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Growth, Solute Accumulation, and Ion Distribution in Sweet Sorghum under Salt and Drought Stresses in a Brazilian Potiguar Semiarid Area

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Abstract: Agriculture in semiarid regions commonly face problems because of salt and availability of irrigation water. Considering this, studies on cultures resistant to salt and water stresses involving sweet sorghum are required. Therefore, the aim was to evaluate the growth and other mechanisms of tolerance to salinity and water deficit in BRS 506 sweet sorghum. The experimental design was conducted in Upanema-RN, Brazil, in randomized blocks, where the isolated and interactive effect of 3 salinity levels, expressed as the electrical conductivity of irrigation water (1.5, 3.8, and 6.0 dS m^{-1}), and 3 irrigation depths (55, 83, and 110% of crop evapotranspiration) were evaluated. During the cycle, sorghum adapted to the salinity and deficit irrigation depth, since stem height reduced only −5.5% with increasing salinity and −11.95% with decreasing irrigation depth, and aerial dry mass was affected by interaction only at the end of the cycle. Proline, total amino acids, and total soluble sugars were not differenced by stresses. Additionally, around 68.71% of total Na^+ was at roots at the end of the cycle. In summary, sorghum BRS 506 was more tolerant to salt than water stress and used Na^+ compartmentalization in root cells as the main tolerance mechanism.

Keywords: salinity; sorghum; stress response; tolerance; water deficit; tolerance

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1. Introduction

In a world of 7.7 billion people, water security is already at risk, and it is expected to aggravate in 2050, especially in developing countries such as Africa and Asia, where clean water is already a major issue [1]. When it comes to agricultural purposes, this lack of both quantity and quality water availability directly affects crop production, since irrigation uses around 25% of groundwaters, serving 38% of the world's irrigated land [2]. Besides this, water scarcity, climate change, and inadequate agricultural management practices have posed a risk to arable land, where 1.5 million ha of production are lost each year due to soil salinity [3]. Therefore, it is interesting to explore crops that are tolerant to salinity and water scarcity, so that there is a balance between social, economic, and environmental aspects of agricultural production.

Among such crops is sorghum, which can be used for different purposes depending on the type (graniferous, biomass, sugar, or broom). In particular, sweet sorghum accumulates a juice rich in sugars in the stalk, so that it can be used in the formation of byproducts such as bioethanol, brown sugar, and molasses, while the rest of the plant can be used for fodder. As it offers greater profitability, the most exploited b-product in saccharin cultivars is bioethanol, whose productivity can be compared to the bioconversion of 12–13 tons of

corn kernels [4]. Considering adjustments due to fermentation efficiency, a biomass yield between 50 and 120 t ha^{-1} can generate about 10,000 L ha^{-1} of bioethanol [4].

Such information is especially necessary for semiarid regions, since the salinity of the soil and irrigation water, as well as the reduced water availability, are common factors in these environments, and therefore, there is a limitation in agricultural development. To promote this development and, consequently, provide an additional source of income in these regions, sweet sorghum stands out in relation to other grasses, such as corn, given that it presents moderate tolerance to salinity, without loss of production when irrigated with 4.5 dS m^{-1} EC water [5], and sped up development under water stress conditions [6]. Therefore, knowing how sweet sorghum metabolism responds to salinity and water scarcity is essential to inform rural producers about what should be prioritized in irrigation water: quality or quantity.

Sweet sorghum growth parameters affected by salt and drought stresses include reduction in leaf area, shoot and root lengths, leaf fresh and dry weights, and total dry mass [7,8]. The presence of salts in soil solution, as well as the unavailability of water, alters its water potential, impairing water absorption and consequently reducing growth. Additionally, both stresses induce ion toxicity in cells followed by an osmotic imbalance, disturbing plant growth and development [9]. However, since sweet sorghum is salt [7] and drought tolerant [4], it means that it adopts physiological, biochemical, and molecular strategies to cope with these stresses [10].

One of the biochemical mechanisms is solute accumulation, as proline, soluble sugars, and amino acids. Proline prevents membrane damage and cell apoptosis by eliminating reactive oxygen species (ROS), OH^- radicals, and signaling redox reactions [11,12]. Soluble sugars have the potential to tampon cell redox potential, protect its structure [13], and maintain photosynthetic activity, ensuring water absorption [14]. On the other hand, amino acids form proteins, are a pool of nitrogen in source and sink tissues, and represent an important precursor of secondary metabolites, promoting crop improvement [15].

Changes in the distribution of toxic ions such as Na^+ and Cl^- are also common in many tolerant crops to deal with abiotic stresses. Ion redistribution acts as a tool for osmotic balance, a process that demands the synthesis of more ATP in order to prevent excessive cation, anion accumulation, and preventing cell membrane damage [16]. Indeed, salt-tolerant plants accumulate excessive Na^+ and Cl^- ions in root tissues to ensure plant growth. This mechanism aims to avoid the translocation of these ions to leaves, where they could damage photosynthetic apparatus and put the survival of the plant at risk [17].

It is common to find in the literature how plant metabolism responds to saline or water stresses isolated, either by changes in growth, electrolyte leakage and relative water content in the leaves, solute accumulation, and/or ionic distribution [7,10,18,19]. However, studies addressing the influence of both stresses are still scarce, and since both can occur simultaneously in nature, it is essential to understand not only the isolated effect but also the interactive effect of both stresses on plant development. In this sense, the innovative approach of this study helps to understand which variables are and which are not affected by the interactive effect of salt and drought stresses. This information provides a better understanding of BRS 506 sweet sorghum tolerance mechanisms and can help other studies involving sweet sorghum cultivars.

Our research seeks to study the sorghum cultivar BRS 506 because, despite already being established in the Brazilian market, it is still not much discussed in the literature. Additionally, it is noteworthy to highlight that no sorghum cultivars were developed specifically for the Brazilian northeast semiarid. In this sense, our research aims to give visibility to this region and help breeding programs to develop cultivars adapted to it, and therefore, addressing its economic development. The study of sorghum responses to abiotic stresses provides a better understanding of the mechanisms adopted by sorghum to resist these stresses. In addition, using lower-quality water for irrigation of this crop appears as an alternative to reduce costs with minimal productivity losses. In this context, the present

study aimed to evaluate how the saccharin sorghum cultivar BRS 506 responds to deficit irrigation depths and high salinity levels.

2. Materials and Methods

2.1. Location and Characterization of the Experimental Area

The research occurred in an open field, in an experimental area of the Cumaru site, at the municipality of Upanema, Rio Grande do Norte, Brazil (5°33′30″ S; 77°11′56″ W). The climate classification in the region is BSh, hot and dry [20], with an annual rainfall of 633 mm over the last 30 years, concentrated in the months of February to May and an average annual temperature of 26 °C [21,22].

During the execution of the experiment, a meteorological station installed at the site monitored daily data referring to temperature, global radiation, wind speed, and air humidity. There was no occurrence of rainfall in the studied period. The data are shown in Figure 1.

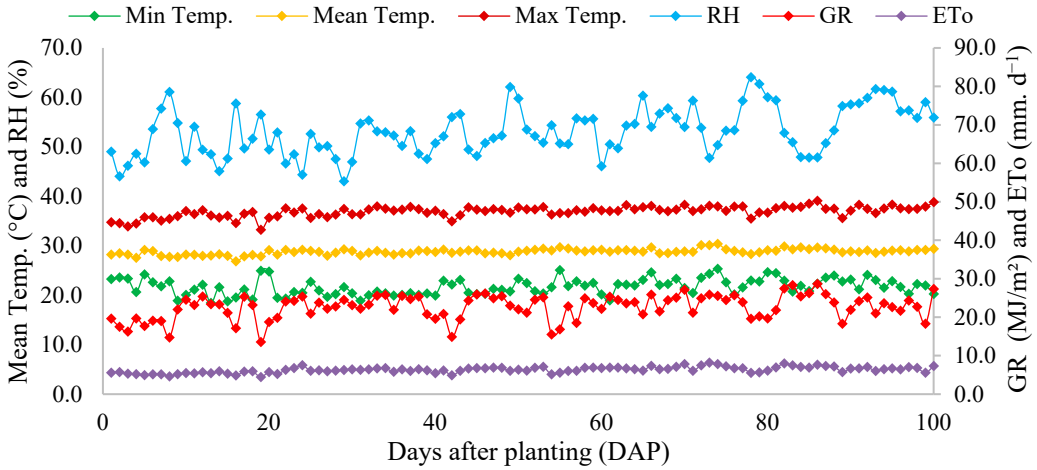

Figure 1. Minimum (Min.), mean, and maximum (Max.) temperatures (Temp.), relative humidity (RH), Global radiation (GR), and reference evapotranspiration (ET_o) registered in the area during the experiment.

The experiment took place on a Cambisol, whose preparation consisted of plowing and harrowing 15 days before planting. Before sowing, we performed foundation fertilization with 17.76 kg ha^{-1} of nitrogen and 65.34 kg ha^{-1} of phosphorus. The physical and chemical characterization of 0–30 and 30–60 cm layers are shown in Table 1.

Table 1. Physical and chemical characterization of the 0–20 cm layer of the soil in the studied area before the experiment was conducted.

Layer	Soil Physics					
	Coarse Sand	Fine Sand	Silt	Clay	Soil Density	
cm	g g^{-1}				g cm^{-3}	
0–30	0.544	0.223	0.044	0.190	1.64	
30–60	0.480	0.218	0.052	0.250	1.62	
	Soil chemistry					
	ECex	pH	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	P
	dS m^{-1}		$cmol_c$ dm^{-3}				mg dm^{-3}
0–30	0.90	7.50	4.80	1.50	0.35	0.46	15
30–60	0.80	7.20	5.10	1.60	0.39	0.45	8

Note: Ecex: Electrical conductivity of extract.

For soil salinity we used saturation paste, where we determined the electrical conductivity and percentage of exchangeable sodium. Electrical conductivity was obtained with the aid of a conductivity meter, whereas for PST, we determined Na^+, K^+, Ca^{2+}, and Mg^{2+}, where Na^+ and K^+ were extracted with Melich-1 and Ca^{2+} and Mg^{2+} with KCl [23].

In the week before planting, we used volumetric rings of approximately 50 cm^3 to collect undisturbed samples in layers 20, 40, and 60 cm to check soil moisture and guarantee the treatments applied. We covered the samples with aluminum foil to preserve the soil structure and took them to the laboratory to be cleaned. With this material, the samples were weighed to determine the soil density (Ds) and subjected to tensions of 1, 3, 6, and 10 kPa in a tension table and 30, 60, 100, 300, and 1500 kPa in a Richards chamber for determination of volumetric humidity. Data were presented as 0–30 and 30–60 cm layers.

At the beginning of the third week of the experiment, tensiometers were installed at depths of 20 cm to represent 0–30 cm layer, and at 40 cm, representing 30–60 cm layer, to verify the water retention in the soil. We recorded the height of the water column on the tensiometers and measured the tension with a tensimeter, twice a week, always before starting irrigation. Based on the observed volumetric humidity, we obtained water retention curves in these layers. The curves, as well as the determined parameters of the Van Genuchten equation can be seen in Figure 2.

