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Characterization and gas exchange in accessions of *Saccharum* complex under salinity in the Sub-middle São Francisco, Brazil[1]

Caracterização e trocas gasosas de acessos do complexo *Saccharum* sob salinidade no Submédio São Francisco

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HIGHLIGHTS:
Accessions of Erianthus arundinaceus with higher photosynthetic rate, sweating and growth under salt stress.
Accessions of Saccharum officinarum present a higher photosynthetic rate under salt stress.
Characterization of accessions with adaptability to salt stress for use in breeding.

ABSTRACT: Salinity is one of the factors that most limit agricultural yield in the Brazilian semi-arid region. In this context, the present study aimed to evaluate the leaf gas exchange and biometric characteristics of accessions of the *Saccharum* complex subjected to salt stress. The experiment was carried out in a greenhouse, installed at Embrapa Semiárido, in Petrolina, PE, Brazil. The experimental design was in randomized blocks, with the treatments represented by 19 accessions belonging to different genera/species, being 10 accessions of *Saccharum officinarum* (BGCN 6, BGCN 91, BGCN 104, BGCN 127, BCGN 90, BGCN 101, BGCN 102, BGCN 118, BGCN 125 and BGCN 122), two accessions of *Saccharum* spp. (BGCN 87 and BGCN 89), one accession of *Saccharum hybridum* (BGCN 88), one accession of *Saccharum robustum* (BGCN 94), four accessions of *Erianthus arundinaceus* (BGCN 117, BGCN 119, BGCN 120 and BGCN 123) and one accession of *Miscanthus* spp., with three repetitions. Biometric characteristics, chlorophyll index and leaf gas exchange of the accessions were evaluated when they were subjected to irrigation with salinized water (6.0 dS m^{-1}). *E. arundinaceus* accessions (BGCN 120 and BGCN 123) showed the highest photosynthetic rate, transpiration rate, plant height and leaf length, indicating greater adaptability to salt stress and could be promising in breeding programs.

Key words: *Erianthus arundinaceus*, *Miscanthus* spp., physiological responses, irrigation, salt stress

RESUMO: A salinidade é um dos fatores que mais limita a produtividade agrícola no Semiárido brasileiro. Neste contexto, o presente estudo foi realizado com o objetivo de avaliar as trocas gasosas e as características biométricas de acessos do complexo *Saccharum* submetidas a estresse salino. O experimento foi realizado em casa de vegetação, instalada na sede da Embrapa Semiárido, em Petrolina, PE. O delineamento experimental foi em blocos casualizados, sendo os tratamentos representados por 19 acessos pertencentes a diferentes gêneros/espécies, sendo 10 acessos de *Saccharum officinarum* (BGCN 6, BGCN 91, BGCN 104, BGCN 127, BCGN 90, BGCN 101, BGCN 102, BGCN 118, BGCN 125 e BGCN 122), dois acessos de *Saccharum* spp. (BGCN 87 e BGCN 89), um acesso de *Saccharum hybridum* (BGCN 88), um acesso de *Saccharum robustum* (BGCN 94), quatro acessos de *Erianthus arundinaceus* (BGCN 117, BGCN 119, BGCN 120 e BGCN 123) e um acesso de *Miscanthus* spp., com três repetições. Foram avaliadas características biométricas, índice de clorofila e as trocas gasosas dos acessos submetidos à irrigação com água salinizada (6,0 dS m^{-1}). Os acessos de *E. arundinaceus* (BGCN 120 e BGCN 123) apresentaram a maior taxa fotossintética, taxa de transpiração, altura de plantas e comprimento das folhas, indicando maior adaptabilidade ao estresse salino e podem ser promissores em programa de melhoramento.

Palavras-chave: *Erianthus arundinaceus*, *Miscanthus* spp., respostas fisiológicas, irrigação, estresse salino

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Introduction

Sugarcane is a crop of great relevance in the semi-arid region, notably in the Sub-middle São Francisco Valley, where the intensive use of irrigated system promotes high yield. However, the practice of irrigation in semi-arid regions has led to soil salinization, due to the low and irregular rainfall, associated with shallow soils and poor natural drainage, or due to the use of low-quality irrigation water (Vasconcelos, 2014; Castro & Santos, 2020).

