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Physiological responses of the beet crop under agricultural environment and saline stress

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ABSTRACT

The deleterious effects of salts on plants exposed to high solar radiation tend to be more accelerated due to the increase of toxic ions in the aerial plant part. Consequently, the physiological and biochemical processes will be affected. These effects can be minimized, however, with the use of management strategies, such as the use of a shading screen and a protected environment. In this sense, the objective of this work was to evaluate the physiological responses of sugar beet cultivated in different environments and irrigated with saline water. The experiment was conducted in an experimental design entirely randomized, using the factorial scheme 3 × 2, equivalent to three environments (FS = full sun; SSOS = shading screen open on the sides and PE = protected environment) and two electrical conductivities of the irrigation water (0.5 and 6.2 dS m^{-1}), with four repetitions. At 45 days after sowing (DAS) the following variables were analyzed: stomatal conductance, liquid photosynthesis, transpiration, internal CO_2 concentration, leaf temperature, instantaneous water use efficiency, instantaneous carboxylation efficiency, intrinsic water use efficiency, and relative chlorophyll index. Irrigation with water of higher salinity negatively affected stomatal conductance, net photosynthesis, leaf temperature and instantaneous water use efficiency of sugar beet plants grown in a full sun environment. The protected environment and open shading on the sides partially mitigated the deleterious effects of salinity.

Keywords: *Beta vulgaris* L., gas exchange, salinity levels.

Respostas fisiológicas da cultura da beterraba sob ambiência agrícola e estresse salino

RESUMO

Os efeitos deletérios dos sais nas plantas expostas a altas radiações solares tendem a ser mais acelerados em razão do aumento de íons tóxicos na parte aérea vegetal, consequentemente,

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os processos fisiológicos e bioquímicos serão afetados. No entanto, esses efeitos podem ser minimizados com a utilização de estratégias de manejo, como o uso de tela de sombreamento e ambiente protegido. Nesse sentindo, objetivou-se avaliar as repostas fisiológicas da cultura da beterraba cultivada em diferentes ambientes e irrigada com águas salinas. O experimento foi conduzido em delineamento experimental inteiramente casualizado, fazendo uso do esquema fatorial 3 × 2, equivalente a três ambientes (PS = pleno sol; TS = telado de sombreamento aberto nas laterais e AP = ambiente protegido) e duas condutividades elétricas da água de irrigação (0,5 e 6,2 dS m^{-1}), com quatro repetições. Aos 45 dias após a semeadura (DAS) foram analisadas as seguintes variáveis: condutância estomática, fotossíntese liquida, transpiração, concentração interna de CO_2, temperatura foliar, eficiência instantânea no uso da água, eficiência da carboxilação instantânea, eficiência intrínseca do uso da água e índice relativo de clorofila. A irrigação com água de maior salinidade afetou negativamente a condutância estomática, a fotossíntese liquida, a temperatura foliar, eficiência instantânea no uso da água de plantas de beterrabas cultivadas em ambiente pleno sol. O ambiente protegido e o telado aberto nas laterais atenuou parcialmente os efeitos deletérios da salinidade.

Palavras-chave: *Beta vulgaris* L., níveis de salinidade, trocas gasosas.

1. INTRODUCTION

Beet (*Beta vulgaris* L.) is a biennial vegetable, component of the Chenopodiacea family, originated in southern European and northern African countries (Filgueira, 2008). The consumption of beet roots in recent years in the world has intensified, due to the chemical properties and bioactive compounds that offer beneficial physiological effects against cardiovascular diseases, diabetes, atherosclerosis and hypertension (Clifford *et al.*, 2015).

The use of irrigated agriculture becomes fundamental for a safe production (Léllis *et al.*, 2022; Lacerda *et al.*, 2020). This practice becomes indispensable in regions that present a high climatic variability, as is the case of the semi-arid region of Brazil; however, the vast majority of water sources available for irrigation in this region present a high content of salts (Fernandes *et al.*, 2016; Pereira *et al.*, 2019; Dias *et al.*, 2020).

