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Growth and photosynthetic parameters of saccharine sorghum plants subjected to salinity

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ABSTRACT. Plants are often exposed to abiotic stresses such as salinity, which represents a barrier to the cultivation of agricultural species mainly in arid and semi-arid regions. This study evaluated the development of four saccharine sorghum genotypes for tolerance to different salinity levels under greenhouse conditions. The experimental design was a randomized complete block arranged in a 5 × 4 factorial scheme, which corresponded to five levels of irrigation water salinity [electrical conductivities of 0.5 (control), 2.5, 5.0, 7.5, and 10.0 dS m^{-1}] and four saccharine sorghum genotypes (CSF 11, CSF 15, P 76 and P 298), with five replicates. The plants were evaluated for dry mass production, leaf area, height, stalk diameter, leaf gas exchange and relative chlorophyll content. The growth and leaf gas exchange measurements of the saccharine sorghum plants were significantly affected by salinity stress. Among the genotypes, CSF 11 and CSF 15 exhibited smaller reductions in growth, whereas P 298 showed the greatest reduction. These findings show that genotype CSF 11 can be classified as the most tolerant to salt stress, and genotype P 298 is the most sensitive.

Keywords: *Sorghum bicolor* (L.); leaf gas exchange; salt stress; salt tolerance.

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Introduction

Plants are often exposed to biotic and abiotic stresses that hinder their growth, development and productivity. Salinity is among the most limiting environmental stresses of plant productivity, affecting the overall metabolism and causing morphological and physiological changes. This problem is most pronounced in arid and semi-arid regions, where vast areas of soil affected by salts are concentrated (Beltrán, 2016).

Salinity stress affects all aspects of plant physiology and metabolism (Munns & Tester, 2008). The deleterious effects of salinity on plant growth are associated with the reduced osmotic potential of the soil solution (water stress), nutritional disturbances, toxic effects of some ions (ionic stress) and combinations of these factors (Saffan, 2008). Salinity affects plant growth, inhibiting both cell division and expansion (Julkowska & Testerink, 2015), which is generally the first effect of salinity on plant development.

The inhibition of plant growth and productivity caused by salinity is attributed to the imbalance in the water and nutritional relationships of plants and to the accumulation of ions considered toxic (Panuccio, Jacobsen, Akhtar, & Muscolo, 2014). However, the mechanisms of salinity tolerance are not yet well elucidated because they are extremely complex and involve morphological, physiological and biochemical alterations (Willadino & Camara, 2010). Thus, the manners by which plants respond to salt stress conditions differ greatly between species and between genotypes of the same species.

Saccharine sorghum [*Sorghum bicolor* (L.) Moench] is a grass with a high forage potential and resembles sugarcane due to the presence of succulent stems with elevated levels of fermentable sugars. This crop species presents advantages such as a rapid cropping cycle (four months), fully mechanizable culture, and grain yields of approximately 2.5 t ha^{-1}, and its bagasse is used an energy source for industrialization or animal feed. Additionally, this crop can be used to supply raw materials during sugarcane harvest to distilleries and thus can be used to strengthen national ethanol production and reduce distillation periods (Parrella, 2011).

Sorghum is cultivated mainly in arid and semi-arid regions and is a species well suited to environments exposed to abiotic stresses, particularly air temperature and soil moisture stresses (Lourenção & Bagega, 2012). It is a crop species that is considered moderately tolerant to salt stress. However, research has shown that the degree of sorghum salt tolerance varies among genotypes (Lacerda, Cambraia, Cano, Ruiz, & Prisco, 2003).

Sorghum is a crop with potential use in Brazil and in the Northeast, the latter of which is characterized by a semi-arid climate. However, for the success of the cultivation of this species, information about salinity tolerance is essential for the development and correct planning of the crop. The objective of this work was therefore to evaluate the salt tolerance of saccharine sorghum genotypes and the effects of this stress on the photosynthetic apparatus of this crop species.

