

## ANTIOXIDANT DEFENSES OF IRRIGATED FORAGE SORGHUM WITH SALINE AQUACULTURE EFFLUENT<sup>1</sup>

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**ABSTRACT** - The objective of this work was to evaluate the biomass production and antioxidant enzymatic system activity of irrigated forage sorghum with saline aquaculture effluent under different leaching fractions. The experiment was conducted in the Caatinga Experimental Field of the Embrapa Semiárido, in Petrolina, State of Pernambuco, Brazil. The experimental design was a complete randomized block in a split-plot arrangement with four replications, consisting of three forage sorghum varieties (Volumax, F305 and Sudan) and four leaching fractions (0, 5, 10 and 15%). The vegetal materials were collected when the plants were at the soft-dough stage. The biomass production and activity of the enzymes superoxide dismutase, catalase and ascorbate peroxidase were evaluated. Irrigation with saline aquaculture effluent with leaching fraction of 15% results in low salinity level in the root zone and higher biomass production of forage sorghum Sudan and F305, in semiarid conditions. The antioxidant system was activated in the three sorghum varieties to prevent accumulation of reactive oxygen species, with the synchrony between the enzymes superoxide dismutase and catalase resulting in a better productive response of the varieties Sudan and F305.

**Keywords:** Enzymes. Oxidative stress. *Sorghum bicolor* (L.). Moench.

## DEFESAS ANTIOXIDATIVAS DO SORGO FORRAGEIRO SOBRE O CULTIVO IRRIGADO COM EFLUENTE SALINO DE PISCICULTURA

**RESUMO** – Objetivou-se com este trabalho avaliar o desempenho da cultura do sorgo forrageiro irrigado com efluente salino da piscicultura sob diferentes frações de lixiviação em relação à produção de biomassa e a atividade do sistema enzimático antioxidativo. O estudo foi realizado no Campo Experimental Caatinga, pertencente à Embrapa Semiárido, em Petrolina – PE. O delineamento experimental foi blocos ao acaso, com quatro repetições, em parcelas subdivididas, composto por três variedades de sorgo forrageiro (Volumax, F305 e Sudão) e quatro frações de lixiviação (0; 5; 10 e 15%). A coleta do material vegetal foi realizada quando os grãos da porção central da panícula apresentaram aspecto leitoso a pastoso. Foi avaliada a produção de biomassa e a atividade das enzimas superóxido dismutase, catalase e ascorbato peroxidase. O uso de 15 % de fração de lixiviação para irrigação com efluentes salinos da piscicultura proporciona um menor nível de salinidade da zona radicular e promove uma melhor produção de biomassa do sorgo forrageiro Sudão e F305 em condições semiáridas. O sistema antioxidativo foi ativado nas três variedades de sorgo para evitar o acúmulo de ROS, sendo a sincronia entre as enzimas superóxido dismutase e catalase que refletiu numa melhor resposta produtiva das variedades Sudão e F305.

**Palavras-chave:** Enzimas. Estresse oxidativo. *Sorghum bicolor* (L.). Moench.

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<sup>1</sup>Received for publication in 07/18/2017; accepted in 03/27/2017.

Paper extracted from the masters dissertation of the first author

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## INTRODUCTION

Saline water is often the only water found in arid and semiarid regions. The greater the amount of salts in the water, the more severe is this abiotic stress and damage to plants, therefore, techniques to minimize this stress through irrigation is very important. Irrigation with saline water considering the leaching fraction aims to leach salts and prevent salinization in the root zone, which limits crop yield (ARAGÜESA et al., 2014).

More tolerant species and cultivars to the adverse conditions of semiarid regions have been used to increase the productive potential of these regions. In this context, forage sorghum production has increasing in recent years in the Brazilian semiarid region, since it is a typical hot climate plant, with xerophilous characteristics, low soil fertility requirement and tolerance to abiotic stresses, such as water deficit and salinity (GUIMARÃES et al., 2016; HEFNY; ABDEL-KADER, 2009).