The effect of salinization on plants is an extremely complex phenomenon that involves morphological (Targino et al., 2017; Braz et al., 2019), physiological (Lira et al., 2018) and biochemical (Simões et al., 2019) changes caused by low water availability to plants and by the toxicity of specific ions, reducing growth, development and yield of the crop.

According to Maas & Hoffmam (1977), sugarcane is considered moderately sensitive to salinity, with threshold of 1.7 dS m^{-1}. However, as this response can vary significantly among cultivated materials, one of the alternatives for the sustainability of sugar-energy production in the semi-arid region may be the identification of genotypes more tolerant to salinity that can be cultivated in saline environments.

Identification of tolerant materials in breeding programs of sugarcane crop is preceded by the characterization of accessions of the genus *Saccharum* and other genera phylogenetically close to *Saccharum*, capable of providing genes of high interest for interspecific crosses, as is the case of the genera *Erianthus*, *Miscanthus*, *Sclerostachya* and *Narenga*, which together form the so-called *Saccharum* complex (Roach & Daniels, 1987; Todd et al., 2014; Oliveira et al., 2017). Thus, knowledge on biometric and physiological characteristics of *Saccharum* complex accessions under saline conditions is of fundamental importance for the recommendation of materials for use in breeding programs of the species with a view to obtaining varieties with tolerance to salinity. In view of the above, the present study aimed to evaluate the gas exchange and biometric characteristics of *Saccharum* complex accessions subjected to salt stress.

Material and Methods

The experiment was conducted in a greenhouse, installed at the Embrapa Semiárido's headquarters, in Petrolina, PE, Brazil (latitude: 9° 09' S, longitude: 40° 22' W, altitude: 365.5 m). According to Köppen's classification, the climate of the region is BSWh, characterized by being a semi-arid region, with a dry and very hot climate (Moura et al., 2007). Rains are concentrated between November and April, with an average annual rainfall of 540 mm, irregularly distributed. The average annual temperature is 26.5 °C, ranging between 21 and 32 °C, with average annual evaporation of 2,000 mm, annual average air relative humidity of 67.8%, with 3,000 hours of solar brightness and average wind speed of 2.3 m s^{-1}.

The experimental design was in randomized blocks, and the treatments were represented by 19 accessions belonging to different genera/species: 10 accessions of *Saccharum officinarum* (BGCN 6, BGCN 91, BGCN 104, BGCN 127, BCGN 90, BGCN 101, BGCN 102, BGCN 118, BGCN 125 and BGCN 122), two accessions of *Saccharum* spp. (BGCN 87 and BGCN 89), one accession of *Saccharum hybridum* (BGCN 88), one accession of *Saccharum robustum* (BGCN 94), four accessions of *Erianthus arundinaceus* (BGCN 117, BGCN 119, BGCN 120 and BGCN 123) and one accession of *Miscanthus spp.*, in three repetitions.

The accessions were obtained at the Germplasm Bank of Embrapa Tabuleiros Costeiros, and were multiplied in the Experimental Field of Mandacaru, Juazeiro, BA, Brazil, belonging to Embrapa Semiárido. At the beginning of the warm period of the region, the accessions, eight months after planting, were collected and planted in a greenhouse, being cultivated in polyethylene pots with capacity of 10 dm^3, filled with Entisol, whose chemical and textural characteristics, analyzed according to the methodology described by EMBRAPA (2017), are presented in Table 1.

Acidity was correct with the application of dolomitic limestone 90 days before planting, besides the application of macro and micronutrients following the recommendation of fertilization for the State of Pernambuco, Brazil (Cavalcanti, 2008). Two buds of each accession were planted in each pot, using uniform cuts of 3 cm of length.

