

Genotypic variation of sugarcane for salinity tolerance: Morphological and physiological responses

Variação genotípica de cana-de-açúcar para tolerância a salinidade: Respostas morfológicas e fisiológicas

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ABSTRACT

Sugarcane (*Saccharum* spp.) is an important crop due to sugar, ethanol and bioenergy production. Its cultivation may occur in tropical regions exposed to high salinity. The aim was to identify cultivars tolerant to salinity to allow the cultivation of sugarcane (*Saccharum* spp.) in saline soils. To test the hypothesis that sugarcane show natural genotypic variation to salinity tolerance, we tested ten cultivars (SP80-3280, RB855453 RB966928, RB855156, SP80-1842, SP80-1816, RB928064, RB867515, RB92579, RB855536) and two sugarcane species: IM76-228 (*S. robustum*) and IN84-82 (*S. spontaneum*) under two concentrations of sodium chloride (NaCl): control (concentration found naturally in the soil used: electrical conductivity of 0.083 dS m⁻¹) and soil enriched with NaCl: EC of 7.2 dS m⁻¹. Biometry and photosynthesis traits were evaluated. The plants were collected and leaf (LDM), stem (SDM), root and total dry matter were used to estimate the tolerance index (TI). A cluster analysis was done to identify phenotypic dissimilarity. Three distinct groups regarding salinity tolerance on biomass-basis were formed. The cultivars RB855156, SP80-1842, SP80-1816 and species IM76-228 showed no reduction in LDM and SDM. Nonetheless, the cultivars SP80-3280, RB928064, RB92579 and species IN84-82 were impaired by salinity. The cultivar SP80-1816 showed the highest biomass accumulation and the highest TI. Therefore, we found a great genotypic variation regarding salinity tolerance in sugarcane, which can be explored by growers to cultivate in saline soils. Also can be used by the Sugarcane Breeding Programs to improve the salinity tolerance.

Index terms: Sodium chloride; saline soils; osmoregulation; abiotic stress.

RESUMO

A cana-de-açúcar (*Saccharum* spp.) é uma cultura importante devido à produção de açúcar, etanol e bioenergia. Seu cultivo ocorre em regiões tropicais, sendo muitas vezes exposta a alta salinidade. O objetivo foi identificar cultivares tolerantes à salinidade para permitir o cultivo da cana-de-açúcar (*Saccharum* spp.) em solos salinos. Para testar a hipótese de que a cana-de-açúcar apresenta variação genotípica natural para tolerância à salinidade, testamos dez cultivares (SP80-3280, RB855453 RB966928, RB855156, SP80-1842, SP80-1816, RB928064, RB867515, RB92579, RB855536) e duas espécies de cana-de-açúcar: IM76-228 (*S. robustum*) e IN84-82 (*S. spontaneum*) sob duas concentrações de sódio: controle [concentração encontrada naturalmente no solo utilizado: condutividade elétrica de 0,083 dS m⁻¹] e solo enriquecido com NaCl: CE de 7,2 dS m⁻¹. Características de biometria e fotossíntese foram avaliadas. As plantas foram coletadas, separadas em folha (MSF), caule (MSC), raiz para determinação da matéria seca total da planta e assim estimar o índice de tolerância (IT). Uma análise de cluster foi feita para identificar a dissimilaridade fenotípica. Três grupos distintos em relação à tolerância à salinidade com base na biomassa foram formados. As cultivares RB855156, SP80-1842, SP80-1816 e espécies IM76-228 não apresentaram redução em MSF e MSC. No entanto, as cultivares SP80-3280, RB928064, RB92579 e as espécies IN84-82 foram prejudicadas pela salinidade. O genótipo SP80-1816 apresentou o maior acúmulo de biomassa e o maior IT. Portanto, encontramos uma grande variação genotípica quanto à tolerância à salinidade em cana-de-açúcar, que pode ser explorada pelos produtores para cultivo em solos salinos. Também podem ser utilizadas pelos Programas de Melhoramento da Cana-de-Açúcar para melhorar a tolerância à salinidade.

Termos para indexação: Cloreto de sódio; solos salinos; osmorregulação; estresse abiótico.

INTRODUCTION

The practice of irrigation is essential to guarantee good agricultural production during episodes of droughts for crops that depend on high water demand (Dalchiavon; Neves; Haga, 2016). However, inadequate irrigation using water with high levels of sodium (Na^+) tends to alter the chemical and physical composition of fertile soils by lowering fertility and making them unsuitable for agriculture (Ahmad et al., 2018).

The negative effects caused by salinity have been reported in several plant species (Cruz; Ferreira; Santos, 2018). It happens because the accumulation of salts interferes with the nutrition concomitantly with a reduction in the plant's ability to absorb water, named osmotic or water-deficit effect of salinity (Machado; Serralheiro, 2017), besides this salt accumulation becomes toxic to the cells.

Sugarcane (*Saccharum* spp.) is considered an important agricultural commodity (Picoli; Machado, 2021) and the interest in this culture has increased because of its importance in the production of renewable energy (Hammer; Sentelhas; Mariano, 2020; Walter et al., 2014). However, it is classified as glycophyte and moderately sensitive to salt stress (Melo et al., 2014). Its cultivation has been expanding to semiarid regions (Begcy et al., 2019), and the success of this expansion depends, in part, on the cultivars that tolerate the adverse conditions of those places such as salinity.

Under salt stress conditions the photosynthetic efficiency of these plants can be affected by a limitation in CO_2 diffusion to the carboxylation site of the enzyme phosphoenolpyruvate carboxylase due to lower stomatal conductance (Maqbool; Wahid; Basra, 2016). Besides compromising the diffusive phase, photosynthesis may also be limited by damage to the photochemical and biochemical apparatus (Moradi; Ismail, 2007). Therefore, the membrane instability, the increase in respiration, the variation in the allocation of nutrients, and ionic toxicity are common changes caused by salinity. Consequently, there is a decrease in the capacity to produce an adequate number of leaves, associated with a reduction in the internodes, compromising the growth and the productive potential of sugarcane (Cruz; Ferreira; Santos, 2018).

The salinity tolerance limits of sugarcane concerning the electrical conductivity of the soil solution (EC) is from 1.7 to 2.3 dS m^{-1} (Brindha et al., 2020), and the irrigation water with EC above those values promotes negative effects on growth. However, plants differ in their responses concerning the salinity tolerance (Ahmed et al., 2020), with inter and intraspecific differences, especially in hybrids with high

genetic variability, such as commercial cultivars of sugarcane, which were not selected for individual characteristics of sodium tolerance (Chiconato et al., 2019; Meena et al., 2020; Kumar et al., 2017). Thus, the development of physiological and growth studies is important to screening for cultivars capable of growing under saline conditions.

