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SALINITY STRESS

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Saline stress affects the growth of *Saccharum* complex genotypes

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Abstract

Soil salinity affects plant growth, compromising sugarcane cultivation in regions with great production potential. Saccharum complex genotypes that respond positively to growth under saline environment can be used in the diversification of sugarcane cultivars to obtain greater economic returns. The objective of this study was to evaluate growth-related traits of *Saccharum* genotypes grown under the presence and absence of salinity. The experiment was carried out in a 32×2 factorial scheme in a randomized block design with three replicates. The first factor consisted of 32 genotypes of the *Saccharum* complex and the second factor consisted of the presence and absence of salinity. The salinity provided higher mean values than the environment without salinity for plant height in the genotypes G9, G11, G13, G22 and G28, leaf number for G9 and G24, leaf area index for G9 and stem diameter for G1, G11 and G24. Among the genotypes tested, G1, G9, G11, G13, G22, G24 and G28 were the most promising genotypes and could be used for breeding new sugarcane cultivars of enhanced salinity tolerance.

KEYWORDS
abiotic stress, growth variables, plant breeding, salt tolerance, sugarcane

Key points

- Sugarcane has high genetic variability under saline stress conditions.
- Some genotypes develop strategies to maintain their development in a saline environment.
- Genus *Erianthus*, *Sclerostachya*, *Miscanthus* and *Narenga* from the *Saccharum* complex maintain their agronomic performance under salinity conditions.
- Saccharum complex genotypes that respond positively in saline environments can be used for new sources of genetic variability.

1 | INTRODUCTION

Sugarcane (*Saccharum officinarum L*) cultivation contributes strongly to Brazil's economic development. Especially because it holds a prominent place in the sugar-energy sector due to the great potential in the production of sugar, ethanol and their respective by-products, representing a market in the constant expansion (Carneiro et al., 2020). In the 2020 harvest, sugarcane cultivation in Brazil occupied an area of 9.74 million hectares, corresponding to an average yield of 76.61 ton ha^{-1} (IBGE, 2020).

Nonetheless, the saline stress also affects plant growth, compromising sugarcane cultivation in regions with great potential for production (Asfaw et al., 2018). Salinity alters the water and nutrient uptake, resulting in decreased cell division and expansion and hence reduced vegetative growth and biomass production (Silveira et al., 2016). Although sugarcane is considered a glycophytic species with moderate sensitivity to salt stress, different responses to salinity tolerance among sugarcane genotypes occur through their high genetic variability (Carvalho et al., 2016).

Chiconato et al. (2019) compared the performance of two sugarcane cultivars and concluded that the cultivars show large differences in growth response, in an environment with a saline concentration of 160 mM NaCl. Simões et al. (2016) found a similar response in ten sugarcane varieties regarding reduced growth in a saline environment with electrical conductivity (EC) of 8.0 dS. Cruz et al. (2018) also showed lower plant growth in an environment with the saline concentration of 160 mM NaCl.

However, certain plants under stress have strategies to promote new stoichiometric homeostasis to balance their metabolism by mitigating environmental restrictions (Prado & Silva, 2017). In this aspect, sugarcane breeding programs in Brazil have sought in the Active Germplasm Bank of the country genetic material with high quality adapted to different growing regions (Oliveira et al., 2017). In this extensive collection of sugarcane germplasm, there is the Saccharum complex formed by several genera phylogenetically close to Saccharum, such as the genera Erianthus, Sclerostachya, Miscanthus and Narenga (Mukherjee, 1957).

Research on the *Saccharum* complex is being improved, mainly because the complex has genes related to resistance to biotic and abiotic stresses. Simões, Oliveira, Tardin, et al. (2021), by analysing the physiological performance of Saccharum complex genotypes grown in environments with and without saline stress, verified a differential response of the genotypes under salinity conditions. Simões, Oliveira, Silva, et al. (2021) also found larger leaf length in *E. arundinaceus* accessions under saline stress. Simões, Oliveira, Silva, et al. (2021) also found larger leaf length in *E. arundinaceus* accessions under saline stress.

The extent of the effect of salt stress on a plant's growth performance depending on the severity of the imposed stress is important for using these environments and obtaining greater economic returns. Our hypothesis is that genotypes that respond positively to growth under saline environment are superior to the others, and may have mechanisms related to saline stress tolerance. Thus, the objective of this research was to evaluate characteristics related to the growth of *Saccharum* genotypes cultivated under the presence and absence of saline stress.

