



DOI: <http://dx.doi.org/10.1590/1807-1929/agriambi.v22n10p673-678>

Chlorophyll a fluorescence in saccharine sorghum irrigated with saline water

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Key words:

salinity
photosynthetic pigments
photochemical efficiency

ABSTRACT

Soil salinity directly affects plants, interfering in the emission of fluorescence, and promoting the degradation of photosynthetic pigments. Thus, estimating the damage to the photosynthetic apparatus caused by saline water is an important tool to detect abiotic stresses. For this purpose, sorghum plants were cultivated in a greenhouse and irrigated with two water sources (NaCl and salt mixture) with six levels of electrical conductivity (EC) (0, 2.5, 5, 7.5, 10, and 12.5 dS m⁻¹), forming a 6 × 2 factorial in randomized blocks with four repetitions. At 60 days after sowing, the photosynthetic pigments were quantified, the chlorophyll a fluorescence parameters (initial fluorescence [F₀]; variable fluorescence [F_v]; maximum fluorescence [F_m]; quantum efficiency of photosystem II - PSII [F_v/F_m]; electron flux per reaction center [ET₀/CR]; quantum energy dissipation [DI₀/CR] and quantum yield for heat dissipation [φD₀]) were evaluated. The photosynthetic pigments were decreased with an increase in the salinity of the irrigation water, and were more expressive at higher electrical conductivities. With the wear on the photosynthetic apparatus by the increase in salinity, a reduction in the emission of the chlorophyll a fluorescence was observed, pointing to a possible photoinhibition of photosystem II.

Palavras-chave:

salinidade
pigmentos fotossintéticos
eficiência fotoquímica

Fluorescência da clorofila a em sorgo sacarino irrigado com água salina

RESUMO

A salinidade do solo afeta diretamente os vegetais interferindo na emissão da fluorescência, além de promover a degradação dos pigmentos fotossintéticos. Assim, estimar o dano no aparato fotossintético provocado por águas salinas é uma ferramenta para detectar o nível de estresses abióticos. Para tanto, plantas de sorgo sacarino foram cultivadas em ambiente protegido e irrigadas com duas fontes de água (NaCl e Mistura de sais) com seis níveis de condutividade elétrica – CE (0; 2,5; 5; 7,5; 10 e 12,5 dS m⁻¹) formando um fatorial de 6 x 2 em blocos ao acaso com 4 repetições. Aos 60 dias após a semeadura, os pigmentos fotossintéticos foram quantificados, além de avaliar os parâmetros da fluorescência da clorofila a: fluorescência inicial (F₀); fluorescência variável (F_v); fluorescência máxima (F_m); eficiência quântica do fotossistema II - PSII (F_v/F_m); fluxo de transporte de elétrons por centro de reação (ET₀/CR); energia dissipada por centro de reação (DI₀/CR) e rendimento quântico da energia dissipada (φD₀). Os pigmentos fotossintéticos apresentaram reduções com o aumento da salinidade da água de irrigação, sendo mais expressivas nas maiores condutividades elétricas. Com o desgaste do aparato fotossintético pelo aumento da salinidade, observou-se a redução na emissão da fluorescência da clorofila a, apontando para possível fotoinibição do fotossistema II.



INTRODUCTION

In semi-arid regions, besides the low water availability and high temperatures, another important factor is the salinity; sorghum is moderately tolerant to this stressing agent (Nabati et al., 2013). This is probably associated with mechanisms such as the control of sodium accumulation in leaves (Deinlein et al., 2014), osmotic adjustment associated with the accumulation of soluble sugars (Yamaguchi & Blumwald, 2005), and maintenance of the synthesis and protection of pigments, such as chlorophyll (Ashraf & Harris, 2013).

According to Tavakkoli et al. (2011), the main ionic constituents of soluble salts in the soil are sodium, calcium, magnesium, and chloride, as well as carbonates and bicarbonates. The action of these salts is directly linked to injuries in the function of photosystem II (PSII) (Ambede et al., 2012). Under stressful environmental conditions, especially in plants under the influence of saline stress, the enzyme chlorophyllase correlates with the degradation of photosynthetic pigments (Neves & Spat, 2013).

