sowing (DAS).

Unlike F_0 (Figure 2A), the increase in irrigation water salinity reduced the maximum fluorescence (Fm) of sour passion fruit (Figure 2B), which decreased by 4.41% per unit increase of ECw. This resulted in a reduction of 10.88% (251.06) between plants irrigated with 3.0 and 0.6 dS m^{-1} water. This effect reflects the slowing down of photosynthetic activity, reducing energy capture to avoid excessive excitation of electrons, which are precursors in the formation of reactive oxygen species (MANAA et al., 2019).

The variable fluorescence (Fv) of sour passion fruit was negatively affected by irrigation water salinity (Figure 2C), with a linear decrease of 6.98% per unit increase in ECw, resulting in a decrease of 17.41% in the Fv of plants cultivated under irrigation with water of 3.0 dS m^{-1} (1472.66) compared to those irrigated with water of 0.6 dS m^{-1} (1781.93). This result demonstrates the lower utilization of the active potential energy of PSII, which would be destined to the production of ATP and NDPH, essential to the Calvin cycle in the biochemical phase of photosynthesis (YANG et al., 2022).

For the quantum yield of photosystem II (Fv/Fm) of sour passion fruit (Figure 2D), a linear decrease of 2.94% was observed per unit increment of ECw. When comparing the Fv/Fm of plants subjected to water salinity of 3.0 dS m^{-1} to that of those irrigated with ECw of 0.6 dS m^{-1}, a reduction of 7.19% was observed. This situation demonstrates the lower activity of P680 under salt stress conditions, which may be associated with the activity of the enzyme chlorophyllase, which reduces chlorophyll levels and, consequently, affects energy capture and transport between reaction centers and free plastoquinone (ÇIÇEK et al., 2018). Linear reduction in the quantum yield of photosystem II in sour passion fruit plants as a function of salt stress was also reported by Andrade et al. (2022), who observed decreases of 6.36% per unit increase in ECw and 13.98% when comparing plants irrigated with 0.7 dS m^{-1} water with those irrigated with 2.8 dS m^{-1} water, standing out as indicative of the occurrence of the photoinhibitory effect caused by salt stress.

There was a significant effect ($p \leq 0.01$) of water salinity levels and proline concentrations on plant height (PH), stem diameter (SD) and leaf area (LA) of sour passion fruit plants (Table 3). However, the interaction between the factors (SL × PRO) did not significantly influence any of the variables measured at 80 days after sowing.

Table 3. Summary of the analysis of variance for stem diameter (SD), plant height (PH) and leaf area (LA) of sour passion fruit plants cultivated under water salinity and proline concentrations, at 80 days after sowing (DAS).

Sources of variation	DF	Mean squares		
		SD	PH	LA
Salinity levels (SL)	4	382.58**	333.27**	79355.29**
Linear regression	1	1498.11**	1289.64**	307856.85**
Quadratic regression	1	28.19ns	8.64ns	1626.05**
Proline concentrations (PRO)	3	89.00**	163.52**	7171.86**
Linear regression	1	5.63ns	10.24ns	11049.79**
Quadratic regression	1	133.70**	427.90**	7380.10**
Interaction (SL × PRO)	12	7.88ns	6.75ns	16.50ns
Residual	12	14.29	30.98	184.84
CV (%)		10.94	18.51	2.07

DF - degrees of freedom; CV (%) - coefficient of variation; (*) significant at $p \leq 0.05$; (**) significant at $p \leq 0.01$; (ns) not significant.

Irrigation water salinity linearly reduced the stem diameter of sour passion fruit plants (Figure 3A) by 11.66%, which is equivalent to 5.1 mm per unit increment of ECw. When comparing the SD of plants cultivated under ECw of 3.0 dS m^{-1} to that of those subjected to the lowest water salinity level (0.6 dS m^{-1}), a reduction of 12.24 mm (30.09%) was observed. The decrease in growth is a consequence of changes in the total water potential in the soil due to the increase in the concentration of salts, which interferes with the absorption of water and nutrients by plants. This response is consistent with lower cell turgidity caused by salinity-induced osmotic damage, which associated with lower photosynthetic activity of plants under salt stress results in lower cell expansion (ZHAO et al., 2020).