The soil in the pots was irrigated until reaching field capacity, using public-supply water. At 20 days after sowing, when plants reached approximate height of 15 cm, irrigations began to be performed with the saline solutions. The water used in the irrigations was artificially salinized using sodium chloride (NaCl), calcium chloride ($CaCl_2.2H_2O$) and magnesium sulfate ($MgSO_4.7H_2O$) salts, to obtain an equivalent 7:2:1 proportion of Na:Ca:Mg (Aquino et al., 2007). The saline waters with electrical conductivity of 6.0 dS m^{-1} were renewed weekly and stored in a cool and shaded place, in order to avoid changes in their values due to possible evaporation and temperature variations.

After 90 days of salt stress, gas exchange was evaluated using a portable infrared gas analyzer (IRGA), Li-6400 Licor® model, with artificial light fixed at 2,000 µmol m^{-2} s^{-1}. The physiological

Table 1. Chemical and physical characteristics of the soil used to cultivate *Saccharum* complex accessions

OM	pH H_2O	EC_{se}	Ca^{+2}	Mg^{+2}	Na^+	K^+	H+Al	T
(g kg^{-1})	(1:2.5)	(dS m^{-1})	(cmol$_c$ dm^{-3})					
10.2	4.8	0.6	1.4	0.4	0.04	0.28	1.48	3.6
P	V	Cu	Fe	Mn	Zn	Sand	Silt	Clay
(mg dm^{-3})	(%)	(mg dm^{-3})				(g kg^{-1})		
4.2	59	0.2	9.1	12.0	1.1	730.0	190.0	80.0

EC_{se} - Electrical conductivity of saturation paste extract; OM - Organic matter by Walkley-Black method; P - Available phosphorus extracted by Mehlich-1 and determined by colorimetry; Ca and Mg extracted by 1 mol L^{-1} KCl solution and determined by flame atomic absorption spectrophotometry (air-acetylene); Na and K extracted by Mehllich-1 solution and determined by flame photometry; T - Cation exchange capacity at pH 7.0; V - Base saturation; Micronutrients (Cu, Fe, Mn and Zn) extracted with Mehlich-1 and determined by flame atomic absorption spectrophotometry (air-acetylene) variables of the plants analyzed were: photosynthesis rate (A), stomatal conductance (gs), leaf temperature and transpiration (E). The different accessions of the *Saccharum* complex subjected to salt stress were also analyzed for the biometric characteristics: plant height (PH), number of leaves (NL), stem diameter (SD), length (LL) and width (LW) of the +3 leaf, which corresponds to the third fully expanded leaf from top to bottom with visible ligule, and chlorophyll index (CI).

Plant height was obtained with a tape measure, from the base of the stem to the insertion of the last visible leaf. These same plants were also evaluated for width and length of the +3 leaf with a tape measure, and the data related to stem diameter were obtained using a digital caliper positioned at the third node, where three measurements were taken per plant. Chlorophyll index was obtained using a portable chlorophyll meter, with readings performed on the +3 leaf, between 8 and 10 a.m.

The obtained data were subjected to analysis of variance using the Sisvar 5.0 program. Comparison between accessions was performed using the Scott-Knott test ($p \leq 0.05$).

Results and Discussion

There were significant differences between genotypes when irrigated with saline water (6.0 dS m^{-1}) for the variables photosynthetic rate, stomatal conductance and transpiration, number of leaves, plant height, stem diameter, leaf length and leaf width. However, leaf temperature and chlorophyll index did not differ among the genotypes (Table 2).

Table 3 shows that two accessions of *E. arundinaceus* (BGCN 120 and BGCN 123) and three accessions of *S. officinarum* (BCGN 6, BCGN 102 and BCGN 101) formed the group that had the highest photosynthetic rate, indicating higher capacity for the formation of photoassimilates compared to the other accessions analyzed, when subjected to salt stress.

According to Simões et al. (2019), soil salinization significantly affects the gas exchange of sugarcane varieties and its detailed evaluation can demonstrate their adaptability to the stress conditions imposed. In addition, Taiz et al. (2017) report that higher rates of stomatal conductance, transpiration and photosynthesis lead to significant increase in crop production.