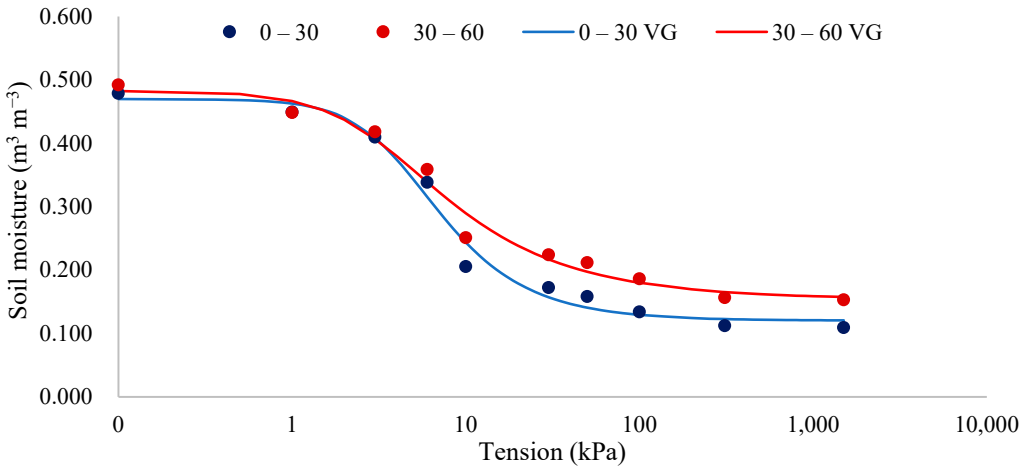

Figure 2. Retention curves at 0–30 and 30–60 cm layers observed in the experimental area.

2.2. Plant Material, Experimental Design, and Treatments

The crop studied was sweet sorghum (*Sorghum bicolor* L. Moench), cultivar BRS 506, whose seeds were donated by the Instituto Agronômico do Pernambuco (IPA). Sorghum was planted in early August 2021, at the beginning of the dry period. We choose cultivar BRS 506 because it has the potential for the economic development of semiarid regions, since sorghum has moderate tolerance to salinity stress [5] and develops well under water scarcity [24]. In addition, it has a dual purpose: while the plant material can be used for fodder, the juice in the stalks can generate byproducts such as bioethanol, honey, or brown sugar.

The experimental design was in randomized blocks, comprising 4 blocks in a 3 × 3 double factorial scheme, with 3 salinity levels and 3 irrigation depths, corresponding to 9 treatments (Figure 3). The area consisted of 36 experimental plots, where each plot comprised 2 double rows of sorghum, 7.0 m long, spaced 1.35 × 0.25 × 0.10 m.

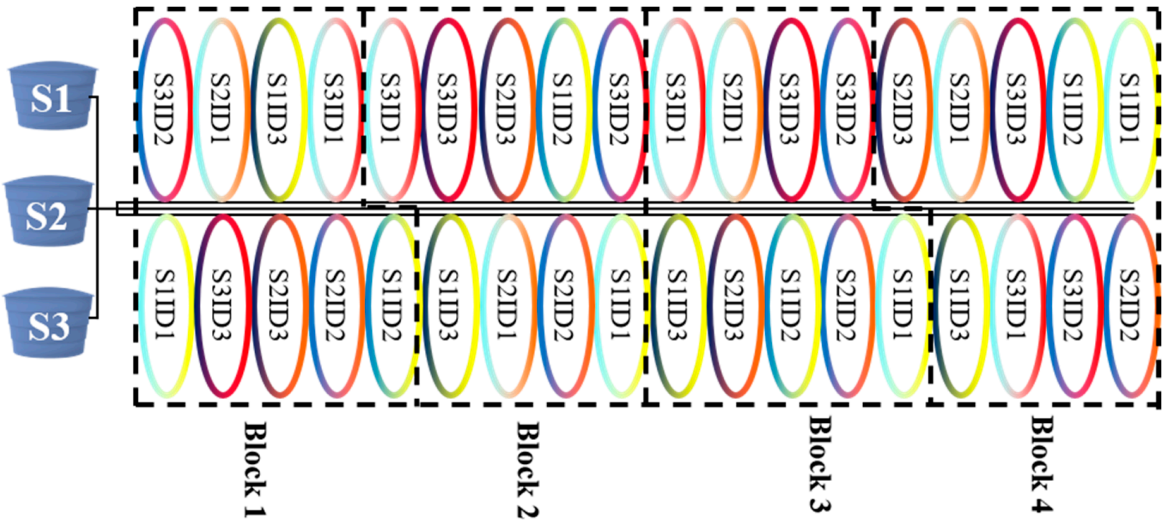

Figure 3. Sketches of the experimental area.

The adopted irrigation was done by dripping, and the water used in the experiment came from a tubular well near, of conductivity 1.5 dS m^{-1}. To establish salinities, conductivities 1.5; 3.8, and 6.0 dS m^{-1}, based on crop tolerance [5]. The salinity levels were got by mixing NaCl, $CaCl.2H_2O$, and $MgSO_4.7H_2O$ salts, until the final molar ratio of loads of 7:2:1 for Na^+, Ca^{2+}, and Mg^{2+}, in order to represent the average composition of the waters of the northeastern in the semiarid region [25].

The irrigation depths were delimited according to crop evapotranspiration (Figure 4). Figure 4 shows the historical series in Mossoró in the last 30 years, where it can be seen that the ETo registered in area was similar to the historical average. ETc was calculated using the Penman–Monteith–FAO equation [26] based on data obtained from the meteorological station installed in the area.

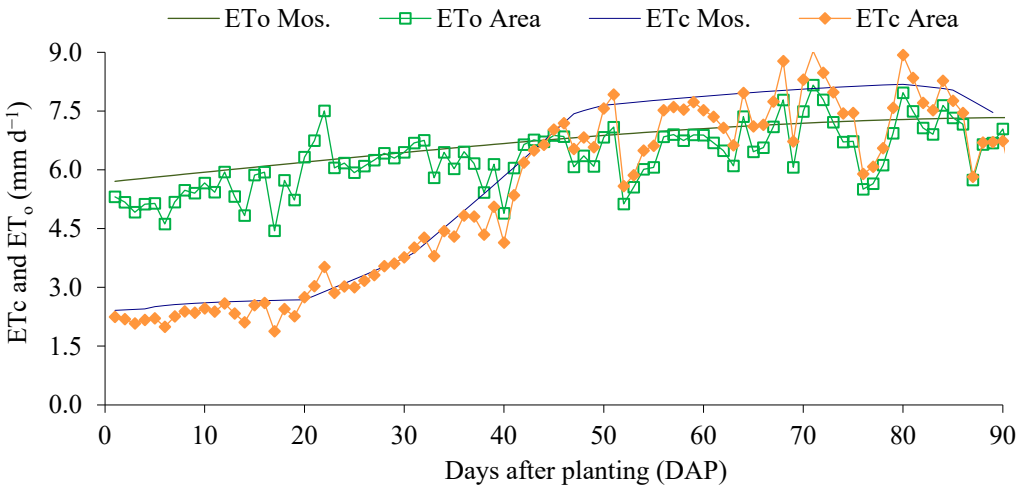

Figure 4. ETc e ETo registered at Mossoró (Mos) and observed in the experiment area (Area) during the experiment.

The flow rates were ascertained according to the spacing between the drippers after flow tests. For the ID1 depth (55% of ETc), the drippers were spaced at 0.30 m, while for ID2 (83% of ETc) and ID3 (110% ETc), 0.20 m, with the difference that for ID3 2 drip tapes were used. To guarantee the applied depths, weekly flow tests were carried out based on water pressure, using a manometer. On average, the outflows of the emitters were

1.5 l h^{-1}, with an emission uniformity coefficient of 95%, based on evaluations carried out at the beginning and middle of the crop cycle.

2.3. Growth Parameters

To measure the sorghum leaf area, we quantified: number of leaves (*NL*), width (W_D), and length (L_D) of the diagnosis leaf at 39 and 60 days after planting (DAP). Leaves were counted manually, considering only the photosynthetically active ones, while WD and LD were measured with a measuring tape, considering only 1 decimal place. The number of leaves and the width and length of the diagnosis leaf were used to calculate the leaf area, according to Equation (1) [27]:

$$LA = NL \times L_D \times W_D \times 0.747 \quad (1)$$

Total height (TH), shoot height (SH), and stem diameter (SD) were measured at 39, 60, and 81 DAP. For TH, we measured from the base of the stem to the apex of the panicle, while SH was measured from the base of the stem to the base of the last leaf, in which a 2 m ruler was used for both. The stem diameter was measured with a digital caliper at approximately 20 cm from the ground, considering 2 decimal places. Data for each plot comprised of the average of data from 5 plants.

Throughout the cycle, 2 plants per plot were collected in the 3 sorghum growth stages: 39 (beginning), 60 (flowering), and 81 (ending) DAP. In the laboratory, the roots were washed and brushed to remove excess soil, and the stem was cut into pieces to facilitate drying in the oven. Then, the samples were taken to the forced circulation oven at 65 °C for 72–96 h, until they reached constant mass. After drying, the root, stem, and leaves were weighed to get the dry mass (DM), used to quantify the content of ions. For growth, the sum of the dry mass of the leaves and stem was considered, and therefore, the aerial dry mass (ADM).

2.4. Electrolyte Leakage and Relative Water Content

For electrolyte leakage (*EL*) analysis, each plot was represented by 10 leaf disks of 0.79 cm^2, cut from the diagnosis leaves of 5 plants at 39, and 60 DAP. The disks were initially immersed in deionized water, from which the initial electrical conductivity (EC_i) was measured, and placed in a water bath for 2 h at 85 °C, where the final electrical conductivity (EC_f) was measured. *EL* was calculated by the ratio of conductivities measured, as elucidated in Equation (2) [11]:

$$EL(\%) = \frac{EC_f}{EC_i} \times 100 \quad (2)$$

The relative water content (*RWC*) was measured in the same 5 diagnosis leaves per plot used in the analysis of electrolyte leakage, from which 15 leaf disks of 0.79 cm^2 were cut, and later weighed on a precision scale to get the fresh mass of the disks (FM_D). The disks were immersed in deionized water for 24 h to get the saturated mass of the disks (SM_D) and weighed again. Subsequently, excess water from the disks was removed with a paper towel, so that they were placed in paper envelopes, identified, and dried in an oven at 65 °C for 24 h to get the dry mass of the disks (DM_D). The RWC calculation was performed according to Equation (3) [28]:

$$RWC(\%) = \frac{FM_D - DM_D}{SM_D - DM_D} \times 100 \quad (3)$$

2.5. Proline, Total Amino Acids, and Total Soluble Sugars

At 81 DAP, 3 diagnosis leaves per plot were collected for proline (PRO), total amino acids (TAA), and total soluble sugars (TSS). At the time of collection, the leaves were stored in plastic bags, identified, placed in a cooler with ice, and immediately taken to the

laboratory, where they were placed in an ultra-freezer at −80 °C until analysis. Analyses were performed using an extract obtained from 400 mg of leaf tissue, which was macerated in liquid nitrogen and dissolved in 6 mL of alcohol, then centrifuged. The analysis of proline used an aliquot of 750 µL [29], total amino acids, 200 µL [30], and total soluble sugars, 20 µL [31].

2.6. Concentration and Content of Na^+, K^+, and Cl^-

With the material dried in an oven at 65 °C obtained in the determination of the dry mass (Topic 2.3), the samples were ground in a Willey SL-31 knife mill and sieved in the 1.00 mm mesh to quantify the Na^+, K^+, and Cl^- concentrations. The extract used in the Na^+ and K^+ analyses comprised 0.5 g of material taken to the muffle at 500 °C for 3 h and later diluted at 25 mL of HNO_3, with readings by flame photometry [32]. For Cl^-, 0.5 g of material was diluted in 50 mL of $Ca(NO_3)_2$, then taken to a shaking table for 15 min and titrated with $AgNO_3$, using 5% K_2CrO_4 as solution indicator [33]. Cl^- concentration was done according to Equation (4):

$$Cl = \frac{V_{Cl} - V_{AgNO_3}}{100} \times 1000 \quad (4)$$

With the values of the concentrations, it was possible to calculate the content and distribution of ions by organ. The content refers to the product between the ion concentration and the dry mass of each organ, in other words, the total of Na^+, K^+, and Cl^- in the roots, shoot, or leaves. The distribution was obtained by the ration between the ion content in the organ (e.g., total Na^+ in the root, shoot, or leaf) and in the whole plant (e.g., Na^+ content in roots relative to the total Na^+ in plant).