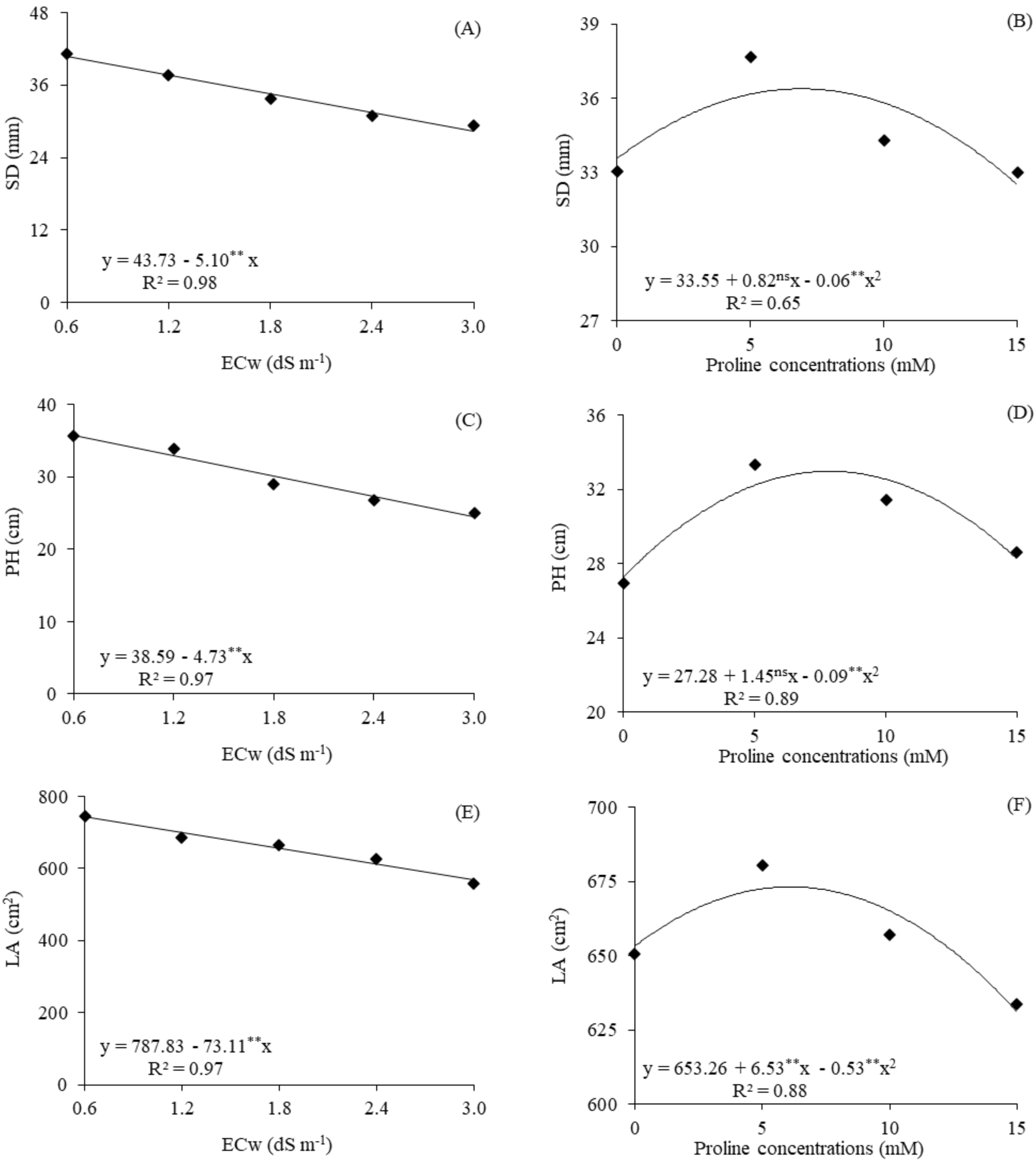
Proline application, in turn, caused an increase in the stem diameter of sour passion fruit plants (Figure 3B), with the maximum estimated value of 36.55 mm obtained in plants cultivated under the concentration of 6.83 mM, being 8.35% higher than the SD of plants that did not receive proline (0 mM). This beneficial effect of proline on growth is associated with its osmoprotective activity, which contributes to the absorption of the soil solution, maintaining turgidity and, consequently, cell division (SHAFI; ZAHOOR; MUSHTAQ, 2019).

Plant height was reduced linearly by irrigation water salinity (Figure 3C), with decreases of 12.25% (24.4 mm) per unit increment of ECw. In relative terms, there was a decrease of 36.91% in the height of plants irrigated with water of 3.0 dS m^{-1} compared to those cultivated under ECw of 0.6 dS m^{-1}. The reduction of growth in plant height also reflects the osmotic and ionic effects caused by the excess of salts and leads to inhibition of meristematic activity as a consequence of the lower flow of photoassimilates for growth. Instead, the energy is used in the maintenance of antioxidants and osmolytes in the plant (LIMA et al., 2021).

