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Abordagem por técnicas estatísticas: crescimento de dois genótipos de cana-de-açúcar submetidos à elevada [CO_2] atmosférica e seca

Statistical techniques approach: two sugarcane genotypes growth under elevated [CO_2] atmospheric and drought

Enfoque por técnicas estadísticas: crecimiento de dos genotipos de caña de azúcar sometidos a alto [CO_2] atmosférico y seco

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Resumo

O objetivo do trabalho foi comparar dois genótipos de cana-de-açúcar frente à elevada concentração atmosférica de dióxido de carbono (CO_2) e deficiência hídrica no solo, através de uma abordagem baseada em análises estatísticas de múltiplas características do crescimento vegetal. Para isso, realizou-se a correlação de Pearson, análise de dissimilaridade e componentes principais (ACP), de alguns parâmetros como o comprimento do caule (colmo) e das raízes, número de folhas, massa seca de tecidos da planta, entre outras características. Os atributos biométricos apresentam correlação positiva e significativa entre si. Entretanto, os valores de massa seca possuem correlação negativa com os demais parâmetros, significando, portanto, haver uma redução do crescimento à medida que também ocorre um decréscimo nas condições hídricas das plantas submetidas ao estresse. Com base nas análises de dissimilaridade e ACP, observa-se que os genótipos divergem geneticamente e a condição ambiental imposta influencia nas respostas de crescimento. Esse estudo preliminar é um indicativo de que essa abordagem é fundamental para o entendimento das variações existentes entre as respostas de genótipos de cana-de-açúcar. Além disso, fica evidente (devido à divergência) que análises acerca das respostas fisiológicas e moleculares desses genótipos nessa condição ambiental contribuirão para a identificação dos processos biológicos regulados e possíveis mecanismos de tolerância.

Palavras-chave: Análise multivariada; Crescimento; Mudanças climáticas; *Saccharum* spp.

Abstract

This article aims was to compare two sugarcane genotypes under elevated atmospheric carbon dioxide (CO_2) concentration and soil water deficit, through an approach based on statistical analysis of plant growth multiple characteristics. For that, Pearson's correlation, dissimilarity and principal components (PC) analysis were performed, of some parameters such as stem length (culm) and roots length, number of leaves, dry mass of plant tissues, among other characteristics. Biometric attributes have a positive and significant correlation with each other. However, the dry mass values have a negative correlation with the other parameters, meaning, therefore, there is a growth reduction as there is also a decrease in plants water conditions subjected to stress. Based on the dissimilarity and PC analysis, observed that genotypes genetically diverge and the imposed environmental condition influences growth responses. This preliminary study is an indication that this approach is essential for understanding the variations between the sugarcane genotypes responses. In addition, is evident (due to divergence) that analyses about the physiological and molecular responses of these genotypes in this environmental condition will contribute to the identification of regulated biological processes and possible tolerance mechanisms.

Keywords: Multivariate analysis; Growth; Climate changes; *Saccharum* spp.

Resumen

El objetivo del trabajo fue comparar dos genotipos de caña de azúcar ante la alta concentración atmosférica de dióxido de carbono (CO_2) y la deficiencia de agua en el suelo, mediante un enfoque basado en el análisis estadístico de múltiples características del crecimiento vegetal. Para ello, se realizó la correlación de Pearson, análisis de disimilitud y componentes principales (ACP), de algunos parámetros como la longitud del tallo y raíces, número de hojas, masa seca de tejidos vegetales, entre otras características. Los atributos biométricos tienen una correlación positiva y significativa entre sí. Sin embargo, los valores de masa seca tienen una correlación negativa con el resto de parámetros, es decir, por lo tanto, hay una reducción del crecimiento ya que también hay una disminución de las condiciones hídricas de las plantas sometidas a estrés. Con base en los análisis de disimilitud y ACP, se observa que los genotipos divergen genéticamente y la condición ambiental impuesta influye en las respuestas de crecimiento. Este estudio preliminar es una indicación de que este enfoque es esencial para comprender las variaciones entre las respuestas de los genotipos de la caña de azúcar. Además, es evidente (debido a la divergencia) que análisis sobre las respuestas fisiológicas y moleculares de estos genotipos en esta condición ambiental contribuirán a la identificación de procesos biológicos regulados y posibles mecanismos de tolerancia.