Generally, the estimation of chlorophyll a fluorescence is used as a way to monitor the electron transfer in the face of environmental adversities, considering that this technique is fast, precise, and non-destructive (Maxwell & Johnson, 2000). Thus, the study of physiological parameters, such as pigment contents and chlorophyll a fluorescence, are important in the determination of the effects of osmotic and water conditions on photosynthetic efficiency in plants (Melo et al., 2017a, b).

Therefore, the objective of this study was to evaluate the foliar photosynthetic pigment contents and chlorophyll a fluorescence in sorghum plants subjected to irrigation with saline solutions of different ionic compositions and increasing levels of electrical conductivity.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse located at the Federal Rural University of Pernambuco, Recife Campus, Brazil, from December 2015 to February 2016, with an experimental period of 60 days. During the conduction of the experiment, the mean values of relative humidity and temperature were 64.5% and 31 °C, respectively, as monitored by datalogger.

The soil used came from the rural area of the city of Pesqueira (08° 21' 28" S and 36° 41' 47" W), located in the semi-arid region of the northeast, in the Upper Ipanema basin,

in the state of Pernambuco, Brazil. The collection was carried out in the 0-30 cm layer, in a soil classified as Neossolo Flúvico (Fluvent), according to EMBRAPA (2013). The soil was air dried, decanted, homogenized, and passed through a 4 mm sieve in order to preserve the micro-aggregates.

In order to characterize the chemical properties of the soil (Table 1), the pH in the water was determined in the proportion of 1:2.5 (soil:water); Ca²⁺ and Mg²⁺ were extracted in 1 M KCl solution and titrated with EDTA, and Na⁺ and K⁺ were extracted by Mehlich-1 and quantified in a flame emission photometer. The analysis was according to the methodology proposed by EMBRAPA (1997). The cation exchange capacity (CEC), the sum of bases (SB), and the exchangeable sodium percentage (ESP) were calculated from the values obtained in the exchange complex. The saturation extract was obtained from the saturation paste (Richards, 1954), and its electrical conductivity and pH were measured.

For the physical characterization, the granulometry and water dispersed clay (WDC) were determined in the air dried soil using the densimeter method, which measures bulk density and particle density (EMBRAPA, 1997) (Table 1).

Sorghum plants of IPA 2502 were cultivated in pots (8 L) filled with Neossolo Flúvico (Fluvent) soil, and irrigation occurred daily, maintaining the soil moisture at 80% of the soil field capacity. The moisture control was performed by daily weighing, and replacement of evapotranspired water. Two solutions were used (Table 2) with six levels of electrical conductivity (0, distilled water; 2.5; 5; 7.5; 10; and 12.5 dS m⁻¹). The solution for the mixture of salts was elaborated according to the ionic proportions of water collected from the artesian wells used for irrigation in the region of soil collection.

The treatment of 12.5 dS m⁻¹ was disregarded, since the experimental units related to it did not resist until the end of the experiment due to the high salt contents of the solutions.

The fluorescence parameters of chlorophyll a (initial fluorescence [F₀], variable fluorescence [F_v], maximum fluorescence [F_m], quantum efficiency of PSII [F_v/F_m], electron flow per reaction center [ET₀/CR], energy [DI₀/CR], and quantum energy dissipation [φD₀]) were measured at 60 days after sowing (DAS) with the aid of the FluorPen F100 fluorometer (Photon Systems Instruments, Drasov, Czech Republic). The measurements were performed on the second fully expanded and healthy upper leaf after the period of

Table 1. Chemical and physical characterization of the Neossolo Flúvico (Fluvent) soil used to fill the pots in the greenhouse experiment

Chemical parameters												
Saturation extract			Exchangeable cations									
EC (dS m ⁻¹)	pH _{es}	pH _{H2O}	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	SB	H	Al	T	ESP	
			(cmol _c kg ⁻¹)								(pH 7.0)	(%)
3.2	7.9	6.5	4.35	2.73	1.48	0.77	9.33	1.43	0.0	10.76	13.75	
Physical parameters												
Sand		Silt	Clay	WDC	BD	PD	TP	FD	DD	FC	PWP	
Fine	Coarse											
		(g kg ⁻¹)			(kg dm ⁻³)			(%)		(g g ⁻¹)		
315	163	378	144	100	1.28	2.52	49.15	30.56	69.44	0.26	0.05	