2 | MATERIALS AND METHODS

2.1 | Experiment conduction

The study was carried out in a greenhouse at Embrapa Semiárido, located in Petrolina-PE (latitude: 9°09′ S, longitude: 40°22′ W, altitude: 365.5 m). The climate of the region, according to the Köppen classification, is BSwh, characterized by scarce and irregular rainfall and high evaporation. The relative humidity varies from 55% to 72%, the air temperature presents average variations between 21°C and 32°C and the average annual rainfall is 500 mm, irregularly distributed, concentrated from November to April.

TABLE 1 Genus and species of the 32 genotypes of the *Saccharum* complex evaluated.

Genotype	Genus	Species
G1, G2, G3, G4, G5, and G6, G13, G15, G16, G17, G18, G19, G20, G21, G25, G30, G31 and G32	*Saccharum*	*officinarum*
G7, G8, G9, G10 and G29	*Erianthus*	*arundinaceus*
G11	*Saccharum*	*hibrido*
G12, G14 and G26	*Saccharum*	*spp.*
G22 and G23	*Miscanthus*	*spp.*
G24	*Saccharum*	*spontaneum*
G27 and G28	*Saccharum*	*robustum*

The experiment was carried out in a 32 × 2 factorial scheme in a randomized block design with three replicates. The first factor consisted of 32 genotypes of the *Saccharum* complex (Table 1) and the second factor consisted of the presence and absence of salinity. Sugarcane genotypes were subjected to saline stress by adding to the irrigation water a saline solution with electrical conductivity (EC) of 8.0 dS with the equivalent proportion 7:2:1 between sodium chloride (NaCl), calcium chloride (CaCl.2HO) and magnesium sulfate (MgSO.7HO) (Aquino et al., 2007). The genotypes were obtained from the Embrapa Tabuleiros Costeiros Germplasm Bank and multiplied in the Mandacaru Experimental Field, belonging to Embrapa Semiarido, in Petrolina-PE.

The plants were grown in 10-L plastic pots, perforated at the base and connected to collection hoses to conduct the leaching water. Each pot corresponded to an experimental unit. Two buds of each genotype were planted per pot. The vases were filled with a gravel layer of approximately two centimetres, and the volume was completed with Neossolo Quartzarênico medium texture soil, collected at a 0 to 0.2 m depth, whose electrical conductivity (EC) determined was 0.6 dS m^{-1}. Then, to avoid the accumulation of salts in the soil profile of the pots, we applied a volume of water higher than necessary to raise the soil to field capacity (FC), obtaining a leaching fraction of approximately 15%. The irrigation of the units was performed every 2 days to maintain the soil close to the FC.

2.2 | Evaluated traits

After 90 days of salt stress, the following plant growth-related variables were measured: plant height (PH), leaf number (NL), stem diameter (SD) and leaf area index (LAI), the latter determined using a Li-3100 C benchtop scanner.

2.3 | Statistical analysis

The data were submitted to analysis of variance and grouping of means by the Scott-Knott test at 5% probability. Subsequently, a correlation plot was constructed between the variables evaluated under presence and absence of salt stress. The analyses were performed on the R software (R Core Team, 2021) using the packages "ExpDes.pt" and "corrplot".

3 | RESULTS

Table 2 contains the mean squares of the variance analysis for each source of variation and growth variables evaluated. There was a significant effect (p-value <.05) for the effects of genotypes, salinity and interaction between these factors for all variables evaluated.

Table 3 shows the significant interaction between genotypes x salinity for the variable plant height. The genotypes G1, G7, G9, G11, G13, G16, G19, G23, G25, G26, G27, G29 and G32 showed the highest mean heights in the presence of salt stress. For the control condition, the genotype G12 stood out by obtaining the highest mean of this variable. Regarding the comparison of environments within genotypes, in general, there was no difference between environments for most genotypes. However, some genotypes showed specific responses as a function of environment. In the genotypes G3, G4, G10, G14, G15 and G30, the absence of salt stress caused higher mean heights. However, in the genotypes G9, G11, G13, G22 and G28, the presence of salinity provided higher means compared to the absence of this stress.

The significant interaction between genotypes x salinity for the variable number of leaves is shown in Table 3. The genotypes G1, G5, G9, G10, G24 and G27 were allocated in the group of the highest means for this variable under saline stress. For the control condition, the genotypes G1, G6, G7, G8, G11, G12, G13, G15, G18, G19, G22, G23, G25, G26, G27, G28, G29 and G31 obtained the highest means. Regarding the comparison of environments within genotypes, the absence of saline stress provided the highest mean number of leaves, except for the genotypes G9 and G24, in which the environment with saline stress obtained the highest mean.

