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Morphophysiology of tomato crop under saline stress in different environments

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ABSTRACT

The use of brackish water can cause severe effects and irreversible damage to the growth and physiological indices of agricultural crops. Thus, the agricultural environment emerges as an alternative to mitigate saline stress in tomato hybrids (*Solanum lycopersicum* L.). This study evaluated the gas exchange and growth of tomato hybrids irrigated with brackish water in different environments. The study was carried out in Aratuba, Ceará, Brazil. The experimental design was a randomized block design with split-split plots. The main plots consisted of two environments (protected environment and full sun); the subplots consisted of five levels of irrigation water electrical conductivity (ECw) (1.0, 1.7, 2.4, 3.1, and 3.8 dS m^{-1}) and the sub-subplots consisted of two tomato hybrids (Itaipava and BS DI0014), with five replicates. Salt stress reduced CO_2 assimilation rate and instantaneous carboxylation efficiency rates more intensely in the full sun environment, while the use of low electrical conductivity in the protected environment promoted greater stomatal conductance in tomato hybrids. The BS DI0014 hybrid was more tolerant to salt stress in terms of transpiration in the protected environment and in full sun than the Itaipava F1 hybrid. The Itaipava F1 and BS DI0014 hybrids grown in a protected environment had higher internal CO_2 concentrations.

Keywords: protected cultivation, salinity, *Solanum lycopersicum* L.

Morfofisiologia da cultura do tomate sob estresse salino em diferentes ambientes

RESUMO

O uso de água salobra pode provocar efeitos severos e danos irreversíveis no crescimento e nos índices fisiológicos das culturas agrícolas. Assim, a ambiência agrícola surge como alternativa para mitigar o estresse salino em híbridos de tomate (*Solanum lycopersicum L.*). Neste sentido, objetivou-se avaliar as trocas gasosas e o crescimento de híbridos de tomate submetida a irrigação com água salobra, em diferentes ambientes. O estudo foi desenvolvido em Aratuba, Ceará, Brasil. O delineamento experimental utilizado foi em blocos ao acaso, em parcelas subsubdivididas, sendo as parcelas formadas por dois ambientes (ambiente protegido e pleno sol), as subparcelas por cinco níveis de condutividade elétrica da água de irrigação (CEa) (1,0; 1,7; 2,4; 3,1 e 3,8 dS m^{-1}) e as subsubparcelas pelos dois híbridos de tomate (Itaipava e BS DI0014), com cinco repetições. O estresse salino reduziu negativamente as taxas fotossintéticas, a condutância estomática, a concentração interna de CO_2 e a eficiência do uso da água, porém com menor intensidade no ambiente protegido. O híbrido BS DI0014 foi mais tolerante ao estresse salino quanto a transpiração no ambiente protegido e a pleno sol em relação ao híbrido Itaipava F1. Os híbridos Itaipava F1 e BS DI0014 cultivados em ambiente protegido apresentam maior concentração interna de CO_2.

Palavras-chave: cultivo protegido, salinidade, *Solanum lycopersicum L.*

1. INTRODUCTION

The tomato (*Solanum lycopersicum L.*) is a plant species belonging to the Solanaceae family and is one of the main vegetables produced and marketed worldwide. It can be eaten fresh or processed and has nutritional functions that are beneficial to human nutrition (Li *et al*., 2022; Alenazi and Khandaker, 2024).

In tropical regions with a hot climate characterized by low rainfall, such as the semiarid, the use of full irrigation is necessary to meet the water demand of agricultural crops and ensure food security. Among the strategies employed to enable agricultural production in this region, the use of brackish water stands out, a practice that has been employed due to the limited availability of better quality water resources (Roque *et al*., 2022; Sousa *et al*., 2024).The use of irrigation with water containing high salt levels can restrict the uptake of water and nutrients by plants and, consequently, the water consumption of plants, affecting metabolism, cell expansion, and the production of photoassimilates, which results in reduced tomato growth, limiting physiological indices, such as transpiration, CO_2 assimilation, and stomatal conductance (Taiz *et al*., 2017; Sousa *et al*., 2021).