Semiarid regions usually have high temperatures, water deficit and high salt rates in soil and water. These factors can cause mild to severe changes in plant metabolism, depending on the intensity and interaction between them (NILSEN; ORCUTT, 1996).

Saline stress causes morphological and physiological changes in plants, reducing their growth and triggering oxidative stress, which increase reactive oxygen species (ROS) (GILL; TUTEJA, 2010). ROS can damage macromolecules and cellular structures and lead to the plant death (BARBOSA et al., 2014). Plants have an efficient antioxidant defense mechanism to these physiological and biochemical effects, with activation of a complex enzymatic system (BARBOSA et al., 2014). Oxidative stress is an imbalance between the endogenous rates of antioxidant and oxidant compounds (ROS) (SHARMA et al., 2012).

The antioxidant system of plants produces a large number of compounds that regulate redox homeostasis, such as the superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (MILLER et al., 2010). According to Gill and Tuteja (2010), SOD is the first defense against ROS, with dismutation of the superoxide radical ( $O_2^{\cdot-}$ ) to form hydrogen peroxide ( $H_2O_2$ ) and molecular oxygen ( $O_2$ ). This  $H_2O_2$  is then converted to  $H_2O$  by the APX and CAT, since  $H_2O_2$  accumulation is also toxic to plants. The balance of these enzymes is essential for homeostasis. However, the increase in the antioxidant system activity alone does not result in high crop yields, as reported by Hefny and Abdel-Kader (2009), who evaluated 26 sorghum genotypes.

In this context, the objective of this work was to evaluate the biomass production and antioxidant

enzymatic system activity of irrigated forage sorghum with saline aquaculture effluent under different leaching fractions.

## MATERIAL AND METHODS

The study was conducted in the Caatinga Experimental Field of the Embrapa Semiarido, in Petrolina, State of Pernambuco, Brazil, in the Sub-mid São Francisco Valley ( $9^{\circ}8'8.9''S$ ,  $40^{\circ}18'33.6''W$  and altitude of 373 m) from February to July 2013. The climate of the region is classified as semiarid, type BSw $h'$ , according to the classification of Köppen. The experimental period had average relative air humidity of 63.86%, average temperature of 25.46 °C, maximum evapotranspiration of 6.97 mm  $dia^{-1}$  (average of 5.85 mm  $day^{-1}$ ), main precipitation events concentrated in the first 10 days after planting (DAP) and at the 74<sup>th</sup> DAP, totaling 32.7 mm.

The experimental design was a complete randomized block in a split-plot arrangement with four replications, consisting of three forage sorghum varieties (Volumax, F305 and Sudan) and four leaching fractions (0, 5, 10 and 15%). Each experimental unit (subplot) was formed by five 5-meter rows in an area of 5.0 x 0.50 m (12.5 m<sup>2</sup>) with 10 plants per linear meter, considering the plants within the central 3-meters of the rows for evaluation.

The soil of the experimental area was classified as a Red Yellow Argissolo (EMBRAPA, 2013) (Ultisol) of medium texture and plane relief. Soil preparation was according to the crop requirements, with plowing and harrowing. Soil fertilization was based on a previous soil analysis (Table 1), with application of nitrogen (30 kg  $ha^{-1}$ ), phosphorus (60 kg  $ha^{-1}$ ) and potassium (20 kg  $ha^{-1}$ ). An additional nitrogen fertilization (30 kg  $ha^{-1}$ ) was performed 30 DAP. Sowing was carried out in April 2013, and seedling emergency occurred at 7 DAP. A manual weeding was performed at 30 DAP and preventive application of insecticide was carried out at 40 and 60 DAP.