2.7. Statistical Analysis

Data were submitted to analysis of variance (ANOVA) with the statistical significance of the F test. Tukey's test at 5% significance was used to compare means when there were isolated effects and when the interaction was significant. The software used for the ANOVA was RStudio, with R version 4.2.2.1 [34], through the ExpDes.pt package, while Statistica [35] was used to perform Pearson correlation matrixes.

For the variables where interaction was significant, we tested eight equation models, using the ExpAnalysis3D package, considering the effects of salinity (Salt) and irrigation depth (ID) at the linear, quadratic level, up to the simple interaction. The equations were chosen by the Akaike Information Criteria (AIC), in which those with the lowest AIC were selected. Equations that presented regression deviation with a significance level below 0.05 were not considered.

3. Results

3.1. Growth Parameters

Salt and ID affected growth parameters mainly at the beginning of the cycle, where both the isolated effect of Salt and ID were significant for LA, SH, and TH (Table 2). During the entire cycle, water stress was more severe than saline stress for plant development, but there was no statistical difference between 1.5 and 3.8 dS m^{-1} ECs, while the reduction of applied irrigation depth reduced progressively SH and TH at 39 and 60 DAP. However, sorghum showed minimal losses in growth parameters at 55% of ETc, compared to 110% ETc, like SH, where the loss was −11.95% at 81 DAP.

The Salt × ID interactive effect was observed only for ADM at 81 DAP. There was no significant difference between the salinities under L1 and L2 irrigation depths, nor between the irrigation depths under S1 salinity (Table 3). On average, at the end of the cycle, the aerial dry mass varied between 160 and 210 g, approximately.

Table 2. F Test values for leaf area (LA), stem diameter (SD), shoot height (SH), total height (TH), and aerial dry mass (ADM) measured at 39, 60, and 81 DAP.

		39 DAP					60 DAP					81 DAP			
SV	**DF**	**LA**	**SD**	**SH**	**TH**	**ADM**	**LA**	**SD**	**SH**	**TH**	**ADM**	**SD**	**SH**	**TH**	**ADM**
Block	3	1.69	3.10 *	1.61	1.61	3.00	0.57	0.89	1.39	1.38	0.23	1.27	0.24	0.67	2.83
Salt	2	3.41 *	3.66 *	15.16 **	15.16 **	2.47	5.00 *	0.32	6.71 **	3.15	0.85	0.23	3.46 *	0.33	1.54
ID	2	10.74 **	3.12	52.86 **	52.86 **	4.24 *	0.41	0.09	37.15 **	34.21 **	0.08	0.69	20.62 **	3.29	0.91
Salt × ID	4	0.44	0.05	0.73	0.73	1.99	0.09	0.54	0.71	0.68	1.44	1.09	0.48	1.22	4.31 **
C. V. (%)		14.2	6.1	10.1	5.2	17.0	19.4	5.9	6.6	5.8	14.3	7.2	5.0	17.2	10.0
									Mean values						
		cm^2	mm	cm		g	cm^2	mm	cm		g	mm	cm		g
1.5 dS m^{-1}		2309.2 a	17.87 ab	68.0 a	132.1 a	51.09 a	2392.0 b	17.81 a	224.7 a	249.3 a	133.08 a	16.69 a	211.6 a	243.0 a	530.55
3.8 dS m^{-1}		2180.2 ab	18.07 a	63.2 a	127.4 a	45.63 a	2512.3 ab	18.04 a	219.7 a	246.5 a	143.53 a	16.47 a	207.1 ab	256.9 a	569.43
6.0 dS m^{-1}		1984.6 b	16.96 b	54.2 b	118.0 b	44.25 a	3014.1 a	18.15 a	204.2 b	235.6 a	139.28 a	16.37 a	200.6 b	248.0 a	556.23
55% ETc		1852.9 b	17.00 a	48.1 c	111.5 c	42.52 b	2719.6 a	18.09 a	190.0 c	218.4 c	140.34 a	16.67 a	194.6 b	237.4 a	545.14
83% ETc		2190.7 a	17.99 a	63.2 b	127.4 b	46.47 ab	2663.9 a	17.91 a	218.5 b	247.3 b	137.22 a	16.18 a	203.7 b	235.3 a	569.44
110% ETc		2430.5 a	17.91 a	74.1 a	138.9 a	51.98 a	2534.8 a	18.00 a	240.1 a	265.7 a	138.34 a	16.67 a	221.0 a	275.2 a	541.63

* 5% significance; ** 1% significance. Different letters in each column indicate significant differences at 0.05 level according Tukey Test.

Table 3. Breakdown of aerial dry mass at 81 DAP (ADM_81).

	ADM_81			
	g			
	55% ETc	83% ETc	110% ETc	Mean
1.5 dS m^{-1}	176.74 aA	183.93 aA	169.88 bA	530.55
3.8 dS m^{-1}	175.38 aB	184.14 aAB	209.91 aA	569.43
6.0 dS m^{-1}	193.02 aAB	201.37 aA	161.84 bB	556.23
Mean	545.14	569.44	541.63	
No tested equations were significant				

Lowercase letters compare column means and uppercase letters compare row means.

3.2. Electrolyte Leakage and Relative Water Content

At 39 DAP, both electrolyte leakage and relative water content were influenced by the Salt × ID interaction (Table 4). At 60 DAP, only salinity affected EL and RWC. At flowering, the 6.0 dS m^{-1} EC resulted in an EL 13.29% higher than that observed under 1.5 dS m^{-1} EC, and 9.29% than observed at 3.8 dS m^{-1}. EC of 6.0 dS m^{-1} reduced RWC by −3.08% when compared to 1.5 dS m^{-1} and by −0.36% compared to 3.8 dS m^{-1}. Despite being statistically significant, the percentage difference between the studied ECs was small, both for EL and RWC, what reinforces the idea of tolerance.

Table 4. F Test values for electrolyte leakage (EL) and Relative Water Content (RWC) at 39 and 60 DAP.

		39 DAP		**60 DAP**	
SV	**DF**	**EL**	**RWC**	**EL**	**RWC**
Block	3	0.79	2.95	14.21 **	0.75
Salt	2	0.48	1.89	3.91 *	4.09 *
ID	2	5.41 *	0.85	2.67	0.31
Salt × ID	4	4.60 **	3.72 *	1.66	1.32
C. V. (%)		19.7	3.2	11.4	2.9
			Mean values		
		%		%	
1.5 dS m^{-1}		11.45 a	89.87 a	22.12 b	95.87 a
3.8 dS m^{-1}		10.68 a	90.54 a	22.93 ab	93.26 ab
6.0 dS m^{-1}		11.44 a	92.09 a	25.06 a	92.92 b
55% ETc		12.36	90.21 a	24.41 a	94.19 a
83% ETc		9.52	90.61 a	23.72 a	94.35 a
110% ETc		11.70	91.68 a	21.98 a	93.51 a

* 5% significance; ** 1% significance. Different letters in each column indicate significant differences at 0.05 level according Tukey Test.

Considering the beginning of the cycle, the highest EL was observed in the treatment of higher water stress combined with less salt stress (S1ID1 = 15.26%) and under higher salt stress and absence of water stress (S3ID3 = 14.13%) (Table 5). The response surface is represented in Figure 5. As for the RWC, it was maximum in the treatment of maximum stress (S3ID1 = 94.75%); however, the RWC in S3ID1 was only 8.57% higher than that observed in the treatment of lesser RWC (S2ID1 = 87.27%) (Table 5). For EL_39, the linear effect of Salt was significant, showing a continuous increase in EL with salinity, and a quadratic effect for ID, with lower EL at 83% of ETc.

Table 5. Breakdown of electrolyte leakage and relative water content variables whose interaction was significant.

	EL_39					RWC_39			
	55% ETc	**83% ETc**	**110% ETc**	**Mean**		**55% ETc**	**83% ETc**	**110% ETc**	**Mean**
1.5 dS m^{-1}	15.26 aA	9.26 aB	9.84 bB	11.45	1.5 dS m^{-1}	88.62 bA	90.18 aA	90.81 aA	89.87
3.8 dS m^{-1}	11.60 abA	9.33 aA	11.13 abA	10.68	3.8 dS m^{-1}	87.27 bB	92.43 aA	91.94 aAB	90.54
6.0 dS m^{-1}	10.21 bB	9.98 aB	14.13 aA	11.44	6.0 dS m^{-1}	94.75 aA	89.22 aB	92.30 aAB	92.09
Mean	12.36	9.52	11.70		Mean	90.21	90.61	91.68	
$EL_39 = 44.72^{***} - 3.12\,EC^{***} - 6.99.10^{-1}\,ID^{***} + 3.30.10^{-3}\,ID^{2\,**} + 3.78.10^{-2}\,ECID^{***}$ ($R^2 = 0.95$)					No tested equations were significant				

EL_39: Electrolyte leakage at 39 DAP; RWC_39: Relative water content at 39 DAP. * 5% significance; ** 1% significance; *** 0.1% significance. Lowercase letters compare column means and uppercase letters compare row means.

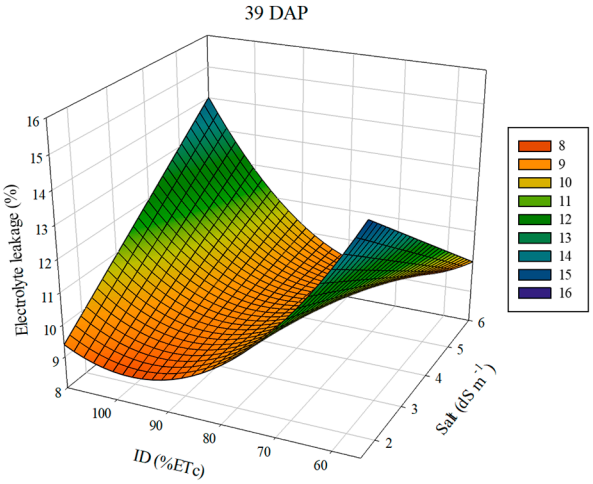

Figure 5. Response surfaces for electrolyte leakage at 39 DAP.

3.3. Proline, Total Amino Acids, and Total Soluble Sugars

According to the F test, even at 81 DAP—ending of the crop cycle—the studied salinities or irrigation depths did not result in a significant difference in the accumulation of organic solutes (Table 6).

Table 6. F Test values for proline, total amino acids, and total soluble sugars at 81 DAP.

SV	**DF**	**Proline**	**Total Amino Acids**	**Total Soluble Sugars**
Block	3	2.87 ns	1.10 ns	0.93 ns
Salt	2	0.72 ns	0.08 ns	1.56 ns
ID	2	0.07 ns	0.36 ns	2.73 ns
Salt × ID	4	1.21 ns	0.26 ns	0.87 ns
C. V. (%)		2.6	11.5	18.1
			General mean	
			µmol g FM^{-1}	µg g FM^{-1}
		3.96	12.21	17.97

ns: Non-significant.