The use of saline water in agriculture and agricultural crops in saline soils significantly modifies the physiological and biochemical processes of plants, resulting in a reduction in the growth, development and productivity of crops (Muhammad *et al.*, 2021). The direct effects of salinity on photosynthesis start with a reduction in stomatal opening, consequently, there will be a reduction in the availability of CO_2 for RUBISCO, resulting in a low fixation of C (Batista-Santos *et al.*, 2015). The combination of all salinity-induced imbalances in photosynthetic mechanisms causes alterations in the Calvin cycle and ATP synthesis (Shahid *et al.*, 2020).

Cultivation in a protected environment mitigates seasonal variations in production, reduces the adverse effects of excess rainfall, high incidence of radiation, and extremes of air temperature (Reis *et al.*, 2012). The shading, partial or total, emerges as an alternative to minimize the damaging effects caused by salts on plants, due to the maintenance of photosynthetic mechanisms, CO_2 assimilation and increased chlorophyll content (Aras *et al.*, 2021; Gálvez *et al.* 2020). Similarly, Nojosa Lessa *et al.* (2022) on passion fruit and Pinho *et al.* (2022) in the culture of *Anadenanthera colubrina* (Vell.) verified positive effects of shading nets on seedling establishment and physiological responses of cultures, especially under salinity conditions.

Therefore, the aim of this study was to evaluate the physiological responses of sugar beet cultivated in different environments and irrigated with saline water.

2. MATERIAL AND METHODS

The experiment was conducted from October to December 2019 in the experimental area of the Auroras Seedling Production Unit (UPMA), belonging to the Universidade da Integração Internacional da Lusofonia Afro-Brasileira (UNILAB) in the municipality of Redenção, CE, Brazil, at the geographical coordinates 4º13'33" S, 38º43'39" W and altitude of 88 m. According to Köppen (1923), the climate of the region is of type Aw', described as a tropical dry winter, with an average temperature of the hottest month above 38ºC and the coldest month below 20ºC.

The experimental design was entirely randomized (DIC), using a 3 × 2 factorial scheme with four repetitions, equivalent to three growing environments (FS = full sun; SSOS = shading screen open on the sides and PE = protected environment) (Table 1) and two electrical conductivity of the irrigation water - ECw (water supply 0.5 dS m^{-1} and saline solution 6.2 dS m^{-1}). The experimental unit consisted of two pots (two plant per pot), totaling forty-eight experimental units.

Table 1. Mean values of temperature and relative humidity of the environments (full sun, open shading screen on the sides and protected environment) during the experiment.

Environments	Relative humidity (%)		Temperature	
	Min	Max	Min	Max
Full sun	45.9	58	32.6	33.7
Open screen on the sides	45.7	52	33.6	34.5
Protected environment	50	57	30.5	39.2

Max - Maximum; Min – Minimum.

Beet seeds (cultivar Early Wonder Tall Top) were used and five seeds were sown 2 cm deep in 8 L pots. To fill the pots, a layer of gravel 2 to 3 mm thick was used to help drainage, then, to supplement the remaining volume, a substrate was used obtained from the mixture of arisco, sand and bovine manure in the proportion of 5:3:2, respectively. To determine the chemical attributes of the substrate, a soil sample was collected before the application of the treatments (Table 2).

At 20 DAS, thinning was performed, leaving only two plants per pot. The saline solution used for irrigation was formulated from the dilution of soluble salts (NaCl, $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$), in the equivalent proportion of 7: 2: 1 between Na, Ca and Mg, obeying the relationship between ECw and its concentration (mmolc L^{-1} = EC × 10), following the methodology contained in Medeiros (1992).

Irrigation with saline water, started at 10 days after sowing and was performed manually each day with leaching blade of 15% according to Ayers and Westcot (1999), calculated according to the drainage lysimeter principle (Bernardo *et al.*, 2019), keeping the soil at field capacity. The volume of water to be applied to the plants was determined by Equation 1:

$$VI = \frac{(Vp - Vd)}{(1 - LF)} \quad (1)$$

Where:

VI – Volume of water to be applied in the irrigation event (mL);

Vp – volume of water applied in the previous irrigation event (mL);

Vd – Volume of water drained (mL); and,

LF – leaching fraction of 0.15.

Table 2. Chemical and physical characteristics of the substrate sample before applying treatments.