Irrigations were carried out daily with saline aquaculture effluent through a surface drip irrigation system connected to fish tanks containing black tilapia (population density of 40 fish  $m^{-3}$ ). Fifty percent of the water of the tanks were pumped daily to storage tanks for irrigation, and the tanks were replenished. The chemical characteristics of the aquaculture effluent used for irrigation were determined and its electrical conductivity (EC) was monitored with a portable digital conductivity meter, which remained at approximately 2.57 dS  $m^{-1}$  (Table 2).

**Table 1.** Soil chemical, physical and granulometry parameters of the experimental area.

Layer cm	EC	pH	OM	P	K	Na	Ca	Mg	Al	H+Al	SB	CEC	V
	dS cm <sup>-1</sup>		g kg <sup>-1</sup>	mg dm <sup>-3</sup>	cmol <sub>c</sub> dm <sup>-3</sup>						%	%	
0 - 10	1.64	5.5	7.7	15.65	0.65	0.8	2.8	1.5	0	2.1	5.8	7.8	73.4
10 - 20	1.99	5.7	5.7	14.25	0.55	0.65	1.9	1.3	0	2.7	4.4	7.1	61.8
20 - 45	2.91	7.4	6.3	3.6	2.05	1.5	1.8	1.4	0	3.7	6.8	10.4	64.7
Layer (cm)	Density (kg dm <sup>-3</sup> )		Total Porosity (%)	Granulometry (g kg <sup>-1</sup> )			Moisture (dag kg <sup>-1</sup> )						
	Soil	Particle		Sand	Silt	Clay	FC	PWP					
0 - 10	1.46	2.59	43.86	729.4	182.9	87.7	0.186	0.075					
10 - 20	1.46	2.51	41.74	789.8	116.9	93.3	0.179	0.070					
20 - 45	1.37	2.52	45.58	613.2	178.1	208.7	0.181	0.110					

EC = electrical conductivity of the saturation extract; OM = organic matter; P = available phosphorus extracted by Mehlich-1; Ca = exchangeable calcium; Mg = exchangeable magnesium; Na = exchangeable sodium; K = exchangeable potassium; Al = exchangeable acidity; SB = sum of bases; CEC = cation exchange capacity at pH 7.0; V = base saturation; FC = field capacity; PWP = permanent wilting point.

**Table 2.** Chemical characteristics of the aquaculture effluent used for irrigation.

Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>	pH	Electrical conductivity at 25°C	Hardness CaCO <sub>3</sub>	Sodium adsorption ratio
mmolc L <sup>-1</sup>						dS m <sup>-1</sup>	mg L <sup>-1</sup>	
12.6	7.7	7.2	0.34	35.2	8.19	2.57	50.75	2.26

Irrigation water depths were calculated according to crop evapotranspiration (ETo×Kc×Kl), using the Kc indicated by FAO (2006), measured in the period between irrigations, according to the water application efficiency of the system and the leaching fractions tested, using the Equation 1,

$$Wd = \frac{(ETo \times Kc \times Kl) - P}{Ef} \times (1 + LF)$$

in which Wd is the water depth (mm), ETo is the evapotranspiration measured in the period (mm), Kc is the crop coefficient, Kl is the coefficient of localization (BERNARDO et al., 2006), P is the precipitation measured in the period (mm), Ef is the efficiency of the irrigation system (0.9) and LF is the leaching fraction applied (decimal).

Soil moisture was monitored with PR2 probes (Profile Probe PR2, Delta-T Devices Ltd), which are based on frequency domain reflectometry (FDR), previously configured to measure soil moisture at depths of 10, 20, 30, 40, 60 and 100 cm. Moisture readings were performed weekly, approximately two hours after each irrigation. Simple soil samples from the layers 0-5, 5-20, 20-40, 40-60 and 60-80 cm were collected when the sorghum was harvested to determine the EC through the saturated soil paste extract in each layer.

The vegetal material was collected for biochemical analyzes in July, when the plants were

at the soft-dough stage. Samples of the leaf blade of the third fully expanded leaf from the apex were collected, stored in aluminum foil envelopes and immersed in liquid nitrogen (N<sub>2</sub>), then, they were stored at -80°C until the enzymatic analyzes.