In view of the exposed, we tested the hypothesis that there are genotypic variation for salinity tolerance for sugarcane cultivars and the plants with lower photosynthesis reduction under salt stress have greater biomass accumulation and, consequently, a higher level of salinity tolerance. In this context, the objective of this work was to evaluate the growth and gas exchange behavior in ten cultivars (*Saccharum* spp.) and two species (*S. robustum* and *S. spontaneum*) of sugarcane exposed to sodium chloride to identify cultivars less sensitive to salt stress and thus present alternatives for crop and forage production in environments conducive to salinity.

MATERIAL AND METHODS

Plant material and treatment

The experiment was conducted in a greenhouse in Lavras, MG – Brazil (21°13'40"S and 44°57'50"W GRW, altitude 960 m). Ten cultivars of *Saccharum* spp.: SP80-3280, RB855453, RB966928, RB855156, SP80-1842, SP80-1816, RB928064, RB867515, RB92579 and RB855536, and two species of sugarcane: IM76-228 (*S. robustum*) and IN84-82 (*S. spontaneum*) were evaluated.

The plants were obtained from the active germplasm bank of the experimental station of Federal University of Lavras - UFPA, located in the Center of Scientific and Technological Development in Agriculture – Muquém Farm in Lavras, Minas Gerais - Brazil. After the harvest, the sugarcane genotypes were cut into short node pieces (eyepieces) with one active bud and then planted in pots (3.5 dm^3) in a sandy soil. The nutritional correction was performed based on chemical analysis according to Raji et al. (1997). A randomized blocks design was adopted with two concentrations of sodium chloride (NaCl) as treatments: control [concentration naturally found in the soil used in the experiment, represented by a conductivity of 0.083 dS m^{-1}] and soil enriched with 100 mM NaCl , resulting in a conductivity (EC) of 7.2 dS m^{-1} , considering $n = 5$ and totaling 120 plants. The amount equivalent to 100 mM of NaCl per pot was weighed and mixed in the soil before planting the buds. The EC was estimated from the solution obtained from washing the soil after collecting the plants and it was determined with a conductivity meter

(Q405M, Quimis, Brazil). To maintain salinity during the experiment, plastic trays were placed under the pots to retain the percolated water after irrigation. This water was returned to the pots to avoid the loss of salt, thus maintaining the amount of salt added, being reduced only by the absorption of the plants.

At 30 and 58 days after planting (DAP), seedling growth evaluations were performed by counting the number of leaves (NL), measuring the plant (PH, cm) and the stem height (SH, cm) with a ruler; and the stem diameter (SD, mm) with a digital caliper.

Gas exchange and apparent electron transport analyses were performed at 45 DAP using a portable infrared CO₂ analyzer (LI-6400xt, Licor, USA) coupled to a fluorometer (6400-40 LCF, Licor, USA). The evaluations were carried out on the leaf +2 from 10 a.m. to 12 p.m. by setting the photosynthetically active radiation (Q) constant at 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, were considered the net photosynthesis (A , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), transpiration (E , $\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) and the apparent electron transport rate (ETR, $\mu\text{mol e}^{-} \text{m}^{-2} \text{s}^{-1}$). The apparent carboxylation efficiency (A/C_i (net photosynthesis/ internal carbon), $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1} \text{Pa}^{-1}$), the intrinsic water-use efficiency (A/g_s , $\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$), the ratio between apparent electron transport rate and CO₂ assimilation (ETR/A , $\mu\text{mol e}^{-1} \mu\text{mol}^{-1} \text{CO}_2$) (Ohkubo et al., 2020) and the effective quantum yield of photosystem II (Genty; Briantais; Baker, 1989) were calculated.

At the end of the experiment, the plants were harvested and separated in roots, stems and leaves, and dried in an oven with air circulation at 70 °C for 72 hours. Then they were weighed in an analytical scale to obtain the leaf dry matter of leaf (LDM, g), stem (SDM, g), root (RDM, g) and total dry matter (TDM, g). These last two were used to calculate the salinity tolerance index (TI, %), on biomass-basis, considering the ratio between the saline treatment and the control, based on the following Equation 1:

$$\text{TI}(\%) = \frac{\text{Production of DM in saline treatment}}{\text{Prroduction of DM in control treatment}} \times 100 \quad (1)$$

The data obtained were subjected to normality tests (Shapiro-Wilk, $p > 0.05$), homogeneity of variance (Bartlett and Levene, $p > 0.05$) and analysis of variance (F test, $p < 0.05$), and when there was a significant effect, the means were compared by Tukey ($p < 0.05$) using the R platform, with the ExpDes package (Ferreira; Cavalcanti; Nogueira, 2014). In addition, a cluster analysis was performed and a dendrogram and heatmap was generated with the

FactoMineR, factoextra, cluster, ggpubr and pheatmap packages (Le; Josse; Husson, 2008; Maechler et al., 2019; Kassambara; Mundt, 2020).

RESULTS AND DISCUSSION

Our results clearly indicate a great genotypic variation regarding sugarcane commercial hybrids and also among species. The most of the sugarcane genotypes under salinity presented a delay in bud sprouting and, after the establishment of the plants, showed decrease in photosynthesis. However, some of the studied cultivar showed favorable response pattern in terms of biometric analyses and biomass production. These responses contributed to the increase in the TI of these cultivars, demonstrating a clear variability for salinity tolerance.

The salinity caused variations in gas exchange in different cultivars (Figure 1). The maintenance of photosynthesis is essential for plant growth. However, under stress conditions, the limitations of the photosynthetic process are variable depending on the genotype, time of exposure and intensity of stress (Marchiori et al., 2017). This was observed in the cultivars evaluated in this study, the cultivars RB855156 and SP80-1842 showed no reduction in A , while the SP80-3280, RB855453 and RB928064 had the greatest limitations in photosynthesis and transpiration (Figure 1a; b).

The cultivars SP80-1816 and SP80-1842, two cultivars highlighted by tolerance to salinity, are sibling genotypes from the same male parent, H57-5028 (Daros; Oliveira; Veríssimo, 2015), while SP80-3280, which was one of the cultivars with a greater reduction in photosynthesis and transpiration, shares both parents of SP80-1816. However, a close genetic relationship between plant cultivars is no showing indication of the same physiological responses, even under similar environmental conditions. It probably did it happen due to the existence of genetic variability between the progenies of the same crossing.