Foliar application of proline also promoted an increase in the growth in plant height for sour passion fruit (Figure 3D), with the maximum estimated value of 33.12 cm obtained under foliar application of 8.05 mM. On the other hand, the minimum estimated value of 27.28 cm was reached in plants that received proline concentration of 0 mM. The increase in plant growth promoted by foliar application of proline reflects improvements in photosynthetic activity, which maintains the metabolic integrity of the plant, resulting in high meristematic activity (BAUDUIN et al., 2022).

The increase in irrigation water salinity resulted in a linear decrease of 9.28% per unit increase in ECw (Figure 3E). When comparing the LA of plants subjected to water salinity of 3.0 dS m^{-1} with that of plants irrigated with ECw of 0.6 dS m^{-1}, a reduction of 23.58% (175.46 cm^2) was observed. Under salt stress conditions, losses in leaf area are an adaptive process of plants, aimed at reducing transpiration flow and mitigating excessive energy capture in the photochemical phase of photosynthesis (ÇIÇEK et al., 2018; KALAJI et al., 2018). Silva et al. (2019), in a study with sour passion fruit under salt stress (ECw ranging from 0.7 to 2.8 dS m^{-1}), observed a linear reduction in LA, which decreased by 13.1% per unit increase in ECw. According to these authors, this reduction in leaf area stands out as a mechanism for the adaptation of plants to salt stress, by reducing the transpiring surface.

As observed for SD and PH (Figures 3B and 3D), foliar application of proline increased the growth in leaf area of sour passion fruit (Figure 3F), with the maximum estimated value of 673.36 cm^2 obtained in plants subjected to foliar application of 6.16 mM, resulting in an increase of 3.07% compared to those that were cultivated without receiving proline (0 mM). In this case, proline maintains the leaf area of sour passion fruit plants, probably because the photochemical area has its efficiency improved by the high concentration of chlorophylls, reflecting gains in carbon assimilation without the need for large leaf expansion (HOSSEINIFARD et al., 2022).



^{ns}, *, ** - non-significant, significant at $p \leq 0.05$ and significant at $p \leq 0.01$ by the F test, respectively.

Figure 3. Stem diameter – SD (A and B), plant height – PH (C and D) and leaf area – LA (E and F) of sour passion fruit plants, as a function of water salinity - ECw and proline concentrations, at 80 days after sowing (DAS).

The results of the analysis of variance (Table 4) show a significant effect of water salinity levels on leaf dry mass (LDM), stem dry mass (StDM), root dry mass (RDM) and shoot dry mass (ShDM), root/shoot ratio (R/S) and Dickson Quality Index (DQI) of sour passion fruit plants, at 80 days after sowing. Proline concentrations also had a significant effect on LDM and ShDM. In addition, the interaction between the factors (SL × PRO) significantly influenced the LDM, ShDM and RDM of sour passion fruit plants at 80 days after sowing.

Table 4. Summary of the analysis of variance for leaf dry mass (LDM), stem dry mass (StDM), root dry mass (RDM) and shoot dry mass (ShDM), root/shoot ratio (R/S) and Dickson Quality Index (DQI) of sour passion fruit plants cultivated under water salinity and proline concentrations, at 80 days after sowing (DAS).

Sources of variation	DF	Mean squares					
		LDM	StDM	ShDM	RDM	R/S	DQI
Salinity levels (SL)	4	14.07**	4.25**	33.40**	0.19**	0.0232**	0.0522**
Linear regression	1	54.52**	14.98**	126.61**	0.06^{ns}	0.0855**	0.1481**
Quadratic regression	1	0.99^{ns}	1.56*	5.06^{ns}	0.70**	0.0044^{ns}	0.0598*
Proline concentrations (PRO)	3	4.56*	0.30^{ns}	6.78*	0.07^{ns}	0.0035^{ns}	0.0023^{ns}
Linear regression	1	0.55^{ns}	0.22^{ns}	1.46^{ns}	0.17*	0.0014^{ns}	0.0051^{ns}
Quadratic regression	1	5.47*	0.01^{ns}	6.06^{ns}	0.003^{ns}	0.0018^{ns}	0.0010^{ns}
Interaction (SL × PRO)	12	5.17**	0.19^{ns}	6.29*	0.08*	0.004^{ns}	0.0075^{ns}
Residual	12	1.02	0.24	1.24	0.098	0.0022	0.0066
CV (%)		12.58	17.50	10.29	10.62	16.78	10.39