Augustine et al. (2015) demonstrated that *E. arundinaceus* has heat shock proteins, known as HSPs, and the expression of the HSP70 protein plays a relevant role in stress tolerance in plants, both water stress and salt stress. Thus, in a process of genetic improvement of sugarcane, this species can be strategic.

For stomatal conductance, it was verified that the accessions BGCN 123 and BGCN 120 of *E. arundinaceus* grouped again and had the highest values of stomatal conductance, which explains the high photosynthetic rate, but under high transpiration rate. On the other hand, the accessions BGCN 6, BGCN 101 and BGCN 102 of *S. officinarum* were part of a third group of reduced stomatal conductance. Probably, salt stress caused the reduction in stomatal conductance, with consequent stomatal closure and reduction in water absorption by the crop, which signals an ability to minimize water loss by transpiration,

Table 2. Summary of the analysis of variance for photosynthetic rate (*A*), stomatal conductance (*gs*), leaf temperature (LT), transpiration rate (*E*), number of leaves (NL), plant height (PH), stem diameter (SD), leaf length (LL), leaf width (LW) and chlorophyll index (CI) at 110 days after planting

Variable	A	gs	LT	E	NL	PH	SD	LL	LW	CI
	F value									
Block	0.418	0.899	0.043	0.269	2.440	0.288	0.874	0.725	0.462	0.775
Treatment	7.294*	13.336*	1.884^{ns}	3.292*	2.726*	7.501*	11.684*	7.045*	2.905*	1.431^{ns}
CV (%)	20.48	28.47	6.17	29.52	17.24	12.18	21.75	11.70	30.50	11.02

CV - Coefficient of variation

Table 3. Means of photosynthetic rate (A), stomatal conductance (gs), leaf temperature (LT) and transpiration rate (E) of *Saccharum* complex accessions subjected to salt stress, at 110 days after planting

Accession	Genus/Species	A (μmol CO_2 m^{-2} s^{-1})	gs (mol m^{-2} s^{-1})	LT (°C)	E (mmol H_2O m^{-2} s^{-1})
BGCN 117	*Erianthus arundinaceus*	9.92 c	0.14 d	31.16 a	1.53 b
BGCN 123	*Erianthus arundinaceus*	24.11 a	0.47 a	31.01 a	4.00 a
BGCN 120	*Erianthus arundinaceus*	26.84 a	0.53 a	29.70 a	4.10 a
BGCN 119	*Erianthus arundinaceus*	12.48 c	0.19 c	31.89 a	2.20 b
BGCN 114	*Miscanthus* spp.	15.02 b	0.30 b	29.63 a	2.56 b
BGCN 88	*Saccharum hybridum*	16.95 b	0.25 c	31.74 a	3.00 a
BGCN 90	*Saccharum officinarum*	9.51 c	0.07 d	34.77 a	2.00 b
BGCN 91	*Saccharum officinarum*	11.76 c	0.18 c	31.17 a	2.66 b
BGCN 104	*Saccharum officinarum*	17.25 b	0.12 d	33.27 a	2.46 b
BGCN 122	*Saccharum officinarum*	12.14 c	0.08 d	34.77 a	1.90 b
BGCN 118	*Saccharum officinarum*	12.51 c	0.05 d	31.23 a	1.50 b
BGCN 6	*Saccharum officinarum*	23.29 a	0.19 c	31.02 a	3.00 a
BGCN 127	*Saccharum officinarum*	16.89 b	0.18 c	32.85 a	4.03 a
BGCN 125	*Saccharum officinarum*	18.08 b	0.20 c	33.60 a	2.80 b
BGCN 102	*Saccharum officinarum*	21.17 a	0.22 c	32.17 a	3.86 a
BGCN 101	*Saccharum officinarum*	22.74 a	0.20 c	32.39 a	3.53 a
BGCN 94	*Saccharum robustum*	9.78 c	0.10 d	32.80 a	1.90 b
BGCN 89	*Saccharum* sp.	17.17 b	0.39 b	29.74 a	3.03 a
BGCN 87	*Saccharum* spp.	14.12 b	0.20 c	33.77 a	4.00 a

Means followed by equal letters in the column do not differ by the Scott-Knott test at $p \leq 0.05$

being a response of plants to salinity. According to Inman-Bamber et al. (2005), reduction in stomatal conductance is a strategy of sugarcane to prevent leaf dehydration. However, the photosynthetic rates for these three accessions were high, suggesting their apparent adaptability to salinity.