Chemical parameters: EC - Electrical conductivity; SB - Sum of exchangeable bases (SB = Ca + Mg + Na + K); T - Cation exchange capacity (CEC = SB + (Al + H)); ESP - Exchangeable sodium percentage (ESP = (100 * Na⁺)/CTC). Physical parameters: WDC - Water dispersed clay; BD - Bulk density; PD - Particle density; TP - Total porosity; FD - Flocculation degree; DD - Dispersion degree; FC - Water content at field capacity; PWP - Water content at the permanent wilting point

Table 2. Quantity of salts (g L⁻¹) required for the formulation of saline solutions, in order to obtain the electrical conductivities of the treatments

CE (dS m ⁻¹)	Mixture of salts						
	NaCl	NaCl	CaCl ₂	Ca(NO ₃) ₂	MgCl ₂	MgSO ₄	KCl
0	-	-	-	-	-	-	-
2.5	1.203	0.751	0.229	0.079	0.258	0.402	0.069
5.0	2.773	1.728	0.528	0.181	0.593	0.925	0.159
7.5	4.461	2.950	0.901	0.309	1.012	1.580	0.272
10.0	6.397	4.256	1.300	0.445	1.460	2.279	0.392
12.5	8.372	5.842	1.785	0.612	2.005	3.129	0.538

adaptation to the dark for 30 min, for total oxidation of the PSII reaction center.

The chemical extraction and determination of the chlorophyll a, b, and carotenoid contents followed the methodology described by Lichtenthaler & Buschmann (2001).

The experiment was carried out in randomized block in a 6 × 2 factorial with four replications, totaling 48 experimental units. The data were submitted to analysis of variance, with the adjustments of regression equations, and the means were compared by Tukey's Test ($p < 0.05$).

RESULTS AND DISCUSSION

There was significant interaction ($p < 0.01$) between the sources of salts and the increasing levels of electrical conductivity (Table 3). The chlorophyll a (Chla) leaf contents decreased, except for in the treatment with 2.5 dS m⁻¹, which presented an increase of 28.25%, for the solution mixture of salts, in relation to the control. The highest decreases were observed for the electrical conductivity of 10 dS m⁻¹ (70.66%), in the NaCl solution, and for the electrical conductivity of 7.5 dS m⁻¹ (68.14%) for the mixing solution of salts.

For the NaCl solution, more precisely, from EC 2.5 dS m⁻¹, the chlorophyll b (Chlb) and carotenoid contents decreased with the increase in the electrical conductivities. However, in the salt mixing solution at 2.5 dS m⁻¹, the highest value was observed for both parameters (Table 3).

In the ratio Chla:Chlb leaf content, a significant difference was observed between the factors (salt source and level of electrical conductivity) at $p < 0.01$ (Table 3). However, from the salt sources, a significant difference only occurred at an EC of 10 dS m⁻¹. The highest decrease (50.19%), when compared to the control, was observed at an EC of 10 dS m⁻¹ (NaCl).

Photosynthetic pigments are responsible for capturing the photons of light and transmitting energy to the reaction centers.

Under stress conditions, such pigments are directly affected as long as stress progresses, and biochemical changes may limit photosynthetic activity more directly, resulting in oxidative damage to cells (Viana et al., 2002). With an increase in NaCl in the irrigation water, the activation of the chlorophyllase enzyme occurs in stressed plants, and is the main factor related to reductions in the contents of photosynthetic pigments (Neves & Spat, 2013).

Lacerda et al. (2003) studied the salinity tolerance in two contrasting sorghum genotypes, and observed a 52% reduction in chlorophyll content for the sensitive genotype (CSF18) and 27% reduction for the salinity tolerant genotype (CSF20), when submitted at 100 mM NaCl. In sugarcane plants submitted to irrigation with a 200 mM saline solution of NaCl, there was a decrease in the levels of Chla, Chlb, and carotenoids by 68.9, 70, and 49.8%, respectively, compared to the levels in the 0 mM concentration (Barreto et al., 2013). Neves & Spat (2013) found reductions of more than 50% in rice cultivars irrigated with saline solutions with concentrations ranging from 0 to 150 mM NaCl.

In a study of six rice cultivars under stress conditions, Cancellier et al. (2011) found a reduction in the chlorophyll a/b ratio. These authors considered that cultivars that presented lower chlorophyll a/b relations were more efficient in the capture of light, since this decrease is not due to the reduction in Chla. This was differently observed in the present study, where Chla reductions were higher than those in Chlb.