Despite the smaller reduction in transpiration observed in the SP80-3280 cultivar (Figure 1b), no improvement was observed in the intrinsic efficiency of water use. This occurrence is probably due to the smaller reduction in stomatal conductance that reduced water loss, however it contributed to the limitation of photosynthesis.

The apparent efficiency of carboxylation (A/C_i), the quantum efficiency of photosystem II (ΦPSII), the relationship ETR/A and the intrinsic water use efficiency (iWUE) have been related to photosynthesis limitations in commercial crops (Ohkubo et al., 2020). It was noticed that the salinity induced increases in ETR/A in genotypes SP80-

3280, RB92579 and IM76-228, whereas other cultivars did not present increases (Figure 2a). It is known that values above $6 \mu\text{mol } \mu\text{mol}^{-1}$ on the ratio ETR/A is an indication of alternative electron sink a parameter widely used to determine whether the flow of electrons is being directed to non-photochemical pathways i.e., a strong (Silva et al., 2011). Now, considering the quantum efficiency of photosystem II (ϕPSII), it was observed that only the cultivars RB867515 and SP92579 did not show a decrease in this parameter due to salinity. The lowest values were recorded for the cultivars SP80-3280, RB855453 and RB928064 with 0.016, 0.035 and 0.045, respectively (Figure 2b). For the apparent efficiency of carboxylation (A/C_i), the cultivars RB966928, RB855156, SP80-1842, SP80-1816, RB867515 and RB855536 were not affected by the salinity condition. The cultivar RB855156 showed higher carboxylation efficiency under high salinity with $0.8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ while the SP80-3280 had the lowest value with $0.08 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ (Figure 2c).

The A/C_i is affected when there are both diffusive and biochemical limitations. It is known that the CO_2 diffusion limitation through the intercellular spaces of the leaves is a consequence of the lower stomatal conductance that leads to a reduced internal CO_2 concentration (C_i) in the leaf mesophile, thus causing a decrease in the efficiency of carboxylation (Maqbool; Wahid; Basra, 2016). The stomatal or diffusive limitation is different from the biochemical one since the former occurs in the first stages of stress or when the stress for the plant

does not represent a very severe limitation. On the other hand, the biochemical limitation is the one that shows up during severe stress or in more sensitive genotypes and is mainly characterized by the decoupling between the photochemical and biochemical phase under stress.

Although the decrease in stomatal conductance is a mechanism to try to maintain the water potential of the plant, it affects the carboxylation efficiency by decreasing the substrate (CO_2). If the duration of the stress is sufficiently prolonged, the excitation energy will be greater than the energy used in the biochemical process leading to a decrease in the renewal rate of ADP and NADP that are normally reduced in the electron transport chain (Buchanan; Jones, 2015). After the over-reduction of the electron transport chain, there is naturally a greater formation of reactive oxygen species (ROS), which, in turn, will further compromise the formation of ATP, leading, this way, to the uncoupling of the two phases of photosynthesis. An important characteristic of the biochemical limitation is that even when C_i increases due to the transpiration process, the assimilation of CO_2 will not recover (Martinez et al., 2015).

The $i\text{WUE}$ is mainly associated with diffusive limitations caused by stomatal closure (Maqbool; Wahid; Basra, 2016), whereas the ϕPSII indicates the efficiency of photosystem II and the main factor affecting that is the ability with which electrons are removed from the PSII receptor quinone, which is directly related to the consumption rate of photosynthetic products from the electron transport chain (Silva et al., 2011).

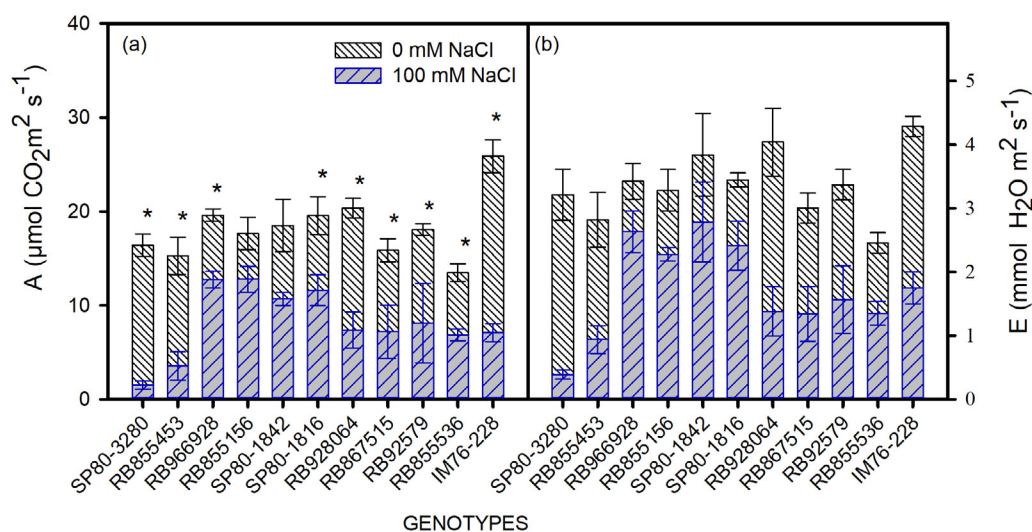


Figure 1: Photosynthesis (A , a) and transpiration (E , b) in different sugarcane genotypes with and without salinity. Each point represents the mean value ($n = 5 \pm \text{se}$). The asterisk (*) indicates significant differences between treatment means by Tukey ($p < 0.05$).