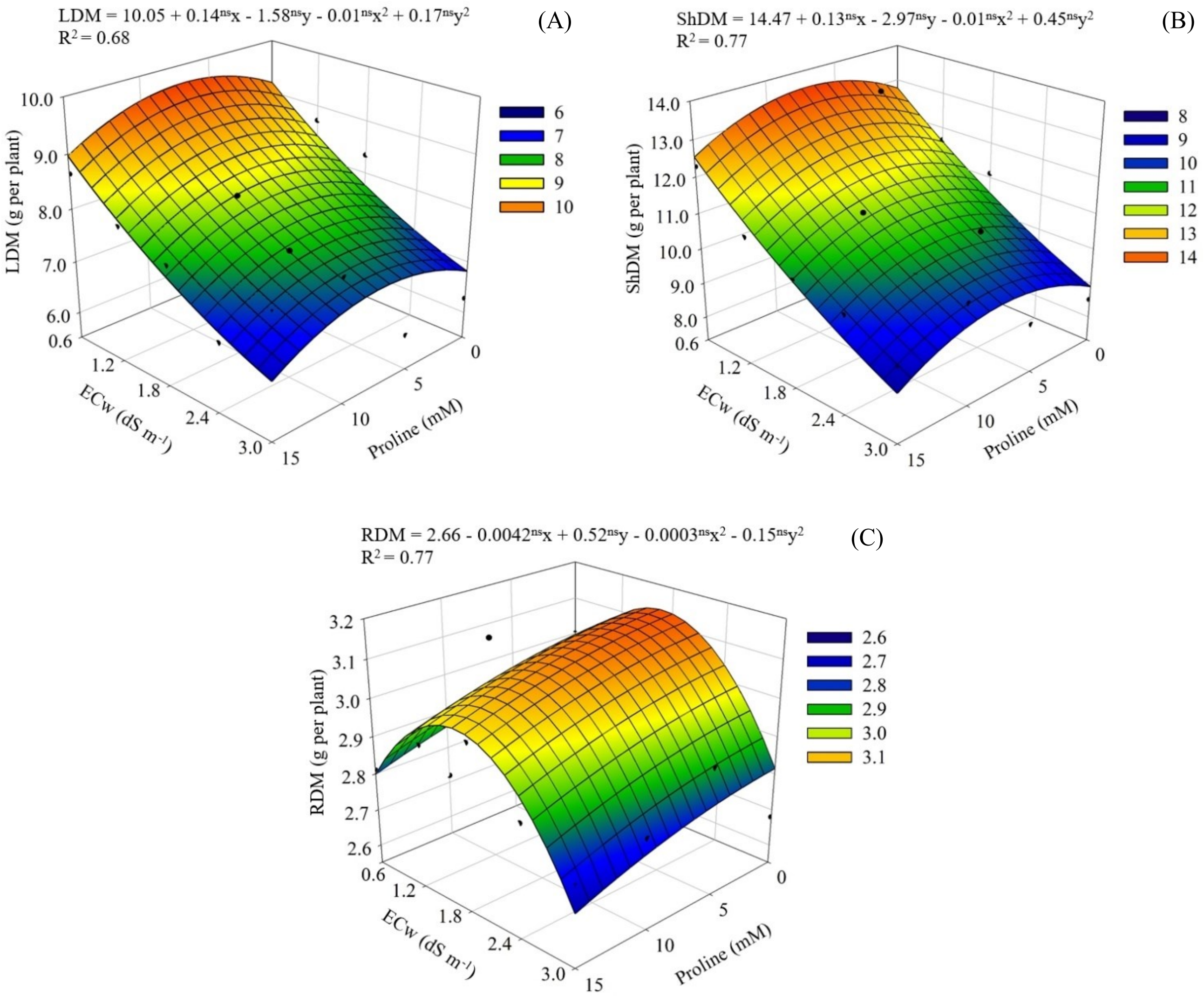
DF - degrees of freedom; CV (%) - coefficient of variation; (*) significant at $p \leq 0.05$; (**) significant at $p \leq 0.01$; (ns) not significant.

