Ciência e Agrotecnologia, 42(5):527-539, Sep./Oct. 2018
http://dx.doi.org/10.1590/1413-70542018425020718

The different response of sugarcane genotypes in multiple stress

Diferente resposta de genótipos de cana-de-açúcar em múltiplo estresse

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Received in August 17, 2018 and approved in September 28, 2018

ABSTRACT

Research focused on identify abiotic stress-tolerant genotypes is highly desirable since their use may reduce costs of soil and crop management and productivity losses. The aim of this study was to determine the behavior of 24 sugarcane genotypes under high levels of Al^{3+} and Mn^{2+} associated with low availability of mineral nutrients. The experiment was carried out under greenhouse condition in a 24 × 2 factorial scheme (24 genotypes × 2 treatments: with and without stress), and four replications in completely randomized design. In the treatment without stress plants were grown in a complete nutrient solution whereas in the treatment with stress a nutrient solution with a high acidity (4.0 ± 0.1) and 5% of its original concentration, as well as a high concentration of aluminum (60 mg L^{-1}) and manganese (700 mg L^{-1}) was used. The genotypes RB966928, RB855443, IACSP96-3060, SP81-3250, RB867515, CTC 21, RB965902, and IAC91-1099 had their biometric characteristics less affected by the stress, possibly due to the ability to continue the process of cell division and elongation and to maintain meristematic viable regions, hence they were considered as the most tolerant. On the other hand, the genotypes RB965917, CTC 15, CTC17, RB855536, CTC 2, CTC 20, and CTC99-1906 were the most sensitive to stress. Root system was the most affected by stress, with most genotypes showing more than 70% reduction in root biomass. No relationship was observed between tolerance level of genotypes and the maturation cycles.

Index terms: Abiotic stress; greenhouse; *Saccharum* spp; hydroponic alternative system.

RESUMO

Pesquisas científicas focadas em identificar genótipos tolerantes a estresse abiótico são altamente desejáveis, pois, o uso desses genótipos permite reduzir custos de manejo do solo, da cultura e perdas de produtividade. O objetivo desse trabalho foi determinar o comportamento de 24 genótipos de cana-de-açúcar sob elevados teores de Al^{3+} e Mn^{2+}, associado a baixa disponibilidade de nutrientes. O ensaio foi instalado e conduzido em casa de vegetação em delineamento inteiramente casualizado, num esquema fatorial 24x2, correspondendo a 24 genótipos, dois tratamentos (com e sem estresse), com quatro repetições. No tratamento sem estresse, as plantas foram cultivadas em solução nutritiva completa e no com estresse foi utilizada solução nutritiva com elevada acidez (4,0 ± 0,1) e com 5% da sua concentração original, além da elevada concentração de alumínio (60 mg L^{-1}) e manganês (700 mg L^{-1}). Os genótipos RB966928, RB855443, IACSP96-3060, SP81-3250, RB867515, CTC 21, RB965902 e IAC91-1099 foram os que tiveram suas características biométricas menos afetadas pelo estresse, possivelmente devido a capacidade de continuarem o processo de divisão e elongação celular e manterem regiões meristemáticas viáveis, dessa forma, foram considerados os mais tolerantes. Por outro lado, os genótipos RB965917, CTC 15, CTC17, RB855536, CTC 2, CTC 20 e CTC99-1906 foram os mais sensíveis ao estresse. O sistema radicular foi o mais afetado pelo estresse sendo que a maioria dos genótipos apresentaram mais de 70% de redução da biomassa da raiz. Não houve relação entre o nível de tolerância dos genótipos com os ciclos de maturação.

Termos para indexação: Estresse abiótico; casa de vegetação; *Saccharum* spp.; sistema alternativo de hidroponia.

INTRODUCTION

Due to the growing demand from domestic and foreign markets for renewable fuels, sugar, and bioenergy, sugarcane (*Saccharum* spp.) has become increasingly important in the Brazilian scenario (Unica, 2017). Currently, along with their derivatives, sugarcane compose the second largest source of primary energy in the Brazilian energy matrix (Maia et al., 2018).

As a result, in recent years there has been a great expansion of sugarcane cultivation in Brazil (Goldfray et al., 2010; Caldarelli; Gilio, 2018). With this expansion, sugarcane has advanced to regions of the west of São Paulo State and areas of the Brazilian Cerrado, which are characterized by acid soils, high levels of toxic elements such as aluminum (Al^{3+}) and manganese (Mn^{2+}), and generalized nutrient deficiency (Sousa; Miranda; Oliveira, 2007).

The stress caused by Al^{3+} is among the most significant to the crop, damaging mainly its root system and reflecting in a low water and nutrient absorption, consequently reducing plant growth and development (Chen et al., 2010; Silva et al., 2010).

The scientific research focused on identifying and understanding genotypes tolerant to these conditions is highly desirable since their use directly in the field or indirectly in breeding processes allows reducing costs of soil and crop management and productivity losses, which is reflected in an increased agricultural and industrial stability and yield (Too et al., 2014).

When there are no limiting climatic and soil factors, sugarcane develops a large part of its metabolically active roots from the soil surface up to about one meter deep and can reach up to two meters deep in the soil (Luchiari Junior, 1986). However, even correcting and fertilizing the soil arable layer, unfavorable chemical characteristics remain in the subsurface of soil, acting as a chemical barrier that limits root development in depth (Raij, 2011).

Considering that plants are routinely subjected to the interaction of different abiotic stresses, it seems coherent to consider that in these studies the genotypes be submitted to their combined effect (Carlin; Rhein; Santos, 2012). However, most studies with the aim at evaluating the behavior of genotypes to abiotic stresses are limited to studying one factor at a time (Fonseca Júnior et al., 2014; Maia et al., 2018). These studies are very important, but not indicated to choose genotypes for limiting environments such as those in crop expansion areas. In this context, this study aimed to determine the behavior of 24 sugarcane genotypes under high contents of Al^{3+} and Mn^{2+}, associated with a low nutrient availability, as well as verify the genetic variability related to this joint stress, the relationship between tolerance and maturation cycle (early, medium, and late), and determine the possible tolerant and susceptible genotypes.