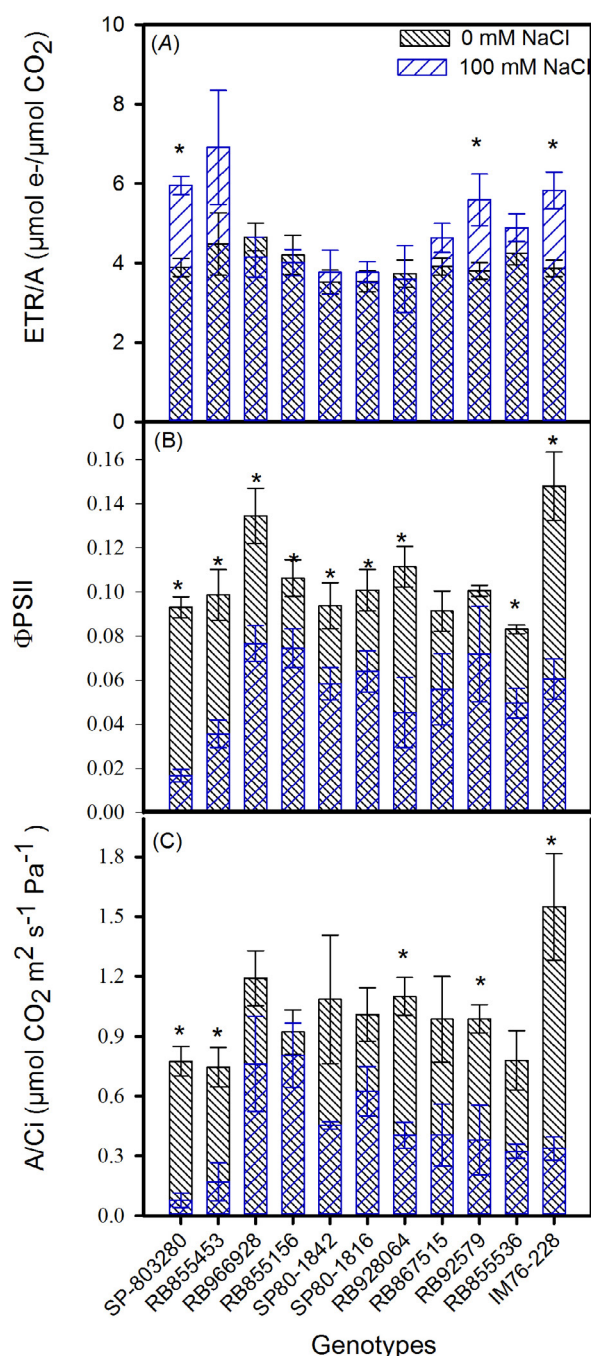


Figure 2: Ratio between apparent electron transport rate and CO₂ assimilation (*ETR/A*) (a), effective quantum yield of photosystem II (ϕ PSII) (b), and apparent efficiency of carboxylation (*A/Ci*) (c) from different sugarcane genotypes with and without salinity. Each point represents the mean value ($n = 5 \pm se$). The asterisk (*) indicates significant differences between means of treatments by Tukey ($p < 0.05$).

Therefore, there is evidence that the genotypes SP80-3280 and IM76-228 had significantly low values in *A* because of biochemical limitations, and besides presenting decreases in *g_s* and *E*, they exhibited significant reductions in *ETR*, ϕ PSII and *A/Ci*, at the same time that the ratio *ETR/A* increased. The salinity also substantially reduces the CO₂ assimilation of the cultivars RB855453 and RB928064, possibly due to the limited CO₂ diffusion to the leaf mesophyll, as a result of the reduced stomatal opening, which consequently promoted an increase in the *iWUE*, without altering the *ETR/A* values.

Concerning the cultivars RB855536 and RB867515, it should be noticed that under high salinity conditions, although *A* may have been affected by stomatal closure (Dabrowska et al., 2007), this fact does not exclude the possibility of metabolic damage due to stress, since it shows signs of decoupling of the photochemical-biochemical phase. Those genotypes showed a decrease in *g_s*, probably as an attempt to maintain the water status of the plant at the expense of CO₂ uptake, which, in turn, led to a decrease in the quantum efficiency of PSII due to a possible imbalance between absorption and use of the luminous energy in PSII.

There is a great variation in *A/g_s* between the cultivars (Figure 3). Under low salinity conditions, the genotypes that showed the higher *A/g_s* are the RB855536, IM76-228 and SP80-1816 with 295, 185 and 155 $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{H}_2\text{O}$, respectively (Figure 3a). However, these same cultivars when submitted to high salinity showed reductions of 83, 51 and 22%, respectively. The cultivar SP80-1842 showed the largest reduction of 73%. In the cultivar RB855156, *A/g_s* increased under salinity conditions, from 127.21 $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{H}_2\text{O}$ in control plants to 366.11 $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{H}_2\text{O}$. Other cultivars that improved *A/g_s* under salinity were the RB92579 that increased 187% and the SP80-3280 with 30% (Figure 3b).

The species IN84-82 was not evaluated in relation to gas exchange analyses because the salinity caused leaf senescence in these plants, resulting in a lack of adequate foliar tissue for carrying out the evaluations.

It was noticed that the cultivar RB855156 maintained photosynthesis, even when exposed to salinity, possibly due to the better capacity to maintain green leaves on the plant (Figure 1a). This is very important trait to allow a larger area to intercept light and assimilate CO₂ (Marchiori et al., 2010); this genotype, besides maintaining *A* under salinity conditions, showed an increase in *iWUE* due to the decrease in transpiration caused by the lower stomatal conductance. Even with a reduction in the diffusion of CO₂, given by the lower *g_s*, this cultivar maintained *ETR* and *A/Ci*. Consequently, there were no reductions in the TDM

for that cultivar, once the photochemical and biochemical aspects of the photosynthesis were maintained. Thus, even under conditions of high salinity, that cultivar has protective mechanisms for the photosynthetic apparatus. On the other side, the cultivar SP80-1842 did not show a reduction in g_s or E , but it did show a reduction in A , which consequently led to a decrease in water use efficiency (Figure 3b). The photosynthesis reduction in this cultivar may have occurred due to photochemical and/or biochemical problems since the diffusive phase was preserved.

The sugarcane growth limitation due to salinity is recognized and has been reported in several studies (Willadino et al., 2011; Simões et al., 2016; Brindha; Vasantha; Arunkumar, 2019; Kasirajan et al., 2020). Similar results were found in this study. Nonetheless, the growth reduction was not widespread in all cultivars, and four cultivars and one species showed no reduction in the growth variables (Table 1). The genotype SP80-3280 reduced the number of leaves (NL) as soon as 30 days after stress and the RB928064 and IN84-82 at 58 days. The other cultivars

did not decrease the NL. Regarding the stem height (SH), the cultivar RB855453 was affected by salinity at 30 days after plantation, while the cultivar SP80-3280 and the species IN84-82 were affected at 58 days when they displayed lower SH. The other genotypes were not affected in relation to stem height. Regarding the stem diameter (SD), the reduction occurred in the cultivars RB855453, RB966928 RB928064 and RB867515, the latter being only affected at 58 DAP.

At 30 days of stress, there was a reduction in plant height (PH) of the cultivars SP80-3280, RB966928 and RB92579. The cultivars RB855453 and IN84-82 were affected at 30 and 58 days after saline stress, respectively. The other cultivars did not reduce the plant height when subjected to salinity. Characteristics such as plant and stem height, stem diameter and leaf area index are influenced by edaphoclimatic factors. Nevertheless, the expression of those characteristics strongly depends on the genetic potential of the cultivar, and the interaction between environment and genotype determines the morphological and growth responses of sugarcane under stress (Simões et al., 2016).