OM	N	C	P	Ca	Mg	Na	Al	H + Al	K	Ecse	ESP	C/N	V	pH
($g\ Kg^{-1}$)			($mg\ Kg^{-1}$)	($cmol_c dm^{-3}$)						($dS\ m^{-1}$)	(%)		(%)	H_2O
14.59	0.93	8.46	27	4.5	0.7	0.67	0.15	1.49	0.78	0.08	8	9	82	6.4

SD ($g\ cm^{-3}$)		CS	FS	Silt	Clay	Textural classification
Bulk	Particle	($g\ kg^{-1}$)				
1.47	2.76	665	201	92	42	Loamy Sand

OM - Organic matter; ESP - Percentage of exchangeable sodium; ECse – Electrical conductivity of the soil saturation extract; V - Base saturation; SD - Soil density; CS Coarse sand; FS - Fine sand.

At 45 DAS, readings of gas exchange and relative chlorophyll index were taken. The measurements took place between 09h00 and 11h00 a.m., only on one plant and on the fully expanded leaves, and the following variables were determined: stomatal conductance (gs) net photosynthesis (A), transpiration (E), internal CO_2 concentration (Ci), leaf temperature (LT), using an infrared gas (IRGA, LI-6400XT, LI-COR, Inc., Lincoln, Nebraska, USA) equipped with a radiation source regulated at 2000 µmol m^{-2} s^{-1}. Using the gas exchange data, instantaneous water use efficiency (A/E), instantaneous carboxylation efficiency (A/Ci) and intrinsic water-use efficiency (A/gs) were determined.

The relative chlorophyll content was measured by the non-destructive method in five readings per leaf, with the aid of a chlorophyll meter (SPAD 502, Minolta Co. Ltd, Osaka, Japan), occurring in the same leaves used to quantify leaf gas exchange. Results were expressed as a mean value referred to as the relative chlorophyll index (RCI).

The variables analyzed in the study were submitted to the Kolmogorov-Smirnov test ($p \leq 0.05$) to assess normality. The data were submitted to analysis of variance (ANOVA). In the occurrence of significance for the interaction between environments versus salinity or single factors, Tukey's test ($p \leq 0.05$) was performed using the program ASSISTAT 7.7 BETA (Silva and Azevedo, 2016).

3. RESULTS AND DISCUSSION

According to the analysis of variance (Table 3), there were significant interactions between the different growing environments and salinity levels for all the variables analyzed, except for the transpiration and intrinsic water use efficiency variable.

Table 3. Summary of analysis of variance for stomatal conductance (gs), net photosynthesis (A), transpiration (E), internal CO_2 concentration (Ci), leaf temperature (LT), instantaneous water use efficiency (A/E), efficiency of instantaneous carboxylation (A/Ci), intrinsic water use efficiency (A/gs) and relative chlorophyll index (RCI) in sugar beet plants cultivated in different environments irrigated with saline waters.

Sources of variation	Mean Square									
	DF	gs	A	E	Ci	LT	A/E	A/Ci	A/gs	RCI
Treatments	5	0.032^{**}	67.30^{**}	0.117^{ns}	1268.85^{*}	3.756^{**}	2.672^{**}	0.001^{**}	90.87^{ns}	113.52^{**}
Environments (E)	2	0.056^{**}	139.33^{**}	0.008^{ns}	1269.38^{**}	6.402^{**}	5.403^{**}	0.002^{**}	49.01^{ns}	154.96^{**}
Salinity (S)	1	0.032^{**}	3.68^{ns}	0.039^{*}	2664.50^{**}	1.125^{*}	0.014^{ns}	0.000^{ns}	270.75^{*}	104.01^{*}
E x S	2	0.009^{*}	27.09^{**}	0.089^{ns}	570.50^{*}	2.426^{**}	1.271^{**}	0.000^{**}	42.79^{ns}	4.05^{*}
Residue	18	0.002	2.23	0.06	131.44	0.16	0.07	0.00005	55.3	18.94
Total	23	-	-	-	-	-	-	-	-	-
CV (%)	-	12.75	11.01	4.89	4.03	1.17	10.19	14.72	19.11	10.09

DF - Degrees of freedom; CV - Coefficient of variation; * - Significant by the F test at $p \leq 0.05$; ** Significant by the F test at $p \leq 0.01$; ns - Not significant.