MATERIAL AND METHODS

Experiments for methodology adjustment

The experiment was carried out in the São Paulo State University, Unesp, School of Agricultural and Veterinary Sciences (FCAV–UNESP), Jaboticabal, SP, located at the geographical coordinates 21°15′22″ S and 48°18′58″ W, with an altitude of 575 m, in the period from April 4 to July 23, 2017.

For carrying out this study, the nutritive solution was provided by means of the system described by Dantas et al. (2001). Briefly, seedlings were obtained from culm billets (3 cm length) containing one bud each and followed the methods described in Carlin, Rhein and Santos, (2012). After cutting, the culm billets were immediately planted in 500 mL capacity plastic containers with holes in the lower part and containing washed and sieved (2 mm) sand. Seedlings were grown without any water restriction for 28 days. After this period, they were selected regarding sanity and homogeneity, being transplanted to 1 L capacity plastic pots (dimensions of 15 × 9 × 9 cm), with small holes at 0.5 cm from the bottom to allow the entrance of the nutrient solution, and containing 750 ml of washed sand. Then, the described plastic pots were placed in a tray with dimensions of 42 × 36 × 11 cm, in which was maintained a layer of 5 cm of nutritive solution, prepared according treatment of each assay. The level of solution was completed daily with distilled water and completely replaced by fresh solution every three days.

To test the mentioned hidroponic alternative system for sugarcane, and to define Al^{3+} dose and to find the suitable solution nutrient strength, three preliminary tests were carried out in order to determine the best conditions to work in the main assay with genotypes. All these experiments were carried out in a greenhouse under the same conditions and with the same genotype (IACSP 95-5000). The IACSP 95-5000 is indicated for favorable environments (A1 - C2) and therefore is sensitive to acid and poor soils (Chaves et al., 2015).

The first one aimed to verify the efficiency of hydroponic alternative system in comparison to the traditional hydroponic system. This assay was carried out in a completely randomized design with two treatments (traditional hydroponic systems, and the alternative system) with 10 replications each. In both systems was used a complete nutrient solution (Furlani; Furlani, 1988) with adaptations for sugarcane, prepared with the following stock solutions: 3.1 ml L^{-1} $Ca(NO_3)_2$ 1.64 mol L^{-1}, 3.1 ml L^{-1} NH_4NO_3 0.42 mol L^{-1}, 2.2 ml L^{-1} KCl 0.25 mol L^{-1}, 2.2 ml L^{-1} K_2SO_4 0.25 mol L^{-1}, 2.2 ml $L^{-1}KNO_3$ 0.24 mol L^{-1}, 1.6 ml L^{-1} $Mg(NO_3)_2$ 0.96 mol L^{-1}, 0.2 ml L^{-1} KH_2PO_4 0.13 mol L^{-1}, 0.6 ml L^{-1} FeDDH 0.16 mol L^{-1}, 0.6 ml L^{-1} $MnCl_2$ 0.08 mol L^{-1}, 0.6 ml L^{-1} H_3BO_2 0.03 mol L^{-1}, 0.6 ml L^{-1} $ZnSO_4$ 0.005 mol L^{-1}, 0.6 ml L^{-1} CuSO4 0.001 mol L^{-1}, and 0.6 ml L^{-1} Na_3MoO_2 0.001 mol L^{-1}, with final pH adjustment to 5.5 ± 0.1 by using HCl 0.1 mol L^{-1} or NaOH mol L^{-1}. For traditional hidroponic system, a layer of 10 cm of nutritive solution was used kipping continuous aeration, while for alternative system, a 5cm layer was maintained in the tray without aeration. In both systems the level of solutions was completed daily with distilled water, and completely replaced by fresh solution every three days.

The other two experiments were conducted with the mentioned alternative system, in a completely randomized design. For the second preliminary assay,

treatments consisted of eight Al^{3+} concentrations in the complete nutrient solution (0, 10, 20, 30, 40, 50, 60, and 70 mg L^{-1}), with three replications. Regarding the assay to define the dilution to be used as low nutrient concentration associated with Al^{3+} and Mn^{2+} toxicity, the concentrations of 0, 5, 10, 15 and 20% of the complete solution (Furlani; Furlani, 1988) were tested, maintaining fixed the doses of 60 and 700 mg L^{-1} of Al^{3+} and Mn^{2+}, respectively, with four replications.

In all three preliminary assays, plants were maintained under respective treatments for 30 days and evaluated for shoot and root dry matter.

Main experiment with 24 genotypes grown with and without stress

Location and experimental design

The experiment was performed in a greenhouse in a completely randomized design, in a 24 × 2 factorial scheme, consisting of 24 genotypes, two treaments (with and without stress), and four replications, totaling 192 experimental units. Seedlings used in this experiment were obtained and selected as mentioned for preliminary assays.

Genotypes tested

The 24 genotypes used in this study belong to three distinct groups regarding maturation, previously identified as early, medium, and late maturation cycle (Table 1). Seedlings were prepared as above mentioned, and propagules were obtained from plants with same age, grown in the FCAV-UNESP experimental farm, thus submitted to the same conditions of management, climate, and soil.

Characterization of treatments with and without stress

For the treatment without stress, plants were grown in a complete nutrient solution (Furlani; Furlani, 1988) with adaptations for sugarcane and a pH of 5.5 ± 0.1. For the treatment with stress, the nutrient solution was only 5% of its original concentration, and with a high acidity (4.0 ± 0.1), containing high concentration of aluminum (60 mg L^{-1}) and manganese (700 mg L^{-1}), both applied in the form of chloride. For determining the ionic strength of nutrient solution (5%) and the aluminum dose (60 mg L^{-1}), the results of the preliminary tests were taken as a basis and manganese concentration (700 mg L^{-1}) was defined based on the literature (Benett et al., 2012).