The significant interaction between genotypes x salinity for the stem diameter is shown in Table 3. The genotypes G1 and G19 obtained the highest means under saline stress. For the control condition, the genotypes G2, G3, G4, G6, G7, G8, G10, G11, G12, G15, G16, G17, G18, G19, G21, G23 G25, G26, G27, G 28, G29, G30, G31 and G32 stood out by obtaining the highest means of this variable. Regarding the comparison of environments within genotypes, there was no difference for most genotypes. However, some genotypes showed specific responses depending on the environment. For G2, G3, G4, G6, G10, G12, G12, G13, G15, G16, G17, G18, G21, G25, G26, G30 and G31, the absence of salt stress caused higher means of stem diameter. However, in the genotypes G1, G11 and G24, the presence of salinity provided higher means compared to the absence of this stress.

Figure 1 shows the Pearson's correlation plot for the growth variables evaluated in the *Saccharum* complex genotypes grown under presence (pink) and absence (blue) of saline stress. Overall, the magnitude and direction of the correlations between the variables were not affected by the presence or absence of salinity. Regardless of the environmental condition, the correlations were weak, and there was no change in their direction.

4 | DISCUSSION

Differential performance of genotypes indicates responsiveness to abiotic stress conditions (Steiner et al., 2021). Thus, *Saccharum* genotypes that showed higher heights under salinity (Table 3) demonstrate superior behaviour and adaptive stress response. Our findings are concordant with the study carried out by Simões et al. (2016), who reported that the increased height of some genera/species of the *Saccharum* complex subjected to irrigation with saline water is also indicative of greater adaptability to saline stress. In this sense, the growth maintenance of these genotypes under saline conditions likely occurs due to their ability to exclude Na^+while removing water from the soil (Chiconato et al., 2019).

In the literature, there are several reports on sugarcane mechanisms to deal with excessive Na^+. These include improved ROS sequestration capacity (Satbhai & Naik, 2014), increased ascorbate peroxidase and catalase activity (Carvalho et al., 2016), lower Na^+ accumulation and higher proline accumulation in leaves (Chiconato et al., 2019) and presence of genes such as sugarcane shaggy-like

TABLE 2 Mean square of the analysis of variance for the variables plant height (PH), number of leaves (NL), leaf area index (LAI) and stem diameter (SD) evaluated in 32 Saccharum complex genotypes grown in the presence and absence of salt stress.

Source of variation	DF	PH	NL	LAI	SD
Blocks	2	1.79^{ns}	1.72^{ns}	80.66^{ns}	0.23^{ns}
Genotypes (G)	31	111.61*	2.56*	8029.49	18.58*
Salinity (S)	1	184.08*	99.91*	288018.74*	344.49*
GxS	31	124.18*	2.74*	8181.77	22.11*
Error	126	22.85	0.92	1379.38	3.65
Coefficient of variation (%)		16.55	15.51	24.65	20.16

Note: ^{ns} and *, not significant and significant at 5% probability by the *F* test, respectively.
Abbreviation: DF, degrees of freedom.

TABLE 3 Unfolding the significant interaction between *Saccharum* genotypes and the presence and absence of saline stress for plant height (cm), number of leaves and stem diameter (mm).