According to Figure 1A, it is possible to observe that gs was statistically superior when irrigated with water of lower salinity (0.5 dS m^{-1}) in the PE environment followed by FS, but did not differ statistically in SSOS. Regarding plants irrigated with high salinity water (6.2 dS m^{-1}), it was found that plants grown in PE and SSOS were statistically different from plants in FS, demonstrating a reduction in stomatal opening.

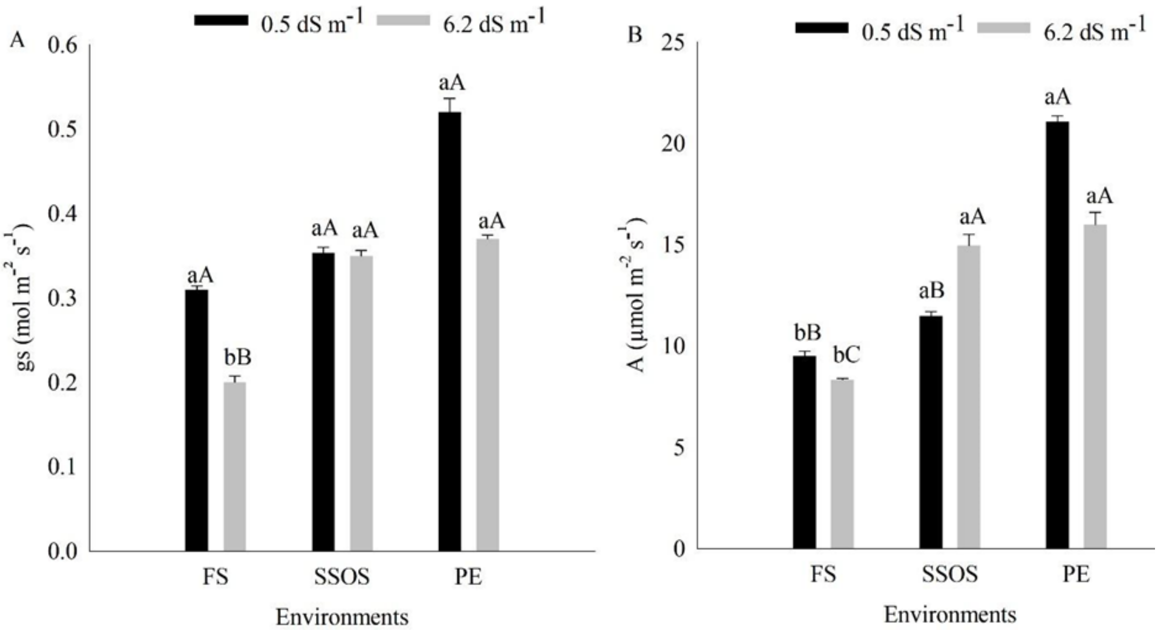

Figure 1. Gs: Stomatal conductance (A) and A: Net photosynthesis (B) in sugar beet plants grown in different environments (FS - Full sun; SSOS - Shading screen open on the sides and PE - Protected environment) irrigated with saline water. Means followed by the same lowercase letters for salinity levels or uppercase letters in the same environment do not differ significantly by Tukey test ($p \leq 0.05$). Vertical bars represent standard error (n = 4).

In an attempt to regulate or reduce the loss of water to the atmosphere due to saline and thermal stress, plants reduce the opening of the stomata, through hormones generated by branches and roots, limiting the net photosynthetic rate and the influx of CO_2 necessary for the assimilation process (Taiz *et al.*, 2017; Pereira *et al.*, 2019; Muhammad *et al.*, 2021). Thus, the decrease in stomatal conductance in the full sun environment in water of higher Ecw is possibly related to the sensitivity of the stomata of C3 plants, such as beetroot, to the increase in the vapor pressure deficit (VPD) (Pinho *et al.*, 2022); and the high concentration of salts in the irrigation water, making it difficult to supply CO_2 to rubisco, consequently, the photosynthetic machinery will increase energy dispersion (Chaves *et al.*, 2009).