Material and methods

The experiment was conducted in a greenhouse at the Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, located at Campus do Pici, Fortaleza, Ceará, Brazil (3°44'S, 38°34'W). Seeds of four saccharine sorghum [*Sorghum bicolour* (L.) Moench] genotypes were used: CSF 11, CSF 15, P 76, and P 298. The seeds were provided by the Instituto Agronômico de Pernambuco (IPA), Recife, Pernambuco State, Brazil.

After seed selection, sowing was performed in 8-L pots that contained a mixture of sand and humus (2:1). After seven days of sowing, thinning was performed, leaving two plants per pot. On the same day, the application of salinity treatments, corresponding to electrical conductivities of the irrigation water (EC_w) of 0.5 (control), 2.5, 5.0, 7.5, and 10.0 dS m^{-1}, was started. The treatments were distributed in a randomized block design in a 5 × 4 factorial scheme. There were five salinity treatments (0.5, 2.5, 5.0, 7.5, and 10.0 dS m^{-1}), four sorghum genotypes (CSF 11, CSF 15, P 76, and P 298), and five replicates; each replicate consisted of of two plants.

For the preparation of the saline solutions, NaCl, $CaCl_2{\cdot}2H_2O$, and $MgCl_2{\cdot}6H_2O$ were dissolved in irrigation water, maintaining an equivalent Na:Ca:Mg ratio of 7:2:1 and following the relationship in which $mmol_c\ L^{-1} = EC_w \times 10$ according to the methods of Rhoades, Kandiah, and Mashali, (1992). To avoid excessive accumulation of salts, the irrigation water was applied according to the drainage principle (Bernardo, Soares, & Mantovani, 2008), maintaining the soil at field capacity and adding leaching fractions that ranged from 0.15 to 0.20. Furthermore, a gravel layer of approximately 2 cm thickness was placed at the bottom of each vase to facilitate drainage. The water was applied in accordance with local practices, using a 2 day irrigation shift. To correct any nutritional deficiencies in the soil, approximately 400 mL of Hoagland nutrient solution was applied once per week to each pot until the end of the experiment.

Before harvest and 40 days after the beginning of treatments, measurements of photosynthesis, transpiration, stomatal conductance and internal CO_2 concentration were performed in the morning (9:00–11:00 a.m.). These measurements were performed using an infrared gas analyser (IRGA, LI-6400XT, LI-COR, Inc., Lincoln, Nebraska, USA) equipped with an artificial radiation source with an intensity adjusted to 2000 μmol m^{-1} s^{-1}. The measurements were performed on the first fully expanded leaf from the apex, with only one plant per pot being used. The relative levels of chlorophyll were determined using a non-destructive method with a portable chlorophyll meter (SPAD-502, Minolta, Osaka, Japan). The measurements were performed on the same leaf used to measure gas exchange. For each plant, an average of three readings were performed on the leaf, and the results were expressed in SPAD index values.

Forty-five days after the beginning of the treatments, the sorghum plants were collected, and height and stem diameter were measured. The plants were then partitioned into leaves, stems + sheaths and roots, after which the leaf area was measured (LI 3100 area meter, Li-Cor, Inc., Lincoln, Nebraska, USA). The material was oven dried to determine the dry mass of the leaves (DML), dry mass of the shoots (DMS), dry mass of the roots (DMR), and total dry mass (DMT).

To define the degree of tolerance to salinity, the method described by Fageria (1985) was used. The per cent reductions in DMT relative to those of the control were calculated and used as indices to compare the tolerance of the four sorghum genotypes. The tolerance was classified as follows: tolerant (0–20% reduction), moderately tolerant (20–40% reduction), moderately sensitive (40–60% reduction), and sensitive (> 60% reduction).

The data were evaluated by analysis of variance (ANOVA) using the F test at 5% probability, and after the regression analysis, the data were analysed using Sisvar 5.0 software.