	Plant height (cm)		Number of leaves		Stem diameter (mm)	
Genotype	Presence	Absence	Presence	Absence	Presence	Absence
G1	33.33 aA	29.67 cA	6.00 aA	7.00 aA	16.13 aA	12.35 aB
G2	28.00 bA	29.33 cA	5.33 bB	6.33 bA	6.11 cB	10.56 aA
G3	24.67 bB	35.33 cA	5.00 bB	6.67 bA	7.77 bB	11.25 aA
G4	26.33 bB	38.33 bA	4.67 bB	5.67 bA	4.84 cB	11.05 aA
G5	21.00 bA	23.33 dA	5.67 aA	5.67 bA	9.89 bA	8.94 bA
G6	26.67 bA	32.33 cA	4.00 bB	7.00 aA	4.51 cB	11.99 aA
G7	33.67 aA	33.33 cA	6.00 aB	7.67 aA	10.26 bA	11.39 aA
G8	22.00 bA	29.33 cA	5.67 aB	7.33 aA	5.21 cA	7.28 aA
G9	32.33 aA	21.67 dB	7.33 aA	5.33 bB	9.95 bA	8.41 bA
G10	22.33 bB	36.67 bA	6.33 aA	6.67 bA	9.92 bB	13.21 aA
G11	34.33 aA	18.00 eB	6.00 aB	8.00 aA	9.62 bA	5.35 bB
G12	25.67 bB	48.33 aA	5.33 bB	7.67 aA	9.63 bB	12.85 aA
G13	34.33 aA	25.67 dB	5.67 aB	8.00 aA	4.62 cB	12.74 aA
G14	24.33 bB	33.00 cA	7.00 aB	6.00 bA	5.56 cA	7.80 bA
G15	22.33 bB	31.33 cA	6.00 aB	7.67 aA	8.11 bB	11.36 aA
G16	29.00 aA	23.33 dA	5.00 bA	5.67 bA	3.64 cB	12.20 aA
G17	21.33 bB	31.00 cA	5.00 bA	5.67 bA	5.12 cB	10.92 aA
G18	25.33 bB	36.67 bA	6.00 aB	7.67 aA	8.59 bB	13.47 aA
G19	31.33 aA	31.33 cA	4.67 bB	7.67 aA	13.97 aA	11.63 aA
G20	26.33 bA	28.00 cA	4.50 bB	6.33 bA	9.01 bA	9.27 bA
G21	24.67 bA	25.00 cA	5.33 bA	5.33 bA	7.92 bB	13.01 aA
G22	27.67 bA	19.00 dB	6.33 aB	8.00 aA	9.30 bA	8.70 bA
G23	40.67 aA	40.00 bA	6.00 aB	9.33 aA	9.40 bA	11.16 aA
G24	20.67 bB	30.33 cA	6.33 aA	5.33 bB	11.22 bA	6.13 bB
G25	32.67 aA	25.00 dA	4.67 bB	7.33 aA	6.87 cB	12.91 aA
G26	37.00 aA	34.67 cA	4.67 bB	7.00 aA	6.95 cB	10.85 aA
G27	34.00 aA	28.67 cA	7.00 aA	7.67 aA	9.87 bA	11.80 aA
G28	28.33 bA	11.67 eB	4.33 bB	8.00 aA	10.06 bA	10.72 aA
G29	30.00 aA	33.33 cA	5.33 bB	7.33 aA	10.25 bA	11.63 aA
G30	18.33 bB	31.00 cA	5.00 bB	6.33 bA	2.77 cB	12.45 aA
G31	23.33 bA	28.00 cA	4.00 bB	8.00 aA	3.07 cB	11.56 aA
G32	31.00 aA	33.00 cA	5.00 bB	6.00 bA	10.35 bA	11.24 aA

Note: Means followed by equal lowercase letters in the same column and equal uppercase letters in the same row do not differ by the Scott-Knott test at 5% probability.

kinase (SuSK) and sucrose transporter (SUT) (Poonsawat et al., 2015). In this respect, *Erianthus arundinaceus*, *Saccharum hibrido*, *Saccharum officinarum*, *Miscanthus spp.* and *Saccharum robustum* present some of these adaptability mechanisms that allow these plants to increase their height under salinity conditions.

Regarding the number of leaves (Table 3), higher means for this variable in non-salinity environments are common, taking into account that the glycophyte plants reduce the emission of leaves as a morphological adaptation to saline stress and minimize water loss transpiration (Taiz et al., 2017). On the other contrary, Costa et al. (2021) observed that salinity-tolerant plants developed more leaves with increasing salinity exposure time and irrigation water salt concentration. In this aspect, the genotypes that showed a higher number of leaves under saline environment indicate less sensitivity to salt stress when compared to other genotypes. This result corroborates those found by Simões, Oliveira, Silva, et al. (2021) when working with a biometric characterization of *Saccharum* complex accessions subjected to saline stress.

It is possible that the saline environment may have led the genotype to develop strategies to increase the leaf area, revealing its greater adaptability to salt stress, which is important in breeding programs for abiotic stress.

Stem diameter was not affected by the presence or absence of salinity in most genotypes (Table 3). This finding agrees with other works