For the leaf dry mass of sour passion fruit plants (Figure 4A), the increase in irrigation water salinity from 0.6 to 3.0 dS m^{-1} resulted in a reduction of 25.93% (2.38 g per plant) at the concentration of 3.18 mM. On the other hand, for proline application, the maximum estimated value (9.67 g per plant) was obtained in plants cultivated under ECw of 0.6 dS m^{-1} and proline concentration of 6.80 mM, with an increase of 5.34% (9.6704 g per plant) compared to those that did not receive proline under the same irrigation condition. At the highest ECw (3.0 dS m^{-1}), proline application reduced the effects of salt stress, promoting an increase of 7.20% at concentration of 7.0 mM (7.29 g per plant) compared to plants that did not receive proline application. Such responses corroborate the adaptive process of plants to salt stress, which reduces leaf biomass to avoid the aggravation of the toxicity by specific ions (YANG et al., 2022), mitigated by proline application, which contributes to the compartmentalization of these ions in the vacuole of cells, maintaining ionic homeostasis and thus leaf expansion (HOSSEINIFARD et al., 2022).

A similar behavior was observed in shoot dry mass (Figure 4B), as the increment in ECw led to a decrease in ShDM accumulation of 27.45% in plants cultivated under water salinity of 3.0 dS m^{-1} (9.31g per plant), when compared to those subjected to water salinity of 0.6 dS m^{-1} (12.83 g per plant). At the lowest level of water salinity, the highest ShDM accumulation (13.28 g per plant) was obtained with the proline concentration of 6.56 mM. Increment in biomass accumulation was also observed with the application of 7.0 mM of proline in plants irrigated with water of 3.0 dS m^{-1} (9.80 g per plant), with an increase of 5.26% compared to plants without proline application (0 mM) and kept under the same irrigation condition. Such reductions show the effects on photosynthetic pigments, fluorescence, and gas exchange, which limit the support necessary to regulate the plant's metabolism, making it necessary to release energy to maintain root homeostasis, hence neglecting shoot growth (ARIF et al., 2020). This situation has already been alleviated in the root system by the application of proline, freeing up energy for shoot growth (BAUDUIN et al., 2022).

Figure 4C shows that the highest RDM accumulation of sour passion fruit plants was obtained under an estimated water salinity of 1.7 dS m^{-1} (3.12 g per plant), with an increase of 6.46% compared to plants irrigated with water of 0.6 dS m^{-1} (2.93 g per plant). Proline application, in turn, caused a decrease in RDM at all salinity levels, and the lowest value was obtained when the highest concentration of proline (15 mM) was associated with the highest water salinity (3.0 dS m^{-1}), leading to a value of 2.72 g per plant, which is 12.82% lower than the maximum estimated value (4.56%) found in plants without proline application and under the same irrigation condition (2.85 g per plant). Increase in root biomass due to increased salt stress is associated with the strategy of increasing the root area as a way to attenuate the osmotic effect of salinity (ZHAO et al., 2020), and at high salinity levels, the osmotic potential decreases to values that limit root expansion, so it becomes necessary to increase osmolyte production to values that do not impair root growth (ARIF et al., 2020). This situation explains the reduction in RDM accumulation caused by the application of proline, which already maintains osmotic regulation with the soil at values that do not require the release of more energy for root growth (SHAFI; ZAHOOR; MUSHTAQ, 2019).

Stem dry mass accumulation in sour passion fruit plants decreased linearly with the increase in ECw levels (Figure 5A), with a decrease of 13.68% per unit increase in ECw. When comparing the StDM of plants irrigated with water of 3.0 dS m^{-1} to that of plants subjected to ECw of 0.6 dS m^{-1}, a decrease of 1.22 g per plant (35.78%) was observed. This reduction in biomass accumulation is a result of changes in gas exchange, due to osmotic effects, leading to losses of turgidity and cell expansion, as found by Lima et al. (2021) in sour passion fruit (*Passiflora edulis Sims*), that inhibited growth in SD and PH.