No significant difference was observed between the salt sources; however, a significant difference in the increasing levels of electrical conductivity ($p < 0.01$) was observed. The F_0 of sorghum plants presented an increase with the increase in electrical conductivity of the irrigation water (Figure 1A). An electrical conductivity of 10 dS m⁻¹ was the most significant, with an increase of 82.23% compared to that of the control.

The initial fluorescence explains the fluorescence emission when the primary electron acceptor quinone (QA) of

Table 3. Leaf content of chlorophyll a, b, and carotenoids in sorghum irrigated with saline solutions with increasing electrical conductivity (EC) at 60 days after sowing (DAS)

EC (dS m ⁻¹)	Chlorophyll a		Chlorophyll b		Ratio Chla/Chlb		Carotenoid	
	(mg g ⁻¹)						(mg g ⁻¹)	
	NaCl	MS	NaCl	MS	NaCl	MS	NaCl	MS
0	1.645 aA	1.319 aAB	0.370 aA	0.349 aBC	4.439 aA	3.783 aA	0.607 aA	0.503 bB
2.5	1.475 aA	1.692 aA	0.406 bA	0.648 aA	3.632 aA	2.611 aB	0.519 bAB	0.659 bA
5.0	1.221 aAB	1.291 aAB	0.341 Aa	0.361 aB	3.581 aA	3.574 aA	0.510 aAB	0.504 aB
7.5	0.893 aB	0.420 bC	0.284 Aa	0.158 aC	3.143 aAB	2.646 aB	0.436 aBC	0.330 bC
10.0	0.483 bC	1.005 aB	0.218 Aa	0.260 aBC	2.211 bB	3.860 aA	0.362 bC	0.507 aB
CV (%)	17.22		23.02		16.17		10.01	

EC - Electrical conductivity; MS - Mixture of salts. Same lowercase letters between water sources, upper case among EC treatments did not differ from each other, by Tukey's test, at 0,05 probability