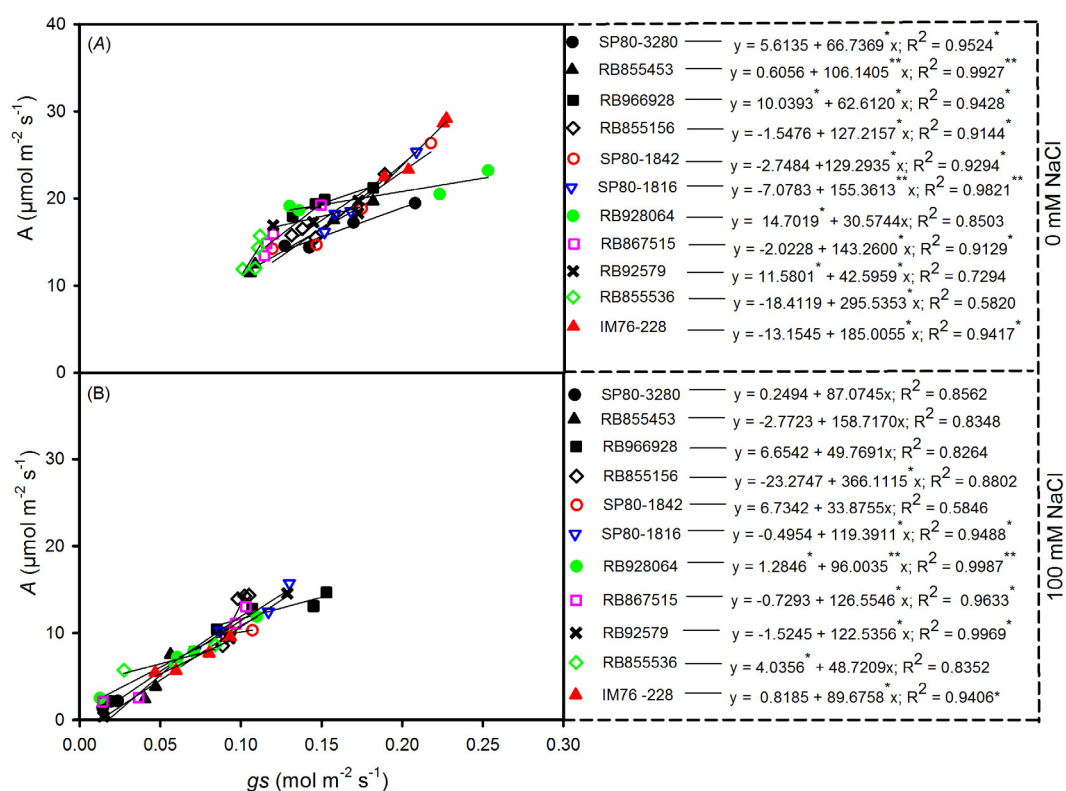


Figure 3: Intrinsic water-use efficiency (A/g_s), under 0 mM NaCl (a) and 100 mM NaCl (b), in different sugarcane genotypes. Each point represents the mean value ($n = 5$). On the right side, the equations that represent the correlation between the variables CO_2 assimilation (A) and stomatal conductance (g_s), with the value of m in the linear equation $Y = mx + b$, corresponding to the value of (A/g_s) of each material.

Table 1: Number of leaves (NL), stem diameter (SD), stem height (SH) and plant height (PH) of sugarcane genotypes with and without salinity at 30 and 58 days after planting (DAP).

Genotype	NaCl (mM)	NL		SD (mm)		SH (cm)		PH (cm)	
		30	58	30	58	30	58	30	58
SP80-3280	0	5.0 ^a	5.0 ^a	6.8 ^a	8.4 ^a	12.2 ^a	14.3 ^a	62.6 ^a	71.9 ^a
	100	3.0 ^b	4.0 ^a	6.1 ^a	6.8 ^a	9.9 ^a	10.9 ^b	42.7 ^b	64.1 ^a
RB855453	0	4.0 ^a	5.0 ^a	7.6 ^a	8.3 ^a	10.8 ^a	12.4 ^a	68.2 ^a	75.9 ^a
	100	4.0 ^a	5.0 ^a	6.1 ^b	6.6 ^a	9.1 ^b	12.8 ^a	52.3 ^b	59.9 ^b
RB966928	0	4.0 ^a	6.0 ^a	6.6 ^a	8.0 ^a	6.2 ^a	12.5 ^a	43.7 ^a	57.3 ^a
	100	4.0 ^a	5.8 ^a	5.8 ^b	7.2 ^a	6.1 ^a	9.9 ^a	38.6 ^b	41.6 ^a
RB855156	0	4.0 ^a	5.0 ^a	6.2 ^a	7.5 ^a	6.8 ^a	13.0 ^a	46.4 ^a	65.7 ^a
	100	4.0 ^a	4.8 ^a	6.0 ^a	7.0 ^a	7.1 ^a	11.7 ^a	44.6 ^a	54.5 ^a
SP80-1842	0	4.0 ^a	4.8 ^a	7.5 ^a	8.4 ^a	10.7 ^a	13.8 ^a	58.7 ^a	73.0 ^a
	100	5.0 ^a	5.0 ^a	7.8 ^a	8.3 ^a	8.8 ^a	14.8 ^a	52.0 ^a	64.3 ^a
SP80-1816	0	4.0 ^a	5.2 ^a	6.3 ^a	7.9 ^a	10.4 ^a	14.2 ^a	55.4 ^a	78.8 ^a
	100	4.0 ^a	4.4 ^a	6.8 ^a	7.8 ^a	11.4 ^a	14.8 ^a	59.3 ^a	65.9 ^a
RB928064	0	5.0 ^a	6.0 ^a	8.3 ^a	10.1 ^a	9.9 ^a	13.6 ^a	64.2 ^a	72.4 ^a
	100	4.0 ^a	4.5 ^b	7.3 ^b	9.3 ^a	7.9 ^a	12.8 ^a	41.9 ^a	62.4 ^a
RB867515	0	4.0 ^a	5.0 ^a	7.4 ^a	9.3 ^a	10.3 ^a	13.6 ^a	60.4 ^a	65.4 ^a
	100	4.0 ^a	4.6 ^a	6.6 ^a	7.2 ^b	8.5 ^a	11.7 ^a	44.6 ^a	55.2 ^a
RB92579	0	3.0 ^a	6.0 ^a	6.9 ^a	8.6 ^a	7.4 ^a	14.2 ^a	45.2 ^a	69.8 ^a
	100	3.4 ^b	4.7 ^b	6.3 ^a	8.3 ^a	6.6 ^a	10.7 ^a	29.9 ^b	48.4 ^a
RB855536	0	4.4 ^a	5.2 ^a	6.3 ^a	7.8 ^a	7.6 ^a	14.4 ^a	58.9 ^a	74.3 ^a
	100	4.0 ^a	4.7 ^a	6.2 ^a	6.7 ^a	8.3 ^a	12.3 ^a	48.7 ^a	69.1 ^a
IM76-228	0	3.0 ^a	5.6 ^a	4.0 ^a	6.4 ^a	7.2 ^a	12.9 ^a	34.1 ^a	57.5 ^a
	100	3.2 ^a	4.6 ^a	4.4 ^a	5.8 ^a	8.3 ^a	11.4 ^a	32.7 ^a	45.7 ^a
IN84-82	0	3.6 ^a	5.8 ^a	4.6 ^a	6.5 ^a	5.2 ^a	15.0 ^a	43.2 ^a	78.7 ^a
	100	2.6 ^a	4.5 ^b	4.3 ^a	5.3 ^a	4.3 ^a	9.8 ^b	25.0 ^b	54.3 ^b