Solution level was corrected daily by adding distilled water, at that time, pH was also corrected. Trays were cleaned and solutions completely replaced every three days when all trays were randomly changed, as well as the seedlings in each tray, in order to keep all experimental units in a completely randomized design.

Analyzed variables

After 50 days, the following non-destructive assessments were performed: number of green leaves, number of dead leaves, stem height, stem diameter, plant height, and leaf area. The live leaves were considered those completely open and with at least 20% green area and the dead leaves were considered those with 20% less green area.

Table 1: Identification of genotypes and classification regarding maturation cycles.

Maturation Cycles of Genotypes					
Early		Medium		Late	
No.	Genotype	No.	Genotype	No.	Genotype
1	SP91-1049	9	SP81-3250*	17	SP83-2847
2	RB855443	10	IAC91-1099	18	SP80-3280
3	RB966928*	11	IACSP95-5094	19	IACSP95-5000
4	RB965902	12	IACSP96-3060	20	CTC 6
5	RB965917	13	CTC 2	21	CTC 15
6	CTC 9	14	CTC 20	22	RB855536
7	CTC17	15	CTC 24	23	RB867515*
8	CTC 21	16	CTC99-1906	24	RB935744*

Source: Agronomic Institute of Campinas (IAC, 2017), RIDESA, (2010), Sugarcane Technology Center (CTC, 2018), * described in Maia et al. (2018).

Leaf area was determined following the methodology proposed by Hermann and Câmara, (1999). Green color index was determined by using a portable chlorophyll meter model CCM-200 (Opti-Scienses, Inc.), with three measurements in the leaf +1 of each plant, obtaining the green color index (GCI) data of leaf.

After non destructive measurements, plants were harvested, and separated into the shoot, roots, and culm billets. Root volume of plants was determined by immersing the roots in a graduated test tube with distilled water and measuring the displaced volume. All samples were then placed in properly identified paper bags, weighed to determine the fresh matter, and dried in a forced air circulation oven at 65 ± 5 °C until constant weight for determining the dry matter.

Considering that different genotypes have different growth potentials, the comparison of absolute values is not suitable to compare tolerance among genotypes. Because of that, for the main experiment, the relative growth (or relative growth imitation) was used to compare data, and tolerance among genotypes (Lima; Peixoto; Ledo, 2007; Maia et al., 2018). Thus, the average value of replications of each genotype without stress was considered to be 100%. Then, this value was used as reference to calculate relative values of all data obtained in all experimental units belonging to the same genotype. So these relative values were submitted to statistical analysis.

Statistical analysis

The results of the preliminary experiments were submitted to analysis of variance by the F-test and mean comparison by the Tukey's test at a 5% probability level. In the experiment in which Al concentrations were tested, when a significant effect was detected, the polynomial regression analysis was applied by using the software AgroEstat (Barbosa; Maldonado Junior, 2015).

In the main experiment, analysis of variance (F-test) was carried out with relative data in a 24 × 2 factorial scheme (24 genotypes × 2 treatments). Mean comparison was carried out by the Scott-Knott test at 5% probability, also using the software AgroEstat (Barbosa; Maldonado Junior, 2015).

In order to observe the similarity among genotypes, a multivariate exploratory analysis was also carried out by using the software Statistica 7.0 (StatSoft. Inc, 2004), since it is commonly applied statistics to provide additional information on the response of genotypes to different environments (Fox; Crossa; Romagosa, 1997). In order to differentiate the clusters and their relationships with the studied variables, a non-hierarchical method was performed by the k-means grouping.

In addition, the factor analysis was used to identify the processes that would respond to the highest variabilities of the measured variables. For this, we first calculated the load values of provisional factors determined by the principal components analysis, which allowed to obtain components which eigenvalues were not lower than 1, following Kaiser (1958) criterion.

The principal component analysis was used as a technique to extract factors (Seal, 1964; Jeffers, 1978), which is based on the correlation matrix between variables. Factors with eigenvalues ≥ 1 were selected also by following also the Kaiser (1958) criterion. In order to identify the factors, the VARIMAX rotation method was adopted (Kaiser, 1958; Hoffmann, 1992), in addition to providing a better interpretation of factors, this method has as objective to obtain a matrix of loads more identifiable regarding the nature of the measured variables (Maxwell, 1977).

RESULTS AND DISCUSSION

Experiments for methodology adjustment

The hydroponic alternative system used in this study provided similar results ($P > 0.05$) to those observed for the traditional hydroponic system for all variables of growth and development of Sugarcane plants (Table 2). A better root development was obtained in the alternative system when compared to the traditional hydroponic system. This result is very important in this type of research since this variable is one of the most important to study the tolerance of plants to Al^{3+} toxicity (Ecco; Santiago; Lima, 2014; Maia et al., 2018).

The great advantage of this hydroponic alternative system is that it allows assessing a large number of experimental units in a much simpler and economical way since it does not need the oxygenation system of the nutrient solution. In addition, it allows a greater management control on the nutrient solution, as well as changing plant position randomly without the risk of damaging its root.

The results for the shoot and root dry matter with increasing Al^{3+} doses showed that the maximum applied concentration (70 mg L^{-1}) was not lethal for plants (Figure 1A). For shoot dry matter (SDM), a decreasing linear effect was observed as the Al^{3+} concentration increased, with an average weight of 5.19 and 4.38 g for the 60 and 70 mg L^{-1} doses, respectively. For root dry matter (RDM), the same effect was also observed as the aluminum doses increased, with a mean weight of 3.11 and 1.80 g for the 40 and 70 mg L^{-1} doses, respectively.