Palabras clave: Analisis multivariable; Crecimiento; Cambios climáticos; *Saccharum* spp.

1. Introduction

Sugarcane (*Saccharum* spp.), from to the Poaceae family, has great importance for the national economy, mainly due to its use as a raw material in the sugar, ethanol, and bioenergy production and also for animal feed (Tejera et al., 2007; Torquato & Ramos, 2013). Brazil is the world's largest sugarcane producer, with annual production above 700 million tons (FAOSTAT, 2018) and stands out as the second largest ethanol producer (USDA, 2019).

This dynamic scenario in the sugar and alcohol sector is directly related to the expansion of new planting areas and / or the development of strategies to improve sugarcane, aiming to increase productivity. Among the strategies, the search for genotypes (cultivars) more adapted to different edaphoclimatic conditions of the country is considered very important.

Adapted to tropical and subtropical climates, sugarcane needs a large volume of water in production system, especially when is in complete vegetative development (Tejera et al., 2007). However, changes in the water availability and global temperature negatively influence plant growth leading to significant reductions in productivity.

These changes are partly a consequence of climate change due to the increased greenhouse gases emission (IPCC, 2007). The CO_2 concentration ($[CO_2]$) has increased considerably since the industrial revolution. Currently, atmospheric $[CO_2]$ is in the range of 409 ppm (Misra et al., 2019), with forecasts for 2100 ranging from 700 to 900 ppm (NASA, 2015).

Increase of CO_2 in the atmosphere affects plants biological processes at different organization levels (Mooney et al., 1999). Researches indicate changes in photosynthetic rates, stomatal conductance, carbohydrate metabolism and other important physiological processes for the sugarcane growth (Vu et al., 2006; Souza et al., 2008).

However, compared to the studies carried out with C_3 metabolism plants, there are few studies with C_4 metabolism plants, (e.g. sugarcane and sorghum) that aimed to investigate the physiological, biochemical, molecular and growth responses under atmospheric conditions of $[CO_2]$ expected in the coming years.

The associated effects of increased $[CO_2]$ and water deficit can induce morphophysiological changes in sugarcane plants. Those changes may become important clues to compare genotypes regarding to their tolerance levels to environmental conditions, and the correlation between the characteristics (biometric, physiological, among others) contributes to the definition of criteria that guide a more efficient selection.

The multivariate analysis techniques as a tool to find patterns and relationships between several variables simultaneously are important for the genetic analysis of the relationship between evaluated characteristics and genetic material (Queiroz et al., 2011). To estimate the genetic divergence biometric models are used and, using statistical methods, with multiple information, it is possible to measure the dissimilarity (Sudré et al., 2005).

Thus, considering that few studies have been done combining the action of different types of stress on plants, especially in sugarcane, in the present study, statistical analyses were carried out of some growth parameters from two sugarcane genotypes under the combined effect of elevated $[CO_2]$ and soil water deficit.

2. Methodology

Sugarcane plants from IACSP94-2094 (genotype A), and IACSP94-2101 (genotype B), 50 days old, were supplied by the Agronomic Institute of Campinas (IAC), Ribeirão Preto - SP, Brazil. Genotypes are contrasting regarding to agronomic characteristics such as: production, planting recommendation, tolerance to unfavorable environments, and others. Genotype A is considered tolerant to water deficit (Machado et al., 2009). Plants were transferred to pots (10 L) containing sand and soil mixture (1:1, v / v). After a 7-day acclimatization period in growth chambers (Fitotron-Weiss Gallenkamp, UK), under controlled temperature (28°C) and humidity (60%) conditions, plants were subjected to the combined stress effects for a period total of 30 days.