Results and discussion

Growth analysis

The growth of sorghum plants was negatively affected by salt the treatments. With increasing salinity level, there was a marked decrease in all growth variables analysed. Regarding the DML, for example, all the genotypes studied were affected by salinity, but CSF 11 and CSF 15 showed the lowest decrease in this variable; in which, at the highest salinity dose, these genotypes exhibited values that were higher than those of the other genotypes (Figure 1A). Salinity also inhibited leaf area expansion in all evaluated genotypes (Figure 1B). However, similar to that observed for the DML, among the genotypes, P 76 and P 298 presented the lowest leaf area values as a function of increasing levels of salinity stress. Freitas, Alencar, Lacerda, Prisco, and Gomes-Filho (2011) also observed reductions in leaf area of sorghum plants exposed to salinity levels whose EC_w values were 4.0 and 8.0 dS m^{-1}. This decrease is probably an adaptive mechanism of the plants to salinity stress; the mechanism conserves water by reducing the transpiration surface area (Tester & Davenport, 2003).

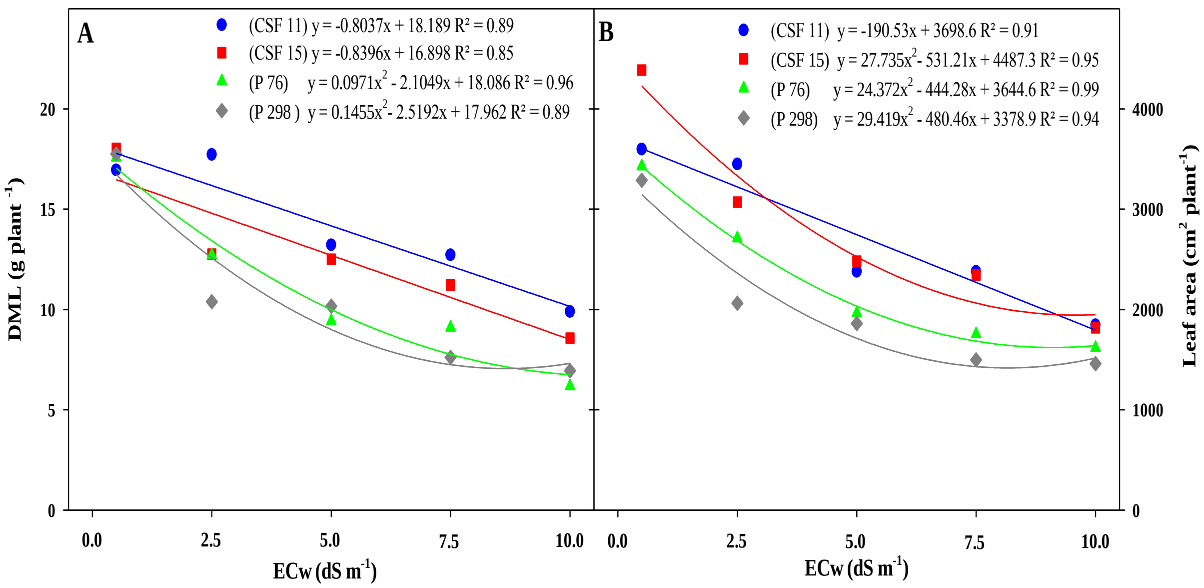

Figure 1. Dry mass of leaves (DML, A) and leaf area (B) of four saccharine sorghum genotypes (CSF 11, CSF 15, P 76, and P 298) exposed to increasing levels of salinity for 45 days; the values are expressed in terms of the electrical conductivity of the irrigation water (EC_w).

For DMS and DMR (Figure 2A and B), a marked decrease was observed in all genotypes studied, but the CSF 11 genotype showed the highest values at the maximum salt stress level investigated. These results were confirmed by the DMT data (Figure 2C), which were similar to the DMS data. The DMS/DMR ratio increased with increasing salinity in all genotypes studied, among which, P 298 and CSF 15 had the highest and lowest ratios, respectively, when exposed to the maximum salinity stress level (Figure 2D).

These results showed a typical response of glycophytes exposed to the salinity, e.g., a significant decrease in dry matter of various organs of the plants (Zhonghua, Yanju, Xiaorui, & Ynangang, 2011; Feijão et al., 2013). Other authors have also reported reductions in the growth of sorghum plants subjected to salinity stress (Aquino, Lacerda, Bezerra, Gomes-Filho, & Costa, 2007; Sousa et al., 2010; Miranda, Alvarez-Pizarro, Araújo, Prisco, & Gomes-Filho, 2013). According to many authors, salinity stress reduces plant growth due to osmotic, toxic and nutritional effects. According to Muuns and Tester (2008), excess salt reduces the development of the plant due to osmotic effects, which hinder the absorption and transport of water in soil-plant systems; these phenomena occur in addition to the energy costs of osmotic and biochemical adjustment mechanisms, which are necessary for the survival of plants under stress.