In relation to the experiment that tested nutrient solution, no effect was observed on shoot dry mass in the

concentration of up to 20% of the original values proposed by Furlani and Furlani (1998), where Al^{3+} and Mn^{2+} remained constant. This was probably due to seedling age, i.e. at this stage, only the reserve still available in the culm piece is sufficient for plant development. In addition, symptoms of Al^{3+} toxicity in the shoot are not always readily identifiable (Vitti; Mazza, 2002) and, unlike the root system, have a little direct effect of this element, mainly in relatively short-time (Rossiello; Jacob-Netto, 2006). The concentration that less affected root system was that of 5 and 10% of the nutrient solution (Figure 1B). Thus, the concentration of 5% is sufficient to lead to a nutritional stress and be assessed with the effect of Al^{3+} and Mn^{2+} without causing root death.

Main experiment with 24 genotypes grown with and without stress

As for the main experiment with the 24 genotypes, the analysis of variance showed a significant effect ($P < 0.05$) for genotypes, treatment, and the interaction genotype × treatment for all 14 biometric variables, except for culm billets fresh, and dry weight. This result shows the existence of genetic variability among the 24 genotypes (Table 3). The significant effect of the treatment on the studied variables also indicates that the conditions were adequate to assess the proposed stress in sugarcane by using this new hydroponic system.

In the principal component analysis (PCA), two components were extracted, which explained 57.46% of the total variation, discriminating the genotypes in four groups (Figure 2A)

The first component (PC1) explains about 42% of the total variation of genotypes and has groups represented in green and blue colors, being strongly influenced positively by the variables of the shoot and root biomass (Figure 2A). Genotypes that are furthest from the origin, farther to the right and more aligned with the horizontal axis, are the most tolerant to stress. In turn, the red group, which presents totally opposite behavior of the genotypes of the green and blue groups, should be the most sensitive to the environment under stress.

Table 2: Means, least significant difference (LSD 5%), and standard error of the means (SE) of the biometric characteristics from sugarcane plants (IACSP95-5000) under traditional (HP) and adapted (AS) alternative system.

Systems	Variables									
	SL	PH	LA	RDW	SDW	NGL	SFW	RFW	RV	SD
	-------(cm)-----		-(cm²)-	-------(g)------		(N°)	-------(g)------		-(ml)-	(cm)
HP	22.85a	103.42a	284.37a	4.61b	8.23a	4.30a	35.98a	33.56a	49.65a	1.09a
AS	21.05a	91.30a	236.94a	9.02a	8.48a	4.10a	32.55a	33.27a	47.75a	0.97a
SE	± 1.01	± 6.56	± 25.71	±0.75	±0.88	± 0.16	± 3.89	± 2.24	± 2.11	±0.05
LSD	3.01	19.51	76.40	2.22	2.63	0.49	11.57	6.65	6.27	0.16
CV (%)	14.59	21.33	31.19	34.79	33.48	12.54	35.94	21.20	13.72	16.97

Stem length (SL), plant height (PH), leaf area (LA), root fresh weight (RFW), shoot dry weight (SDW), number of green leaves (NGL), shoot fresh weight (SFW), root dry weight (RDW), root volume (RV), and stem diameter (SD), Means followed by the same letter in the column do not differ statistically from each other by the Tukey's test at 5% probability level.

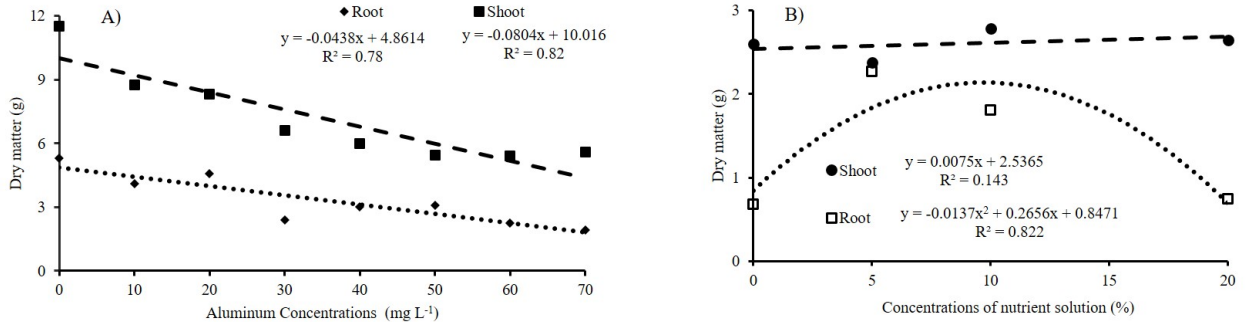

Figure 1: Values of the shoot and root dry matter of the genotype IACSP95-5000 submitted to Al doses (A) and different concentrations of nutrient solution (B).

Table 3: Analysis of Variance for Major Effects and Interaction for all variables with relativized data.

CV	Shoot						
	SDW	LA	NGL	NDL	PH	GCI	SFW
	F Calculated						
Genotype (G)	3.52 NS	1.35 NS	1.43 NS	1.38 NS	1.63 NS	0.27 NS	1.29 NS
Treatment (T)	401.98**	383.25**	457.99**	114.79**	894.75**	933.63**	677.64**
Interaction G × T	3.50 NS	1.31 NS	1.43 NS	1.38 NS	1.64 NS	0.27 NS	1.28 NS
SE	± 2.71	± 12.93	± 4.75	± 13.44	± 4.55	± 9.05	± 5.58
CV (%)	5.87	30.97	11.15	33.93	11.33	30.15	14
CV	Root, Stem and culm billets						
	RV	RFW	RDW	CBFW	CBDW	SD	SH
	F Calculated						
Genotype (G)	0.36 NS	0.22 NS	1.11 NS	1.96 NS	1.69 NS	1.98 NS	1.33 NS
Treatment (T)	1230.86**	1046.8**	1352.8**	0.11 NS	7.29**	280.86**	292.86**
interaction G × T	0.36 NS	0.22 NS	0.99 NS	1.96 NS	1.69 NS	1.98 NS	1.30 NS
SE	± 7.70	± 8.77	± 8.13	± 8.92	± 10.20	± 5.60	± 6.29
CV (%)	25.26	29.42	27.12	17.76	19.62	12.97	14.88