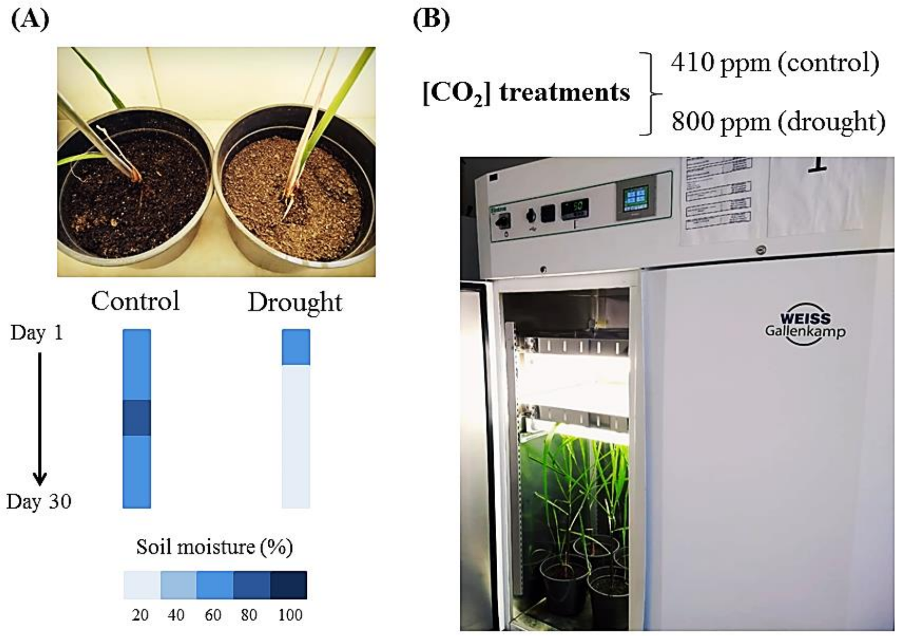
The methodology used is it of a quantitative nature (Pereira et al., 2018). Experimental design was completely randomized, 2x2 (two genotypes and two stress conditions) with three biological replicates. The established stress conditions consisted of plants in pots properly irrigated in a chamber with an atmospheric [CO_2] of 410 ppm (control); and plants subjected to water deficiency (irrigation suppression) in a chamber with [CO_2] of 800 ppm (Figure 1).

After 30 days plants were evaluated for growth and physiological parameters, including stem length (SL), stem diameter (SD), green leaves (GL or number of leaves), roots length (RL), roots area (RA), roots volume (RV), dry mass leaves (DML), dry mass stem (DMS), and dry mass roots (DMR). RA and RV measurements were determined in three plants per treatment, using SAFIRA software (Root and Fibers Analysis System) (Jorge & Rodrigues, 2008). DML, DMS, and DMR were measured using an analytical balance. For the dry mass (DM) determination, we obtained the dry mass of the samples (1 gram of leaves, stem, and roots) after drying at 65 °C for 24 h in an oven. DM calculation followed the formula DM (leaves, stem, and roots) = [(dry weight/ fresh weight)] × 100.

Data of the evaluated parameters were submitted to variance analysis (ANOVA) by the Agroestat software - System for Statistical Analysis of Agronomic Tests, version 1.1.0 (Barbosa & Maldonado, 2014). Pearson's correlation, Dissimilarity, and Principal components analysis (PCA) were carried out using XLSTAT software package (Addinsoft, France).

Dissimilarity (Agglomerative hierarchical clustering – AHC) between treatments was measured by the Euclidean distance and the agglomeration method was performed by the Ward’s method, to determine the number of clusters previously. In the PCA, the eigenvalues were extracted from the covariance matrix that generated the eigenvectors called principal components.

Figura 1. Experimental Design. (A) Soil moisture monitored over the 30 stress days; (B) Control pot plants were subjected to treatment with 410 ppm [CO_2] and plants in drought pots were subjected to treatment with 800 ppm [CO_2].



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3. Results and Discussion

The correlations analysis between the biometric (SL, SD, GL, RL, RA, and RV) and physiological attributes (DML, DMS, and DMR) showed significant values in the correlation matrix. Strong and positive correlation (≥0.730) between SL, SD, and GL with all biometric parameters; RL with SL, SD, and GL; RV with SL, SD, GL, and RA; RA with all parameters except RL, was observed (Figure 2A). These results indicate that plants of both genotypes under the imposed stress condition (elevated [CO_2] and drought) showed changes in the growth and development of the aerial part and the root system.