The plants were harvested shortly after the leaf material was collected to determine their shoot biomass production. Plants from the evaluation area of the plots were cut at 10 cm from the soil, weighted to determine their fresh biomass, and dried in an oven at 60 °C to a constant weight, to determine their dry biomass.

The catalase (CAT) activity was assessed by a method based on Havir and Mchale (1987). A solution of 1.0 mL of potassium phosphate buffer (100 mM at pH 7.5) and 25 µL of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (1.0 mM) reacted with 25 µL of the protein extract. The CAT activity was determined by the decomposition of H<sub>2</sub>O<sub>2</sub> for 60 seconds with spectrophotometric readings at 240 nm and 25 °C.

The ascorbate peroxidase (APX) activity was determined as described by Nakano and Asada (1981). A solution of 650 µl of potassium phosphate buffer (80 mM at pH 7.5), 100 µl of ascorbate (5.0 mM), 100 µl of EDTA (1.0 M), 100 µL of H<sub>2</sub>O<sub>2</sub> (1.0 mM) reacted with 50 µL of the protein extract. The APX activity was determined by monitoring the ascorbate oxidation rate for 60 seconds using a spectrophotometer at 290 nm and 30 °C.

The superoxide dismutase (SOD) activity was determined by the protocol of Giannopolitis and Ries

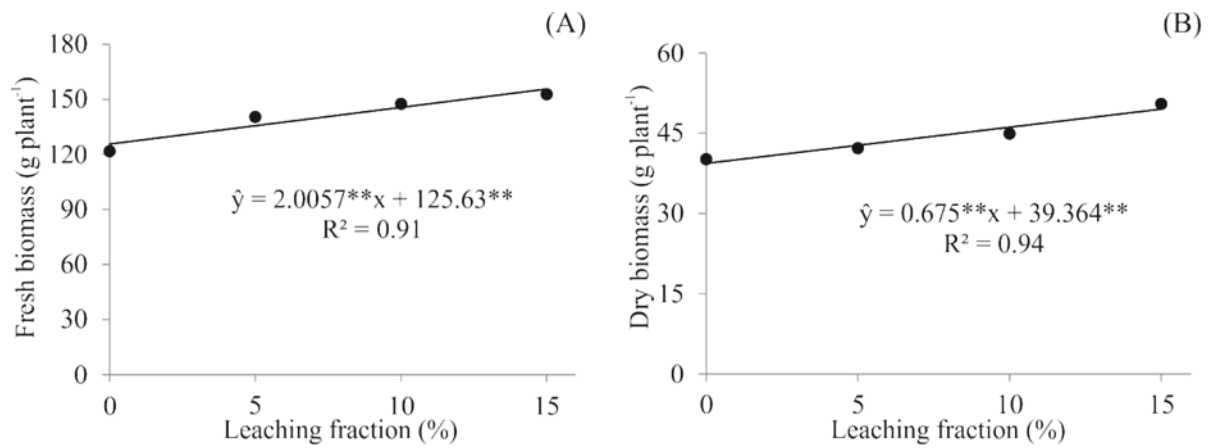
(1977), determining the inhibition of the reduction of nitro blue tetrazolium (NBT) by the enzyme extract to avoiding formation of chromophore. A solution (3.0 mL) of consisted of 85 mM phosphate buffer (pH 7.8), 75 µM of NBT, 5.0 µM of riboflavin, 13.0 mM of methionine, 0.1 mM EDTA reacted with 50 µl of the enzyme extract. This solution was placed in glass tubes, irradiated by a white light (15-W fluorescent lamp) for 5 min and then analyzed in a spectrophotometer at 560 nm.

The data were subjected to analysis of variance (ANOVA), test of means (Tukey) and regression, using the program Sisvar 5.0. When significant interactions between varieties and leaching fractions were found, the variables within each factor were considered, otherwise the independent effect of the factors on the variables was

considered.