Plant height (PH), leaf area (LA), root fresh weight (RFW), shoot dry weight (SDW), number of green leaves (NGL), number of dead leaves (NDL), shoot fresh weight (SFW), root dry weight (RDW), green color index (GCI), root volume (RV), stem diameter (SD), stem height (SH), cause of variation (CV), culm billet fresh weight (CBFW), culm billet dry weight (CBDW), standard error of the means (SE) and coefficient of variation (CV). * significant at 5% probability, ** significant at 1% probability e NS not significant.

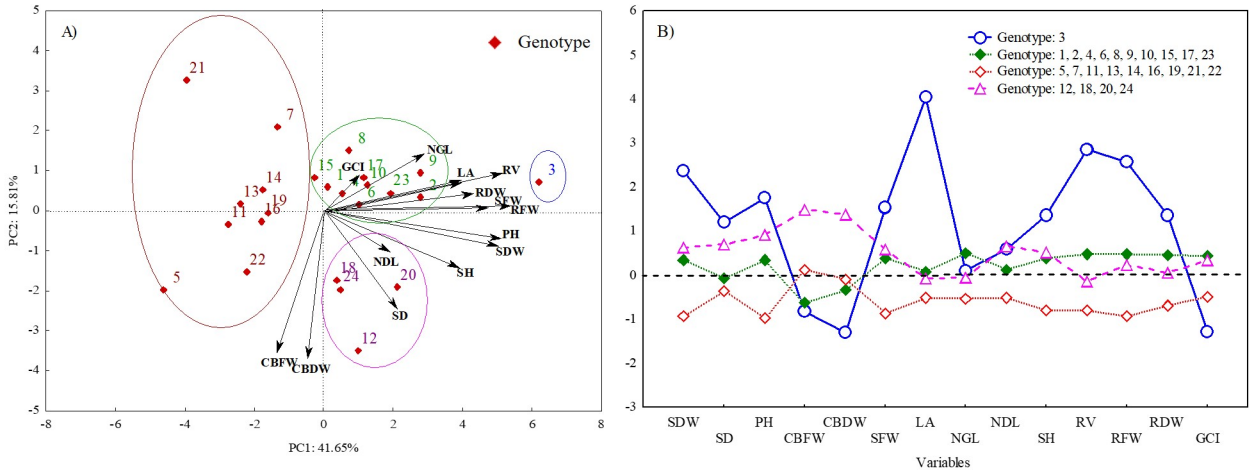

Figure 2: Biplot of scattering distribution of genotypes, and variables by the principal component analysis (A), Non-hierarchical k-means clustering method (B). SP91-1049(1), RB855443(2), RB966928(3), RB965902(4), RB965917(5), CTC 9(6), CTC17(7), CTC 21(8), SP81-3250(9), IAC91-1099(10), IACSP95-5094(11), IACSP96-3060(12), CTC 2(13), CTC 20(14), CTC 24(15), CTC99-1906(16), SP83-2847(17), SP80-3280(18), IACSP95-5000(19), CTC 6(20), CTC 15(21), RB855536(22), RB867515(23), and RB935744(24). Plant height (PH), leaf area (LA), root fresh weight (RFW), shoot dry weight (SDW), number of green leaves (NGL), number of dead leaves (NDL), shoot fresh weight (SFW), root dry weight (RDW), green color index (GCI), root volume (RV), stem diameter (SD), stem height (SH), culm billet fresh weight (CBFW) and culm billet dry weight (CBDW).

The second component (PC2) is responsible for 15.81% of total variation, presenting more relation with variables whose arrows are more aligned with the vertical axis of the graph. Therefore, the genotypes 12, 20, 18, and 24 are also influenced by stem height (SH), stem diameter (SD), and number of dead leaves (NDL). The variables GCI, number of dead leaves (NDL), and number of green leaves (NGL) did not present a strong influence on the groups discriminated by PC1, despite being indicators of tolerance (Inman-Bamber et al., 2008; Silva et al., 2011) (Figure 2A). The component PC2 was also responsible for dispersing some genotypes of the red group. The genotypes 5 and 22 were strongly influenced by the same variables responsible for the separation of the lilac group, while the genotypes 7 and 21 tended to present an opposite response to that observed by the components of the lilac group.

By analyzing the non-hierarchical k-means clustering results, we decided to define four clusters based on the number of groups determined by principal component analyzes. This analysis allows better observing the separation of groups regarding the genetic variability, as well as their relationships with the evaluated variables (Figure 2B).

As for the pattern of division of genotypes within each group, no relation was observed with maturation cycles, that is, precocious, medium and late. The first cluster, formed only by the genotype 3 (Figure 2B) stood out with the highest variations for the variables of the shoot, root system, and GCI. The second cluster, formed by genotypes 1, 2, 4, 6, 8, 9, 10, 15, 17 and 23, presented the lowest variation and certainly formed by more tolerant and moderately tolerant genotypes to the tested stress, because it was above average for almost all biometric variables analyzed. The third cluster was formed by the genotypes 5, 7, 11, 13, 14, 16, 19, 21, and 22 and was well below the average for the variables. In this cluster, the genotypes 11, 13, 21, 7, and 5, which represent the cultivars IACSP95-5094, CTC2, CTC 15, CTC 17, and RB965917, respectively, were the most sensitive in the treatment with stress. The variation pattern of each cluster is closely related to the average of each group. Therefore, the highest variation was observed for the genotype 3.