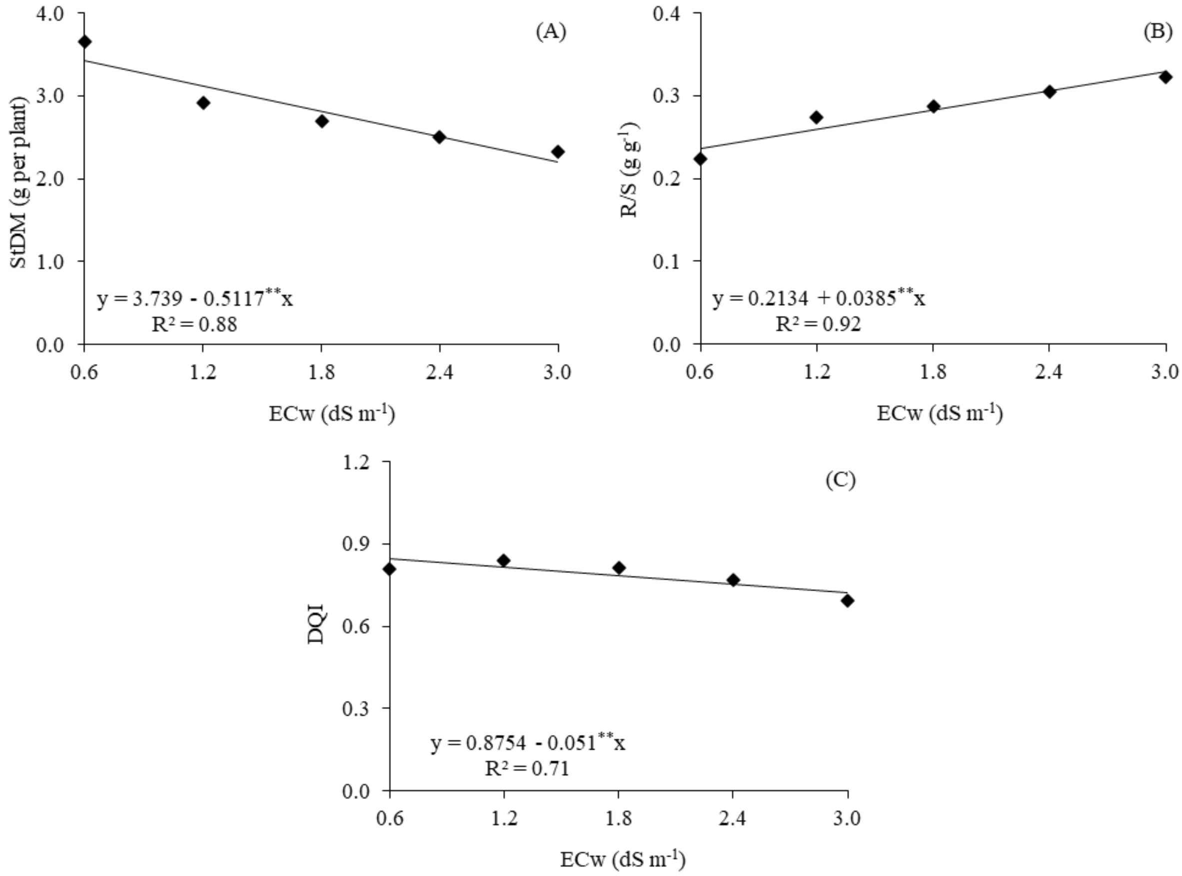
X and Y - electrical conductivity of water – ECw and proline concentration, respectively;
ns, *, ** - not significant, significant at $p \leq 0.05$ and significant at $p \leq 0.01$ by the F test, respectively.

Figure 4. Leaf dry mass - LDM (A), shoot dry mass - ShDM (B) and root dry mass - RDM (C) of sour passion fruit plants, as a function of the interaction between water salinity and proline concentrations, at 80 days after sowing (DAS).

Water salinity linearly increased the root/shoot ratio of sour passion fruit plants (Figure 5B), by 18.04% per unit increment of ECw. When comparing the R/S of plants subjected to ECw of 3.0 dS m^{-1} to that of plants cultivated under the lowest water salinity level (0.6 dS m^{-1}), an increase of 0.092 g g^{-1} (39.06%) was observed. This result corroborate those obtained for RDM under salt stress, as plants transfer the resources from shoot growth to root expansion in order to try to maintain the absorption of the soil solution (ZHAO et al., 2020).

Diniz et al. (2021), in a study with 'BRS GA1' sour passion fruit cultivated with saline waters (ECw ranging from 0.3 to 3.1 dS m^{-1}), also observed that the R/S increased linearly with the increment in the water electrical conductivity levels, with increase of 91.16% in plants that received ECw of 3.1 dS m^{-1} compared to those that received the lowest salinity level (0.3 dS m^{-1}). According to the authors, the increase in R/S suggests that the aerial part is more sensitive to salt stress than the root system, which is important for optimizing the absorption of water and nutrients, since salinity limits the growth and development of plants, as the reduction of growth parameters is the result of defense strategies of the plant itself, such as decrease in cellular turgidity, metabolic processes and biochemical processes.

The Dickson quality index (Figure 5C) of sour passion fruit plants was also reduced linearly with the increase in water electrical conductivity levels, with a decrease of 5.82% per unit increment of ECw. There was a decrease of 14.48% (0.122), in relative terms, in the DQI of plants irrigated with water of 3.0 dS m^{-1} compared to those subjected to the lowest salinity level (0.6 dS m^{-1}). Despite the reduction, sour passion fruit plants obtained DQI values > 0.2, which gives these seedlings acceptable quality for transplanting to the field (SOUZA et al., 2017). It is important to highlight that the DQI indicates the sturdiness and balance of biomass distribution in the plant.