**RESULTS AND DISCUSSION**

The shoot fresh and dry biomass of the plants increased with increasing leaching fractions (Figure 1). Increases in dry biomass as a function of leaching fractions has been reported in peanut (SANTOS et al., 2012), sorghum (GUIMARÃES et al., 2016) and maize (CARVALHO et al., 2012), and increases in fresh and dry biomass was also found by Carvalho Junior et al. (2010), who describe the irrigation management with leaching fractions as an efficient alternative to make irrigation with saline water possible.



**Figure 1.** Shoot fresh (A) and dry (B) biomass of irrigated forage sorghum with saline aquaculture effluent, subjected to different leaching fractions.

However, the crop tolerance level to salt to determine the appropriate leaching fraction are directly related to the irrigation water salinity and the efficiency of this application depends on physical-chemical and biological interactions of agricultural systems (LETEY et al., 2011). A proper irrigation management is essential for crop efficiency, since high leaching fractions can reduce crop yield, as observed by Carvalho et al. (2012),

who evaluated irrigated corn yield with saline water (3.3 dS m<sup>-1</sup>), applying leaching fractions of up to 20%.

The variety Sudan stood out with the highest fresh biomass production, and the variety F305 with the highest dry biomass production. The variety Volumax had the lowest fresh and dry biomass (Table 3).

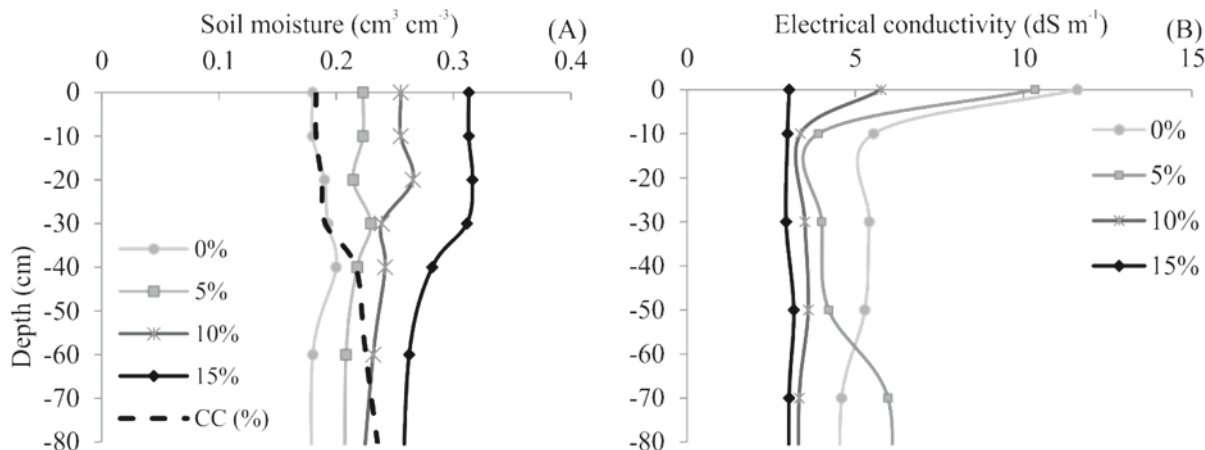
**Table 3.** Average fresh and dry biomass production of irrigated forage sorghum varieties with saline aquaculture effluent.

Variety	Fresh Biomass	Dry Biomass
	g plant <sup>-1</sup>	
Volumax	126.19 b	38.86 b
F305	141.40 ab	49.88 a
Sudão	154.42 a	44.55 ab

Means followed by the same letter in the columns do not differ by the Tukey's test at 5% probability.

Treatments with leaching fractions of 5, 10 and 15% increased the water availability in the soil layer 0-40 cm to 0.02, 0.05 and 0.11  $\text{cm}^3 \text{cm}^{-3}$ , respectively, above the field capacity (FC) (Figure 2). This result denotes the low water flow in this soil, which hinders the drainage and leaching of salts,

thus making the application of leaching fractions an even more important factor for irrigated sorghum crops with saline water, since about 80% of its effective root system is distributed in the first 30 cm of soil (MAGALHÃES et al., 2000).