Modulating the agricultural environment, especially in protected systems, combined with the use of tolerant hybrids, constitutes an efficient strategy for mitigating the effects of saline stress. In these environments, factors such as temperature and luminosity can be controlled, thus reducing direct radiation favors an increase in CO_2 concentration and possibly the accumulation of phytochemicals in tomato plants (Alshami *et al*., 2023). Studies by Rodrigues *et al*. (2024) found that tomato plants subjected to salinity under high temperature conditions resulted in plants with lower chlorophyll and carotenoid content, decreased leaf area, and lower yield. El-Mogy *et al*. (2018) observed that under protected environment conditions and moderate saline stress, it is possible to satisfactorily produce plants without a significant reduction in productivity.

This study is based on the hypothesis that the agricultural environment can mitigate the effects of saline stress on the morphophysiology of tomato hybrids. Accordingly, the study evaluated the gas exchange and growth of tomato hybrids irrigated with brackish water in different environments.

2. MATERIAL AND METHODS

The research was conducted at Sítio Flexeiras, Aratuba, Ceará, Brazil (4°07'48.6” S; 38°49' 30.6” W; altitude 830 m). The region's climate is classified as Tropical Sub-Humid, with average temperatures of 24 to 26ºC (Alvares *et al*., 2013). The daily average temperature and relative humidity data were monitored by a humidity sensor (HO-BO® U12-012 Temp/RH/Light/Ext), shown in Figure 1.

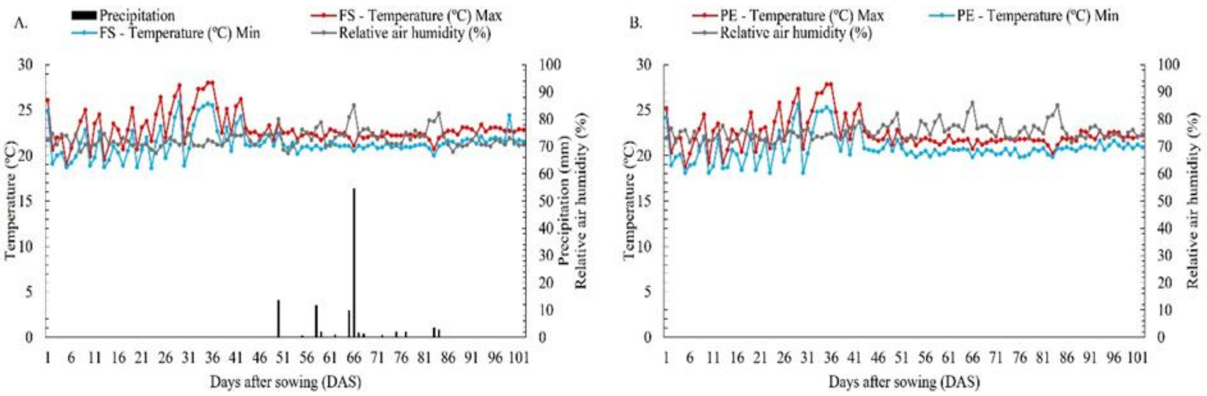

Figure 1. Data related to mean values of temperature, precipitation, and relative humidity of the air in full sun (A) and protected environment (B) during the experimental period (August to December 2022). FS – Full sun; PE – Protected environment.

The experimental design used was a randomized complete block design in split plots, with the plots consisting of two environments (protected environment and full sun), the subplots consisted of five levels of electrical conductivity of the irrigation water (ECw) (1.0, 1.7, 2.4, 3.1, and 3.8 dS m^{-1}) and the sub-subplots consisted of two tomato hybrids (Itaipava and BS DI0014), with five replicates. The electrical conductivities of water used were based on the threshold salinity (2.5 dS m^{-1}) proposed by Maas and Hoffman (1977), that is, two levels below and two above the threshold.

The crop used in the study was tomato (*Solanum lycopersicum L.*), hybrids Itaipava Topseed Premium® and BS DI0014 Blueseeds®. Sowing was carried out in a polypropylene tray with 200 cells of 40 cm^3 in volume, where each cell received a seed placed 2 cm deep. Transplanting took place 25 days after sowing (DAS) into 49 dm^3 pots in a protected environment with a 50% shade screen and in full sun.