In relation to the factor analysis, we first calculated the load values of provisional factors determined by the principal components analysis, which allowed to obtain components which eigenvalues were not lower than 1, following Kaiser (1958) criterion. Approximately 77% of the data variability is explained by four principal factors. Thus, the results of the fourteen original variables were distributed into only four factors, each of them representing an independent physiological process (Table 4).

In the first factor, shoot dry mass, plant height, shoot fresh mass, root volume, root dry mass, and fresh root mass were the variables with the highest factor loads, explaining 41.65% of the total variation of the data (Table 4). This factor is related to the Al^{3+} and Mn^{2+} toxicity processes and the low nutrient content that must be occurring on the genotypes since the variables SDM, WPH, SFM, RV, RDM, and RFM are positively correlated with this factor (Table 5).

In the factor 2, the variables with the highest factor loads were culm billets fresh and dry weight, which presented an inverse correlation with Factor 2. This factor was responsible for 15.81% of the total variation of the 14 variables measured. Because they were included in a factor distinct from the Factor 1, they should not be related to Al^{3+} and Mn^{2+} toxicity associated with low nutrient availability. However, these variables might be more related to the process of preparation of the culm billets for seedling production, possibly due to the variation in the diameter of the collected stems and to the imprecision of stem cutting (length) to obtain the culm billet.

In the third factor, the highest loads are related to the variables NGL and GCI, with values of − 0.826445 and − 0.722021, respectively, influencing 11.10% of the total variation. In addition, the fourth factor explained about 8% of the total variation and was composed of the variables NGL and GCI. Similarly, to the second factor, the third and fourth factors also appear to be unrelated to the stress process here studied. However, they are possibly related to

Table 4: Eigenvalues and percentage of variation explained by the first four principal components of biometric variables of the 24 genotypes under the stress treatment.

Factors	Eigenvalues	Variation explained (%)	Accumulated Eigenvalues	Var. explained Accumulated (%)
1	5.8314	41.65	5.8314	41.65
2	2.2127	15.81	8.0441	57.46
3	1.5545	11.10	9.5986	68.56
4	1.1647	8.31	10.7634	76.88

specific characteristics of the genotypes which responses are not related to the studied stress. Several factors can affect GCI index values, including the cultivar (Coelho et al., 2010). Moreover, the differential development and/or growth index may present different values of GCI unit (Fontes; Araujo, 2007) and NGL.

As for the groups formed by the genotypes in the results of Scott-Knot clusters was not found clear relation with the maturation cycles of the genotypes (Table 6), confirming the results obtained by the non-hierarchical grouping method (Figure 2).

Taking into account that tolerance to abiotic stress is the plant ability to maintain stable growth (and values of other physiological parameters) when subjected to stressful conditions (Maia et al., 2018), it is suitable to say that genotypes with a lower variation of growth (and other variables) when comparing different environments can be characterized as tolerant. And, great variation indicates low tolerance. Therefore, the genotypes 3, 2, 12, 9, 23, 8, 4, and 10 had their biometric characteristics less affected under the proposed stress, and hence they were considered as the most tolerant genotypes (Table 6). This tolerance possibly is due to the ability to continue the process of cell division and elongation and to maintain meristematic viable regions (Foy,1984). On the other hand, the genotypes 5, 21, 7, 22, 13, 14, and 16 were the most sensitive to the proposed stress, especially the first three genotypes.

The behavior presented by these two groups of genotypes showed great coherence with the reality in the field. For example, the genotypes of the group identified as tolerant are indicated and are actually being cultivated in acid-poor, nutrient-poor and drought-prone environments (Silva et al., 2012; Ridesa, 2017). The genotypes 3, 9 and 23 correspond to almost 50% of the planted area in the northeast region of the country and most of the Brazilian cerrado (Ridesa, 2015a; Ridesa, 2017), regions characterized by acid and nutrient poor soils. On the other hand, the genotypes identified as sensitive are indicated to production environments with good soil and climatic conditions (Ridesa, 2015a; Ridesa, 2017; CTC, 2018). In fact, genotypes 13, 21 and 22 do not reach 3% of the planted area of the Brazilian cerrado, and in the Northeast this percentage is less than 1% (Ridesa, 2015a; Ridesa, 2017). Therefore, it seems that the tolerance level presented by the genotypes may be related to the tolerance to aluminum.

Table 5: Factor matrix determined by the Varimax orthogonal rotation method.

Variables	Factor 1	Factor 2	Factor 3	Factor 4
SDW	0.855719	-0.207812	0.103510	-0.015527
SD	0.363250	-0.571107	0.414618	-0.056875
PH	0.870576	-0.152480	-0.052741	0.117518
CBFW	-0.222720	-0.814872	-0.213728	-0.002336
CBDW	-0.033604	-0.880080	0.053417	0.221009
SFW	0.832106	0.029983	-0.027889	0.341067
LA	0.690493	0.160176	-0.062728	-0.430157
NGL	0.527029	0.332616	0.313223	0.607166
NDL	0.329085	-0.234881	-0.826445	-0.197130
SH	0.663707	-0.326518	0.110182	0.134335
RV	0.863993	0.207380	-0.012937	-0.215616
RFW	0.927236	0.015774	-0.020385	-0.203106
RDW	0.724444	0.089433	0.027531	-0.150444
GCI	0.178192	0.176984	-0.722021	0.511168
Expl.Var	5831409	2.212706	1.554565	1.164723
Prp.Totl	0.416529	0.158050	0.111040	0.083194

Shoot dry weight (SDW), stem diameter (SD), plant height (WPH), culm billet fresh weight (CBFW), culm billet dry weight (CBDW), shoot fresh weight (SFW), leaf area (LA), stem height (SH), root volume (RV), root fresh weight (RFW), root dry weight (RDW), number of green leaves (NGL), number of dead leaves (NDL), and green color index (GCI).