3.4. Concentration and Content of Na^+, K^+, and Cl^- at Plant Tissue

At 39 DAP Salt influenced the concentration of K^+ in the roots and Na^+ in the shoot, with no effect on leaves (Table 7). During flowering time (60 DAP), increasing EC promoted higher Na^+ concentration in roots and leaves, whereas decreasing ID increased Na^+ concentration in leaves. At 81 DAP, the isolated effect of Salt was observed on K^+ in roots and leaves, with no isolated effect of ID for the ions in the organs studied. In all variables, the isolated effects of Salt or ID were disregarded when the interaction was significant.

Table 7. F test values for Na^+, K^+ and Cl^- concentration in roots, shoot and leaves at 39, 60, and 81 DAP.

		39 DAP			60 DAP			81 DAP		
SV	**DF**	**Na^+**	**K^+**	**Cl^-**	**Na^+**	**K^+**	**Cl^-**	**Na^+**	**K^+**	**Cl^-**
					Roots					
			mg g^{-1}			mg g^{-1}			mg g^{-1}	
Block	3	0.72	0.43	0.05	1.66	0.34	0.68	0.33	0.69	2.17
Salt	2	129.43 **	10.77 **	17.75 **	223.17 **	1.30	97.08 **	184.44 **	16.47 **	42.73 **
ID	2	1.30	1.90	16.24 **	0.68	6.80 **	1.65	4.78 *	1.69	3.46 *
Salt × ID	4	12.62 **	2.76	3.53 *	0.64	11.67 **	16.83 **	3.49 *	2.63	3.34 *
C. V. (%)		16.4	14.9	11.9	17.7	12.0	11.7	13.5	16.9	13.5
1.5 dS m^{-1}		1.64	13.19 a	13.56	1.00 c	10.00 a	10.42	2.55	10.71 a	15.92
3.8 dS m^{-1}		4.93	10.30 b	16.58	6.89 b	9.48 a	17.44	7.73	7.17 c	22.50
6.0 dS m^{-1}		5.92	13.41 a	18.11	9.90 a	10.24 a	21.08	8.89	8.87 b	26.92
55% ETc		4.06 a	13.00 a	18.64	5.94 a	10.00	16.75 a	6.98	9.49 a	23.00
83% ETc		4.42 a	12.35 a	14.67	5.68 a	10.75	15.50 a	6.30	8.90 a	22.33
110% ETc		4.00 a	11.54 a	14.94	6.18 a	8.97	16.69 a	5.90	8.36 a	20.00
					Shoot					
SV	DF		mg g^{-1}			mg g^{-1}			mg g^{-1}	
Block	3	1.54	6.05	0.23	0.15	2.59	3.20*	0.32	0.72	1.47
Salt	2	42.52 **	1.45	0.43	78.00 **	2.83	0.75	97.64 **	13.05 **	1.60
ID	2	0.07	3.16	0.23	17.56 **	6.77 **	3.07	18.03 **	10.15 **	2.41
Salt × ID	4	1.45	2.41	0.65	5.85 **	4.90 **	1.74	13.63 **	4.47 **	3.32 *
C. V. (%)		15.5	7.3	8.8	17.0	11.4	10.3	20.6	10.6	10.5
1.5 dS m^{-1}		0.55 c	56.98 a	46.83 a	0.27	18.74	24.00 a	0.15	14.61	18.08 a
3.8 dS m^{-1}		0.84 b	54.20 a	47.17 a	0.69	20.89	25.17 a	0.42	12.26	17.33 a
6.0 dS m^{-1}		1.01 a	55.32 a	48.33 a	0.65	19.55	24.17 a	0.60	15.20	18.72 a
55% ETc		0.80 a	55.38 a	46.83 a	0.51	17.85	23.00 a	0.44	12.51	18.17 a
83% ETc		0.80 a	53.50 a	48.00 a	0.44	20.26	24.92 a	0.46	14.38	18.83 a
110% ETc		0.79 a	57.62 a	47.50 a	0.65	21.08	25.42 a	0.28	15.19	17.14 a
					Leaves					
SV	DF		mg g^{-1}			mg g^{-1}			mg g^{-1}	
Block	3	0.52	3.22 *	3.96 *	1.95	0.56	6.42 **	1.50	2.70	7.37 **
Salt	2	0.90	0.41	1.57	5.53 *	0.30	0.86	0.00	6.78 **	2.66
ID	2	1.40	0.32	0.17	6.79 **	3.03	2.42	2.01	0.90	1.44
Salt × ID	4	1.39	1.14	1.84	1.85	1.47	4.02 *	0.37	0.84	2.93 *
C. V. (%)		17.2	6.8	9.1	18.2	9.7	6.5	19.4	9.0	8.6
1.5 dS m^{-1}		0.41 a	27.74 a	16.83 a	0.14 b	13.62 a	14.58 a	0.12 a	11.65 ab	15.33 a
3.8 dS m^{-1}		0.39 a	27.08 a	16.00 a	0.17 ab	14.04 a	15.08 a	0.12 a	10.86 b	14.75 a
6.0 dS m^{-1}		0.43 a	27.27 a	15.83 a	0.17 a	13.82 a	14.94 a	0.12 a	12.45 a	16.00 a
55% ETc		0.43 a	27.70 a	16.08 a	0.18 a	14.42 a	15.28 a	0.12 a	11.37 a	15.58 a
83% ETc		0.38 a	27.13 a	16.17 a	0.14 b	13.96 a	14.92 a	0.13 a	11.94 a	15.67 a
110% ETc		0.41 a	27.26 a	16.42 a	0.15 ab	13.09 a	14.42 a	0.11 a	11.65 a	14.83 a

* 5% significance; ** 1% significance. Different letters in each column indicate significant differences at 0.05 level according Tukey Test.

The lowest K^+ concentration in the root at 39 DAP was observed under an EC of 3.8 dS m^{-1} (10.30 mg g^{-1}), equivalent to a content of 78.21 mg (Table 8), 4.16% of the total K^+ in the plant. In the shoot, the effect of salinity on the Na^+ concentration was gradual, in which under EC of 1.5 dS m^{-1} the content was 15.15 mg, (41.85% of the total sorghum), 3.8 dS m^{-1} was 18.74 mg (28.67%), and under 6.0 dS m^{-1} was 23.54 mg (34.86%). In leaves, the isolated effect of salinity did not occur for Na^+, K^+ or Cl^-. Regarding the effect of irrigation depth for Na^+ in leaf, the irrigation depth at 55% of ETc resulted in a concentration of 0.18 mg g^{-1}, about 28.57% higher than that observed at 83% of ETc (0.14 mg g^{-1}).

Table 8. F test values for content of Na^+ (CNa^+), content of K^+ (CK^+), and content of Cl^- (CCl^-) in roots, shoot, and leaves at 39, 60, and 81 DAP.

		39	DAP		60	DAP		81	DAP
Trat	**CNa^+**	**CK^+**	**CCl^-**	**CNa^+**	**CK^+**	**CCl^-**	**CNa^+**	**CK^+**	**CCl^-**
					Roots				
		mg			mg			mg	
S1ID1	17.2 ± 1.9	77.0 ± 6.1	110.7 ± 8.4	12.2 ± 1.1	161.6 ± 11.9	174.6 ± 18.5	45.8 ± 6.1	223.6 ± 33.3	233.4 ± 33.7
S1ID2	10.4 ± 1.7	113.2 ± 11.1	93.9 ± 4.9	16.8 ± 1.7	210.0 ± 15.1	169.1 ± 20.3	76.9 ± 5.8	273.6 ± 17.3	327.6 ± 25.7
S1ID3	7.2 ± 0.7	116.1 ± 13.3	96.2 ± 11.5	24.3 ± 2.3	162.4 ± 22.6	214.6 ± 28.2	60.0 ± 7.8	260.4 ± 22.7	284.5 ± 15.6
S2ID1	23.4 ± 1.4	61.2 ± 3.9	105.1 ± 4.8	121.3 ± 11.0	153.1 ± 16.6	316.7 ± 24.7	153.6 ± 6.0	149.0 ± 16.1	429.1 ± 27.8
S2ID2	36.1 ± 3.8	82.3 ± 4.7	112.9 ± 14.9	102.3 ± 13.6	146.2 ± 5.9	212.2 ± 18.9	155.8 ± 13.3	171.6 ± 18.9	362.7 ± 13.6
S2ID3	60.7 ± 2.5	91.1 ± 5.8	171.2 ± 10.2	129.4 ± 16.2	186.9 ± 14.9	368.1 ± 37.7	159.8 ± 22.7	114.1 ± 14.7	360.1 ± 33.8
S3ID1	27.1 ± 2.9	80.9 ± 7.9	100.4 ± 10.0	174.0 ± 5.8	201.6 ± 16.5	377.3 ± 15.5	212.1 ± 18.9	190.0 ± 14.9	532.7 ± 43.8
S3ID2	38.1 ± 3.7	62.5 ± 6.0	91.0 ± 3.0	152.8 ± 7.0	185.2 ± 10.1	377.1 ± 21.5	165.9 ± 11.2	172.2 ± 22.2	416.0 ± 24.5
S3ID3	42.0 ± 4.7	100.4 ± 7.8	138.3 ± 15.1	136.3 ± 12.3	100.8 ± 10.1	243.8 ± 25.6	141.8 ± 12.5	154.4 ± 17.7	320.5 ± 15.7
					Shoot				
		mg			mg			mg	
S1ID1	12.6 ± 1.8	1175.5 ± 38.5	1045.7 ± 45.4	21.3 ± 1.9	1578.0 ±123.3	2103.7 ± 48.9	19.2 ± 1.6	1668.9 ± 151.2	1582.9 ± 21.7
S1ID2	18.5 ± 2.2	1713.6 ± 158.5	1445.7 ± 164.1	25.2 ± 2.2	1664.3 ± 245.3	2267.2 ± 206.4	19.3 ± 1.9	2097.7 ± 252.6	1843.1 ± 173.3
S1ID3	14.4 ± 2.3	1835.3 ± 199.3	1397.3 ± 169.1	29.8 ± 3.7	2110.4 ± 242.1	2458.4 ± 256.3	22.5 ± 2.2	2291.6 ± 160.7	1751.8 ± 307.8
S2ID1	15.3 ± 1.1	1064.2 ± 139.2	876.7 ± 115.7	70.6 ± 3.5	1809.8 ± 144.5	2358.4 ± 198.2	63.1 ± 8.0	1410.7 ± 151.6	1875.9 ± 160.5
S2ID2	16.5 ± 1.6	1126.6 ± 145.6	1087.2 ± 123.2	64.3 ± 7.8	2599.5 ± 289.4	2893.0 ± 166.4	60.7 ± 5.1	1975.0 ± 121.4	1936.5 ± 213.2
S2ID3	24.5 ± 2.4	1704.8 ± 264.7	1402.9 ± 205.8	79.4 ± 9.2	2130.8 ± 127.0	2553.9 ± 67.2	61.0 ± 8.6	2098.4 ± 90.1	1614.5 ± 174.2
S3ID1	22.7 ± 1.1	1287.5 ± 122.1	1075.3 ± 122.3	64.9 ± 7.8	2073.6 ± 163.9	2584.6 ± 278.5	109.1 ± 11.5	2291.7 ± 86.5	2103.5 ± 148.1
S3ID2	21.3 ± 1.3	1235.7 ± 108.4	1102.7 ± 105.4	41.7 ± 1.9	1789.6 ± 115.0	2145.8 ± 82.2	128.2 ± 15.3	2318.8 ± 145.9	1761.7 ± 131.6
S3ID3	26.6 ± 3.7	1345.8 ± 93.3	1235.5 ± 157.7	96.2 ± 8.1	2200.9 ± 301.0	2733.6 ± 252.0	35.5 ± 4.5	1878.7 ± 204.2	1927.4 ± 222.5
					Leaves				
		mg			mg			mg	
S1ID1	9.2 ± 0.6	589.0 ± 51.4	357.5 ± 24.9	6.7 ± 0.7	534.7 ± 25.2	586.9 ± 54.2	4.9 ± 0.5	439.8 ± 34.8	586.0 ± 33.8
S1ID2	9.6 ± 1.7	650.3 ± 33.6	374.5 ± 37.5	4.6 ± 1.6	519.0 ± 61.4	544.7 ± 47.0	4.9 ± 0.4	481.8 ± 46.9	623.4 ± 52.3
S1ID3	9.9 ± 1.3	720.2 ± 103.2	454.2 ± 53.9	4.3 ± 0.6	483.0 ± 25.2	515.0 ± 43.8	3.9 ± 0.2	442.7 ± 33.7	582.5 ± 18.2
S2ID1	7.9 ± 1.0	515.8 ± 54.7	279.1 ± 20.4	6.1 ± 0.7	506.0 ± 52.8	522.6 ± 46.9	4.0 ± 0.4	378.0 ± 7.7	544.1 ± 34.0
S2ID2	8.2 ± 0.6	574.9 ± 36.2	350.4 ± 33.0	7.1 ± 0.6	676.3 ± 81.0	740.9 ± 84.0	5.8 ± 0.4	493.6 ± 33.0	669.0 ± 33.5
S2ID3	9.4 ± 0.6	675.6 ± 72.8	418.5 ± 40.0	6.3 ± 0.7	490.2 ± 19.5	533.0 ± 25.4	4.2 ± 0.4	433.4 ± 17.4	552.6 ± 56.4
S3ID1	10.1 ± 0.8	662.0 ± 83.1	383.0 ± 45.4	8.4 ± 0.4	636.0 ± 73.3	666.7 ± 61.8	4.7 ± 0.5	499.3 ± 18.7	679.9 ± 63.5
S3ID2	6.8 ± 0.7	495.2 ± 51.4	295.7 ± 12.1	4.5 ± 0.8	425.0 ± 36.1	444.0 ± 19.0	5.2 ± 0.6	530.7 ± 34.7	679.3 ± 48.5
S3ID3	9.5 ± 0.9	544.2 ± 43.8	299.7 ± 30.6	6.3 ± 0.8	451.1 ± 25.4	519.7 ± 24.2	4.9 ± 0.7	550.0 ± 26.5	677.3 ± 27.8