** - significant at $p \leq 0.01$ by the F test.

Figure 5. Stem dry mass - StDM (A), root/shoot ratio - R/S (B) and Dickson quality index - DQI (C) of sour passion fruit plants, as a function of water salinity - ECw, at 80 days after sowing (DAS).

The irrigation water salinity threshold for 'BRS GA1' sour passion fruit observed by the plateau followed by linear decrease model (MAAS; HOFFMAN, 1977) is 0.6 dS m^{-1} (Figure 6), with a decrease of 10.49% per unit increment above this water salinity level. However, irrigation with ECw of 3.46 dS m^{-1} can result in a yield of up to 70% of its total dry mass per plant. On the other hand, irrigation using water with electrical conductivity of 5.36 dS m^{-1} allows obtaining a relative production in total dry mass of 50%. Based on the degree of tolerance defined by Maas and Hoffman (1977), which takes into account the relative production in total dry mass and the decrease per unit increment above the threshold level, the genotype 'BRS GA1' is classified as sensitive to irrigation water salinity. This behavior is similar to that observed by Lima et al. (2023), who studied the tolerance of sour passion fruit cultivars to salt stress conditions in the semi -arid region of Paraíba and established ECw of 0.3 dS m^{-1} as the threshold of the 'BRS GA1' genotype.

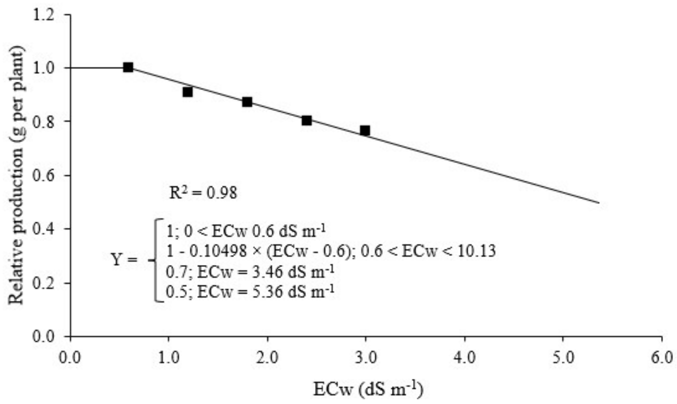


Figure 6. Relative production of biomass by 'BRS GA1' sour passion fruit, as a function of the electrical conductivity of irrigation water – ECw described by the plateau followed by linear decrease mathematical model of Maas and Hoffman (1977).

CONCLUSION

Water salinity from 0.6 dS m^{-1} reduces the maximum fluorescence, variable fluorescence, quantum yield of photosystem II and growth of 'BRS GA1' sour passion fruit seedlings. Foliar application of proline at concentrations ranging from 6 to 8.05 mM increases the growth in plant height, stem diameter and leaf area of sour passion fruit seedlings. Water with electrical conductivity of up to 3.0 dS m^{-1} enables the formation of sour passion fruit seedlings with acceptable quality for transplanting to the field. The sour passion fruit genotype 'BRS GA1' is sensitive to water salinity.

REFERENCES

ANDRADE, E. M. G et al. Hydrogen peroxide as attenuator of salt stress effects on the physiology and biomass of yellow passion fruit. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 26: 571-578, 2022.

ARIF, Y. et al. Salinity induced physiological and biochemical changes in plants: Anatomic approach towards salt stress tolerance. **Plant Physiology and Biochemistry**, 156: 64-77, 2020.

BAUDUIN, S. et al. Interplay between proline metabolism and ROS in the fine tuning of root-meristem size in arabidopsis. **Plants**, 11: e1512, 2022.

CAVALCANTE, L. F. et al. Germinação de sementes e crescimento inicial de maracujazeiros irrigados com água salina em diferentes volumes de substrato. **Revista Brasileira de Fruticultura**, 24: 748-751, 2002.

ÇIÇEK, N. et al. Salt stress effects on the photosynthetic electron transport chain in two chickpea lines differing in their salt stress tolerance. **Photosynthesis Research**, 136: 291-301, 2018.

CORRÊA, R. C. G. et al. The past decade findings related with nutritional composition, bioactive molecules and biotechnological applications of *Passiflora spp.* (passion fruit). **Trends in Food Science & Technology**, 58:79-95, 2016.