Nojosa Lessa *et al.* (2022), state that plants exposed to sunlight under salt stress show higher rates of uptake and translocation of potentially toxic ions when compared to shaded plants. In a study evaluating the responses of sweet cherry to salt stress under different shading, Aras *et al.* (2021), found that the stomatal conductance of the plants reduced when grown under salt stress in a non-shaded environment.

Net photosynthesis of sugar beet was statistically reduced by the higher salinity water in the FS, but there was an increase in the SSOS and PE (Figure 1B). In the full sun environment, the plants, when irrigated with high salinity water (6.2 dS m^{-1}), showed a reduction in the net photosynthetic rate of 48% when compared to plants cultivated in PE and 44% when compared to cultivated plants.

The increase in net photosynthesis with the increase in salinity in the protected environment and open shading on the sides demonstrates that it possibly promoted a physiological acclimation, partially alleviating thermal stress and excess light, (Mupambi *et al.*,

2018) and salinity (decreasing the translocation of toxic ions to leaves and favoring CO_2 assimilation in the biochemical phase, even under stress) (Dias *et al.*, 2018; Shahid *et al.*, 2020).

Similar results were found by Gálvez *et al.* (2020), in peppers shaded with red screens and not shaded under salt stress, where they found an increase in net photosynthetic rate under salinity conditions in shaded pepper plants.

The transpiration of sugar beet plants (Figure 2) was negatively influenced by increasing the electrical conductivity of irrigation water (6.2 dS m^{-1}), causing a drop in transpiration equivalent to 5.62%, when compared to low salinity water (0.5 dS m^{-1}). The increase in the concentration of salts in the soil caused a decrease in osmotic potential, driving plants to use a strategy to decrease water loss to the environment, since the transpiration rate is higher than water uptake by plants (Silva *et al.*, 2019). Linked to the strategy of reducing water loss to the environment, the reduction of transpiration in saline environments decreases the absorption of salts; consequently, the translocation to part of the area occurs (Pinho *et al.*, 2022).

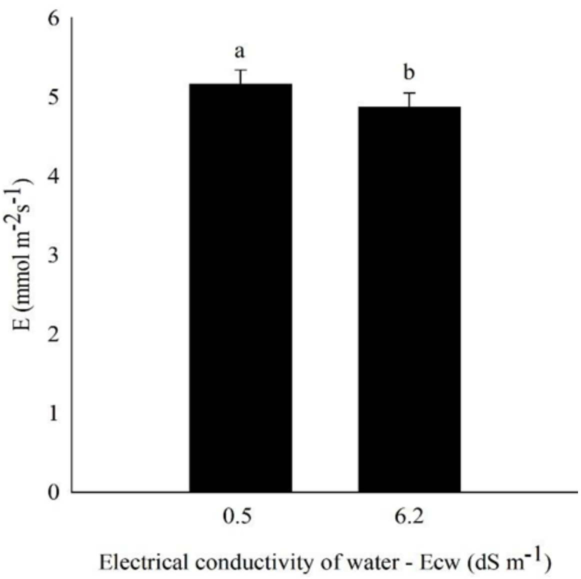

Figure 2. Transpiration (E) in sugar beet plants irrigated with saline water. Lowercase letters compare the means by Tukey test ($p \leq 0.05$). Vertical bars represent standard error (n = 4).

In studies with sugar beet, Tahjib-UI-Arif *et al.* (2019) and Zou *et al.* (2019) found that under high salt stress conditions there was a decrease in plant transpiration. However, contrary results to the studies were observed by Melo Filho *et al.* (2020), who, working with table beet, observed that stomatal conductance had an average increase at 30 DAI, keeping transpiration unchanged, with a decrease occurring in both variables only at 60 DAI.

Regarding the internal concentration of CO_2 (Figure 3A), the lower salinity water was statistically superior in full sun and shade cloth open on the sides compared to the higher salinity water and was not statistically different from plants irrigated with high salinity water in PE. This increase in Ci, demonstrates that CO_2 accumulation supposedly occurred in the leaf mesophyll in these environments; however, it happened as a result of microclimate and biochemical effects, generating damage to the carbon metabolism (Neves *et al.*, 2019; Dias *et al.*, 2020).