The material used to compose the substrate was obtained from a mixture of a Red-Yellow Argisol + goat manure + washed sand in a 3:1:1 (v/v) ratio, respectively, whose physicochemical analysis (Table 1) was carried out in the soil laboratory of the Federal University of Ceará (UFC), following the methodologies described by Teixeira *et al*. (2017).

To prepare the irrigation water, the salts NaCl, $CaCl_2.2H_2O$, and $MgCl_2.6H_2O$ were used to obtain the desired ECw in a 7:2:1 equivalent ratio, following the relationship between ECw and its concentration (mmolc L^{-1} = EC × 10) (Rhoades *et al.,* 2000). Irrigation was carried out following the methodology of Bernardo *et al.* (2019), based on the principle of the drainage lysimeter on a daily basis, applying a 15% leaching fraction.

Table 1. Chemical and physical characteristics of the substrate sample before applying treatments.

Chemical characteristics									
OM	N	P	Ca^{2+}	K^+	Mg^{2+}	Na^+	pH	ESP	ECse
($g\ kg^{-1}$)		($mg\ kg^{-1}$)	($cmol_c\ dm^{-3}$)				(H_2O)	%	($dS\ m^{-1}$)
22.03	1.37	83	6.1	1.72	2.6	0.3	6.8	3	2.14

Physical characteristics							
Coarse sand	Fine sand	Clay	Silt	SD ($g\ cm^{-3}$)		DF	Textural classification
($g\ kg^{-1}$)				Bulk	Particle	$g\ 100g^{-1}$	Loamy sand
612	205	44	139	1.36	2.63	4	

OM - Organic matter; SD - Soil density; DF - Degree of flocculation; ESP - Exchangeable sodium percentage; ECse - Electrical conductivity of the saturation extract.

The irrigation system used was drip irrigation with drippers with a discharge rate of 8 L h^{-1}. The irrigation time was quantified according to the volume to be applied in each irrigation event, determined according to Equation 1.

$$VI = (Vp - Vd)/(1 - Lf) \tag{1}$$

Where: VI - volume of water to be applied in the irrigation (mL); Vp - volume of water applied in the previous irrigation (mL); Vd - volume of water drained (mL); and, FL - leaching fraction of 0.15, applied in each irrigation event.

The brackish water treatments began 15 days after transplanting (DAT), and the following physiological indices were assessed at 30 DAT: CO_2 assimilation rate (A, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mol m^{-1} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), and internal CO_2 concentration (Ci, μmol CO_2 mol^{-1}). These data were then used to calculate the instantaneous water use efficiency (WUE, [(μmol CO_2/mmol H_2O m^{-2} $s^{-1)-1}$]) and instantaneous carboxylation efficiency (iCE, μmol CO_2 m^2 s^{-1}/μmol CO_2 mol^{-1} air).

The measurements were carried out using an infrared gas analyzer (IRGA - LI 6400 XT, LICOR), in an open system, with an air flow of 300 μmol s^{-1}, on the limb of the first leaf below the inflorescence. The measurements were made between 08 and 10 a.m., using an artificial radiation source (around 1,200 μmol m^{-2} s^{-1}), and under ambient temperature and CO_2 concentration conditions.

During this same period, the following growth variables were assessed: plant height (PH, cm), measured from the base to the apex of the plant with a graduated ruler, stem diameter (SD, mm), measured 2 cm above the substrate with a digital caliper, and leaf area (LA, cm^2), estimated by the ratio between leaf width and length, using a correction factor of 0.59, (Reis *et al*., 2013), according to Equation 2.

$$LA = C\ x\ L\ x\ f \tag{2}$$

Where: LA - leaf area, cm^2; C - leaf length, cm; L - leaf width, cm; and, f - shape factor (0.59).

Data regarding gas exchange and growth variables were analyzed using the Kolmogorov-Smirnov test ($p \leq 0.05$) to assess normality. After verifying normality, the data were subjected to analysis of variance using the F-test. In cases of significance, for electrical conductivity, regression analysis was performed, while data on tomato hybrids and environments were subjected to the Tukey test ($p \leq 0.05$), using the Assistat 7.7 Beta software (Silva and Azevedo, 2016).

