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Effects of saline and water stress on sweet sorghum

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Abstract
Sweet sorghum (*Sorghum bicolor* [L.] Moench) is a plant that can be an alternative for the production of bioethanol in semi-arid regions. The objective of this work was to evaluate sweet sorghum 'BRS 506' under salt and water stress. The experimental design was in randomized blocks, in a factorial scheme (4x4), with the first factor referring to the electrical conductivities of the irrigation water (1.5; 3.0; 4.5; and 6.0 dS m^{-1}) and the second refers to irrigation depths (53, 67, 85 and 95% of crop evapotranspiration). Gas exchange, leaf water status, leaf sugars and plant growth were evaluated. Salt and water stress cause negative effects on the growth of sweet sorghum 'BRS 506'. Salt stress causes disturbances in gas exchange and sugar levels. Sweet sorghum 'BRS 506' is tolerant to combined salt and water stress.

Keywords: *Sorghum bicolor*; water deficit; salinity; gas exchange.

Efectos del estrés salino e hídrico en el sorgo dulce

Resumen
El sorgo dulce (*Sorghum bicolor* [L.] Moench) es una planta que puede ser una alternativa para la producción de bioetanol en regiones semiáridas. El objetivo de este trabajo fue evaluar el sorgo dulce 'BRS 506' bajo estrés hídrico y salino. El diseño experimental fue en bloques al azar, en esquema factorial (4x4), siendo el primer factor referido a las conductividades eléctricas del agua de riego (1.5; 3.0; 4.5; y 6.0 dS m^{-1}) y el segundo a las aspas de riego (53, 67, 85 y 95% de la evapotranspiración del cultivo). Se evaluaron el intercambio de gases, el estado hídrico de las hojas, los azúcares de las hojas y el crecimiento de las plantas. El estrés salino e hídrico provoca efectos negativos en el crecimiento del sorgo dulce 'BRS 506'. El estrés salino provoca alteraciones en el intercambio de gases y en los niveles de azúcar. El sorgo dulce 'BRS 506' es tolerante al estrés salino y hídrico conjunto.

Palabras clave: *Sorghum bicolor*; déficit de agua; salinidad; intercambio de gases.

1 Introduction

Sweet sorghum (*Sorghum bicolor* [L.] Moench) is a C4 plant widely cultivated in the world, having high concentrations of soluble sugars (sucrose, glucose and fructose) contained in its stems [1]. Most of the worlds ethanol production is extracted from corn and sugarcane [2]. However, sweet sorghum presents itself as an alternative for the production of bioethanol of plant origin from its sugary stem [3,4]. Since it has greater drought tolerance and requires less water compared to the other two crops [5].

Sorghum is generally cultivated in arid and semi-arid regions, where there are often limiting factors for the development of agriculture, such as irregular rainfall and water with inferior qualities [6,7]. However, it is a plant adapted to these regions and produces even under adverse conditions of low water availability and brackish water [8].

Salt and water stress cause a variety of problems in sorghum plants, which can unbalance cellular homeostasis, leading to morphological, physiological and molecular changes [9,10]. These changes have a negative impact on plant growth and development, with a decrease in production [11,12].

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Some studies were carried out to evaluate the physiology and growth of sorghum under saline stress conditions. The authors reported that the variables of: membrane damage [13], relative water content [14-16], gas exchange [11,17] and growth [15] are negatively affected.

On the other hand, other studies addressed the effects of water stress in sorghum, where the results showed that membrane damage, relative water content, sugars [12], gas exchange and growth [18] suffered reductions as water stress was high. However, plants develop some protective mechanisms to reduce the harmful effects of stress [10,13], such as the accumulation of sugars in response to salt and water stress [11,19].

Increasingly, water resources, whether by quality or quantity, are becoming limited for agriculture, and it is essential to understand plant responses to saline and water stress, in order to develop alternatives for the production of agricultural crops in adverse conditions [20,21]. Increasing tolerance to abiotic stresses (saline and water) in sorghum is of great importance, and it is essential to obtain information on how stresses influence physiological and growth processes in sorghum plants [10,19].