At 60 DAP there was a sharp increase in Na^+ concentration in the root according to salinity. Under 1.5 dS m^{-1} EC, the Na^+ content in root was 17.8 mg, increasing to 117.7 mg at 3.8 dS m^{-1} and 154.4 mg under 6.0 dS m^{-1}, representing 36.05, 59.80, and 68.09% in the root in relation to the whole plant, respectively. In leaves, the Salt effect was also significant, but only between ECs 1.5 and 6.0 dS m^{-1}, whose distribution was 11.33 and 2.75%, respectively. As for the irrigation depths, the isolated effect was observed for the Na^+ concentration in leaves, between 55 and 83% of ETc, representing a content of 7.0 and 5.4 mg, respectively.

At the end of the cycle, at 81 DAP, Salt affected the K^+ concentration in roots and leaves, while ID effects were disregarded since they were significant when the interaction was significant. In roots, the highest K^+ concentration occurred under 1.5 dS m^{-1} EC (10.70 mg g^{-1}), representing a content of 252.23 mg (Table 8), while in leaves, maximum concentration was observed at 6.0 dS m^{-1} (12.45 mg g^{-1}), equivalent to a content of 526.7 mg.

Analyzing the interaction effect at the beginning of the cycle, it was significant for Na^+ and Cl^- concentration in roots (Table 7). The lowest Na^+ concentration in roots was in the least stressful treatment S1ID3 (0.80 mg g^{-1}) (Table 9), corresponding to a content of 7.2 mg. It is worth mentioning that in the treatments under lower salinity (1.5 dS m^{-1}), the distribution of Na^+ in roots regarding the whole plant was 31.65% on average, being 44.23% in S1ID1, and decreasing as the irrigation depth increased, being 27.42% in S1ID2 and 23.31% in S1ID3.

For Cl^- in roots at 39 DAP (Figure 6), the regression analysis showed the linear and quadratic effect of ID, as well as the simple interaction (Table 9). The highest Cl^- concentration was observed in S3ID1 (19.33 mg g^{-1}), corresponding to a content of 100.4 mg (Table 8). However, the highest Cl^- contents were observed in S2ID3 (171.2 mg) and S3ID3 (138.3 mg) (Table 8), this was due to the increase in irrigation depth significantly increasing dry mass, and therefore, the larger irrigation depths presented greater content.

Table 9. Breakdown of ion concentration variables at 39 DAP whose interaction was significant.

	Na_R39					Cl_R39			
	55% ETc	83% Etc	110% Etc	Mean		55% Etc	83% Etc	110% Etc	Mean
1.5 dS m^{-1}	2.83 bA	1.28 cB	0.80 bB	1.64	1.5 dS m^{-1}	18.33 aA	11.67 bB	10.67 bB	13.56
3.8 dS m^{-1}	4.08 aB	4.81 bAB	5.88 aA	4.93	3.8 dS m^{-1}	18.25 aA	15.00 abA	16.50 aA	16.58
6.0 dS m^{-1}	5.27 aB	7.16 aA	5.33 aB	5.92	6.0 dS m^{-1}	19.33 aA	17.33 aA	17.67 aA	18.11
Mean	4.06	4.42	4.00		Mean	18.64	14.67	14.94	
No tested equations were significant					Cl_R39 = 42.81 *** − 9.98.10^{-1} EC − 6.15.10^{-1} ID *** + 2.77.10^{-3} ID^2 ** + 2.43.10^{-2} ECID ** (R^2 = 0.93)				

Na_R39: Na^+ concentration in roots at 39 DAP; Cl_R39: Cl^- concentration in roots at 39 DAP. * 5% significance; ** 1% significance; *** 0.1% significance. Lowercase letters compare column means and uppercase letters compare row means.

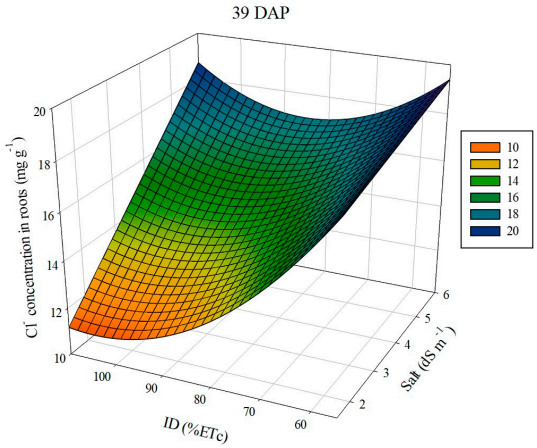

Figure 6. Response surface for Cl^- concentration in roots at 39 DAP.

At 60 DAP, the interaction affected K^+ and Cl^- in roots, Na^+ and K^+ in the shoot, and Cl^- in leaves (Table 7). In roots, the K^+ concentrations were maximum in S3ID1 and S3ID2 (11.75 and 11.91 mg g^{-1}) (Table 10), corresponding to contents of 201.6 and 185.2 mg, respectively, slightly above 7% of the K^+ was allocated in roots in these treatments. Likewise, the Cl^- concentration was maximum in roots in S3ID1 and S3ID2, corresponding to a content of 377.3 and 377.1 mg, just over 10% in the root concerning the whole plant.

In shoot at 60 DAP, the Na^+ concentrations were minimum in the 1.5 dS m^{-1} EC, regardless of the depth, representing an average Na^+ content of 25.4 mg, while for the other salinities the ID at 110% of ETc (content = 68.5 mg g^{-1}, on average) resulted in a higher concentration of Na^+ than IDs at 55 and 83% ETc (Table 8). For the K^+ concentration, the effect of ID was significant for 1.5 and 3.8 dS m^{-1} ECs, while the effect of Salt was only observed at 83% ETc (content = 2017.8 mg, on average). It is worth mentioning that K^+ was distributed more in the shoot than in other organs, where between 69.10 and 79.48% of the total K^+ in sorghum was in this organ. In leaves, the Cl^- concentration was maximum in the greater stress treatment: S3ID1 (16.33 mg g^{-1}), while the lowest concentrations (14.00 mg g^{-1}) were observed at S3ID2 and S1ID3. There was no effect of ID at 1.5 and 3.8 dS m^{-1} salinities, and there was no effect of Salt at 110% of ETc.

At the end of the cycle, the interaction was significant for Na^+ and Cl^- concentrations in roots, Na^+, K^+, and Cl^- concentrations in shoot, and Cl^- in leaves (Table 7). The increase in salinity resulted in a gradual increase in the content of Na^+ in roots at ID of 83% of ETc, where the content was 76.9, 155.8, and 165.9 mg for S1ID2, S2ID2, and S3ID2, respectively (Table 8). In addition, the effect of the irrigation depth was significant for the Na^+ concentration in roots at 3.8 and 6.0 dS m^{-1} salinities, but not for 1.5 dS m^{-1}, where the content was 60.9 mg on average. The Cl^- concentration in roots was maximum in the treatment of greater stress, S3ID1 (31.00 mg g^{-1}) (Table 11), in which under an ID at 55% of ETc, the effect of Salt was gradual, corresponding to a content of 45.8, 153.6, and 212.1 mg for S1ID1, S2ID1, and S3ID1, respectively (Table 8).

Table 10. Breakdown of ion concentration variables at 60 DAP whose interaction was significant.

		K_R60					**Cl_R60**		
		mg g^{-1}					mg g^{-1}		
	55% ETc	83% ETc	110% ETc	Mean		55% ETc	83% ETc	110% ETc	Mean
1.5 dS m^{-1}	9.73 abAB	11.31 aA	8.97 abB	10.00	1.5 dS m^{-1}	10.50 cA	9.00 cA	11.75 cA	10.42
3.8 dS m^{-1}	8.52 bB	9.02 bAB	10.88 aA	9.48	3.8 dS m^{-1}	17.75 bB	13.25 bC	21.33 aA	17.44
6.0 dS m^{-1}	11.75 aA	11.91 aA	7.05 bB	10.24	6.0 dS m^{-1}	22.00 aA	24.25 aA	17.00 bB	21.08
Mean	10.00	10.75	8.97		Mean	16.75	15.50	16.69	
No tested equations were significant					No tested equations were significant				
		Na_S60					**K_S60**		
		mg g^{-1}					mg g^{-1}		
	55% ETc	83% ETc	110% ETc	Mean		55% ETc	83% ETc	110% ETc	Mean
1.5 dS m^{-1}	0.22 bA	0.27 bA	0.32 bA	0.27	1.5 dS m^{-1}	15.94 aB	17.50 bB	22.78 aA	18.74
3.8 dS m^{-1}	0.72 aAB	0.59 aB	0.75 aA	0.69	3.8 dS m^{-1}	18.43 aB	23.90 aA	20.35 aAB	20.89
6.0 dS m^{-1}	0.60 aB	0.45 aB	0.89 aA	0.65	6.0 dS m^{-1}	19.16 aA	19.38 bA	20.12 aA	19.55
Mean	0.51	0.44	0.65		Mean	17.85	20.26	21.08	
No tested equations were significant					No tested equations were significant				
		Cl_L60							
		mg g^{-1}							
	55% ETc	83% ETc	110% ETc	Mean					
1.5 dS m^{-1}	15.00 abA	14.75 abA	14.00 aA	14.58					
3.8 dS m^{-1}	14.50 bA	16.00 aA	14.75 aA	15.08					
6.0 dS m^{-1}	16.33 aA	14.00 bB	14.50 aB	14.94					
Mean	15.28	14.92	14.42						
No tested equations were significant									

K_R60: K^+ concentration in roots at 60 DAP; Cl_R60: Cl^- concentration in roots at 60 DAP; Na_S60: Na^+ concentration in the shoot at 60 DAP; K_S60: K^+ concentration in shoot at 60 DAP; Cl_L60: Cl^- concentration in leaves at 60 DAP. Lowercase letters compare column means and uppercase letters compare row means.