DICKSON, A.; LEAF, A. L.; HOSNER, J. F. Quality appraisal of white spruce and white pine seedling stock in nurseries. **The Forest Chronicle**, 36:10-13, 1960.

DINIZ, G. L. et al. Irrigation with saline water and silicate fertilization in the cultivation of 'Gigante Amarelo' passion fruit. **Revista Caatinga**, 34: 199-207, 2021.

GHARSALLAH, C. et al. Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. **AoB Plants**, 8: 1-21, 2016.

HOSSEINIFARD, M. et al. Contribution of exogenous proline to abiotic stresses tolerance in plants: a review. **International Journal of Molecular Sciences**, 23: 1-20, 2022.

KALAJI, H. M. et al. Can chlorophyll-a fluorescence parameters be used as bio-indicators to distinguish between drought and salinity stress in Tilia cordata Mill. **Environmental and Experimental Botany**, 152: 149-157, 2018.

LIMA, G. S. et al. Gas exchange, chloroplast pigments and growth of passion fruit cultivated with saline water and potassium fertilization. **Revista Caatinga**, 33: 184-194, 2021.

LIMA, G. S. et al. Tolerance of sour passion fruit cultivars to salt stress in a semi-arid region. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 27: 785-794, 2023.

MAAS, E.V.; HOFFMAN, G. J. Crop Salt Tolerance-Current Assessment. **Journal of the Irrigation and Drainage Division**, 103: 115-134, 1977.

MANAA, A. et al. Salinity tolerance of quinoa (*Chenopodium quinoa* Willd) as assessed by chloroplast ultrastructure and photosynthetic performance. **Environmental and Experimental Botany**, 162: 103-114, 2019.

MOHAMED, I. A. A. et al. Stomatal and photosynthetic traits are associated with investigating sodium chloride tolerance of Brassica napus L. cultivars. **Plants**, 9: 1-19, 2020.

NOVAIS, R. D.; NEVES, J. C. L.; BARROS, N. D. Ensaio em ambiente controlado. In: OLIVEIRA, A. J. et al. (Eds.). **Métodos de pesquisa em fertilidade do solo**. Brasília, DF: EMBRAPA, 1991. v. 1, cap. 2, p. 89-253, 1991.

PINHEIRO, F. W. A. et al. Gas exchange and yellow passion fruit production under irrigation strategies using brackish water and potassium. **Revista Ciência Agronômica**, 53: e20217816, 2022.

PIRES, A. A. et al. Efeito da adubação alternativa do maracujazeiro amarelo nas características químicas e físicas do solo. **Revista Brasileira de Ciência do Solo**, 32: 1997-2005, 2008.

RAMOS, J. G. et al. Hydrogen peroxide as salt stress attenuator in sour passion fruit. **Revista Caatinga**, 35: 412-422, 2022.

RICHARDS, L. A. **Diagnosis and improvement of saline and alkali soils**. Washington: U.S, Department of Agriculture. 1954. 160 p.

SHAFI, A.; ZAHOOR, I.; MUSHTAQ, U. Proline accumulation and oxidative stress: Diverse roles and mechanism of tolerance and adaptation under salinity stress. **Salt Stress, Microbes, and Plant Interactions: Mechanisms and Molecular Approaches**, 2: 269-300, 2019.

SILVA, A. A. R. et al. Gas exchanges and growth of passion fruit seedlings under salt stress and hydrogen peroxide. **Pesquisa Agropecuária Tropical**, 49: 1-10, 2019.

SOUZA, L. P. et al. Produção de porta-enxerto de goiabeira cultivado com águas de diferentes salinidades e doses de nitrogênio. **Revista Ciência Agronômica**, 48: 596-604, 2017.

TEIXEIRA, P. C. et al. **Manual de métodos de análise de solo**. 3. ed. Brasília, DF: Embrapa Solos, 2017. 573 p.

VELOSO, L. L. S. A. et al. Morphophysiology of guava cv. Paluma with water of different salt concentrations and proline doses. **Semina: Ciências Agrárias**, 39: 1877-1886, 2018.

WANG, C. P. et al. Proline inhibits plant growth by reactive oxygen species signaling. **Plant Physiology Communications**, 53: 1788-1794, 2017.

YANG, Y. et al. Trehalose alleviates salt tolerance by improving photosynthetic performance and maintaining mineral ion homeostasis in tomato plants. **Frontiers in Plant Science**, 13: 1-22, 2022.

ZHAO, C. et al. Mechanisms of plant responses and adaptation to soil salinity. **The innovation**, 1: 1-41, 2020.