In general, the studies have focused on the development of research on salt and water stress in isolation and not jointly. Thus, the objective of this work was to evaluate the variables of leaf water status, sugars, gas exchange and growth in sweet sorghum (*Sorghum bicolor* [L.] Moench) 'BRS 506' under salt and water stress.

2 Material and methods

2.1 Experimental area

The experiment was carried out between September and December 2020, in the experimental area of the Cumaru farm (5°33'30" S, 37°11'56" W), located in the rural area in Upanema, Rio Grande do Norte, Brazil (Fig. 1).

According to the Köppen classification, the climate in the region is BSh – hot semi-arid with autumn rains and monthly average air temperature consistently above 18 °C [22]. During the experimental test, the temperature and relative humidity averaged around 28 ºC and 60%, respectively (Fig. 2).

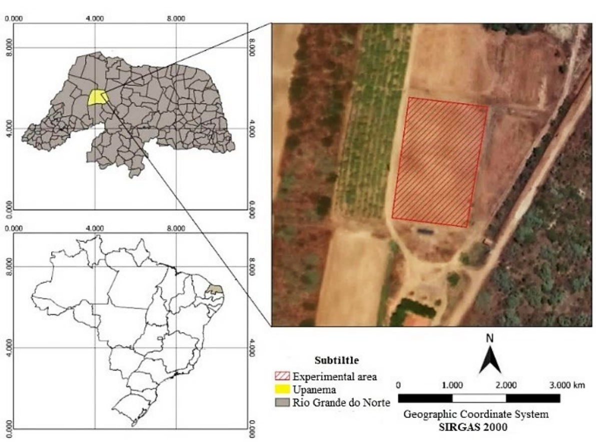


Figure 1. Location of the experimental area at the Cumaru farm, Upanema, Rio Grande do Norte, Brazil.
Source: The authors.

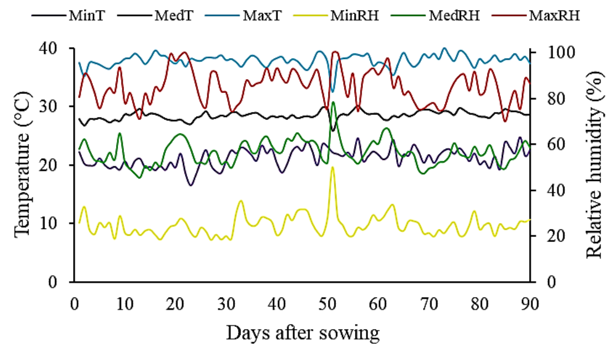


Figure 2. Climatic conditions of temperature and relative humidity during the experimental test period.
Source: The authors.

The soil of the experimental area is classified as Cambisol [23], before the installation of the experiment, composite soil samples were taken, where physical and chemical soil analyzes were carried out. For the 0-20 cm layer, the following physical characteristics were presented: sand = 71.77%; silt = 6.54%; clay = 21.69%. While for the 20-40 cm layer, the results were: sand = 61.67%; silt = 4.95%; clay = 33.38%. For the 0-20 cm layer, the following results were obtained for the chemical characteristics: pH = 7.56; ECs = 0.57 dS m^{-1}; organic matter = 1.87%; P = 0.05 $cmol_c\ dm^{-3}$; K^+ = 0.20 $cmol_c\ dm^{-3}$; Na^+ = 1.11 $cmol_c\ dm^{-3}$; Ca^{2+} = 7.00 $cmol_c\ dm^{-3}$; Mg^{2+} = 1.91 $cmol_c\ dm^{-3}$; and SB = 10.22 $cmol_c\ dm^{-3}$. For layer 20-40 cm the chemical characteristics obtained were: pH = 7.28; ECs = 0.36 dS m^{-1}; organic matter = 1.40%; P = 0.03 $cmol_c\ dm^{-3}$; K^+ = 0.18 $cmol_c\ dm^{-3}$; Na^+ = 0.95 $cmol_c\ dm^{-3}$; Ca^{2+} = 5.96 $cmol_c\ dm^{-3}$; Mg^{2+} = 1.77 $cmol_c\ dm^{-3}$; and SB = 8.86 $cmol_c\ dm^{-3}$.