3. RESULTS AND DISCUSSION

Based on the results obtained, there was a triple interaction between the factors studied, cultivation environment, brackish water and tomato hybrids only for the transpiration variable (Table 2). There was also an interaction between the factors brackish water and growing environment for the variables CO_2 assimilation rate, stomatal conductance, and instantaneous water use efficiency. There was also an isolated effect for the growing environment factor for leaf temperature.

Table 2. Summary of analysis of variance of CO_2 assimilation rate (A), stomatal conductance (gs), transpiration (E), internal carbon concentration (Ci), and instantaneous water use efficiency (WUE) of tomato hybrids under different electrical conductivities of irrigation water and growing environments.

Sources of variation	Mean Square					
	DF	*A*	*gs*	*E*	Ci	*WUE*
Block	4	13.55	0.024	0.11	966.17	0.33
Growing environment (GE)	1	0.13^{ns}	1.53**	1.21^{ns}	18115.66**	1.66^{ns}
Residue (a)	4	2.88	0.009	0.16	68.84	0.58
Brackish water (BW)	4	31.68*	0.24**	1.10^{ns}	778.72^{ns}	0.26^{ns}
GE×BW	4	41.31**	0.12**	2.14**	2,179.88**	3.49*
Residue (b)	32	9.96	0.03	0.48	523.14	1.14
Hybrid (H)	1	2.04^{ns}	0.10^{ns}	0.73^{ns}	$1,089.71^{ns}$	1.49^{ns}
GE × H	1	0.11^{ns}	0.04^{ns}	0.18^{ns}	0.0089**	0.51^{ns}
BW × H	4	11.46^{ns}	0.008^{ns}	0.54^{ns}	483.68^{ns}	1.75^{ns}
GE × BW × H	4	16.76^{ns}	0.031^{ns}	1.05*	403.51^{ns}	1.14^{ns}
Residue (c)	40	7.78	0.03	0.34	658.69	0.72
Total	99					
CV (a)	-	7.60	15.21	8.88	3.24	15.5
CV (b)	-	14.14	27.7	15.15	8.24	21.68
CV (c)	-	12.5	29.88	12.82	10.04	17.23

DF - Degree of freedom; CV - Coefficient of variation; **, * Significant at $p \leq 0.01$ and $p \leq 0.05$, respectively by F test; and ns - Not significant ($p > 0.05$).

The decreasing linear model was the best fit, reducing the CO_2 assimilation rate by 13.04% from the lowest to the highest electrical conductivity of the irrigation water for the full sun environment (Figure 2A). For the protected environment, the quadratic polynomial model was the best fit, with a maximum CO_2 assimilation rate of 23.24 $\mu mol\ m^{-2}\ s^{-1}$ with an electrical conductivity of 1.4 $dS\ m^{-1}$. It is noteworthy that saline stress in plants grown in a full-sun environment provided greater CO_2 assimilation rate. This effect possibly promoted physiological acclimatization, partially relieving heat stress and excess light, reducing the translocation of toxic ions to the leaves and favoring CO_2 assimilation in the biochemical phase (Alshami *et al*., 2023; Jiang *et al*., 2024).