Table 11. Breakdown of ion concentration variables at 81 DAP whose interaction was significant.

		Na_R81					**Cl_R81**		
		mg g^{-1}					mg g^{-1}		
	55% ETc	83% ETc	110% ETc	Mean		55% ETc	83% ETc	110% ETc	Mean
1.5 dS m^{-1}	2.32 bA	2.71 cA	2.63 bA	2.55	1.5 dS m^{-1}	14.00 cA	17.75 bA	16.00 bA	15.92
3.8 dS m^{-1}	8.67 aA	7.04 bB	7.48 aAB	7.73	3.8 dS m^{-1}	24.00 bA	22.50 abA	21.00 abA	22.50
6.0 dS m^{-1}	9.94 aA	9.15 aA	7.60 aB	8.89	6.0 dS m^{-1}	31.00 aA	26.75 aAB	23.00 aB	26.92
Mean	6.98	6.30	5.90		Mean	23.00	22.23	20.00	
Na_R81 = 4.84 ** + 5.27 EC *** − $3.96.10^{-1}$ EC^2 *** + $2.07.10^{-2}$ ID − $1.07.10^{-2}$ ECID ** (R^2 = 0.99)					Cl_R81 = 4.54 + 5.80 EC *** + $9.77.10^{-2}$ ID * − $4.0.10^{-2}$ ECID ** (R^2 = 0.97)				
		Na_S81					**K_S81**		
		mg g^{-1}					mg g^{-1}		
	55% ETc	83% ETc	110% ETc	Mean		55% ETc	83% ETc	110% ETc	Mean
1.5 dS m^{-1}	0.14 cA	0.13 cA	0.17 bA	0.15	1.5 dS m^{-1}	12.21 bB	14.50 aB	17.14 aA	14.61
3.8 dS m^{-1}	0.45 bA	0.44 bA	0.36 aA	0.42	3.8 dS m^{-1}	10.12 bB	14.12 aA	12.55 bAB	12.26
6.0 dS m^{-1}	0.72 aA	0.80 aA	0.30 abB	0.60	6.0 dS m^{-1}	15.22 aA	14.52 aA	15.87 aA	15.20
Mean	0.44	0.46	0.28		Mean	12.51	14.38	15.19	
No tested equations were significant					K_C81 = 9.72 *** − 2.36 EC ** + $5.22.10^{-1}$ EC^2 *** + $1.14.10^{-1}$ ID *** − $1.73.10^{-2}$ ECID ** (R^2 = 0.84)				
		Cl_S81					**Cl_L81**		
		mg g^{-1}					mg g^{-1}		
	55% ETc	83% ETc	110% ETc	Mean		55% ETc	83% ETc	110% ETc	Mean
1.5 dS m^{-1}	16.00 bB	19.75 aA	18.50 aAB	18.08	1.5 dS m^{-1}	14.50 aA	15.50 aA	16.00 aA	15.33
3.8 dS m^{-1}	19.00 abA	17.75 aAB	15.25 aB	17.33	3.8 dS m^{-1}	16.00 aA	15.25 aAB	13.00 bB	14.75
6.0 dS m^{-1}	19.50 aA	19.00 aA	17.67 aA	18.72	6.0 dS m^{-1}	16.25 aA	16.25 aA	15.50 aA	16.00
Mean	18.17	18.83	17.14		Mean	15.58	15.67	14.83	
Cl_C81 = 5.94 + $1.28.10^{-2}$ EC + $2.11.10^{-1}$ EC^2 + $3.07.10^{-1}$ ID * − $1.57.10^{-3}$ ID^2* − $1.76.10^{-2}$ ECID * (R^2 = 0.62)					Cl_F81 = 17.86 ** − 1.21 EC * + $1.81.10^{-1}$ EC^2 − $1.35.10^{-2}$ ID (R^2 = 0.36)				

Na_R81: Na^+ concentration in roots at 81 DAP; Cl_R81: Cl^- concentration in roots at 81 DAP; Na_S81: Na^+ concentration in shoot at 81 DAP; K_S81: K^+ concentration in shoot at 81 DAP; Cl_S81: Cl^- concentration in shoot at 81 DAP; Cl_L81: Cl^- concentration in leaves at 81 DAP. * 5% significance; ** 1% significance; *** 0.1% significance. Lowercase letters compare column means and uppercase letters compare row means.

In the shoot, the interaction influenced the content of all ions studied (Table 7), response surfaces are shown in Figure 7. The salinity considerably increased the Na^+ concentrations in the treatments under 55 and 83% of ETc IDs, in which the 6.0 dS m^{-1} EC increased the Na^+ concentration in 414.29% for ID1 and 515.39% for ID2 compared to 1.5 dS m^{-1} EC (Table 11). The irrigation depth was only significant at 6,0 dS m^{-1} EC, in which the lowest Na^+ concentration occurred at S3ID3 (0.30 mg g^{-1}). As for K^+, the levels ranged from 10.12 to 17.14 mg g^{-1} (Table 11) (content = 72.4 and 76.3 mg), while for the Cl^- concentrations, they were between 15.25 and 19.75 mg g^{-1} (Table 11) (content = 1614.5 and 1843.1 mg). It is worth mentioning that K^+ and Cl^- were more distributed in the shoot, between 71.32 and 79.30% for K^+, and 61.55 and 65.99% for Cl^-. In leaves, the Cl^- concentration was influenced by the salinity only in the treatments under 110% of ETc ID, being the 3.8 dS m^{-1} the EC that showed lowest content (13.00 mg g^{-1}, content = 552.6 mg), whereas the effect of ID was significant only at treatments under 3.8 dS m^{-1} EC (Table 11).

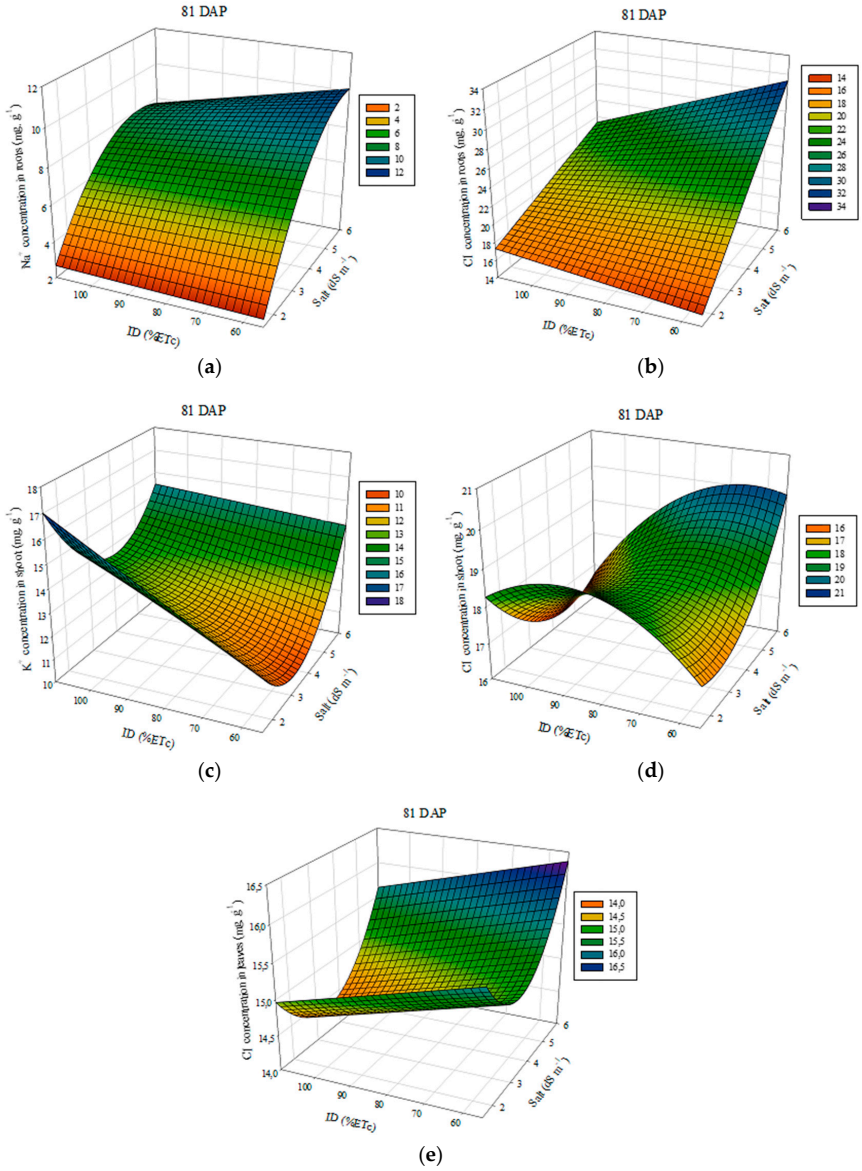

Figure 7. Response surfaces for: Na^+ concentration in roots (**a**); Cl^- concentration in roots (**b**); K^+ concentration in shoot (**c**); Cl^- concentration in shoot (**d**); and Cl^- concentration in leaves (**e**) at 81 DAP.

3.5. Pearson Correlation Matrix

At 39 DAP, regarding variables related to growth, SH and TH were strongly influenced by leaf area (r = 0.76; r = 0.76) and also showed strong correlation with each other (r = 1.00) (Table 12). As for the variables related to ion content, Na^+ in roots was strongly correlated with Cl^- in roots (r = 0.64), and with Na^+ in the shoot (r = 0.72), while Cl^- in roots, besides Na^+ in roots, correlated negatively with total (r = −0.68) and shoot (r = −0.68) heights. Among plant parts, the ions correlated with each other from very weak to weak.

Table 12. Pearson correlation matrix for variables measured at 39 DAP.