The results are similar to those found by Simões et al. (2019), who studied sugarcane varieties subjected to different values of water electrical conductivity and observed that the variety RB 867515 maintains the same photosynthetic rate with increasing salinity, despite showing a reduction in its stomatal conductance, demonstrating relative tolerance to salinity. Nonetheless, Lira et al. (2018) in a study with the cultivar RB 867515, concluded that the increase in irrigation water salinity inhibited the stomatal conductance and photosynthetic capacity of the plants.

The accessions did not show differences in leaf temperature, which averaged 32.04 °C. Regarding the transpiration rate, two groups were formed, and the accessions BGCN 123 and BGCN 120 of *E. arundinaceus* and BGCN 88, BGCN 6, BGCN 102, BGCN 127, BGCN 101, BGCN 89 and BGCN 87 of the genus *Saccharum* were grouped among those with highest transpiration rates.

Regarding biometric characteristics, among the accessions studied, three of *E. arundinaceus* (BGCN 117, BGCN 123 and BGCN 119), one of *Miscanthus* spp. (BGCN 114), one of *Saccharum hybrid* (BGCN 88), one of *Saccharum officinarum* (BGCN 6) and one of *Saccharum robustum* (BGCN 94) obtained the highest numbers of leaves (Table 4).

Regarding plant height, the highest values were observed for the accessions of *E. arundinaceus* (BGCN 123, BGCN 120, and BGCN 119), *Miscanthus* spp. (BGCN 114), *Saccharum hybrid* (BGCN 88), *Saccharum officinarum* (BGCN 90, BGCN 118, BGCN 6), *Saccharum robustum* (BGCN 94) and *Saccharum* spp. (BGCN 87).

The behavior of the accessions in relation to plant height and number, length and width of the living leaves resulted in different clusters as a function of the variables analyzed. However, the analysis of stem diameter made it possible to identify the accessions of *Saccharum officinarum* (BGCN 6 and BGCN 118) as the ones with largest diameter.

The highest values of leaf length were observed for the accessions of *E. arundinaceus* (BGCN 123), *Miscanthus* spp. (BGCN 114), *Saccharum hybridum* (BGCN 88), *Saccharum officinarum* (BGCN 90 and BGCN 91), *Saccharum robustum* (BGCN 94) and *Saccharum* spp. (BGCN 87).

The highest values of leaf width were observed for the accessions *E. arundinaceus* (BGCN 117, BGCN 123, BGCN 120 and BGCN 119), *Miscanthus* spp. (BGCN 114), *Saccharum officinarum* (BGCN 90, BGCN 91, BGCN 118, BGCN 6), *Saccharum robustum* (BGCN 94) and *Saccharum* spp. (BGCN 87). Considering that salinity tends to reduce leaf width (Taiz et al., 2017), this variable should also be taken into account for this type of evaluation, since plants with larger leaf area may have greater production potential due to the greater capacity to intercept solar radiation, resulting in greater biomass accumulation.

It should also be considered that the chlorophyll index when correlated with chlorophyll and carotenoid contents in sugarcane can be used as a technique in the selection of water deficit-tolerant cultivars in breeding programs (Silva et al., 2014). However, salinity did not cause differences in the chlorophyll content of the different accessions evaluated in the present study.