Each value represents the mean (n = 5). The averages are representative for two days of assessments. Different letters in the column indicate significant differences between treatment means (Tukey, p <0.05).

In relation to dry mass, the genotypes SP80-3280, RB92579, RB855536 and IN84-82 had reduced dry matter in all tissues due to salinity, demonstrating that they are sensitive to salinity. On the other hand, the genotypes RB855156 and SP80-1816 were not affected (Figure 4). The cultivars RB855453 and RB966928 reduced only the LDM, whereas the RB928064 and RB867515 reduced the SDM and the cultivar SP80-1842 and the species IM76-228 only the RDM. Regarding the accumulation of TDM, the genotypes RB855156, SP80-1816, SP80-1842, RB867515 and IM76-228 were not affected (Figure 4d). The genotypes SP80-3280, RB928064, RB92579 and IN84-82 are among

the ones with the greatest reduction in TDM with 71.4%, 62.1%, 64% and 73.9% of reduction, respectively (Figure 4d), thus contributing to the lowest TI observed (Figure 5).

The genotype determines the plant's defense strategies, and there are plants whose defense strategies or systems may be more efficient than others, therefore, the effects of stress sometimes cannot be observed in biometric parameters (Verma et al., 2020), as it was observed for five of the twelve cultivars evaluated though. Likewise, it is known that the maintenance of stem growth under stress is positively correlated with greater productivity potential (Simões et al., 2016). This fact can be linked

to the results obtained for the cultivar SP80-1816 that did not show growth modifications while maintaining the production of biomass together with the RB855156 (Table 1). It is worth noticing that this former material already has characteristics of high productivity, medium maturation and high sucrose content, while the latter is described as early maturation and has medium flowering and productivity (Simões et al., 2016), which comes from the crossing between RB72454 x TUC71-7. It also presents high average heritability values for the sucrose, fiber content and production variables, indicating a predominance of the genetic component instead of the environmental component (Ferreira et al., 2010).

Among the species evaluated, the *S. robustum* IM76-228 stands out for the maintenance of growth and biomass accumulation in both root and shoot, plus a high

TI (Table 1, Figure 4 and 5). Whereas, the species *S. spontaneum* IN84-82 displayed reduction in growth and biomass, and one of the lowest TI. Studies carried out with seven *S. spontaneum* clones have already reported that there are differences in morphophysiological responses among them under salinity conditions; and for that study, the genotype IND-16-1762 was classified as tolerant under the saline condition of 8 dS m⁻¹ (Kasirajan et al., 2020), a level very similar to the one used in this study (7.2 dS m⁻¹).

The biomass accumulation observed in the cultivar SP80-1816 in the saline treatment indicated that this cultivar has the highest TI for both the root and total biomass (Figure 6a and b). The cultivars SP80-3280, RB92579 and IN84-82 showed the lowest TI and demonstrated that these cultivars do not tolerate the salinity level applied in the study.

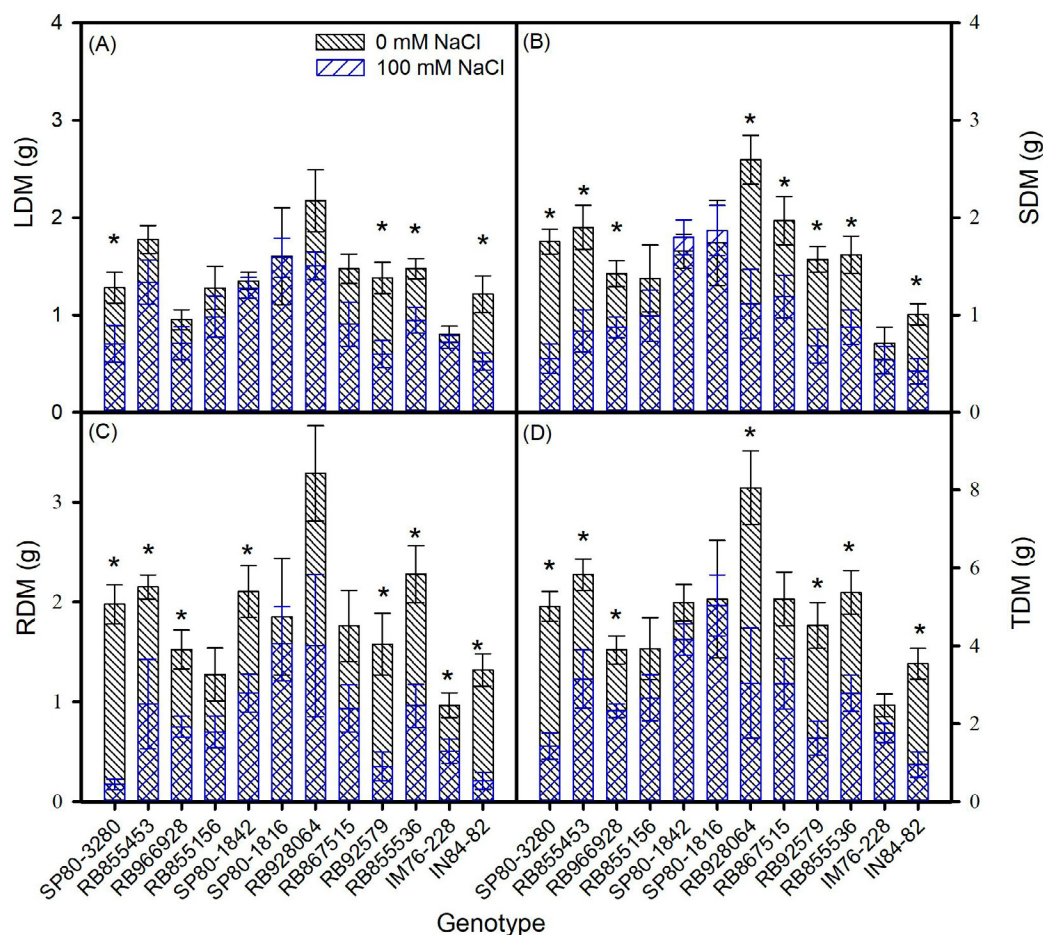


Figure 4: Leaf dry matter (LDM, a), stem dry matter (SDM, b), root dry matter (RDM, c) and total dry matter (TDM, d) in different sugarcane genotypes with and without salinity. Each point represents the mean value (n = 5) ± se. The asterisk (*) indicates a significant difference between the means by Tukey (p < 0.05).