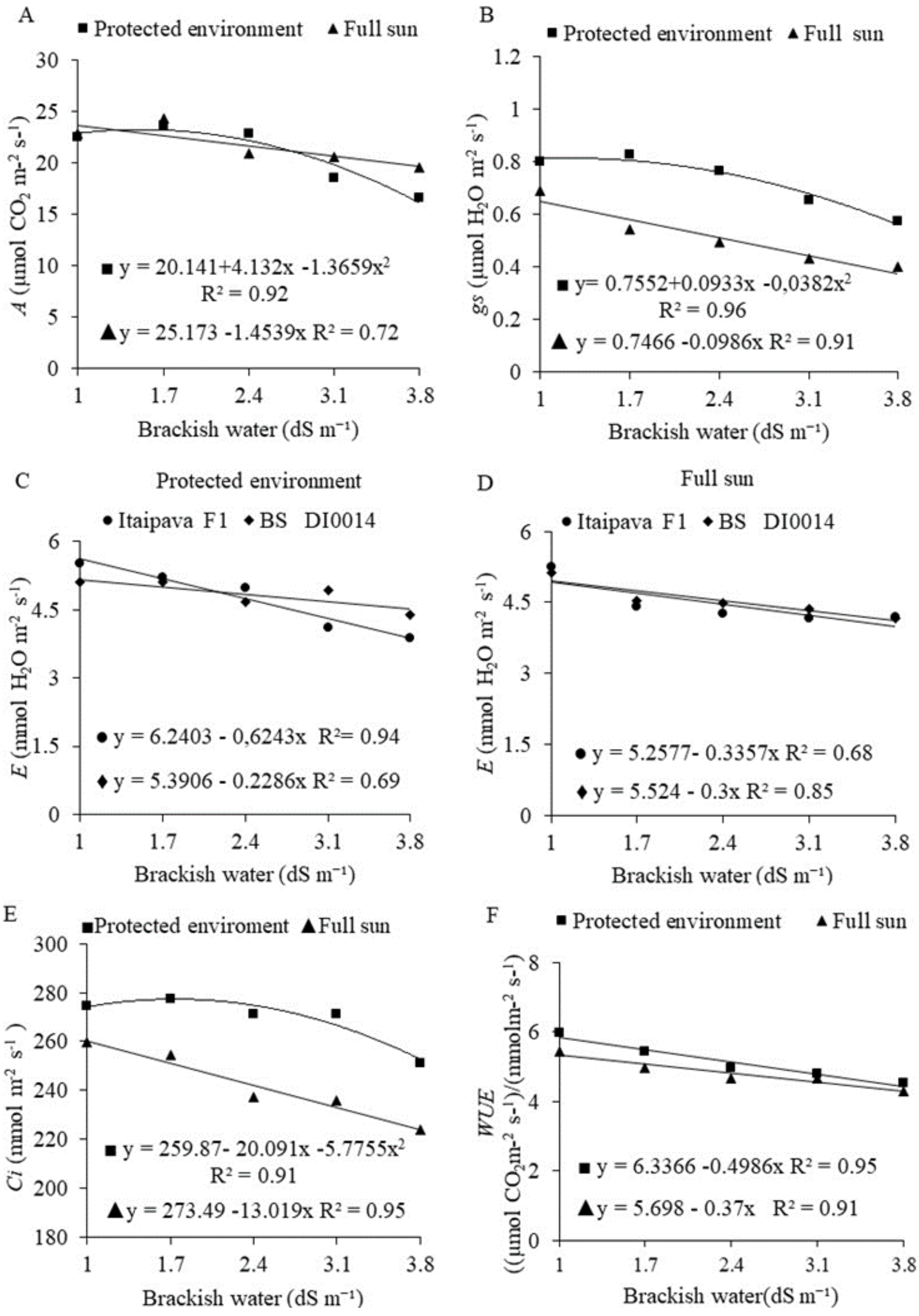

Figure 2. CO_2 assimilation rate - A (A) and stomatal conductance – gs (B) of tomato hybrid plants irrigated with brackish water in a protected environment (■) and in full sun (▲), Transpiration - E (C e D) of tomato hybrid plants (● Itaipava F1) and (♦ BS DI0014), Internal CO_2 concentration - Ci (E), and instantaneous water use efficiency – WUE (F) of tomato hybrid plants irrigated with brackish water in a protected environment (■) and in full sun (▲).