Regni *et al.* (2019), describe that plants under stress present a decrease in stomatal conductance, due to an increase in internal CO_2 concentration and a decrease in transpiration. Results contrary to the present study were observed by Sales *et al.* (2021), who verified that the increase in irrigation water salinity reduced the internal CO_2 concentration in okra culture.

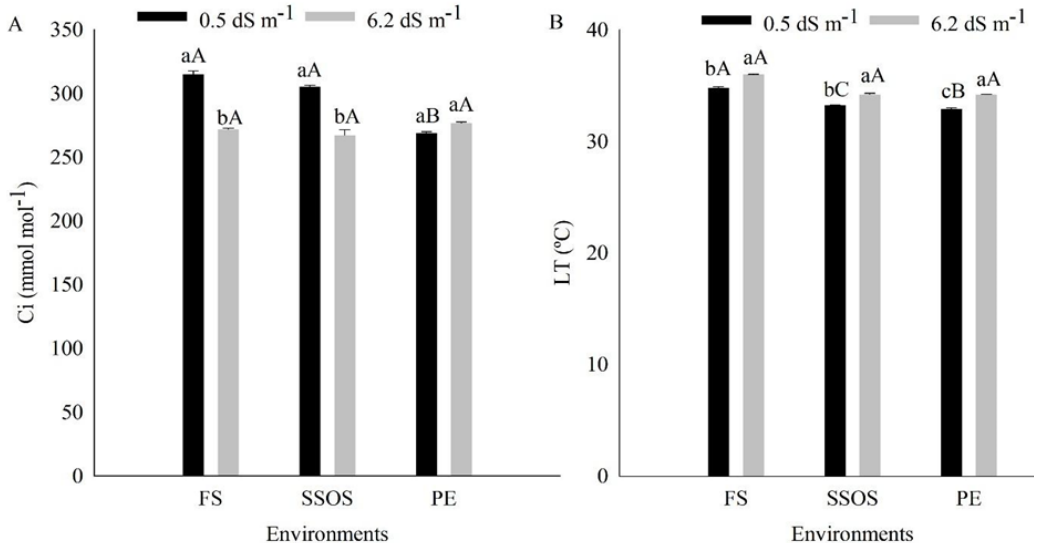

Figure 3. Ci: Internal concentration of CO_2 (A) and LT: Leaf temperature (B) in sugar beet plants grown in different environments (FS - Full sun; SSOS - Shading screen open on the sides and PE - Protected environment) irrigated with saline water. Means followed by the same lowercase letters for salinity levels or uppercase letters in the same environment do not differ significantly by Tukey test ($p \leq 0.05$). Vertical bars represent standard error (n = 4).

When analyzing the average comparison test (Figure 3B) for the interaction between factors in leaf temperature, it was verified that the beet plants irrigated with water with a lower saline level (0.5 dS m^{-1}) differed statistically between the environments, presenting lower leaf temperatures (34.83; 33.23 and 32.90ºC, respectively). On the other hand, the increase in the electrical conductivity of the irrigation water increased the leaf temperature in the different types of environments, showing higher temperature values (36.00; 34.20 and 34.20ºC, respectively) and not differing statistically between the environments.

The combined effect of the high concentration of salts and the direct radiation in the full sun environment caused an increase in stomatal resistance, hindering the main method responsible for adjusting leaf temperature, transpiration (Taiz *et al.*, 2017). Excess latent heat in leaves of C3 plants, as is the case of sugar beet, causes deleterious effects on photosynthesis. Consequently, there is a lower activation in rubisco (Deva *et al.*, 2020).

Costa Freire *et al.* (2021) studied the gas exchange of bean varieties under irrigation water salinity conditions in a protected environment and found that all bean varieties showed a linear increase in leaf temperature with an increase in irrigation water salinity.

For the lowest salinity level on instantaneous water use efficiency (A/E), significant differences were found between environments, where the highest instantaneous water use efficiency was observed in plants grown in protected environment (4.16 ((μmol m^{-2} s^{-1}) (mol H_2O m^{-2} s^{-1}))). In contrast, the increase in irrigation water salinity in plants grown in the protected environment and open shading screen on the sides promoted an increase in A/E (3.19 and 3.10 ((μmol m^{-2} s^{-1}) (mol H_2O m^{-2} s-^{1}), respectively)) when compared to the full sun environment irrigated with low salinity water and not statistically different from the protected environment (Figure 4A).