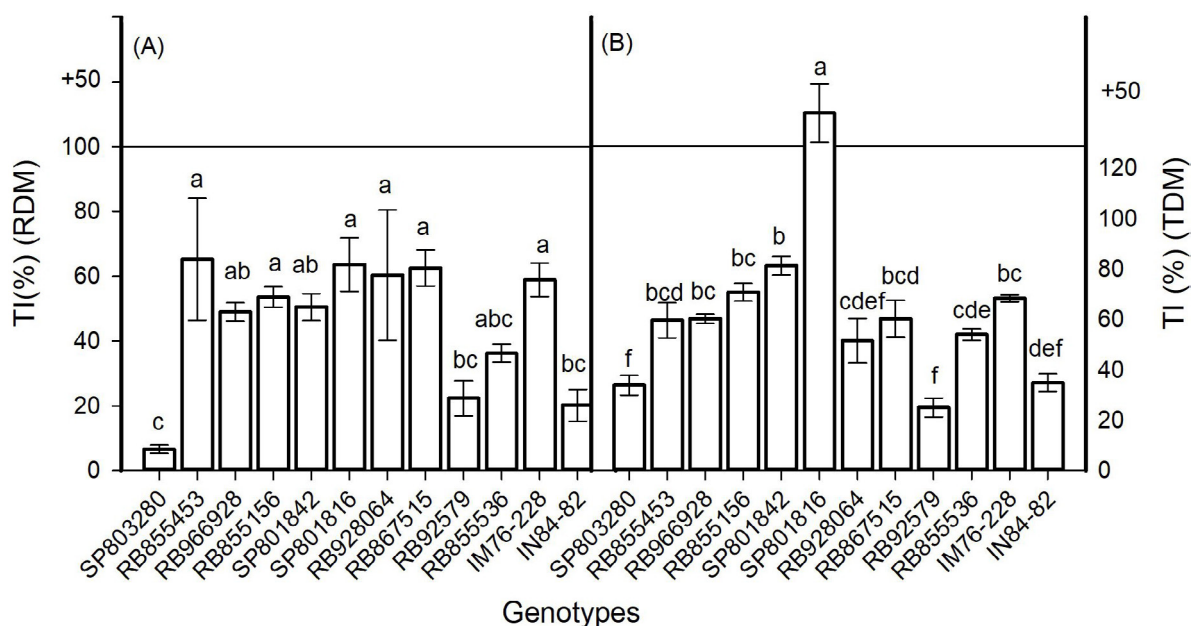


Figure 5: The tolerance index (TI) of the root (a) and total biomass (b) in different sugarcane genotypes with and without salinity. Each histogram represents the mean value ($n = 5$) \pm se. Different letters indicate significant differences between treatment means (Tukey, $p < 0.05$).

The responses to water deficit and salinity show similarities in biometric, physiologic, and molecular levels (Vasanthi; Gomathi; Brindha, 2017), and the plant's defense mechanisms against these stresses are also similar. Therefore, eventual drought-tolerant cultivars could also be tolerant to salinity. In this study, the cultivar RB867515 described by the CCA/UFSCar Genetic Improvement Program for sugarcane as a drought-tolerant cultivar showed a decrease in stem diameter under salinity. Nonetheless, it displayed high performance in all the other variables when compared to the remaining cultivars, probably due to its genotype of high productivity.

On the other hand, the cultivars SP80-3280 and RB966928 showed lower tolerance to salinity due to their lower biomass accumulation, growth reduction and low TI. It probably happened first because of the accumulation of toxic ions that may be accompanied by a decrease of sucrose production in the roots, as observed by Kasirajan et al. (2020) and reported by Verma et al. (2020) in sugarcane plants subjected to salt stress, and second by the requirements of those cultivars, since the SP80-3280 is described as a cultivar that demands good soil, and the RB96-6928 is an early maturation cultivar also demanding fertility and water availability from the soil (Daros; Oliveira; Verissimo, 2015).

The generated dendrogram formed three distinct clusters based on the Euclidean distance among the different genotypes in relation to the biomass accumulation and TI in the salinity conditions (Figure 6a). The first group was the largest, where most of the genotypes was located, because they had the shortest distances, the cultivars SP80-3280, RB92579 and the species IN84-82 belonged in this group. The intermediate group was represented by the cultivars RB855453 and RB928064. The cultivars SP80-1842 and SP80-1816 formed the third group for having the greatest distances.

The scale on the right side of Figure 6b represents a score that each cultivar presented according to the evaluated characteristics. The higher the value on the scale, the higher the value of the evaluated characteristic. The heatmap showed how these groups were formed based on the Euclidean distances between the cultivars for each characteristic (Figure 6b). Therefore, it was possible to verify that the cultivars SP80-3280, RB92579 and the species IN84-82 had the shortest distances among themselves for the analyzed characteristics. The cultivars RB855453 and RB928064 displayed the second longest distances for the dry matter characteristics. On the other hand, the cultivars SP80-1842 and SP80-1816 were the ones that obtained the greatest distances, mainly for TI