This negative effect of saline stress in a protected environment may be associated with lower light reaching the plants, reducing transpiration and increasing partial closure of stomata, thus promoting oxidative stress with harmful effects on lipid peroxidation, membrane degradation and lower chlorophyll concentrations and consequently lower CO_2 assimilation rate (Jiang *et al*., 2024). Roque *et al*. (2022) also showed a decline in the CO_2 assimilation rate of tomato crops subjected to high saline concentrations in a protected environment. The salt stress linearly reduced the stomatal conductance of the tomato crop grown in full sun, reducing 42% from the lowest to the highest electrical conductivity of water. As for the protected environment, the quadratic polynomial model was the one that best fitted the data, obtaining a maximum of (0.81 mol m^{-1} s^{-1}) for the electrical conductivity of 1.2 dS m^{-1}. This behavior can be attributed to the lower light intensity within the protected environment, which favored greater interaction between water below the threshold salinity, evidencing greater stomatal regulation and water transport within cells, increasing cell turgidity and resulting in greater stomatal opening (Taiz *et al*., 2017). However, higher saline levels reduced gs, promoting partial closure of stomata, since the osmotic effect triggered by saline stress reduces cell turgidity, promoting ostiole closure (Roque *et al*., 2022). Corroborating this study, Batista *et al*. (2021) showed a reduction in the stomatal conductance of tomato plants grown in a protected environment and subjected to irrigation with brackish water in a nutrient solution.

Rising salt levels in irrigation water led to a reduction in transpiration of the tomato hybrids Itaipava F1 and BS DI0014 in the protected environment (Figure 2C) and in full sun (Figure 2D). The superiority of the BS DI0014 hybrid compared to the Itaipava F1 in the protected environment at the highest salt levels may be related to an acclimatization mechanism of this hybrid, which possibly reduced the water potential gradient between the soil and the roots, decreasing stomatal conductance and consequently the transpiration rate.

This result may be related to the fact that salinity triggers processes such as an increase in abscisic acid, which is a hormonal signal of stressful conditions. This increase in ABA concentration promotes stomatal closure and consequently a decrease in perspiration (Pei *et al*., 2024). It is worth noting that the agricultural environment can promote improved tomato cultivation as a response mechanism for water retention, thus maintaining the water potential for easy absorption and regulating the amount of water absorbed as a response mechanism to minimize water loss (Batista *et al*., 2021; Alshami *et al*., 2023). A similar trend to this study was reported by Roque *et al*. (2022) when irrigating tomato crops with brackish water in a protected environment.

The model for internal carbon concentration was the quadratic polynomial, with a maximum of 277.342 μmol mol^{-1} at an electrical conductivity of 1.73 dS m^{-1} in the protected environment and a decreasing linearity in full sun (Figure 2E). The protected environment relieved saline stress and provides a higher internal concentration of CO_2 compared to full sun, increasing stomatal conductance, positively interfering in the photosynthesis process, as it raises the partial pressure of this gas in the intracellular spaces (Sousa *et al*., 2024).

The data described in this study reveal that the water deficit induced by the increase in salinity of irrigation water caused stomatal closure, which limits the increase in internal CO_2 concentration in the plant. A similar trend was reported by Rodríguez-Ortega *et al*. (2019), who found that the internal CO_2 concentration decreased with increasing salinity in a protected environment. The authors emphasize that these responses may be related to stomatal behavior, but may also be metabolic factors, which vary according to tomato varieties.

The instantaneous water use efficiency showed a linear decrease with saline stress in the protected environment and in full sun, with a reduction of 23.95 and 21.95% from the lowest to the highest salinity, respectively (Figure 2F). This result describes that saline stress showed a reduction in water availability for plants, evidencing partial closure of stomata, restricting the entry of water and photosynthetic pigments, which limits the absorption of CO_2 in the leaf mesophyll cells, consequently lower instantaneous water use efficiency (Fatima *et al*., 2024).

This behavior may be linked to the plant's ability to reduce evapotranspiration as a defense mechanism, since a reduction in water consumption implies a reduction in the absorption of specific ions, avoiding toxic effects on the plants (Lessa *et al*., 2022). Similarly, Oliveira *et al*. (2022) also showed greater instantaneous water use efficiency in sugar beet crops irrigated with lower salinity water under protected growing conditions.

The summary data from the analysis of variance for initial growth are shown in Table 3. It can be seen that there was a significant effect for the interaction between the factors growing environment, salinity and tomato hybrids for the variable plant height at $p \leq 0.05$. There was an interaction between the factors growing environment and tomato hybrids for the leaf area variable and an isolated effect for the brackish water factor ($p \leq 0.01$ and ≤ 0.05). In the stem diameter variable, there was an interaction between the salinity and tomato hybrid factors and an isolated effect for the growing environment factor ($p \leq 0.01$ and ≤ 0.05).