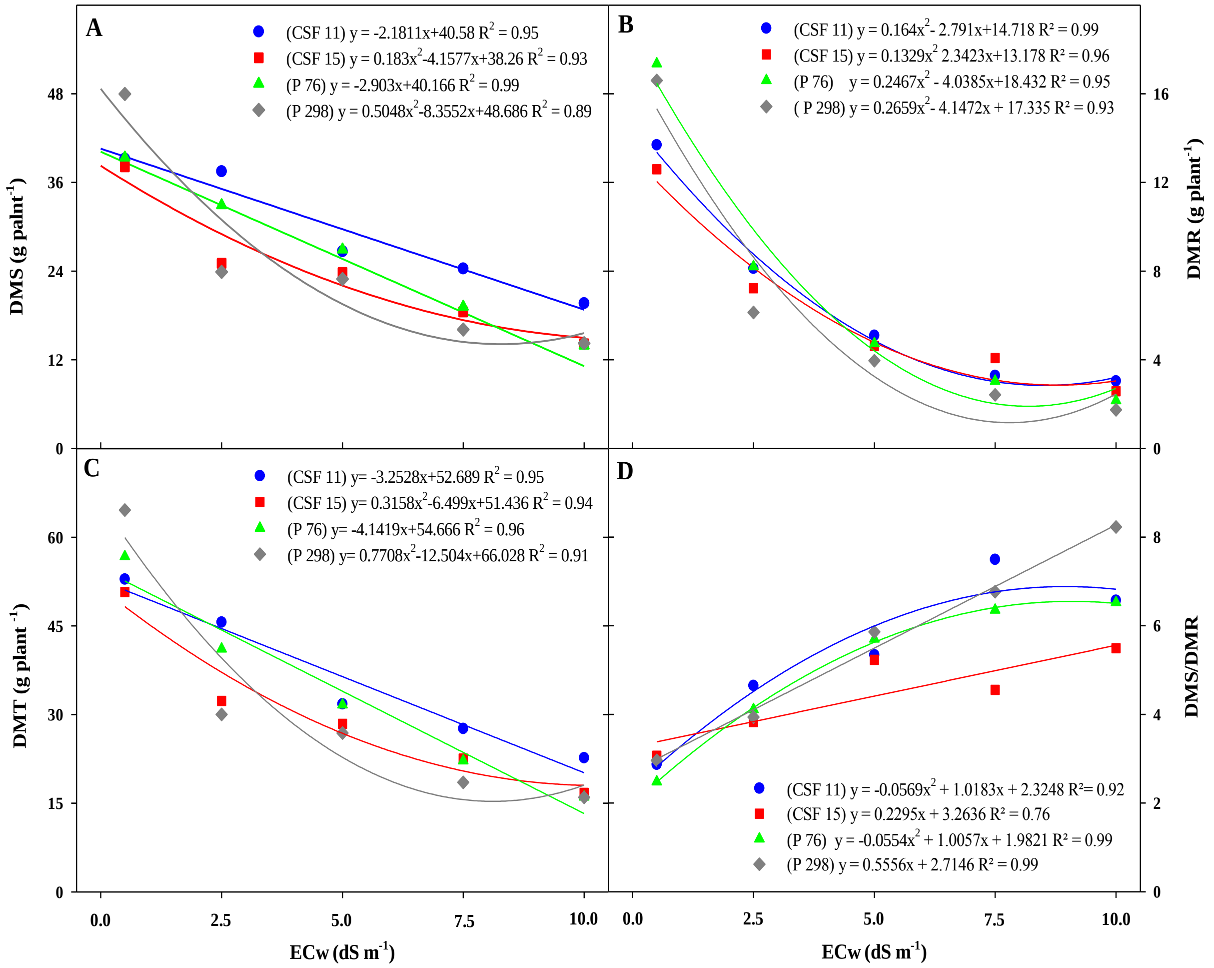

Figure 2. Dry mass of shoots (DMS, A), dry mass of roots (DMR, B), total dry mass (DMT, C) and the relations between the dry mass of shoots and roots (DMS/DMR, D) of four saccharine sorghum genotypes (CSF 11, CSF 15, P 76, and P 298) exposed to increasing levels of salinity for 45 days; the values are expressed in terms of the electrical conductivity of the irrigation water (EC_w).

The gradual increase in the DMS/DMR ratio with increasing irrigation water salinity (Figure 2D) conflicts with the results of several studies carried out under controlled conditions or in field conditions, in which it is accepted by most authors that the aerial parts of the plants are usually more sensitive to salt stress (Cruz, Coelho Filho, Coelho, & Santos, 2017). Conversely, a greater reduction in the root growth of plants exposed to salinity stress was also observed in the sorghum genotype CSF 20 (Sousa et al., 2010) and in rice (Rodrigues et al., 2005), and it is possible to suggest that the mechanisms of acclimatization to stress differ between species and possibly depend on the growth conditions.