2.2 Plant material and soil preparation

The sorghum cultivar [*Sorghum bicolor* (L.) Moench] used in the experiment was the saccharin cultivar 'BRS 506'. This cultivar can be used to complement the production of ethanol, having as characteristics succulent stems and a high concentration of sugars, with emphasis on its short cycle (90 to 130 days), with wide edaphoclimatic adaptation and high efficiency in water use [3,24].

Before the installation of the experiment, the soil was prepared, consisting of plowing and harrowing and a foundation fertilizer with 104 kg ha^{-1} of P_2O_5 and 22 kg ha^{-1} of N, using MAP as a source. In addition, cover fertilization was carried out via fertirrigation, applying 30 kg ha^{-1} of K_2O, using potassium chloride as source and 40 kg ha^{-1} of N, using urea as source.

2.3 Salt stress management

The application of saline stresses started 21 days after sowing. The water used in irrigation management was prepared as follows: the lowest concentration (1.5 dS m^{-1}) came from a tubular well that supplies the Cumaru farm, originating from the calciferous sandstone aquifer. The waters with the other electrical conductivities (3.0; 4.5 and

Table 1.
Chemical characteristics of the water used in the irrigation of the experiment.

EC	Na^+	Ca^{2+}	Mg^{2+}	K^+	Cl^-	SO_4^{2-}	HCO_3^-
dS m^{-1}				$mmol_c$			
1,5	5,0	8,0	2,0	0,12	8,1	0,3	7,0
3,0	19,0	8,0	3,0	0,12	22,1	1,3	6,9
4,5	28,5	12,0	4,5	0,12	35,6	2,8	6,9
6,0	38,0	16,0	6,0	0,12	49,1	4,3	6,8

EC: electrical conductivity of irrigation water; Na^+: sodium; Ca^{2+}: calcium; Mg^{2+}: magnesium; K^+: potassium; Cl^-: chlorine; SO_4^{2-}: sulfates; HCO_3^-: bicarbonates.
Source: The authors.

6.0 dS m^{-1}) were prepared from the addition of NaCl, $CaCl.2H_2O$ and $MgSO_4.7H_2O$ salts [25]. The water with the highest concentration was based on the salinity tolerance of the sorghum crop for a potential yield of 50% [26], while the other waters had intermediate electrical conductivities (Table 1).

2.4 Water stress management

The application of the different irrigation depths started at 7 days after sowing (DAS). The irrigation depths were applied based on the maximum evapotranspiration of the sorghum crop (ETc), adjusting the conditions of the experiment and the irrigation system (53, 67, 85 and 95% of the total estimated ETc for the crop cycle). ETc was estimated daily from the estimate of daily reference evapotranspiration (ETo), by the Penman-Monteith method according to [27]. ETo was estimated daily from data collected at a meteorological station installed in the experiment area. Whereas, the daily crop coefficient (Kc) was determined by the dual Kc method, assuming the length of phenological phases I, II, III and IV to be 28, 32, 20 and 10 days, respectively, and the crop coefficients baseline for phase III and end of the cycle, 1.00 and 0.70.

2.5 Experimental design

The experimental design was in randomized blocks (DBC), in a factorial scheme (4x4), with the first factor referring to the electrical conductivities of the irrigation water (ECw - 1.5; 3.0; 4.5; and 6.0 dS m^{-1}), and the second factor refers to the irrigation depths (ETc - 53, 67, 85 and 95%). Using two blocks with two replications within the blocks, totaling 16 treatments and 64 experimental plots.