**Figure 2.** Moisture (A) and electrical conductivity (B) along the soil profile under crops of irrigated forage sorghum varieties with saline aquaculture effluent, subjected to leaching fractions of 0, 5, 10 and 15%. FC = moisture at field capacity.

The largest leaching fractions had the lowest electrical conductivity (EC) (Figure 2). Studies on beetroot (SIMÕES et al., 2016) and peanut (SANTOS et al., 2012) crops also showed increases in leaching fraction reducing EC and sodium accumulation in the surface layer, and improving salt distribution in the soil profile, confirming the use of leaching fractions as an effective practice to reduce excess soluble salts in the root zone of crops. Reducing EC of the soil increases the area available to plant roots, thus reducing the stress caused by the accumulation of salts.

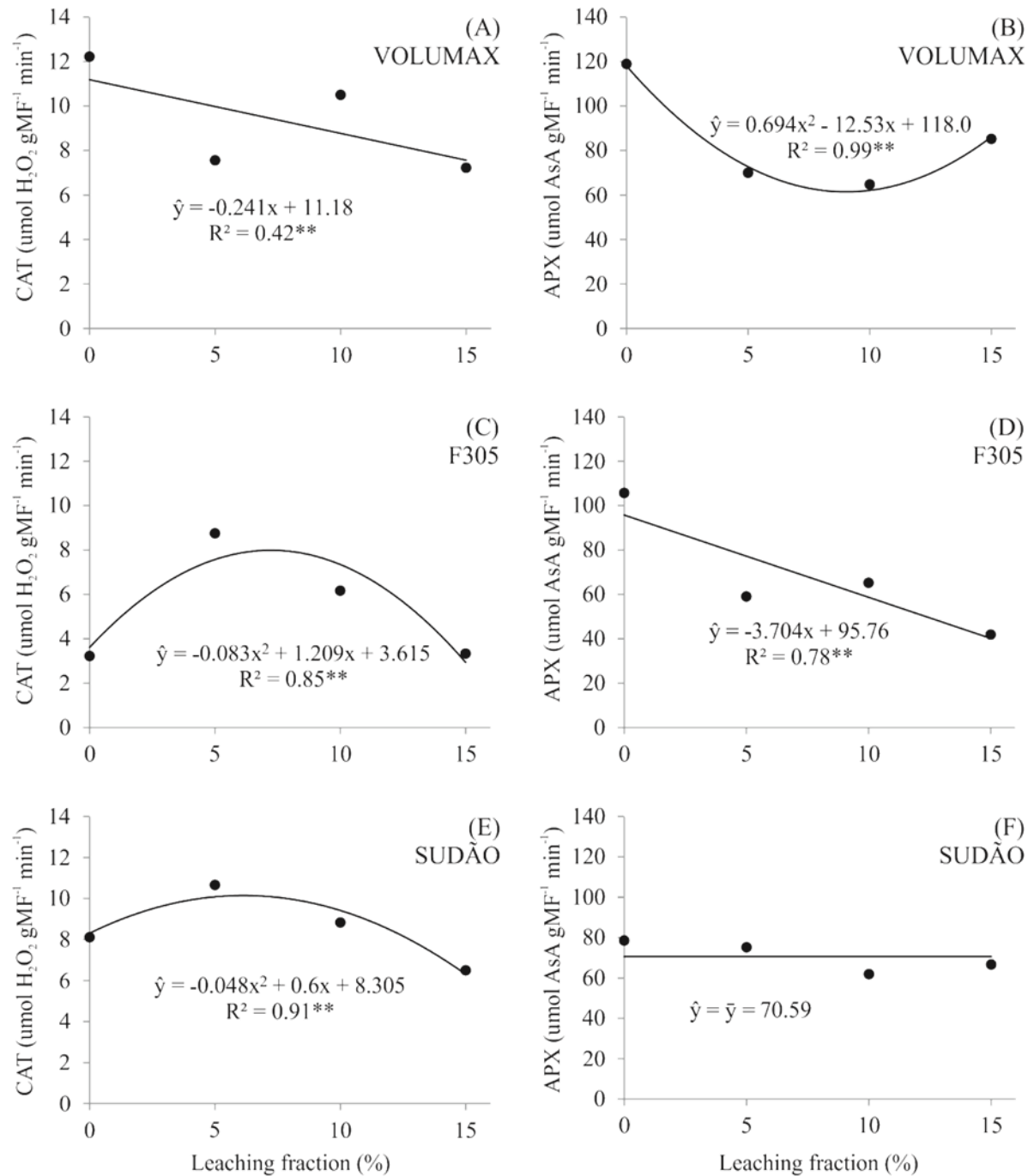
Reducing EC of the soil with high leaching fractions resulted, in general, in a lower activity of the antioxidant enzymes catalase (CAT) and ascorbate peroxidase (APX) (Figure 3). An additional daily irrigation leach salts accumulate in the root zone, since the soil retains water only until reaching field capacity (FC) (ASSOULINE; OR, 2014). Thus, this decrease in soil salinity reduces the stress intensity and consequently, the generation of reactive oxygen species (ROS), decreasing the need for activation of the enzyme antioxidant defense systems.