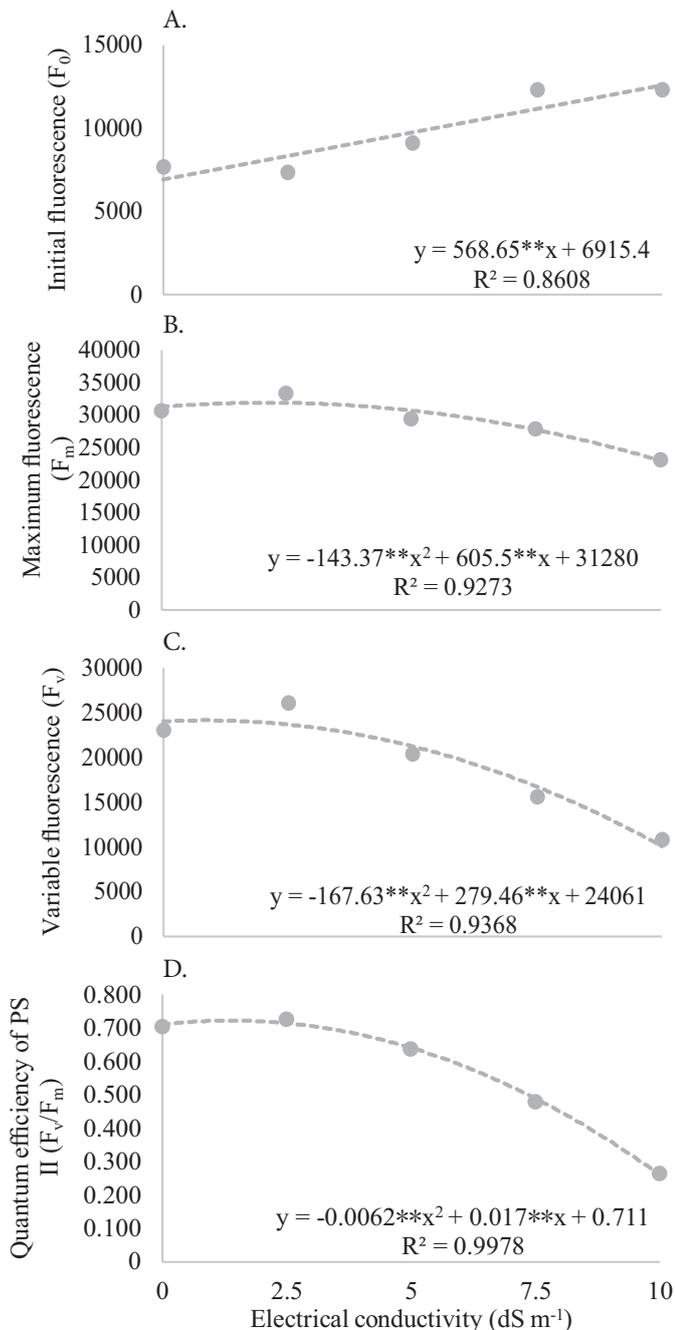


Figure 1. Parameters of chlorophyll a fluorescence emissions in saccharine sorghum plants under abiotic stress conditions at 60 days after sowing (DAS). Initial fluorescence - F_0 (A); maximum fluorescence - F_m (B); variable fluorescence - F_v (C); quantum efficiency of photosystem II - F_v/F_m (D)

photosystem II (PSII) is fully oxidized and the reaction center (P680) is open, indicating the activation of the photochemical reactions (Baker & Rosenqvist, 2004). In a study with sorghum and salinity, the emission of the initial fluorescence (F_0) increased with an increase in the electrical conductivity (Nabati et al., 2013). The increase in F_0 in plants subjected to some type of stress may be related to the increase in leaf thickness (Munns & Tester, 2008) due to lipid peroxidation by reactive oxygen species (Yamane et al., 2008).

The F_m and F_v presented with a significant differences ($p < 0.01$) in electrical conductivity levels. With the increase in the

electrical conductivity of the irrigation water, the emission of both F_m and F_v decreased by 26.48 and 58.05%, respectively, when the EC of 10 dS m⁻¹ was compared to the control (Figures 1B and C).

The maximum fluorescence emission represents the maximum intensity of the fluorescence, when practically all the quinone is reduced and the reaction centers reach the maximum capacity for photochemical reactions (Baker & Rosenqvist, 2004; Silva et al., 2015). According to Nabati et al. (2013), sorghum plants submitted to irrigation with increasing levels of electrical conductivity (5.2; 10.5; 23.1 dS m⁻¹) presented a reduction in F_m with the elevation of salinity. They also reported that the decrease in the maximum fluorescence was related to the photoinhibition caused by saline stress.

The quantum efficiency of PSII (F_v/F_m) presented significant difference only in the electrical conductivity levels. The saccharine sorghum plants reduced the quantum efficiency of PSII with elevated salinity levels (Figure 1D), showing reductions from the EC of 5 dS m⁻¹, reaching values 63.3% lower in EC of 10 dS m⁻¹ when compared to the control.

Coelho et al. (2011) studied sorghum plants subjected to irrigation with saline solution with 15 g L⁻¹ NaCl concentration, and they observed reductions of 35% in the quantum efficiency of PSII. According to Tezara et al. (2005), plants that present an F_v/F_m below 0.8 are subjected to stressful conditions and a reduction in the maximum quantum efficiency of PSII, a situation similar to the plants of the present study that presented with F_v/F_m values of below 0.728 (2.5 dS m⁻¹) to 0.266 (10 dS m⁻¹). In stress-prone situations, plants do not present changes in the primary photochemistry of PSII, but a reduction in the quantum efficiency of electron transport is observed (Lu & Zhang, 1998).

As the EC levels increased, the electron transport flux decreased progressively (Figure 2A) and presented a statistical difference. The greatest reduction compared to the control (0 dS m⁻¹) occurred for the EC of 10 dS m⁻¹ (45.70%). The electrical conductivity of 2.5, 5, and 7.5 dS m⁻¹ decreased by 2.28, 10.66, and 25.13%, respectively.

With increasing levels of saline concentration (0, 50, 100, and 250 mM), reductions in the electron transport rate of 20 and 29% were observed for two sorghum varieties, according to Netondo et al. (2004). This reduction in electron transfer rates can be attributed to the ions that cause deleterious effects on the thylakoid membranes, disrupting the electron transport in the lipid-protein bilayer, and damaging the activity of the complex (Ambede et al., 2012).