2.6 Variables analyzed

2.6.1 Gas exchange

Leaf gas exchange was measured by a portable infrared gas analyzer (IRGA, GFS-300, WALZ, Germany). Measurements were made between 08:00 and 10:00 am, in two plants per plot on the flag leaf. The IRGA settings were defined as follows: temperature of 25 ºC; relative humidity of 60%; flow rate of 750 µmol/min; light intensity of 1200 µmol m^{-2} s^{-1}; impeller speed of 7; CO_2 concentration of 400 ppm and reading area of 8 cm^2.

The gas exchange variables were evaluated at 62 DAS. Leaf temperature (LT - ºC), vapor pressure deficit (VPD - kPa), stomatal conductance (*gs* - mmol H_2O m^{-2} s^{-1}), transpiration rate (*E* - mmol H_2O m^{-2} s^{-1}), CO_2 assimilation rate (*A* - µmol CO_2 m^{-2} s^{-1}), water use efficiency (WUE - *A*/*E*) and intrinsic water-use efficiency (WUEi - *A*/*gs*) were measured.

2.6.2 Leaf water status

At 59 days after sowing (DAS), the end of phenological phase II, beginning of flowering, the leaf water status variables were evaluated: membrane damage (MD) and relative water content (RWC). MD was determined by the electrolyte extravasation method, according to the methodology proposed by [28].

Where:

$$MD = \frac{C1}{C2} \times 100 \quad (1)$$

MD: membrane damage, %;
C1: initial electrical conductivity, dS m^{-1};
C2: final electrical conductivity, dS m^{-1}.

While, RWC was evaluated through leaf discs, by the equation proposed by [29]. Where:

$$RWC = \frac{FM - DS}{TM - DS} \times 100 \quad (2)$$

RWC: relative water content, %;
FM: fresh mass, g;
DS: dry mass, g;
TM: turgid mass, g.

2.6.3 Sugars

From leaf samples collected at 69 DAS, measurements of total sugars, reducing sugars, non-reducing sugars and starch were made. The samples were placed in paper bags and placed to dry in an oven with forced air circulation at 65 ºC for 72 hours, and subsequently ground in a Willye-type mill.

Total sugars (TS): were determined by the Antrona method, according to the methodology proposed by [30].

Reducing sugars (RS): determined by the Somogyi-Nelson method [31].

Non-reducing sugars (NRS): obtained through the equation proposed by [32]. On what:

$$NRS = (TS - RS) \times 0{,}95 \quad (3)$$

Where:
NRS: non-reducing sugars, %;
TS: total sugars, %;
RS: reducing sugars, %.

Starch (ST): determined by the Somoghy-Nelson method [31], with values expressed in mg/g.

2.6.4 Plant growth

At 73 DAS, the following variables were evaluated: plant height (cm): measuring from the base of the plant to the apex of the leaf meristem; and stem height (cm): measuring the entire length of the stem up to the last leaf insertion.

The leaf, stem, root and total dry mass (g) were evaluated at 69 DAS, where the parts of the plants were separated and then placed in paper bags and placed to dry in an oven with circulation of forced air temperature for 72 hours at 65 °C.

2.7 *Statistical analysis*

Data were submitted to analysis of variance by F test at a 5% significance level, in case of significant effects, regression analysis was performed using the R® software version 4.1.3 [33], and the graphics produced with SigmaPlot® software version 12.3 [34].

3 Results

According to the variance analysis (Table 2), for the leaf gas exchange variables, there were isolated effects of the data for the different electrical conductivities of the irrigation water (ECw) in the variables leaf temperature, water use efficiency and intrinsic water-use efficiency.

For leaf temperature (Fig. 3A), the highest values were observed in the lowest ECa of 1.5 dS m^{-1}, while the highest value was obtained in the treatment containing ECa of 3.0 dS m^{-1}, however there was no trend defined, although there was a statistical difference between the means.

Similar effects occurred for WUE and WUEi gas exchange variables, subjected to different ECws, with the highest values (23.96 and 0.41 µmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}) being obtained in ECw of 4.5 dS m^{-1}, while the lowest values (20.16 and 0.34 µmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}) were observed at an intermediate ECw of 3.0 dS m^{-1}, for WUE and WUEi, respectively. However, the values obtained did not fit the linear or quadratic model, despite the significant effects obtained (Figs. 3B and 3C).