Plant height was also affected by salinity stress, decreasing as stress levels increased (Figure 3A). Genotype P 298 was affected the most by salinity stress and showed a considerable decrease in plant height when subjected to increasing salinity levels. The results showed that this genotype presented a greater height under control conditions (0.5 dS m^{-1}), but at the highest salt concentration (EC_w of 10 dS m^{-1}), its height practically equalled that of the other genotypes. According to Tavakkoli et al. (2011), the most common effect of salinity on plants is growth limitation, due to the increase in osmotic pressure of the medium and, consequently, the reduction in water availability, which affects cell division and elongation.

At the maximum level of salinity stress, the genotypes P 76 and P 298 expressed the smallest stem diameters, whereas the genotypes CSF 11 and CSF 15 presented the largest diameters. Nonetheless, genotype CSF 15 was the most affected by salinity and presented the greatest decrease in stem diameter among the genotypes, with a 36.6% reduction at the highest level of stress compared to that of the control (Figure 3B).

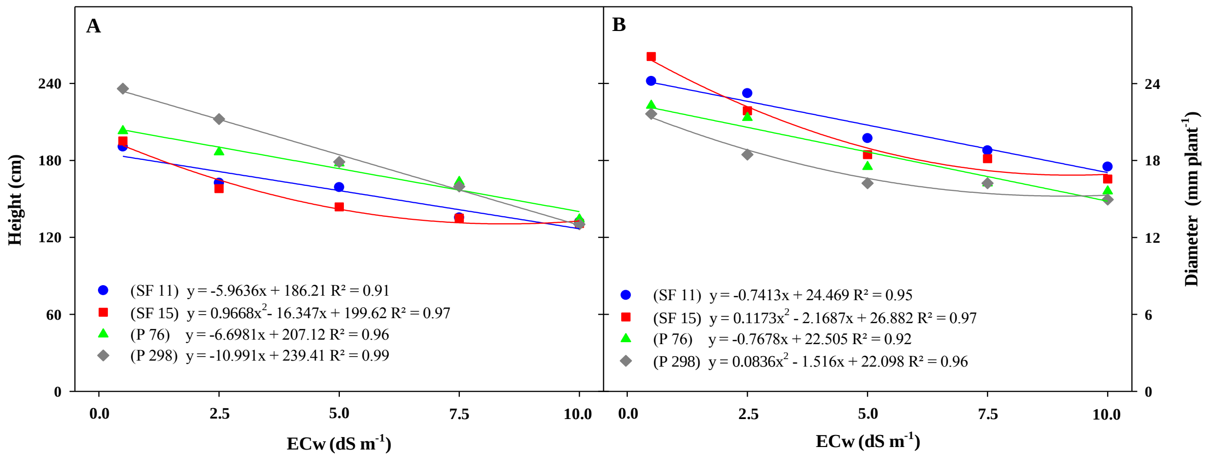

Figure 3. Height (A) and stem diameter (B) of four saccharine sorghum genotypes (CSF 11, CSF 15, P 76, and P 298) exposed to increasing levels of salinity for 45 days; the values are expressed in terms of the electrical conductivity of the irrigation water (EC_w).