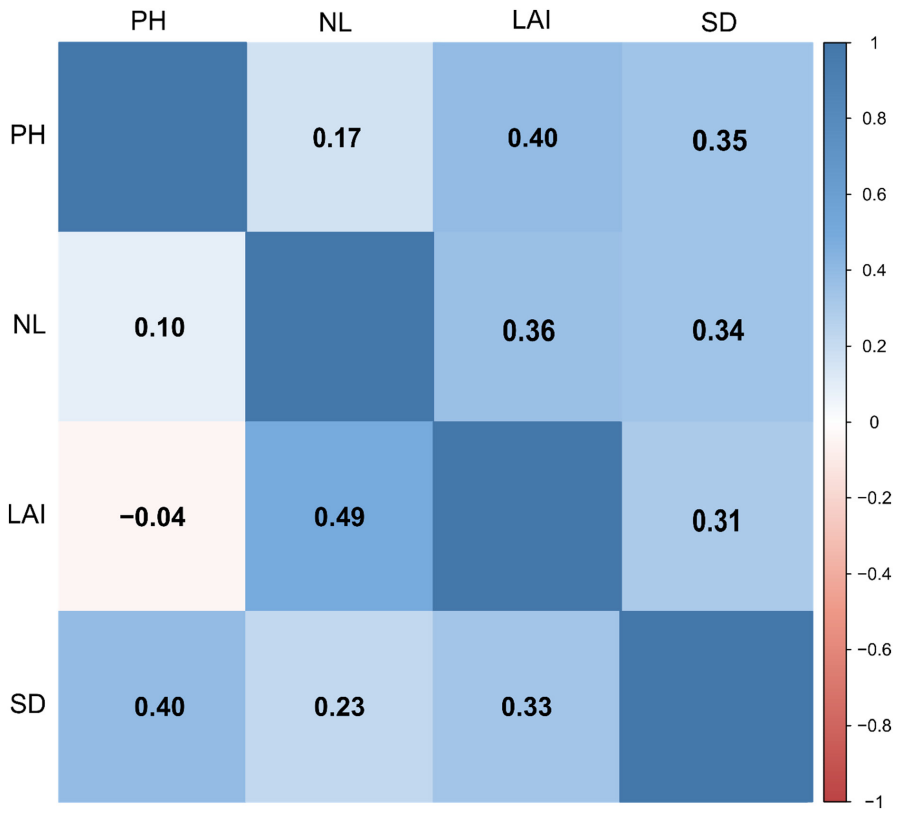

FIGURE 1 Scatterplot containing Pearson's correlation between the variables plant height (PH), number of leaves (NL), leaf area index (LAI) and stem diameter (SD) evaluated in 32 Saccharum complex genotypes grown under presence (upper diagonal) and absence (lower diagonal) of saline stress.

in the literature, which have reported that stem diameter is a growth trait with slight variation since it depends more on the genetic traits of the genotype than environmental factors (Capone et al., 2011; Oliveira et al., 2011). Thus, the larger stem diameter observed in the genotypes G1, G11 and G24 under salinity is possibly related to these genotypes' intrinsic characteristics. Different responses to salt tolerance within sugarcane genotypes occur through their high genetic variability, limiting the persistent selection pressure for individual traits related to salinity tolerance (Chiconato et al., 2019).

In Figure 1, the low magnitude and non-significant correlations among the variables indicate that plant height, number of leaves, leaf area index and stem diameter independently influence the growth of the *Saccharum* complex genotypes under the presence and absence of saline stress. The lack of correlation may be associated with sugarcane growth mainly being determined by varietal characteristics (Oliveira et al., 2011). Thus, possibly the genotypes have metabolites and genes regulating salt tolerance, favouring the growth of these plants in different environmental conditions.

However, it is important to emphasize that these findings do not exclude the importance of these growth parameters in selection. Among the variables studied, cane height is perhaps the best screening tool since it has a low coefficient of variation and is relatively easy to determine. Moreover, taller genotypes tend to have a higher mass production per stem (Carmo et al., 2013). Thus, as observed in Table 3, plant height is a tool that can help understand the tolerance of plants to abiotic stresses.

By the growth-related traits observed in this study, it is evident that the *Saccharum* complex genotypes respond differently to saline stress. The higher means for plant height, number of leaves, leaf area index and stem diameter observed in some genotypes grown in saline environment reveals a superior performance compared to others, as well as the importance of these variables as an adaptive response to stress. This behaviour may have significant genetic value since these measures are associated with growth rates (Li et al., 2017). In this regard, our findings may contribute to sugarcane breeding programs targeting abiotic stresses. However, for practical applications in breeding programs, further investigations should now focus on establishing the narrow-sense heritability of these growth traits.

5 | CONCLUSIONS

Saccharum complex genotypes maintain their growth under salinity conditions, indicating the presence of genes related to salinity tolerance. G1, G9, G11, G13, G22, G24 and G28 can be used in the diversification of sugarcane cultivars to obtain a greater productive potential in saline conditions.

AUTHOR CONTRIBUTIONS

Welson Lima Simões: Methodology; project administration; resources; writing – original draft. **Anderson Ramos de Oliveira:** Resources; visualization. **Flavio Dessaune Tardin:** Visualization. **Cintia Patrícia Martins de Oliveira:** Writing – original draft. **Lizz Kezzy de Morais:** Visualization. **Larissa Pereira Ribeiro Teodoro:** Writing – review and editing. **Paulo Eduardo Teodoro:** Writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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