	EL39	RWC39	ADM_39	Na_R39	K_R39	Cl_R39	Na_S39	K_S39	Cl_S39	Na_L39	K_L39	Cl_L39	LA39	SD39	SH39	TH39
EL39	1.00															
RWC39	−0.07	1.00														
ADM_39	−0.31	0.09	1.00													
Na_R39	0.07	0.14	−0.30	1.00												
K_R39	0.07	0.18	−0.12	−0.21	1.00											
Cl_R39	0.37	−0.00	−0.38	0.64	0.04	1.00										
Na_S39	0.02	0.19	−0.37	0.72	−0.03	0.50	1.00									
K_S39	−0.08	0.11	0.04	−0.31	0.05	−0.22	−0.16	1.00								
Cl_S39	−0.19	0.23	−0.10	0.09	0.06	0.12	0.26	0.29	1.00							
Na_L39	0.37	−0.04	−0.16	0.01	0.15	0.32	0.33	0.03	0.14	1.00						
K_L39	0.09	−0.00	0.24	−0.22	0.21	−0.09	−0.18	0.28	0.18	0.08	1.00					
Cl_L39	−0.26	−0.13	0.19	−0.21	0.04	−0.13	−0.09	−0.12	0.19	0.15	0.00	1.00				
LA39	0.17	0.11	0.29	−0.16	−0.16	−0.49	−0.38	0.13	0.03	−0.26	0.21	−0.10	1.00			
SD39	−0.09	0.15	0.23	−0.17	−0.14	−0.26	−0.33	0.00	−0.03	−0.32	0.06	−0.06	0.52	1.00		
SH39	−0.13	0.13	0.56	−0.36	−0.14	−0.68	−0.39	0.14	−0.07	−0.23	0.12	0.04	0.76	0.52	1.00	
TH39	−0.13	0.13	0.56	−0.36	−0.14	−0.68	−0.39	0.14	−0.07	−0.23	0.12	0.04	0.76	0.52	1.00	1.00

EL39: Electrolyte leakage; RWC39: Relative water content; ADM_39: Aerial dry mass; Na_R39: Na^+ concentration in roots; K_R39: K^+ concentration in roots; Cl_R39: Cl^- concentration in roots; Na_S39: Na^+ concentration in shoot; K_S39: K^+ concentration in shoot; Cl_S39: Cl^- concentration in shoot; Na_L39: Na^+ concentration in leaves; K_L39: K^+ concentration in leaves; Cl_L39: Cl^- concentration in leaves; LA39: Leaf area; SD39: Stem diameter; SH39: Shoot height; TH39: Total height. All variables at 39 DAP.

Compared to 39 DAP, at flowering (60 DAP), the variables linked to growth were much less correlated, except for total height and shoot height (r = 0.99) (Table 13). However, Na^+ concentration in roots remained strongly correlated with Cl^- in roots (r = 0.83) and Na^+ in shoot (r = 0.74). There was also a correlation between K^+ and Cl^- in shoot (r = 0.63). As with flowering, for the growth variables at the end of the cycle, only SH and TH showed a strong correlation (r = 0.84) (Table 14). Root Na^+ continued to be linked to root Cl^- (r = 0.82) and shoot Na^+ (r = 0.83), root Cl^- also correlated strongly with shoot Na^+ (r = 0.74).

Table 13. Pearson correlation matrix for variables measured at 60 DAP.

	EL60	RWC60	ADM_60	Na_R60	K_R60	Cl_R60	Na_S60	K_S60	Cl_S60	Na_L60	K_L60	Cl_L60	LA60	SD60	SH60	TH60
EL60	1.00															
RWC60	−0.16	1.00														
ADM_60	0.03	−0.09	1.00													
Na_R60	0.17	−0.32	0.16	1.00												
K_R60	−0.14	0.18	−0.03	0.06	1.00											
Cl_R60	0.13	−0.18	0.03	0.83	0.36	1.00										
Na_S60	0.08	−0.46	0.21	0.74	−0.34	0.52	1.00									
K_S60	0.12	−0.12	0.18	0.13	−0.18	0.05	0.26	1.00								
Cl_S60	−0.09	0.05	−0.18	0.08	−0.24	−0.10	0.17	0.63	1.00							
Na_L60	0.15	−0.16	0.16	0.40	0.02	0.34	0.35	−0.06	−0.04	1.00						
K_L60	−0.07	0.15	0.25	0.06	0.37	0.10	−0.04	0.10	0.19	0.24	1.00					
Cl_L60	0.26	−0.12	0.42	0.10	0.07	0.06	0.07	0.16	0.11	0.09	0.45	1.00				
LA60	0.27	−0.15	0.07	0.42	0.11	0.42	0.29	−0.06	−0.18	0.24	0.25	0.15	1.00			
SD60	0.05	−0.17	−0.05	0.01	−0.04	0.03	−0.07	0.06	0.08	−0.01	0.29	0.05	0.35	1.00		
SH60	−0.18	−0.00	−0.02	−0.29	−0.26	−0.33	−0.03	0.43	0.40	−0.34	−0.38	−0.18	−0.38	0.09	1.00	
TH60	−0.15	−0.03	−0.03	−0.25	−0.22	−0.28	−0.03	0.42	0.39	−0.34	−0.37	−0.15	−0.29	0.14	0.99	1.00

Note: EL60: Electrolyte leakage; RWC60: Relative water content; ADM_60: Aerial dry mass; Na_R60: Na^+ concentration in roots; K_R60: K^+ concentration in roots; Cl_R60: Cl^- concentration in roots; Na_S60: Na^+ concentration in shoot; K_S60: K^+ concentration in shoot; Cl_S60: Cl^- concentration in shoot; Na_L60: Na^+ concentration in leaves; K_L60: K^+ concentration in leaves; Cl_L60: Cl^- concentration in leaves; LA60: Leaf area; SD60: Stem diameter; SH60: Shoot height; TH60: Total height. All variables at 60 DAP.

Table 14. Pearson correlation matrix for variables measured at 81 DAP.

	Pro	TAA	TSS	ADM_81	Na_R81	K_R81	Cl_R81	Na_S81	K_S81	Cl_S81	Na_L81	K_L81	Cl_L81	SD81	SH81	TH81
Pro	1.00															
TAA	0.25	1.00														
TSS	−0.02	−0.12	1.00													
ADM_81	−0.13	0.41	−0.14	1.00												
Na_R81	0.15	−0.07	0.27	0.13	1.00											
K_R81	0.14	0.23	−0.11	−0.19	−0.48	1.00										
Cl_R81	0.13	0.08	0.23	0.10	0.82	−0.17	1.00									
Na_S81	0.06	−0.01	0.16	0.31	0.83	−0.25	0.74	1.00								
K_S81	0.25	−0.06	−0.30	−0.10	−0.12	0.24	−0.01	0.01	1.00							
Cl_S81	−0.00	−0.35	0.16	−0.17	0.12	0.22	0.22	0.20	0.33	1.00						
Na_L81	−0.12	−0.23	0.13	−0.42	0.08	−0.06	0.23	0.11	−0.11	−0.01	1.00					
K_L81	0.10	−0.22	0.26	−0.04	0.10	−0.07	0.01	0.21	0.40	0.38	0.06	1.00				
Cl_L81	−0.06	−0.25	0.02	−0.43	0.14	0.29	0.33	0.21	0.18	0.47	0.31	0.06	1.00			
SD81	−0.15	0.17	0.03	0.03	−0.10	−0.03	−0.14	−0.05	−0.13	−0.25	0.04	−0.06	0.07	1.00		
SH81	0.13	0.12	−0.49	−0.13	−0.31	−0.02	−0.39	−0.45	0.37	−0.21	−0.30	−0.14	−0.12	0.04	1.00	
TH81	−0.04	0.05	−0.55	−0.15	−0.32	0.05	−0.32	−0.40	0.47	−0.07	−0.14	−0.12	−0.04	0.18	0.84	1.00

Note: Pro: Proline; TA: Total amino acids; TSS: Total soluble sugars; ADM_81: Aerial dry mass; Na_R81: Na^+ concentration in roots; K_R81: K^+ concentration in roots; Cl_R81: Cl^- concentration in roots; Na_S81: Na^+ concentration in shoot; K_S81: K^+ concentration in shoot; Cl_S81: Cl^- concentration in shoot; Na_L81: Na^+ concentration in leaves; K_L81: K^+ concentration in leaves; Cl_L81: Cl^- concentration in leaves; LA81: Leaf area; SD81: Stem diameter; SH81: Shoot height; TH81: Total height. All variables at 81 DAP.

3.6. Soil Parameters

3.6.1. Soil Salinity

Salt was significant in ECex and exchangeable sodium percentage (ESP) in the soil for both the 0–20 and 20–40 cm layers. The irrigation depth was significant only in the 20–40 cm layer for ECex and ESP of the soil (Table 15). Soil ECex reduced when water with a salinity of 6.0 dS.m^{-1} was applied to the two layers studied, this can be explained because the plant absorbs a higher concentration of Na^+ in the roots, reducing the concentration of soluble salts in the soil as can be seen in Table 15.

Table 15. Electrical conductivity (EC) and Exchangeable sodium percentage (ESP) of soil solution in the 0–30 and 30–60 cm layers.

		0–20 cm		20–40 cm	
SV	**DF**	**EC**	**ESP**	**EC**	**ESP**
Block	3	1.75	0.22	0.52	0.57
Salt	2	7.93 **	71.26 **	20.94 **	46.21 **
ID	2	0.90	1.63	12.57 **	0.07
Salt × ID	4	0.52	0.39	1.81	0.40
C. V. (%)		51.4	24.4	34.5	36.7
1.5 dS m^{-1}		1.58	2.85	1.72	1.99
3.8 dS m^{-1}		3.84	9.76	4.77	8.46
6.0 dS m^{-1}		2.71	12.50	4.20	13.38
55% ETc		2.98	9.24	4.71	7.78
83% ETc		2.87	7.93	3.76	7.85
110% ETc		2.27	7.94	2.22	8.20

* 5% significance; ** 1% significance.

Under different irrigation depths, ECex reduced with the greater availability of water being leached into deeper soil layers. In this sense, the use of water with a higher salt content, using 10% more of the water requirement of the crop, in addition to providing a development condition for the crop with salts more dissolved in the root zone, allows soil salts to be leached, avoiding the soil degradation process.

Salinity was significant for ESP in both layers when different salinities of irrigation water were used, with an increase in ESP as water electrical increased. Considering that soil becomes sodic for ESP greater than 15%, even using a salinity of 6.0 dS m^{-1}, no sodification process was observed in the studied layers, remaining below the conditions for sodification process.

3.6.2. Soil Water Retention

Under tensions of 0, 1, and 3 kPa, the moisture content was practically the same in both layers. Under 6, 10, 30, and 60 kPa, higher moisture was observed in the surface layer, whereas with increasing tensions to 100, 300, and 1500 kPa, the 0–30 cm layer showed higher soil water content. Regardless of the depth used, the increase in salinity promoted higher moisture content in the 0–30 and 30–60 cm soil layers (Table 16). The highest moisture content was observed when EC 6.0 dS m^{-1} water was used. However, this higher moisture content does not mean that water is available for the plant. This is because of the reduction of the osmotic potential of the soil solution caused by salts, the plant needs to direct a lot of energy to absorb water.

Table 16. Moisture observed at 0–30 and 30–60 cm layers according to the treatments applied.

Treatment	Period from Sowing (Days)						Period from Sowing (Days)					
	15–21	**22–35**	**36–49**	**50–63**	**64–77**	**78–91**	**15–21**	**22–35**	**36–49**	**50–63**	**64–77**	**78–91**
	Moisture at 0–30 cm Layer (m^3 m^{-3})						**Moisture at 30–60 cm Layer (m^3 m^{-3})**					
S1ID1	0.125	0.121	0.121	0.121	0.122	0.121	0.178	0.161	0.160	0.159	0.160	0.160
S1ID2	0.138	0.121	0.121	0.121	0.122	0.121	0.189	0.162	0.160	0.160	0.160	0.160
S1ID3	0.136	0.130	0.127	0.133	0.133	0.129	0.200	0.165	0.177	0.192	0.186	0.182
S2ID1	0.128	0.123	0.123	0.124	0.124	0.122	0.178	0.161	0.160	0.159	0.159	0.159
S2ID2	0.137	0.126	0.125	0.129	0.137	0.125	0.191	0.166	0.169	0.177	0.188	0.170
S2ID3	0.137	0.132	0.125	0.131	0.136	0.128	0.197	0.188	0.171	0.191	0.196	0.185
S3ID1	0.133	0.130	0.128	0.129	0.126	0.124	0.184	0.161	0.177	0.170	0.178	0.171
S3ID2	0.132	0.127	0.128	0.131	0.125	0.127	0.181	0.163	0.173	0.177	0.173	0.167
S3ID3	0.135	0.134	0.125	0.132	0.134	0.131	0.199	0.197	0.188	0.197	0.192	0.187

4. Discussion

Sorghum resisted to salinity, water scarcity, and their interaction. The main mechanism observed was the compartmentalization of Na^+ ions in the root cells, preventing them from being transported to the leaves, which could cause damage to the photosynthetic apparatus. We also noted that the Salt × ID interaction was significant for most variables associated with ion concentration, especially at the end of the cycle. The combined effects of these stresses are poorly discussed in the literature, but they can occur naturally, which makes it interesting to understand how this interaction affects plant metabolism.