During salt stress, photosynthesis and cell growth are directly affected by the reduction in the availability of carbon dioxide and changes in photosynthetic metabolism, or indirectly affected by oxidative stress (Simões et al., 2018). In this context, the higher adaptability to salt stress of the accessions of *E. arundinaceus* (BGCN 120 and BGCN 123) can be observed through the higher values of photosynthetic rate and transpiration, plant height and leaf length, compared to the others, under the imposed conditions of salt stress. This result corroborates those obtained by Munns (2011) and Munns & Gilliham (2015), who report that salt stress directly affects the photosynthetic process, with consequent losses of growth and

Table 4. Means of number of leaves (NL), plant height (PH), stem diameter (SD), leaf length (LL), leaf width (LW) and chlorophyll index (CI) of the different accessions of the *Saccharum* complex subjected to salt stress, at 110 days after planting

Accession	Genus/Species	NL	PH (cm)	SD (cm)	LL (cm)	LW (cm)	CI
BGCN 117	*Erianthus arundinaceus*	6.3 a	22.3 c	9.9 b	83.6 b	3.5 a	37.0 a
BGCN 123	*Erianthus arundinaceus*	7.3 a	32.3 a	9.9 b	109.6 a	2.6 a	32.9 a
BGCN 120	*Erianthus arundinaceus*	5.3 b	30.0 a	10.2 b	83.3 b	3.0 a	30.7 a
BGCN 119	*Erianthus arundinaceus*	6.0 a	33.6 a	10.3 b	83.0 b	2.6 a	36.7 a
BGCN 114	*Miscanthus* spp.	6.0 a	40.6 a	9.4 b	106.0 a	2.3 a	35.1 a
BGCN 88	*Saccharum hybridum*	6.0 a	34.5 a	9.6 b	119.5 a	1.5 b	34.6 a
BGCN 90	*Saccharum officinarum*	5.6 b	34.3 a	4.6 c	97.6 a	2.6 a	37.1 a
BGCN 91	*Saccharum officinarum*	5.3 b	28.0 b	6.1 c	105.0 a	2.2 a	34.6 a
BGCN 104	*Saccharum officinarum*	4.6 b	26.3 b	4.8 c	67.0 c	1.6 b	34.7 a
BGCN 122	*Saccharum officinarum*	5.0 b	18.3 c	2.8 c	65.6 c	1.1 b	31.8 a
BGCN 118	*Saccharum officinarum*	4.6 b	31.3 a	14.0 a	85.6 b	1.8 b	35.3 a
BGCN 6	*Saccharum officinarum*	6.0 a	33.3 a	16.1 a	80.0 b	2.5 a	33.6 a
BGCN 127	*Saccharum officinarum*	4.0 b	26.6 b	4.5 c	64.0 c	1.6 b	33.9 a
BGCN 125	*Saccharum officinarum*	5.3 b	24.6 b	7.9 b	80.0 b	3.0 a	32.2 a
BGCN 102	*Saccharum officinarum*	5.0 b	22.0 c	5.3 c	90.0 b	2.5 a	32.2 a
BGCN 101	*Saccharum officinarum*	5.0 b	29.0 b	3.6 c	80.0 b	1.0 b	26.4 a
BGCN 94	*Saccharum robustum*	7.6 a	34.6 a	10.5 b	104.3 a	1.6 b	34.4 a
BGCN 89	*Saccharum* sp.	5.3 b	25.6 b	9.6 b	76.6 b	2.4 a	36.1 a
BGCN 87	*Saccharum* spp.	4.6 b	37.0 a	6.9 c	102.3 a	2.0 b	32.0 a

Means followed by equal letters in the column do not differ by the Scott-Knott test at $p \leq 0.05$

yield. Therefore, for having higher photosynthetic efficiency, these materials can be used in studies for increments of yield.

Due to cellular changes that occur in plants under salt stress, studying plant physiology is extremely important, in order to understand how stress occurs and its consequences, since each species can respond differently. Knowledge on possible physiological indicators contributes to the selection of more tolerant cultivars and to sugarcane breeding programs.

Therefore, each variety of sugarcane can respond differently to salt stress (Sengar et al., 2013). The selection of cultivars that develop satisfactorily in saline environments is a challenge in terms of the expansion of sugarcane production, both in Brazil and in the world, due to the large area affected by salinity.

Conclusion

The accessions of *E. arundinaceus* (BGCN 120 and BGCN 123) have higher photosynthetic rate, transpiration rate, plant height and leaf length, indicating greater adaptability to salt stress and may be important in breeding programs.

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