Leaf gas exchange and relative chlorophyll content

Both photosynthesis and cell growth are affected primarily by abiotic stresses such as drought and salinity stresses (Gupta & Huang, 2014). In general, except the internal CO_2 concentration (*Ci*), the leaf gas exchange measurements decreased for all sorghum genotypes as the salinity levels increased (Figure 4). For example, in all genotypes evaluated, the stomatal conductance (*gs*) decreased as salinity increased (Figure 4A). However, it can be observed that the CSF 11 genotype presented the greatest reduction, which may have occurred because it was a point measurement. Similarly, the transpiration rate (*E*) also decreased with salinity in all genotypes studied (Figure 4B). Regarding the photosynthetic rate (*A*) of the plants, the sorghum genotypes were negatively affected by increasing salinity rates (Figure 4C). For the three gas exchange variables mentioned above, genotype P 298 presented the highest values at the maximum salinity level. Regarding *Ci*, the sorghum genotypes responded very similarly, showing a slight linear increase in this variable with increasing irrigation water salinity levels (Figure 4D). An increase in *Ci* inside the leaves indicates that CO_2 is not being used for the synthesis of sugars by photosynthesis, suggesting that some non-stomatal factor is negatively interfering in this process (Larcher, 2000), particularly in plants irrigated with high-salinity water.

Aquino et al. (2007) also reported a decrease in the photosynthetic variables in sorghum plants after 40 days of salinity stress. Several other plant species, including jatropha (Silva, Ribeiro, Ferreira-Silva, Viégas, & Silveira, 2011), citrus (Lopéz-Climent, Arbona, Peréz-Clemente, & Goméz-Cadenas, 2008), cowpea (Assis Junior et al., 2007) and cassava (Cruz et al., 2017), have shown limitations of these variables as a function of increased salinity. In addition to the decrease in the photosynthetic rate, the reduction in leaf area (Figure 1B) caused by increasing salinity levels considerably decreased the area destined for photosynthesis, which in turn reduced the production capacity of the genotype (Munns & Tester, 2008).

Figure 5 shows that the relative chlorophyll index presented a quadratic response to increasing levels of salinity, in which genotype P 76 reached its maximum chlorophyll content (SPAD index of 47.9) at an ECw of 9.32 dS m^{-1}. The other genotypes presented the highest value at approximately 6.0 dS m^{-1}. It was also observed that genotype P 76, followed by P 298, presented higher SPAD index values than did the other genotypes, both under the control and stress conditions. Typically, plants subjected to abiotic stress display reduced chlorophyll content (Lacerda et al., 2003; Debouba, Gouia, Valadier, Ghorbel, & Suzuki, 2006). However, there may also be increases in chlorophyll concentration, depending on the level of salt to which the plant is exposed (Ma, Fung, Wang, Altman, & Huttermann, 1997). Jamil et al. (2007) claimed that the chlorophyll content is normally reduced in salinity-sensitive plants, whereas the opposite occurs in more salinity-tolerant plants. Graciano, Nogueira, Lima, Pacheco, and Santos (2011) also verified that there was an increase in the chlorophyll indices of peanut leaves with increasing salinity; this phenomenon was considered a compensatory mechanism to maintain growth and production by allowing increased photosynthetic efficiency.