The quantum of energy dissipation by the reaction center showed a significant effect ($p < 0.01$) on the electrical conductivity levels of the irrigation water. At 60 DAS, it was observed that the energy dissipated by the reaction center increased with the increasing salinity levels (Figure 2B). The electrical conductivity of 10 dS m⁻¹ presented an increase of 179.29% in relation to the electrical conductivity of 0 dS m⁻¹ group (Figure 2B). According to Zegada-Lizarazu et al. (2015), the dissipation of excess energy is a mechanism of release and regulation of PSII, allowing the closure of the reaction centers and preserving the cycles of xanthophyll and lutein.

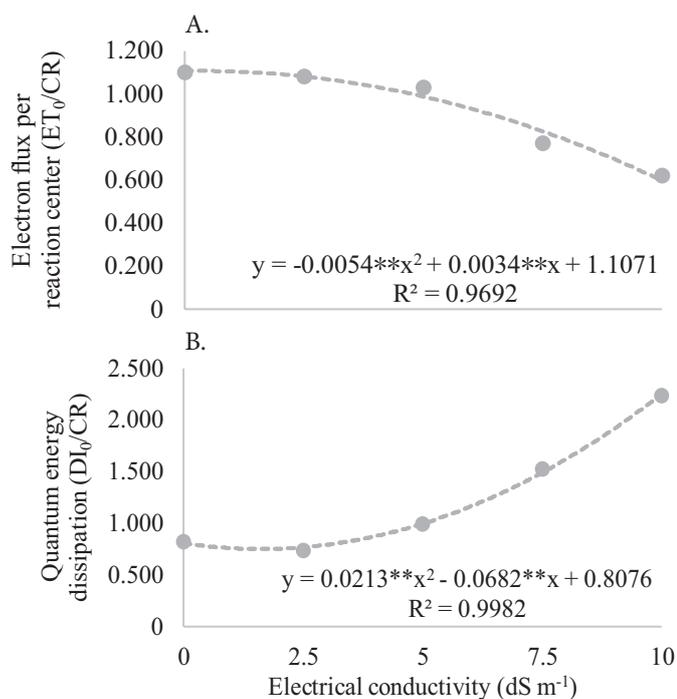
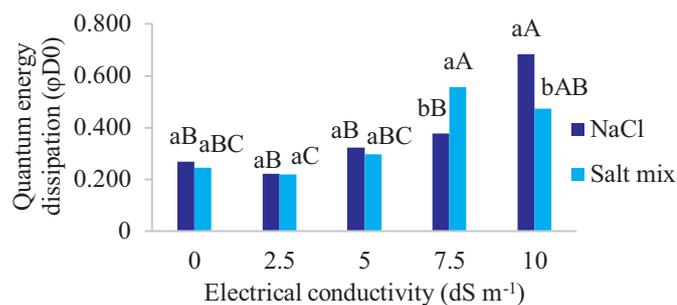


Figure 2. Electron flux per reaction center in sorghum plants submitted to irrigation with saline solutions at 60 days after sowing (DAS) (A); Energy dissipated by reaction center in sorghum plants under abiotic stress conditions at 60 DAS (B)

The quantum yield for heat dissipation (ϕD_0) showed significant interaction between salt sources and levels of electrical conductivity ($p < 0.05$). With the increase in salinity, it was observed that for both salt sources there was an increase in ϕD_0 (Figure 3), except for the EC of $2.5 dS m^{-1}$, which presented reductions of 17.29% (NaCl) and 10.82% (salt mix). The highest increments were observed for EC $10 dS m^{-1}$ (NaCl) and in EC $7.5 dS m^{-1}$ (salt mix), corresponding to 152.96 and 125.50%, respectively.



The same lowercase letters between water sources and upper case letters between treatments did not differ from each other, by Tukey's test ($p < 0.05$)

Figure 3. Quantum yield for heat dissipation in sorghum plants at 60 days after sowing (DAS) irrigated with saline solutions with increasing electrical conductivity levels

CONCLUSIONS

1. High salinity causes the degradation of photosynthetic pigments, both chlorophyll a and b and carotenoids in sorghum plants, in response to severe abiotic stress.
2. The increase in the electrical conductivity of the saline solutions used in the irrigation of the plants negatively affected the quantum efficiency of photosystem II and the electron flow

per reaction center, thus modifying the emission of chlorophyll a fluorescence, implying severe damage to the photosynthetic apparatus of the sorghum plants.

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