4.1. Concentration and Content of Na^+, K^+, and Cl^-

At the beginning of the cycle, under salinity of 1.5 dS m^{-1}, the irrigation depth reduction caused an increase in Na^+ concentration in roots, reaching 2.83 mg g^{-1} in S1ID1, equivalent to a content of 17.2 mg in which approximately 44.23% of the Na^+ was allocated in roots. This suggests that there is a limit to the concentration of Na^+ in the soil solution that induces the compartmentalization of Na^+ in root cells. In S1ID3, Na^+ was translocated to the shoot (45.30%), probably because, due to S1ID3 being the lowest stress condition, the concentration of this ion did not generate a physiological response of compartmentalization in roots. This compartmentalization of Na^+ can also be evidenced because the increase in salinity resulted in higher levels of Na^+ in roots, reaching a maximum of 7.16 mg. g^{-1} in S3ID2 (content = 39.1 mg).

The K^+/Na^+ ratio is an excellent parameter for identifying sorghum genotypes that are tolerant or sensitive to salinity [36]. Furthermore, in the roots, it was higher in treatments under 1.5 dS m^{-1} EC, showing a greater allocation of Na^+ to the other organs as salinity increases. The chemical similarity of the K^+ and Na^+ ions can cause an exchange of K^+ for Na^+ in biochemical reactions and a consequent change in the structure of proteins [37], impairing plant development.

Regarding Cl^- in roots, higher irrigation depths also increased the amount of Cl^- in the soil solution because more Cl^- ions are dissolved in the irrigation water, which is

reinforced because two of the three salts used in the irrigation water are a source of Cl^-, contributing to a more expressive content of this anion.

At 60 DAP, only salinity interfered with the Na^+ concentration in roots, with an average of 1.00 mg g^{-1} for irrigation at 1.5 dS m^{-1}, corresponding to a content of 17.8 mg, and therefore, 36.05% allocated in roots. This is possibly related to the absorption of Na^+, considering that compared to 39 DAP the Na^+ content under 1.5 dS m^{-1} in the entire plant was lower. Apparently, at lower salinity it is possible for the sorghum the non-absorption of Na^+ ions as a tolerance mechanism; at other salinities, the higher concentration of solutes in the soil solution may prevent this selectivity from occurring.

The K^+ content at flowering was influenced by the interaction of stresses in roots and shoot, whereas in leaves there was no isolated or interactive effect. In roots, Cl^- was also affected by the interactive effect of stresses, and in the shoot, the interaction was significant for Na^+. These responses show an excess of Na^+ and Cl^- in the root zone, in order to interfere with the absorption of K^+ and generate nutrient imbalance, reduction of enzymatic activity, ionic stress and formation of ROS [38]. It is also worth mentioning Cl^-, which has high mobility [39], and probably interfered with its content in the leaves, since the reduction in irrigation depth was significant for the increase in Cl^- content at a salinity of 6.0 dS m^{-1}.

At 81 DAP, Na^+ remained concentrated in roots, representing between 55.59 and 77.61% of the total Na^+ in sorghum, although it was expected that there would be an exclusion of these ions by Na^+/H^+ antiporters [10]. However, the salt content in roots can be a positive aspect for the survival of the plant, since it helps to maintain an osmotic gradient favorable to water absorption [18].

There was no significant difference in the Na^+ content in leaves, even at the end of the cycle, when the plant had been under poor irrigation for a long time, the sorghum continued to prioritize the non-translocation of Na^+ ions to the leaves, which could damage the photosynthetic apparatus [36]. In leaves, the high cytosolic balance of the K^+/Na^+ ratio, varying between 86.68 and 115.63, leads to greater plant growth and tolerance to saline stress [40].

4.2. Growth Parameters

At the beginning of the cycle (39 DAP), the highest concentration of salts in the 6.0 dS m^{-1} EC water can lead to changes in the water potential of sorghum metabolism and reduction of the osmotic potential of the soil solution [10,41], reducing LA, SD, SH and TH. It is noteworthy that the increase in EC from 1.5 dS m^{-1} to 3.8 dS m^{-1} did not statistically reduce any of the variables associated with growth, showing tolerance. In addition, germination is a sensitive period for plant development, and salinity at this stage may be even more critical for sorghum development.

The ID effect at 39 DAP is also linked to changes in soil and plant water potential because, under water scarcity, sorghum closes stomata to reduce water loss. This stomatal closure, however, despite reducing losses via transpiration, also reduces photosynthesis and consequently the production of biomass [18]. It is interesting to point out that SD was not affected by ID in any of the seasons studied, possibly because—since BRS 506 sorghum is a saccharin cultivar—there is a preference to keep SD for broth storage. However, there was a reduction in SH according to ID at all times, which may be linked to lower lignin production under water stress [42]. In this sense, considering that the lignin production decreased according to ID, the shoot height reduced, but the sorghum sought to maintain the stem diameter.

At 60 DAP, it was possible to observe that fewer variables associated with growth were affected by the isolated effects of Salt and ID, so the sorghum developed stress response mechanisms to tolerate them. It is interesting to emphasize this, since the flowering period is a period more sensitive to water scarcity for sorghum [43].

At 81 DAP, only SH was affected by the isolated effects of Salt and ID, reinforcing the idea of tolerance. Under abiotic stresses, tolerant cultivars seek to keep the photosynthetic

system stable and increase the efficiency of CO_2 fixation, minimizing the damage caused by these through apoplastic barriers [10]. The Salt × ID interaction was significant only at the end of the cycle, for ADM_81, in which the salinity effect was not significant in the 55 and 83% of ETc irrigation depths, showing that more brackish waters can be used even in smaller irrigation depths.

4.3. Electrolyte Leakage and Relative Water Content

Electrolyte leakage refers to the integrity of the plasma membrane, the disruption of which results in increased permeability, and therefore, increased leakage. This permeability results from changes in the composition and structure of the membrane, caused by the action of ions from salts when the plant is subjected to salinity, probably being the first sign of response to saline stress [44]. However, greater extravasation of electrolytes does not mean that the plant is sensitive to salinity. Hniličková et al [45], studying *P. oleracea* under saline stress, found EL between 86.7% and 92.4%; however, these values were attributed to the high content of K^+ in the cytoplasm, given that K^+ is abundant in plant cells and whose efflux is mainly responsible for the extravasation of electrolytes [46].

The relative water content is associated with the turgor of plant cells, being an excellent indicator of the water status of the plant. Here, the combined effect of stresses was observed only at 39 DAP, which leads to the idea that the beginning of the cycle is more sensitive to applied stresses. During cycle, at 60 DAP, only salinity changed the RWC, while the isolated or interactive effect of water depth was not significant, which is possibly linked to the sorghum roots. When comparing sorghum with less drought-tolerant crops such as maize, the sorghum root system is deeper, with possibly greater hydraulic conductivity and water transport from the root to the other organs [47].

4.4. Proline, Total Amino Acids, and Total Soluble Sugars

Proline production can occur both under the isolated effects of salinity or deficient irrigation depth, but Wang et al. (2022), studying germination of sweet sorghum cultivars, found that the combination of these stresses resulted in greater gene expression of P5CS, P5CR, and OAT linked to proline production. In sorghum, proline production occurs under moderate stress [48], but according to the results obtained in this study, the applied stresses were not sufficient to differentiate the production of this solute, which may indicate tolerance of the BRS 506 cultivar. There are still controversies about the production of proline by sorghum, since a sensitive cultivar can accumulate the same amount of proline as a tolerant one [17].

The accumulation of amino acids is linked to a better development of the culture because they are constituents of proteins and an important precursor of secondary metabolites [15], and as for proline, the accumulation was not significant for this study. Sugar production is also a common strategy used by sorghum as osmoregulation, as it maintains the plant's water potential and water absorption capacity [10], but it was not observed here either. Although the accumulation of organic solutes as a means of tolerance is common, in the studied sorghum, their production was not observed, indicating that the application of treatments did not result in significant stress for the leaves. However, other studies with the cultivar BRS 506 involved with the expression of genes linked to the production of proline, amino acids, and sugars are recommended, to explain what was observed here.

4.5. Pearson Correlation Matrix

Pearson's correlation showed greater interdependence between the variables linked to growth at the beginning of the cycle. Whatever the effects of saline and/or water stress on one of the sorghum organs, the others are affected. This indicates the crop's sensitivity to abiotic stresses at the time of plant establishment, both in terms of drought sensitivity [43] and salinity. At the other times, only TH and SH showed correlation, for obvious reasons, given that the shoot height is inherent to the total height.

As for ions, the relationship between Na^+ in the root and shoot, as well as Na^+ with Cl^- in roots, was the only strong correlation observed in the three studied periods. The fact that both Na^+ and Cl^- are correlated in the root is linked to the moment of water absorption, given that they are the constituent ions of NaCl, one of the salts applied in irrigation water. It is worth mentioning that throughout the cycle, there was a trend towards a greater distribution of Na^+ in the root and of Cl^- in the stem, that is, the correlation of both in the root is not linked to a greater distribution in the root than in the other organs.

As for the relationship between Cl^- and total and shoot height at 39 DAP, sorghum is a fast-growing plant, especially in the vegetative stage, so the energy from photosynthesis is used intensively in cell elongation and division, growth promoters. At flowering, sorghum intensifies transpiration, which promotes the translocation of Cl^- from free spaces, so more nutrients are absorbed [49]. During flowering, K^+ and Cl^- in the shoot may be related to the fact that the shoot is the organ with the highest biomass, and therefore, the probability of ions accumulating in the shoot is greater. Here, Na^+ was not translocated to the shoot because it is being stored in the roots, whereas Cl^-, although similar to Na^+ and can confer toxicity to the plant, is a highly mobile anion [39], being easily carried from roots to shoot. For the same reason, at the end of the cycle, the relationship between Cl^- in the root and Na^+ in the leaf suggests a preference in the distribution of these ions in different parts of the plant because of their toxicity, requiring further studies to verify this hypothesis.

5. Conclusions

Our research showed that the main tolerance mechanism adopted by the BRS 506 sorghum was the distribution of Na^+ ions to the root cells so the sorghum adapted to salt and water stress. In fact, when aiming at deficit irrigation in order to reduce water costs, the sorghum showed that even under a depth of 55% of the ETc, waters with EC of up to 6.0 dS m^{-1} can be adopted without loss in the aerial dry mass. However, when irrigation waters are EC of 3.8 dS m^{-1}, irrigation at 110% ETc is preferable, and under EC of 6.0 dS m^{-1}, 83% of ETc.

According to our study, BRS 506 sorghum can be irrigated with lower-quality water and in smaller quantities. This information is especially beneficial to small rural producers in semiarid regions who live in areas with water scarcity and salinity problems. As future prospects, we seek to highlight BRS 506 sorghum as a crop to be explored in the Brazilian semiarid region. In addition, crop development programs can also benefit from this research, given that we observed ionic redistribution as sufficient to tolerate stress, without altering the production of osmoregulatory substances such as proline, soluble sugars, and total amino acids.

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