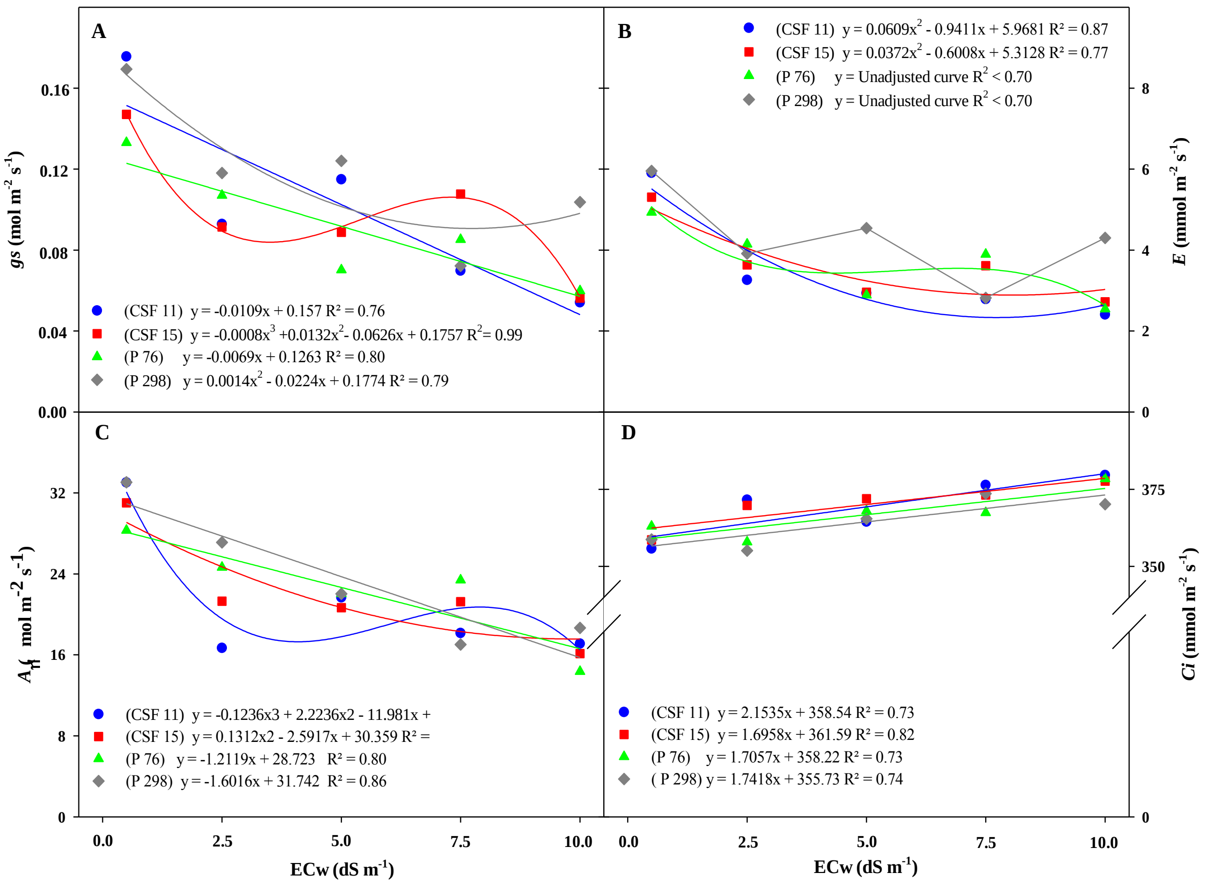

Figure 4. Stomatal conductance (*gs*, A), transpiration (*E*, B), photosynthesis (*A*, C) and internal CO_2 concentration (*Ci*, D) of four saccharine sorghum genotypes (CSF 11, CSF 15, P 76, and P 298) exposed to increasing levels of salinity; the values are expressed in terms of the electrical conductivity of the irrigation water (EC_w).