The increase in A/E in water use in plants irrigated with low salinity water in the protected environment is possibly related to quantitative effects of water in the leaf and qualitative effects on osmotic potential, because the shaded plants are not directly exposed to the external environment (Gálvez *et al.*, 2020). Furthermore, Fernandes *et al.* (2016) describe that in saline environments, the A/E increase is a strategy of moderately tolerant crops, which aims to decrease high salt concentrations in the plant area.

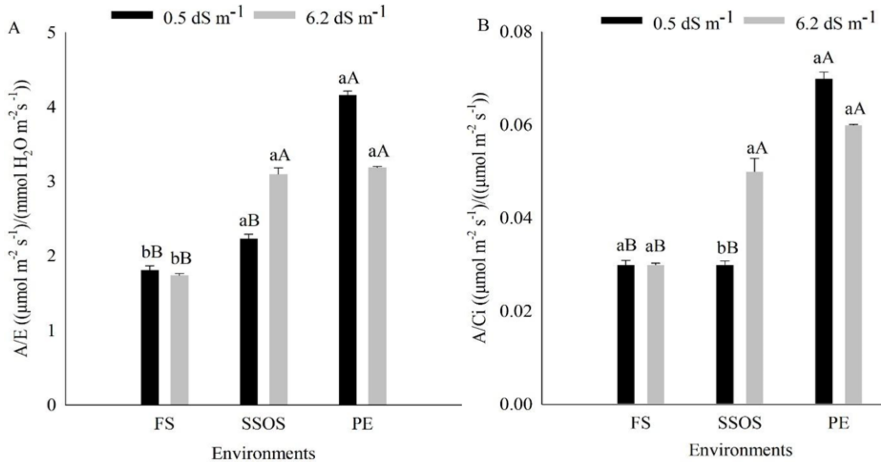

Figure 4. A/E: Instantaneous water use efficiency (A) and A/Ci: Instantaneous carboxylation efficiency (B) in sugar beet plants grown in different environments (FS - Full sun; SSOS - Shading screen open on the sides and PE - Protected environment) irrigated with saline water. Means followed by the same lowercase letters for salinity levels or uppercase letters in the same environment do not differ significantly by Tukey test ($p \leq 0.05$). Vertical bars represent standard error (n = 4).

Similar to the results obtained in the present study, Nojosa Lessa *et al.* (2022) analyzed the production of yellow passion fruit seedlings under saline stress and agricultural environment, and found that A/E was superior when irrigated with low salinity water in certain types of environments.

Figure 4B shows the values of instantaneous carboxylation efficiency (A/Ci) under the salinity levels and different environments. It was found that for lower salinity water the environments differed statistically from each other, with an increase in A/Ci in the protected environment of 160.00 and 110.81% compared to full sun and shade screen open on the sides, respectively. In water with a higher salinity, it was found that the environments differed statistically, where the plants grown in full sun showed a reduction of 50% when compared with the protected environment.

Under salinity conditions, the instantaneous carboxylation efficiency is influenced by the increase in internal CO_2 concentration, due to the decrease in photosynthetic rate (Dias *et al.*, 2018). Opposite results to the present study were found by Lacerda *et al.* (2020) when analyzing the morphophysiological responses and salt tolerance mechanisms in four perennial ornamental species grown in a greenhouse, found a decrease in A/Ci with increasing electrical conductivity of irrigation water.

The intrinsic water-use efficiency (A/gs) (Figure 5) increased significantly by 22.15% when irrigated with high salinity water, differing statistically from plants irrigated with low salinity water. This result, corroborates with Oliveira *et al.* (2017), when they detected that high intrinsic water-use efficiency in plants under salt stress, is a mechanism arising from a greater reduction in stomatal conductance when compared to net photosynthesis.

Similarly, Moles *et al.* (2016) also found an increase in intrinsic water-use efficiency in tomato varieties subjected to salt stress. Boari *et al.* (2019) observed that saline stress increased osmotic effects and caused a reduction in xylem potential in the toment culture, consequently increasing the intrinsic efficiency of water use.