The stem growth and the number of leaves of both genotypes were affected by environmental condition imposed, and these changes are significantly related to the root length (e.g., correlation coefficient between SL and RL higher than 0.800) (Figure 2A). In contrast, there is a negative correlation between physiological measures (dry mass) and the biometric parameters evaluated (red correlation values) (Figure 2B). Dry mass analysis of 1 gram of samples is directly related to the water content available in plant tissues (water loss). Therefore, higher DML, DMS, and DMR values represent an increase water loss.

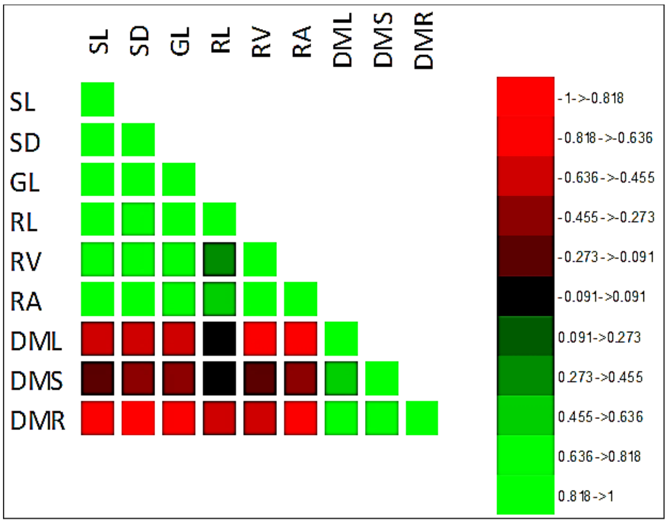
According to the correlation coefficients (Figure 2), the sugarcane genotypes under elevated [CO_2] and drought, have reduced measures of growth attributes and DM values increased, consequently due to water loss. As these characteristics can be associated with more expressive productivity, they are then considered valid during the breeding procedure, since they contribute to selection of sugarcane genotypes more tolerant to water deficit conditions (Silva et al., 2008).

Figure 2. Pearson's correlation coefficients of biometric and physiological traits evaluated in two sugarcane genotypes under elevated [CO_2] and drought. (A) Correlation matrix (Pearson’s) considering the data for the two genotypes. Values in bold are different from 0 with a significance level alpha=0.05; (B) Heatmap representing the negative (red) and positive (green) correlation values.

(A)

Variables	SL	SD	GL	RL	RV	RA	DML	DMS	DMR
SL	**1**	**0.962**	**0.945**	**0.828**	**0.803**	**0.879**	-0.498	-0.147	**-0.721**
SD		**1**	**0.954**	**0.816**	**0.730**	**0.834**	-0.548	-0.382	**-0.869**
GL			**1**	**0.830**	**0.769**	**0.816**	-0.514	-0.283	**-0.770**
RL				**1**	0.401	0.494	0.008	0.018	**-0.553**
RV					**1**	**0.938**	**-0.795**	-0.206	**-0.578**
RA						**1**	**-0.781**	-0.283	**-0.672**
DML							**1**	**0.628**	**0.662**
DMS								**1**	**0.762**
DMR									**1**

(B)



SL	stem lenght
SD	stem diameter
GL	green leaves
RL	roots lenght
RA	roots area
RV	roots volume
DML	dry mass leaves
DMS	dry mass stem
DMR	dry mass roots

Source: Authors.

Additionally, it appears that based on the characteristics evaluated, the four treatments (three biological replicates per treatment) are distributed in 3 groups according to dendrogram that shows the hierarchy groups (Figure 3) and distribution of the cluster centroids (average distance centroid ranging from 4531.166 to 2977.588) (Table 1).

Cluster 1 was formed by genotype A, control plants. The plants of genotype B (control) and one plant of genotype A (under stress) constitute the second cluster. Cluster 3 was formed by two plants of genotype A under stress and plants of genotype B under stress. Therefore, there is a clear difference between genotypes and an approximation of these taking into account all parameters evaluated and the interaction with the environment (genotype x environment) (Figure 3). That is, the genotypes under stress (high [CO_2] and drought) show some similar growth responses.