According to the analysis of variance (Table 3), there was a significant isolated effect only for the variable of total sugars when subjected to different electrical conductivities of irrigation water (ECw).

Table 2.
Analysis of variance summary (ANAVA) for leaf temperature (LF), vapor pressure deficit (VPD), stomatal conductance (*gs*), transpiration rate (*E*), CO_2 assimilation rate (*A*), water use efficiency (WUE) and intrinsic water-use efficiency (WUEi) as a function of electrical conductivities of irrigation water (ECw) and irrigation depths (ETc) in sweet sorghum (*Sorghum bicolor* L.) 'BRS 506'.

		Variables						
SV	DF	LF	VPD	*gs*	*E*	*A*	WUE	WUEi
		F Statistic						
ECw	3	$3,91^{*}$	$2,10^{ns}$	$1,90^{ns}$	$2,60^{ns}$	$1,41^{ns}$	$3,82^{*}$	$2,92^{*}$
ETc	3	$1,42^{ns}$	$0,16^{ns}$	$0,03^{ns}$	$1,35^{ns}$	$0,78^{ns}$	$0,94^{ns}$	$1,16^{ns}$
ECwxETc	9	$1,95^{ns}$	$0,61^{ns}$	$0,55^{ns}$	$1,95^{ns}$	$0,59^{ns}$	$0,98^{ns}$	$0,91^{ns}$
CV%	-	1,64	18,19	19,45	4,14	14,08	25,86	21,53

SV: source of variation; DF: degree of freedom; CV: coefficient of variation; ECw: electrical conductivity of irrigation water; ETc: irrigation depth; (^{ns}) not significant; (*) significant at the 5% level; (**) significant at the 1% level; (***) significant at the 0.1% level.
Source: The authors.

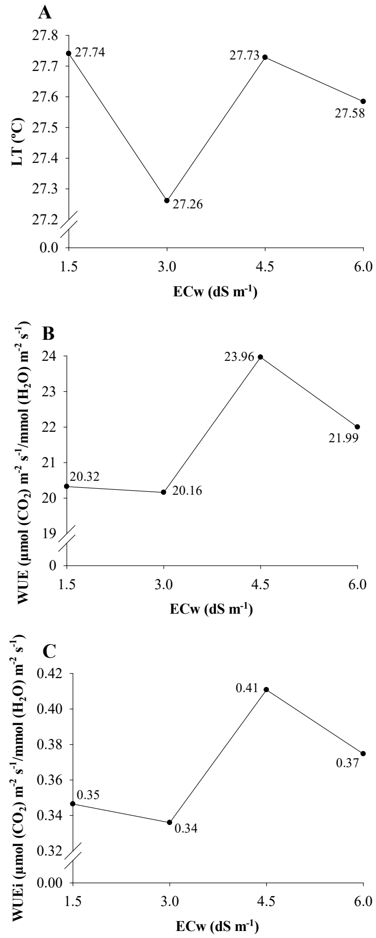


Figure 3. Leaf temperature (A), water use efficiency (B) and intrinsic water-use efficiency (C) as a function of electrical conductivities of irrigation water (ECw) in sweet sorghum (*Sorghum bicolor* L.) 'BRS 506'.
Source: The authors.

Table 3.
Analysis of variance summary (ANAVA) for membrane damage (MD), relative water content (RWC), total sugars (TS), reducing sugars (RS), non-reducing sugars (NRS) and starch (ST) as a function of electrical conductivities of irrigation water (ECw) and irrigation depths (ETc) in sweet sorghum (*Sorghum bicolor* L.) 'BRS 506'.