Table 6: Mean comparison of genotypes under stress with the data transformed into a percentage for biometric variables by the Scott & Knott test at 5%.

Genotypes	Variables							
	RV	RFW	RDW	CBFW	CBDW	GCI	NGL	NDL
	---------- % ----------							
SP91-1049(1)	17.96A	15.26A	7.89A	95.14B	91.40B	27.98A	68.00B	83.33A
RB855443(2)	25.77A	23.65A	20.10A	97.25B	105.70B	13.71A	86.96A	37.50B
RB966928(3)	40.54A	33.51A	26.19A	86.80B	83.33B	11.51A	71.43B	71.42A
RB965902(4)	28.57A	24.21A	21.43A	93.84B	102.10B	14.85A	64.70B	50.00B
RB965917(5)	13.57A	11.78A	10.02A	119.47A	123.30B	6.07A	50.00B	50.00B
CTC 9(6)	21.97A	20.70A	19.92A	92.56B	105.28B	22.40A	76.92A	42.85B
CTC17(7)	19.31A	15.54A	18.51A	87.51B	86.65B	11.02A	83.33A	22.22B
CTC 21(8)	28.41A	20.56A	23.32A	92.73B	98.74B	31.92A	75.00A	62.50B
SP81-3250(9)	30.00A	24.87A	30.86A	68.59B	117.01B	18.60A	82.61A	40.00B
IAC911099(10)	27.78A	23.42A	21.04A	88.57B	98.34B	26.61A	72.41B	50.00B
IACSP95-5094(11)	17.55A	12.08A	10.60A	102.44B	109.85B	18.32A	65.52B	37.50B
IACSP96-3060(12)	14.37A	18.69A	9.04A	113.87A	145.53A	19.71A	70.36B	62.50B
CTC 2(13)	18.62A	11.71A	6.09A	106.66A	104.27B	19.61A	68.97B	50.00B
CTC 20(14)	19.23A	13.32A	9.76A	104.65B	91.01B	27.03A	68.00B	62.50B
CTC 24(15)	20.00A	13.58A	12.80A	91.99B	96.37B	19.29A	76.92A	62.50B
CTC99-1906(16)	16.50A	12.66A	16.31A	101.72B	112.50B	13.39A	60.00B	25.00B
SP83-2847(17)	22.22A	21.60A	17.63A	85.24B	99.89B	25.46A	78.26A	62.50B
SP80-3280(18)	19.69A	21.69A	19.97A	146.20A	111.56B	22.66A	70.83B	80.00A
IACSP95-5000(19)	18.80A	15.77A	12.60A	111.18A	113.85B	22.85A	71.43B	50.00B
CTC 6(20)	26.80A	23.19A	27.11A	123.56A	131.26A	21.76A	66.67B	100.00A
CTC 15(21)	12.40A	10.52A	6.87A	72.34B	74.50B	21.68A	64.00B	50.00B
RB855536(22)	15.43A	14.82A	18.26A	116.85A	139.08A	11.10A	65.38B	83.33A
RB867515(23)	27.92A	25.69A	24.30A	94.57B	102.22B	29.88A	64.00B	116.66A
RB935744(24)	23.65A	16.32A	12.57A	127.21A	147.82A	25.36A	72.73B	50.00B

Root volume (RV), root fresh weight (RFW), root dry weight (RDW, culm billet dry weight (CBDW), green color index (GCI), number of green leaves (NGL), and number of dead leaves (NDL) and culm billet weight (CBFW). Means followed by the same letter in the column do not differ statistically from each other by the Scott & Knott test at a 5% probability level.

Among the biometric variables, the root system was the most affected by the stress caused by Al^{3+}. In most genotypes, stress limited to root biomass accumulation by more than 70% (Table 6). This result is higher than that found by Watt (2003) when assessing the growth of roots exposed to high Al^{3+} concentrations, who observed an inhibition of root growth, with a variation between 36 to 46%.

This high limitation of root biomass production may be associated not only with Al^{3+} effect but also with Mn^{2+} effect. In fact, although symptoms of manganese toxicity in plants are more pronounced in leaves than in roots, when wheat tolerance to aluminum toxicity was determined together with that of manganese in nutrient solutions, all the genotypes showed a reduction in root growth ranging from 59 to 68% as Mn^{2+} concentrations in nutrient solutions increased from 0.11 to 1200 mg L^{-1} (Camargo, 1995). The reduction of root dry matter due to the toxic effect of Mn^{2+} has also been observed in other crops such as Rice (Lindon; Barreiro; Ramalho, 2004) and bean (Soratto et al., 2005).

Although no statistical difference has been observed among genotypes regarding the reduction of root dry mass, those less affected also stood out in the other variables. This result reinforces the importance of this variable in the selection of genotypes to the stress caused by Al^{3+}, Mn^{2+} and low nutrient avalilability.

Regarding GCI readings, no statistical difference was observed among genotypes under stress, even though it is considered as a good tool for genotype selection (Silva et al., 2011). Despite this result, pigment content in leaves was affected by the stress when compared to the treatment without stress, indicating that only GCI reading does not seem to be enough to select genotypes for this variable at that stress level. Therefore, other analyses are recommended in future researches with the same focus.

The genotype 3 stood out with the best results for root volume, root fresh weight, and the second highest average for stem diameter and plant height. In addition, this genotype did not present a reduction in leaf area and shoot dry weight in the treatment with stress. A similar result for this latter variable was also observed for the genotype 2, composing the more tolerant group "A" to stress imposed (Table 7).

Table 7: Mean comparison of genotypes in the treatment with stress with data transformed into a percentage for shoot variables by the Scott & Knott test at 5%.