The low activity of CAT in high EC, as found with the sorghum variety F305 in treatment without leaching fraction ( $\text{CE} > 5 \text{ dS m}^{-1}$ ) was also found with the variety CSF-20 subjected to salinity of 4.0 to 8.0  $\text{dS m}^{-1}$  (FREITAS et al., 2011). Similar results were found in *Boehmeria nivea* plants subjected to progressive levels of NaCl (2 to 8  $\text{g kg}^{-1}$ ) in the soil (HUANG et al., 2014). The activity of CAT in the varieties Sudan and F305 increased with salinity, however, subsequently decreasing with increasing

salt stress, confirming that the activity of CAT in low saline stress (4  $\text{g kg}^{-1}$  NaCl) could extinguish  $\text{H}_2\text{O}_2$ , while under high stress (6  $\text{g kg}^{-1}$  NaCl), the capacity of this enzyme to eliminate  $\text{H}_2\text{O}_2$  is reduced (HUANG et al., 2014). Very high concentrations of NaCl, especially above 100 mM, cause inhibition of a wide range of enzymes (MUNNS et al., 2002).

The enzyme APX is also responsible for eliminate  $\text{H}_2\text{O}_2$ . The difference in the activity of the enzymes CAT and APX in the three varieties evaluated denotes the different behavior of these varieties in saline environments.

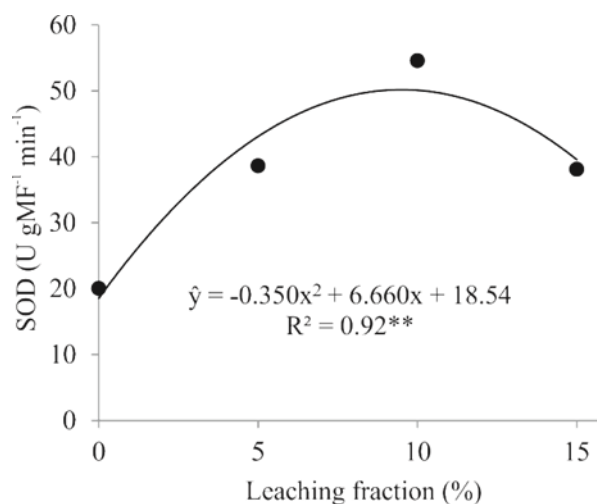
The APX activity in the variety F305 decreased with increasing leaching fractions, denoting a gradual reduction in plant stress. The APX activity did not vary depending on the treatments in the variety Sudan, denoting that this enzyme was not affected in these saline levels. That APX activity in the variety Volumax, which had the lowest biomass production, was inversely proportional to SOD (Figure 4), this asynchrony between the enzymes APX and SOD indicates a probable accumulation of  $\text{O}_2^{\cdot-}$ . The low activity of SOD in this cultivar (Table 4) and the high activity of APX and CAT, which eliminate  $\text{H}_2\text{O}_2$ , indicate the existence of other sources of  $\text{H}_2\text{O}_2$ , especially without leaching fraction. The low SOD activity without leaching fraction can be an important parameter, since it results in accumulation of  $\text{O}_2^{\cdot-}$  and, like other ROS, causes oxidation of proteins, amino acids, nucleic acids and carbohydrates, resulting in cell damage (SHARMA et al., 2012) and reduced crop yields.