		Variables					
SV	DF	MD	RWC	TS	RS	NRS	ST
		F Statistic					
ECw	3	$1,43^{ns}$	$1,39^{ns}$	$2,96^{*}$	$2,60^{ns}$	$1,29^{ns}$	$1,20^{ns}$
ETc	3	$1,24^{ns}$	$1,12^{ns}$	$0,23^{ns}$	$0,57^{ns}$	$0,57^{ns}$	$2,76^{ns}$
ECwxETc	9	$1,38^{ns}$	$1,81^{ns}$	$0,95^{ns}$	$1,16^{ns}$	$0,73^{ns}$	$0,96^{ns}$
CV%	-	39,35	7,55	23,79	25,49	41,13	13,32

SV: source of variation; DF: degree of freedom; CV: coefficient of variation; ECw: electrical conductivity of irrigation water; ETc: irrigation depth; (^{ns}) not significant; (*) significant at the 5% level; (**) significant at the 1% level; (***) significant at the 0.1% level.
Source: The authors.

In Fig. 4, it is possible to observe that there was a significant effect for total sugars, with the highest value (4.75%) being obtained at the ECw of 4.5 dS m^{-1}, on the other hand, the lowest value (3.77%) was observed at ECw of 3.0 dS m^{-1}. Despite this, the values did not fit into a defined model, even with statistical differences between the means.

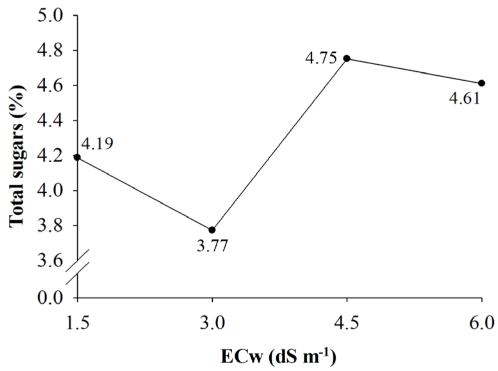


Figure 4. Total sugars as a function of electrical conductivities of irrigation water (ECw) in sweet sorghum (*Sorghum bicolor* L.) 'BRS 506'.
Source: The authors.

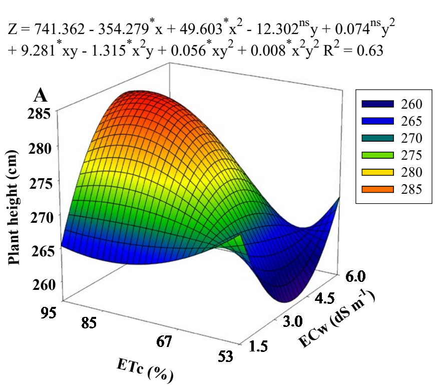
Table 4.
Analysis of variance summary (ANAVA) for plant height (PH), stem height (SH), leaf dry mass (LDM), stem dry mass (SDM), root dry mass (RDM) and total dry mass (TDM) as a function of electrical conductivities of irrigation water (ECw) and irrigation depths (ETc) in sweet sorghum (*Sorghum bicolor* L.) 'BRS 506'.

		Variables					
SV	DF	PH	SH	LDM	SDM	RDM	TDM
		F Statistic					
ECw	3	1,63^{ns}	0,82^{ns}	1,99^{ns}	1,07^{ns}	1,50^{ns}	2,74^{ns}
ETc	3	1,98^{ns}	2,28^{ns}	0,67^{ns}	0,53^{ns}	0,20^{ns}	1,13^{ns}
ECwxETc	9	2,69*	2,12*	0,55^{ns}	1,06^{ns}	0,89^{ns}	0,78^{ns}
CV%	-	5,08	7,87	20,56	20,71	38,96	21,11

SV: source of variation; DF: degree of freedom; CV: coefficient of variation; ECw: electrical conductivity of irrigation water; ETc: irrigation depth; (^{ns}) not significant; (*) significant at the 5% level; (**) significant at the 1% level; (***) significant at the 0.1% level.
Source: The authors.

For the growth variables, there was a significant effect for the interaction electrical conductivities of irrigation water (ECw) x irrigation depths (ETc) for plant height and stem height (Table 4).

Plant height (Fig. 5A) was affected by combined abiotic stresses (saline and water), with the highest values being obtained in the combination ECw 3.83 dS m^{-1} and ETc 87.76% (284.51 cm), by on the other hand, the lowest values (257.97 cm) were found in the combination ECw 3.98 dS m^{-1} and ETc 53%.