Genotypes	Variables					
	SDW	SD	SH	SFW	LA	PH
	%					
SP91-1049(1)	87.98B	68.75C	72.76A	56.52A	62.68B	60.77B
RB855443(2)	100.47A	73.07C	74.72A	72.62A	37.19C	68.53A
RB966928(3)	101.35A	86.36B	82.27A	77.66A	112.08A	75.04A
RB965902(4)	84.11C	76.00B	78.81A	65.63A	19.04C	57.73B
RB965917(5)	77.12D	79.41B	64.18B	46.27B	11.26C	47.31B
CTC 9(6)	86.04B	78.57B	62.97B	60.90A	21.18C	62.97A
CTC17(7)	79.78C	68.00C	55.60B	52.06B	21.15C	53.37B
CTC 21(8)	84.24C	67.74C	56.58B	57.62A	28.38C	61.03B
SP81-3250(9)	83.88C	77.77B	77.83A	63.24A	25.45C	72.80A
IAC911099(10)	84.86C	83.87B	70.78A	62.21A	22.85C	60.55B
IACSP95-5094(11)	72.89D	78.12B	60.90B	45.49B	16.82C	52.96B
IACSP96-3060(12)	92.05B	104.54A	83.45A	67.94A	25.18C	68.92A
CTC 2(13)	78.05D	72.41C	54.92B	48.66B	15.53C	57.98B
CTC 20(14)	81.08C	76.00B	61.71B	51.86B	21.83C	49.97B
CTC 24(15)	86.98B	67.85C	65.47B	60.82A	21.33C	63.24A
CTC99-1906(16)	82.81C	66.67C	68.67B	53.71B	14.67C	58.52B
SP83-2847(17)	82.70C	67.64C	91.6A	57.39A	37.24C	58.76B
SP80-3280(18)	87.07B	76.66B	75.43A	63.84A	23.93C	60.95B
IACSP95-5000(19)	77.66D	70.27C	61.90B	51.18B	23.52C	54.72B
CTC 6(20)	87.44B	77.14B	67.59B	61.90A	36.07C	75.21A
CTC 15(21)	71.38D	65.71C	51.22B	39.55B	22.67C	51.44B
RB855536(22)	78.27D	43.24D	64.86B	49.82B	13.49C	46.81B
RB867515(23)	86.87B	58.33C	73.14A	60.24A	28.95C	68.57A
RB935744(24)	88.73B	64.52C	78.95A	64.63A	22.88C	67.75A

Shoot dry weight (SDW), stem diameter (SD), plant height (PH), shoot fresh weight (SFW), leaf area (LA), and stem height (SH). Means followed by the same letter in the column do not differ statistically from each other by the Scott & Knott test at a 5% probability level.

The most sensitive group "D" consisted of genotypes 5, 11, 13, 19, 21 and 22. The lowest averages for shoot dry weight were presented by the genotypes 21, 11, and 5 and, consequently, they presented the highest growth restrictions, which values that reached 28.62, 27.11 and 22.88%, respectively, while genotypes of the other groups showed reductions less than 20% (Table 7). This result is below that found by Ecco, Santiago and Lima, (2014) studying the interaction between types of abiotic stress (water deficit and soil acidity) in sugarcane under greenhouse conditions, and observed a reduction of 23% in shoot biomass production under stress caused by Al^{3+} and 69 % under water stress combined with Al^{3+} toxicity. Maia et al. (2018) also found that the stress caused by Al^{3+} led to an average reduction of 44% in shoot weight. Restrictions on shoot biomass accumulation due to Al^{3+} effect, among other factors, occur due to a reduction in photosynthetic activity. Specifically, Al^{3+} interfering with the absorption process of nutrients such as nitrogen and manganese may limit the formation and function of chloroplasts (Konrad et al., 2005; Mendes et al., 2018).

The genotypes 9, 23, 8, 4, and 10, even with a good performance, had a production below 100% for shoot biomass. Similar results were obtained by Maia et al. (2018), that assessed the phenotypic plasticity of 11 sugarcane genotypes under Al^{3+} stress and classified the genotype 3 (RB966928) as the most tolerant, even presenting shoot with dry weight below the average, under both cultivation conditions.

The performance of more tolerant genotypes may be associated with their ability to exclude Al^{3+} from root apex and/or with its accumulation through mechanisms such as Al^{3+} chelation in the cytosol, Al^{3+} compartmentalization in the vacuole or in aluminum-protein bonds that are some mechanisms of tolerance to Al^{3+} (Hartwig et al., 2007; Inostroza-Blancheteau et al., 2012).

In relation to stem length, the genotypes were classified into two groups. The genotypes 17, 12, and 3 were the least affected, presenting a limitation of only 8.36, 16.55, and 17.73%, respectively, while the genotypes 21, 13, 7, and 8 presented a higher restriction in stem growth (about 50%) in the treatment with stress (Table 7). This variable is considered as excellent indicators of tolerance to the proposed stress since they present a higher correlation with sugarcane production (Silva et al., 2008).

CONCLUSIONS

There is variability regarding the tolerance of the 24 sugarcane genotypes under the stress conditions caused by Al^{3+}, Mn^{2+}, and low nutrient availability. No relationship was observed between tolerance level of genotypes and the maturation cycles. The genotypes RB966928, RB855443, IACSP96-3060, SP81-3250, RB867515, CTC 21, RB965902, and IAC91-1099 were identified as the most tolerant whereas the genotypes RB965917, CTC 15, CTC17, RB855536, CTC 2, CTC 20, and CTC99-1906 were identified as the most sensitive to high levels of Al^{3+} and Mn^{2+}, associated to low nutrient availability.

AKNOWLEDGMENTS

The first author thanks to CNPq (National Council for Scientific and Technological Development) for grantinf a graduate scholarship. The second author also are grateful to the CNPq for the granting of research productivity scholarship.

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