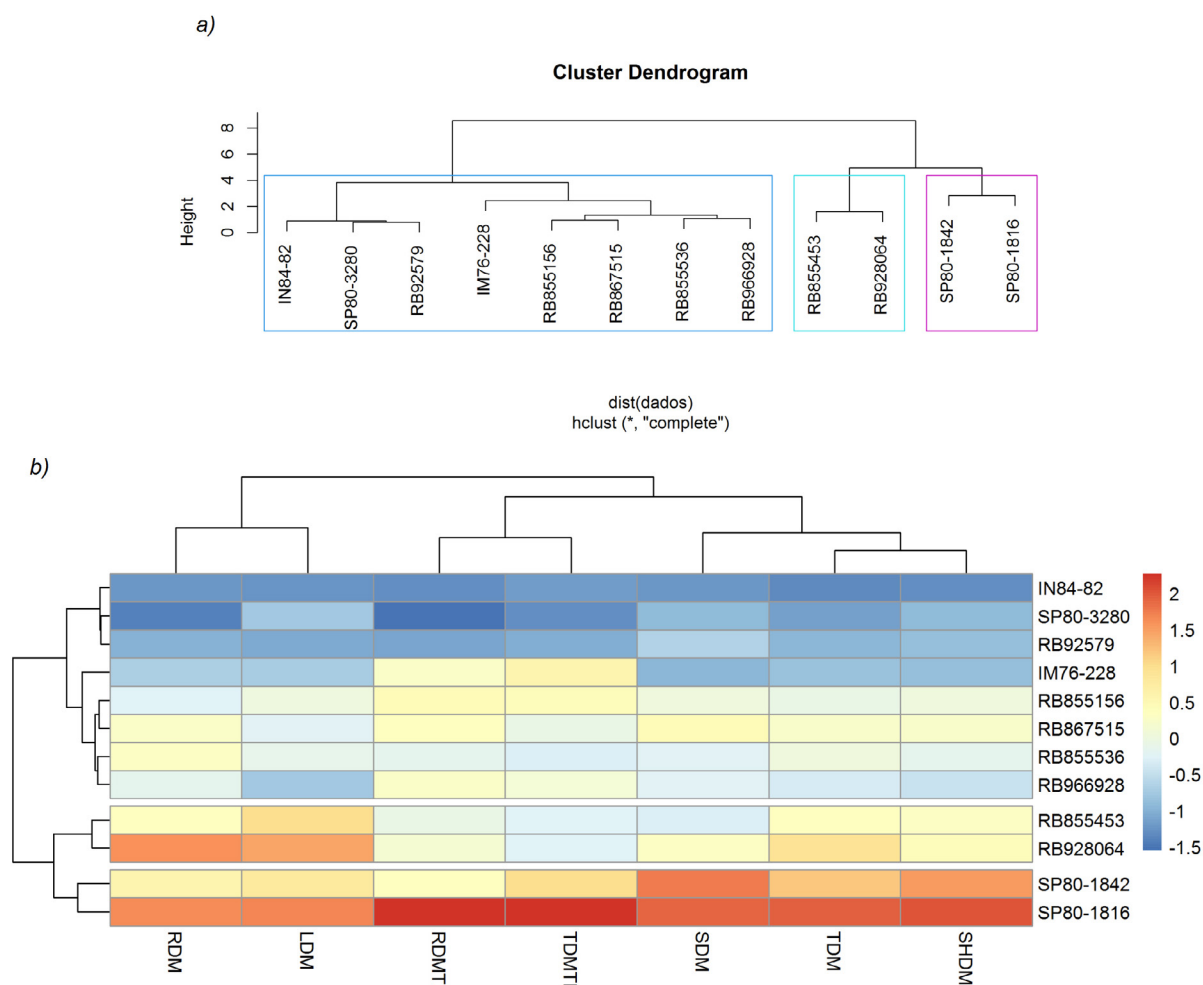


Figure 6: Dendrogram of phenotypic dissimilarity (a) and heatmap of grouping (b) of sugarcane genotypes under salinity. RDM = dry root matter; LDM = leaf dry matter; RDMTI = stress tolerance index of root dry matter; TDMTI = stress tolerance index of total dry matter; SDM = stem dry matter; TDM = total dry matter and SHDM= shoot dry matter.

In the salinity condition, plants adopt different strategies to deal with stress, one of the first responses being detoxification through ion compartmentation, restoring homeostasis through the synthesis of osmolytes and, finally, resuming growth (Kasirajan et al., 2020). Thus, the deleterious effects of salinity will be observed at the last level, as noted for some cultivars. A study by Melo et al. (2014) indicate that the pre-conditioning of plants for 24 - 36 hours in 25 mM NaCl prevented the reduction of plant growth for the cultivar RB98710 when subjected to gradual salt stress up to 60 days, which could be an interesting line of research.

Among the cultivars studied here, those that were affected by salinity revealed results that demonstrate the

importance of plants to avoid growth limitations during the tillering phase and stem growth, which, in turn, also corresponds to the phase of greatest water demand for the plants. The growth processes are particularly sensitive to salinity, therefore the growth rates and other growth variables along with the photosynthetic capacity are good parameters for assessing the effects of salinity, as well as the plant's ability to tolerate this stress (Simões et al., 2021). As previously described and according to the results found for the cultivars SP80-1816 and RB855156 and the species IM76-228, which express their adaptive characteristics to survive in stressful conditions, such results make these individuals stand out as tolerant cultivars and constitute potential targets for evaluation in saline regions.

The variation in gas exchange, biometric and growth characteristics of the cultivars allowed different responses to the TI. Thus, some plants behaved as tolerant to the salinity level and others as sensitive, in addition to those that remained as intermediaries, which were not very affected, but which also did not obtain significant gains in biomass and TI. These results were confirmed by the cluster analysis, in which, the greater the phenotypic difference of one cultivar in relation to another, based on a characteristic, the greater its Euclidean distance or phenotypic dissimilarity. Thus, the cultivars SP80-1842 and SP80-1816 were the most tolerant to salinity, as they accumulated more biomass in saline conditions.

CONCLUSIONS

The cultivars with the lowest reductions in photosynthesis did not have the dry matter affected, including the RB855156 and SP80-1816. On the other hand, others significantly reduced the total biomass, such as the SP80-3280, RB928064, RB92579 and IN84-82, demonstrating their sensitivity to the salt stress. The cultivar SP80-1816 showed the highest biomass accumulation and the highest tolerance index based on the root and total biomass, which constitutes this cultivars a potential one to be explored in saline environments.

AUTHORS CONTRIBUTION

Conceptual idea: Silva, A. A.; Marchiori P. E. R.; Methodology design: Silva, A. A.; Pimentel, G. V.; Marchiori, P. E. R.; Data collection: Silva, A. A.; Linhares, P. C. A.; Rubio, Z. C. C.; Silva, K. R.; Data analysis and interpretation: Silva, A. A.; Linhares, P. C. A.; Rubio, Z. C. C.; Marchiori, P. E. R.; Writing and editing: Silva, A. A.; Linhares, P. C. A.; Rubio, Z. C. C.; Silva, K. R.; Pimentel, G. V.; Marchiori, P. E. R.

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