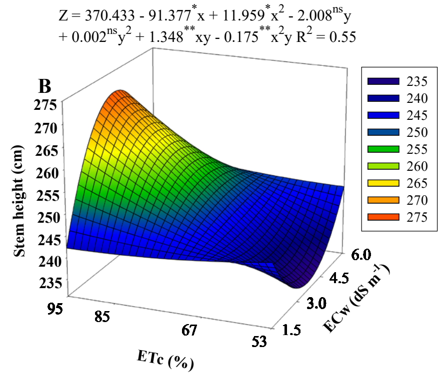


Figure 5. Plant height (A) and stem height (B) as a function of electrical conductivities of irrigation water (ECw) and irrigation depths (ETc) in sweet sorghum (*Sorghum bicolor* L.) 'BRS 506'.
Source: The authors.

Similarly to plant height, for stem height the highest value (270.18 cm) was observed in the combination with ECw of 3.98 dS m^{-1} and 95% of ETc, at the same time that the highest height (232. 81 cm) was found in the combination of ECw of 3.67 dS m^{-1} and 53% ETc (Fig. 5B).

4 Discussion

Plants subjected to high levels of abiotic stresses, such as saline and water, generally show damage to the water conditions of the leaves. In some previous studies in sorghum, increases in membrane damage were reported under conditions of water [12] and salt [13]. On the other hand, the relative water content is reduced in sorghum leaves under saline [14,16] and water [12] stress. However, in the present study, leaf water status variables (membrane damage and relative water content) were not affected by salt and water stress.

Salt and water stress cause reductions in soil water potential and leaf water potential, altering water relations and reducing plant turgor, leading to osmotic stress, and consequently reductions in leaf transpiration and gas exchange [35,36].

Generally, strategies for the mechanism of tolerance to abiotic stresses involve actions at the cellular level, such as maintenance of osmotic balance through ion absorption, preservation of hydrolase enzymes, accumulation of compatible ions, in order to maintain active photosynthesis [37-39]. In addition, these plants may have greater osmotic homeostasis compared to sensitive plants, through the accumulation of soluble sugars, in response to salt and water stress [10,15,19].

In our work, the total sugars showed an increase in the sorghum plants submitted to the highest electrical conductivities of the irrigation water. Corroborating the results found by [11], who reported greater accumulation of total soluble sugars in sorghum plants in response to salt stress. Total soluble sugars, especially non-reducing ones, are the main agents that contribute to the osmotic adjustment in sorghum leaves, being indispensable for the acclimatization of sorghum under conditions of abiotic stress [19].

Osmotic adjustment is essential for the acclimatization of sorghum to saline and water stress by maintaining turgor pressure and relative water content, as well as regulating physiological processes [15,17]. Changes in physiology and growth caused by salt and water stress generally depend on specific conditions, such as duration and intensity of stress, in addition to factors such as plant age and genotype [40].

In the present work, the gas exchange variables LT, WUE and WUEi were altered under salt stress, while the variables plant height and stem height were negatively influenced by salt and water stress together. However, there were marked reductions in both gas exchange variables and growth variables.

This behavior can be explained because the water potential in the leaves remained stable, not being affected by salt and water stress, resulting in the maintenance of gas exchange processes and the growth of sorghum plants. Showing that the sorghum cultivar ‘BRS 506’ can be cultivated under conditions of salt and water stress in semi-arid regions, without major losses in production [39].

5 Conclusions

The results indicate that combined salt and water stress cause negative effects on the growth of sweet sorghum [*Sorghum bicolor* (L.) Moench] plants. Salt stress generates disturbances in gas exchange and sugar content in plant leaves. The sorghum plants subjected to salt and water stress under the conditions of our work did not suffer major changes in the variables studied (gas exchange, leaf water status, sugars in the leaf and growth). Being able to show that the sweet sorghum cultivar 'BRS 506' is tolerant to the abiotic stresses studied.

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