Table 3. Summary of analysis of variance for plant height (PH), stem diameter (SD), number of leaves (NL), and leaf area (LA) of two tomato hybrids under different growing environments and electrical conductivity of irrigation water.

Source of variation	Mean Square				
	DF	PH	SD	LN	LA
Block	4	7721.83	0.9511	0.6342	398.66
Growing environment (GE)	1	7646,40**	5,98*	4,23ns	1187,96**
Error a	8	9.42	0.71	0.98	181.73
Brackish water (BW)	4	181,87**	6.77	1,74ns	406.57
GE x BW	4	99,33*	1,08ns	4,57**	169,41 ns
Error b	32	22.12	1.15	0.85	87.08
Hybrid (H)	1	1021,20**	1,39ns	8,07*	8283.92
GE × H	1	48,38ns	1,16ns	0,47ns	1077,25*
BW × H	4	47,98ns	1,99**	2,01ns	226,95ns
GE ×BW × H	4	73,76**	0,09ns	3,30*	330,40ns
Error c	40	18.89	0.50	1.10	226.39
Total	99				
CV (a)	-	4.64	14.01	12.97	28.60
CV (b)	-	7.11	17.76	12.07	19.80
CV (c)	-	6.57	11.79	13.73	31.92

SV - Sources of variation; DF - Degree of freedom; CV - Coefficient of variation; **, * Significant at $p \leq 0.01$ and $p \leq 0.05$, respectively by F test; and ns - Not significant ($p > 0.05$).

For the data presented in Figure 3A, it can be seen that the quadratic polynomial model was the one that best adjusted the data for plant height in the protected environment, obtaining a maximum height of 82.49 cm for the Itaipava hybrid in an ECw of 2.65 dS m^{-1} and for the BS DI0014 of 73.91 cm in an ECw of 1.90 dS m^{-1}. Similarly, the model that best adjusted the data for the full sun environment (Figure 3B) was also the quadratic polynomial with a maximum plant height value of 61.81 cm for the Itaipava hybrid in an ECw of 1.93 dS m^{-1} and for the BS DI0014 of 60.22 cm in an ECw of 1.60 dS m^{-1}.