The chlorophyll index of beet culture was influenced by the interaction between the salinity levels of irrigation water and the different environments (Figure 6), with significant differences between the protected environment and shading screen open on the sides to the full sun environment in low salinity water. On the other hand, when the plants were irrigated with high salinity water, it was observed that there were no statistical differences between the environments and an increase of 19.06, 6.47 and 27.68% was observed when compared to the plants grown in the three types of environments irrigated with 0.5 dS m^{-1} water.

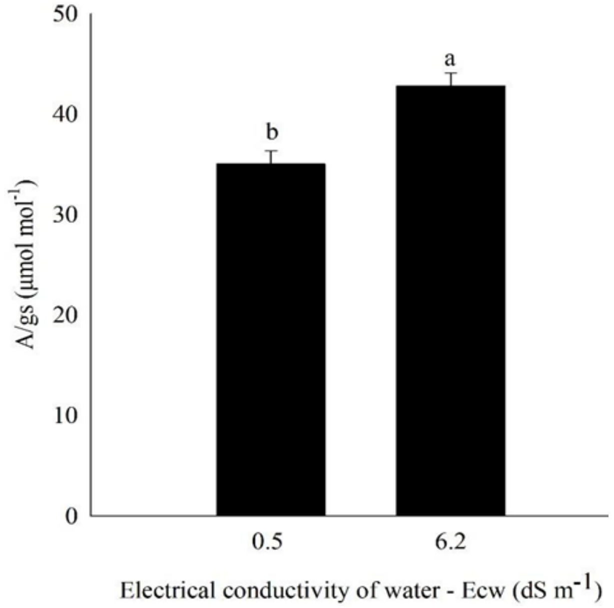

Figure 5. Intrinsic water use efficiency (A/gs) in sugar beet plants irrigated with saline water. Lowercase letters compare the means by Tukey test ($p \leq 0.05$). Vertical bars represent standard error (n = 4).

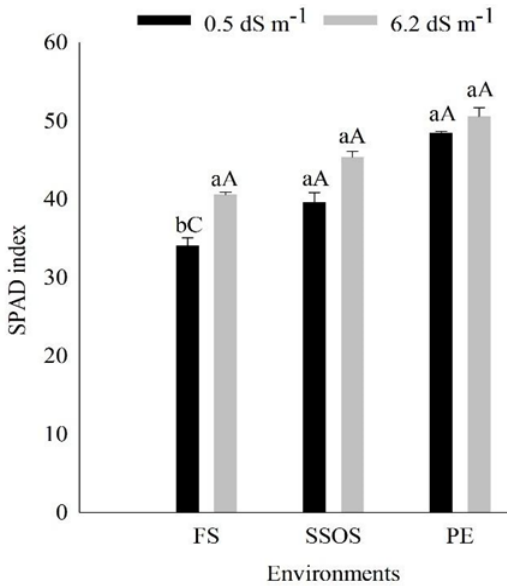

Figure 6. Relative chlorophyll index (RCI) in sugar beet plants grown in different environments (FS - Full sun; SSOS - Shading screen open on the sides and PE - Protected environment) irrigated with saline water. Means followed by the same lowercase letters for salinity levels or uppercase letters in the same environment do not differ significantly by Tukey test ($p \leq 0.05$). Vertical bars represent standard error (n = 4).

The increase in the relative chlorophyll index in beet plants irrigated with high salinity water is possibly related to a process of acclimation to salt stress and ambience by the crop, in order to ensure photosynthetic rates according to physiological needs and growth (Adhikari *et al.*, 2019). Furthermore, Fan *et al.* (2019) described that shading plants contain more chlorophyll for efficient light capture. Similar results to the present study were observed by Aras *et al.* (2021) in the sweet cherry tomato crop grown in a protected environment and under salt stress.

4. CONCLUSIONS

Irrigation with water of higher salinity negatively affected stomatal conductance, net photosynthesis, leaf temperature and instantaneous water use efficiency of sugar beet plants grown in full sun.

The protected environment and open side-shade partially attenuated the deleterious effects of salinity on stomatal conductance, net photosynthesis and the relative chlorophyll index.

Salt stress negatively affects the transpiration of beet plants, but increases the intrinsic water use efficiency and the relative chlorophyll index in the growing environments.

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