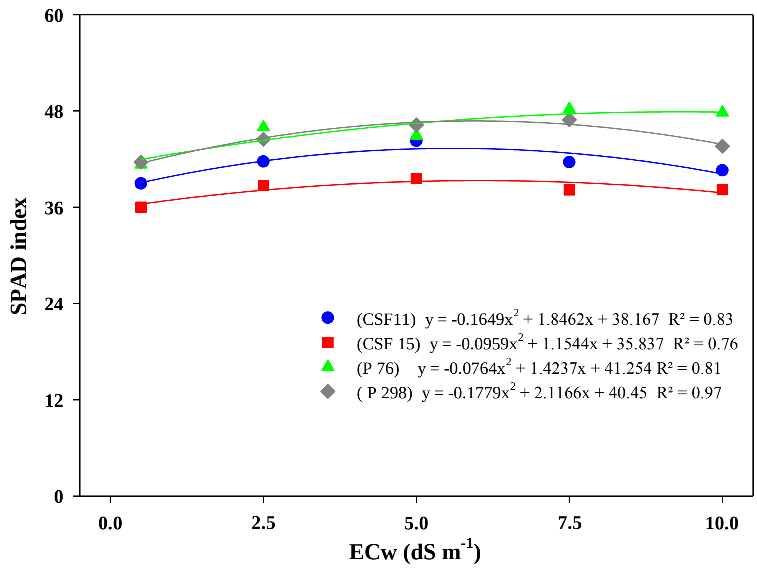

Figure 5. Relative chlorophyll content (SPAD index) of four saccharine sorghum genotypes (CSF 11, CSF 15, P 76, and P 298) exposed to increasing levels of salinity; the values are expressed in terms of the electrical conductivity of the irrigation water (EC_w).

Indices of tolerance to salinity

The classification of the tolerance to salinity of the four saccharine sorghum genotypes, which was based on the reduction of DMT relative to that of the control (Fageria, 1985), is shown in Table 1. The CSF 11 genotype had the lowest per cent DMT loss and was classified as tolerant and moderately tolerant at EC_w values of 2.5 and 5.0 dS m^{-1}, respectively. At an EC_w of 7.5 dS m^{-1}, this genotype was classified as moderately sensitive, and at the highest level of salinity stress (EC_w of 10.0 dS m^{-1}), it was classified as sensitive. The low reduction in DMT recorded for CSF 11 may be related to the ability of this species to develop mechanisms of tolerance to salinity, providing a better acclimatization to the saline culture medium.

Table 1. Per cent reductions in the total dry mass of four saccharine sorghum genotypes exposed to increasing levels of salinity.

Genotype	Equations	R^2	Reduction relative to that of the control (0.5 dS m^{-1}) (%)			
			2.5 dS m^{-1}	5.0 dS m^{-1}	7.5 dS m^{-1}	10.0 dS m^{-1}
CSF 11	y = 6.1512x + 0.3609	0.95	15.7^{T}	31.1^{MT}	46.5^{MS}	61.9^{S}
CSF 15	y = 6.278x + 8.5023	0.88	24.2^{MT}	40.0^{MS}	55.6^{MS}	71.3^{S}
P 76	y = 7.3021x + 3.5994	0.96	21.85^{MT}	40.1^{MS}	58.4^{MS}	76.6^{S}
P 298	y = 6.8452x + 16.149	0.75	33.26^{MT}	50.4^{MS}	67.5^{S}	84.6^{S}

T, MT, MS and S – Tolerant, moderately tolerant, moderately sensitive and sensitive, respectively. The classification was according to that of Fageria (1985).

The genotypes CSF 15 and P 76 were moderately tolerant to an EC_w of 2.5 dS m^{-1}, moderately sensitive to EC_w values of both 5.0 and 7.5 dS m^{-1} and sensitive to an EC_w of 10.0 dS m^{-1}, with reductions in growth of up to 71.3 and 76.6%, respectively. Compared with CSF 11, P 298 had the highest per cent DMT loss, with losses of up to 84.6% at the maximum salinity level. Consequently, P 298 was classified as sensitive to the highest levels of stress (7.5 and 10.0 dS m^{-1}), moderately tolerant to an EC_w of 2.5 dS m^{-1} and moderately sensitive to an EC_w of 5.0 dS m^{-1}.

Conclusion

Salinity adversely affected the growth and photosynthesis variables evaluated in the four sorghum genotypes studied. This effect was more pronounced in the plants of genotype P 298 than in those of the other genotypes. P 298 plants showed greater reductions in growth measurements, indicating their relatively greater susceptibility to salt stress.

The CSF 11 genotype showed the smallest reductions in all the growth and photosynthesis variables when subjected to increasing levels of salinity. Therefore, among the genotypes studied, CSF 11 presented better indices of tolerance to salinity.

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