**Figure 3.** Activity of catalase (CAT-A, C and E) and ascorbate peroxidase (APX-B, D and F) in irrigated forage sorghum varieties with saline aquaculture effluent, subjected to different leaching fractions.

The SOD regression curve of the varieties Sudan and F305 (Figure 4), as well as the curve of the CAT (Figure 3) were parabolic, denoting the synchrony of these enzymes. The highest biomass production of these varieties (Table 3) coincided with the highest SOD activity (Table 4), and the lowest biomass production coincided with the lower activities of SOD and CAT, which occurred without leaching fraction, resulting in a higher accumulation of salts in the soil. Carrasco-Ríos and Pinto (2014) and Gill and Tuteja (2010) also found high SOD activities for more tolerant genotypes to salinity.

Although SOD is not the only H<sub>2</sub>O<sub>2</sub>-producing enzyme in plant tissues, the balance between the activity of this enzyme and those responsible for the elimination of H<sub>2</sub>O<sub>2</sub> in the cells is considered essential for the balance between O<sub>2</sub><sup>•-</sup> and H<sub>2</sub>O<sub>2</sub> levels, preventing the formation of hydroxyl radical (OH<sup>•</sup>) (GILL; TUTEJA, 2010). The relationship between SOD activity and H<sub>2</sub>O<sub>2</sub> elimination in sorghum is an important biochemical marker to determine the tolerance of this species to salinity (COSTA et al., 2005).



**Figure 4.** Enzymatic activity of superoxide dismutase (SOD) in irrigated forage sorghum varieties with saline aquaculture effluent, subjected to different leaching fractions.

**Table 4.** Enzymatic activity of superoxide dismutase (SOD) in irrigated forage sorghum varieties with saline aquaculture effluent.

Variety	SOD Activity (U gMF <sup>-1</sup> min <sup>-1</sup> )
Volumax	25.03 b
F305	45.97 a
Sudão	42.44 a

Means followed by the same letter in the columns do not differ by the Tukey's test at 5% probability.

The enzymatic synchrony of SOD and CAT, observed in the varieties Sudan and F305, is essential to regulate the level of ROS produced in the plant cell, since as the O<sub>2</sub><sup>•-</sup> are generated, dismutation occurs by SOD to H<sub>2</sub>O<sub>2</sub>, which is eliminated by the CAT, converting it to water and oxygen.

The efficiency of this process reduces the level of oxidative stress (ASHRAF, 2009). Researches with the sorghum variety IPA-1011 in saline conditions indicate that activity of APX is not important to protect this variety against oxidative stress damage (OLIVEIRA et al., 2012).

## CONCLUSIONS

Irrigation with saline aquaculture effluent with leaching fraction of 15% results in lower salinity level in the root zone and higher biomass production of forage sorghum Sudan and F305, in semiarid conditions. The antioxidant system was activated in the three sorghum varieties evaluated to prevent accumulation of reactive oxygen species, with the synchrony between the enzymes superoxide dismutase and catalase resulting in a better productive response of the varieties Sudan and F305.

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