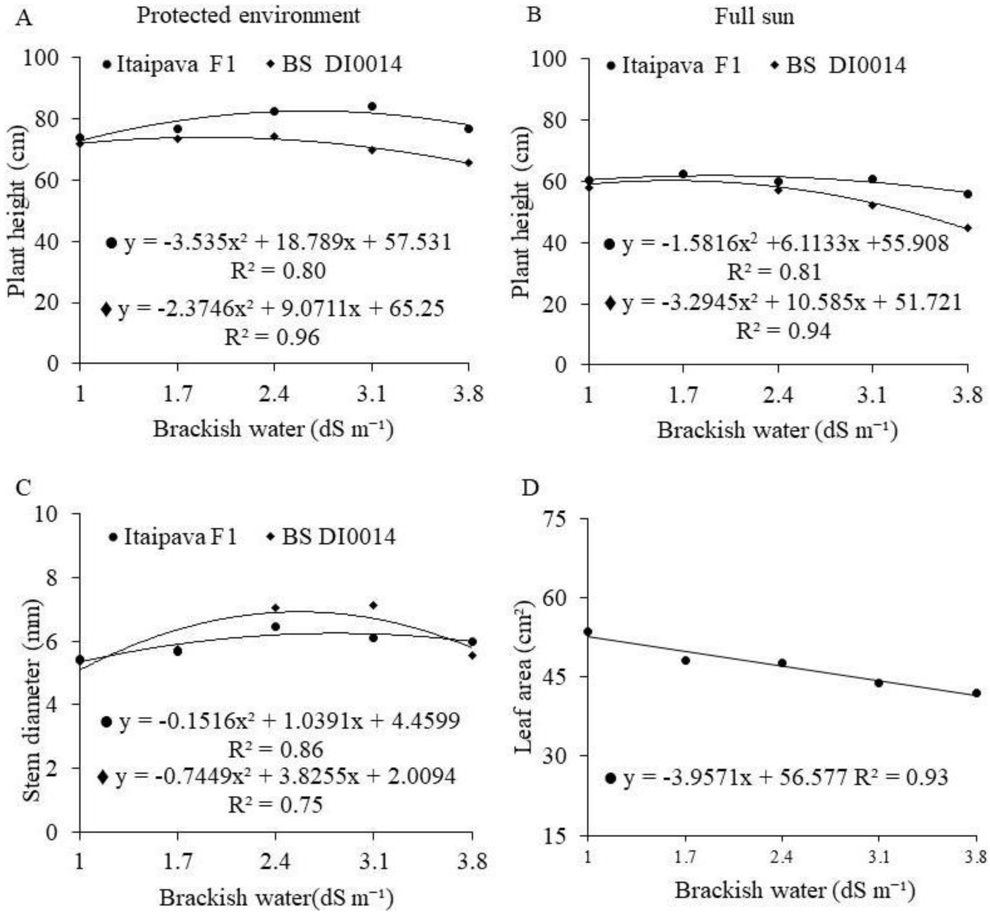

Figure 3. Plant height of tomato hybrid plants (● Itaipava F1) and (♦ BS DI0014), irrigated with brackish water in a protected environment (A) and in full sun (B), Stem diameter (C) of two tomato hybrids, and leaf area (D) irrigated with brackish water.

The results presented in Figure 3A are in agreement with the data described by Santiago *et al*. (2018). These authors report that cultivation in a protected environment reduces the incidence of radiation due to shading screens, influencing the interior radiation flow and consequently the morphophysiological dynamics of crops. Corroborating the present study, El-Mogy *et al*. (2018), observed a 12.1% increase in height of tomato plants under salt stress and grown in a protected environment.

The greater reduction in plant height under saline stress and growth in full sun (Figure 3B) may be associated with the ionic and osmotic effects triggered by salinity, which reduce cell expansion and impair photochemical processes and carbon metabolism, contributing to reduced plant growth (Zhang *et al*., 2022). Similar to the present study, Zhang *et al*. (2017) found a reduction in the height of tomato plants subjected to increasing electrical conductivity of irrigation water.

Regarding stem diameter, the quadratic polynomial model best fitted the data in the two tomato hybrids when subjected to the salinity of irrigation water (Figure 3C). The maximum diameter observed was 6.24 and 6.92 mm for the Itaipava and BS DI0014 hybrids, at electrical conductivities of 3.42 and 2.56 dS m^{-1}, respectively. According to Oliveira *et al*. (2022), the salinity causes a reduction in osmotic potential in the root zone, which limits the absorption of water and nutrients, causing a delay in crop development. Corroborating the results of this study, El-Mogy *et al*. (2018) found that increased salinity of irrigation water caused a reduction in the stem diameter of tomato plants. Souza *et al*. (2019) also found a decrease in the stem diameter of tomato plants under the effect of saline stress.

The leaf area decreased by 21.78% from the lowest to the highest electrical conductivity of irrigation water, regardless of the genotype and cultivation environment (Figure 3D). The increase in the concentration of salts in irrigation water results in an increase in osmotic pressure in the root zone, inhibiting the water absorption process, which directly interferes with cell elongation (Rodriguez-Ortega *et al*., 2019). Similar trends were observed by Huang *et al*. (2016), who found a reduction in the leaf area of tomato plants under salt stress. El-Mogy *et al*. (2018), when evaluating tomato cultivation irrigated with brackish water, concluded that increasing the concentration of salts in irrigation water reduced leaf area.

4. CONCLUSIONS

Salt stress reduced CO_2 assimilation rate and instantaneous carboxylation efficiency rates more intensely in the full sun environment, while the use of low electrical conductivity water in the protected environment promoted greater stomatal conductance in tomato hybrids.

The BS DI0014 hybrid was more tolerant to salt stress in terms of transpiration in the protected environment and in full sun than the Itaipava F1 hybrid.

The Itaipava F1 and BS DI0014 hybrids grown in a protected environment had higher internal CO2 concentrations.

5. DATA AVAILABILITY STATEMENT

Data availability not informed.

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