However, it is emphasized that the existing genetic variability is a crucial point to identify the studied genotypes as divergent (genetic divergence expressed as a measure of dissimilarity). Considering this genetic divergence between the evaluated genotypes, it becomes necessary to understand how other physiological responses (photosynthetic apparatus), biochemical and molecular mechanisms (carbohydrate metabolism, antioxidant defense, etc.) are regulated in the predicted climatic condition. Queiroz et al. (2011), evaluated two sugarcane cultivars (IAC91-2195 e IAC91-5155), and presented information's on drought tolerance, based on the responses of biochemical and physiological indicators to water stress by assessing the levels of osmoprotectors and biometric variables of initial growth.

The variability can be explained by the analysis of the main components, resulted in two principal components (PC), which explained 87.316% of the variance (cumulative variability) (Figure 4A). The two-dimensional plane formed by the components F1 (69.34%) and F2 (17.97%) retained this original variance above 87% (Figure 4B). The treatments are distributed on a coordinate factor plan considering the relationship between the active variables.

Variables related to the biometric parameters (SL, SD, GL, RL, RA, and RV) explain much of the variability between the genotypes. DM variables (DML, DMS, and DMR) differentiate genotypes under stress (A800 and B800) and contribute to the least variability (Figure 4B). However, plants of genotype B (B800) have higher DM values. This difference is an important factor that must be taken in consideration, as it is a good reference of water conditions in plants and other vital processes at the cellular level.

Figure 3. Dendrogram of dissimilarity indices based on 12 treatments plus GE (genotype x environment) interaction. A410 (genotype A control); A800 (genotype A under stress); B410 (genotype B control); and B800 (genotype B under stress).

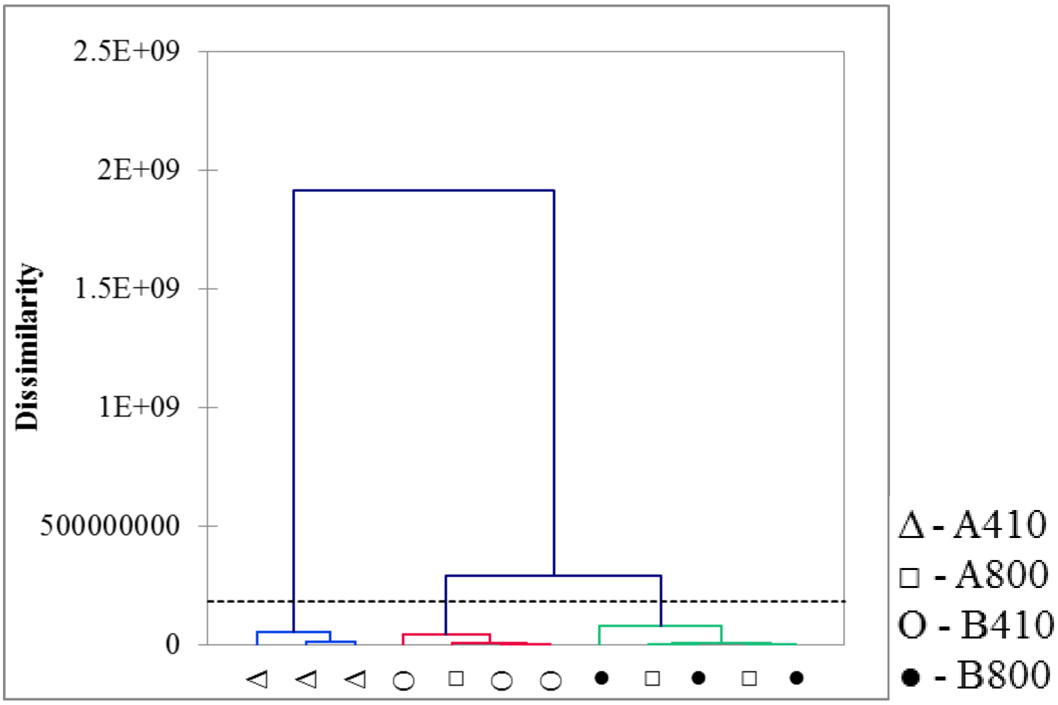


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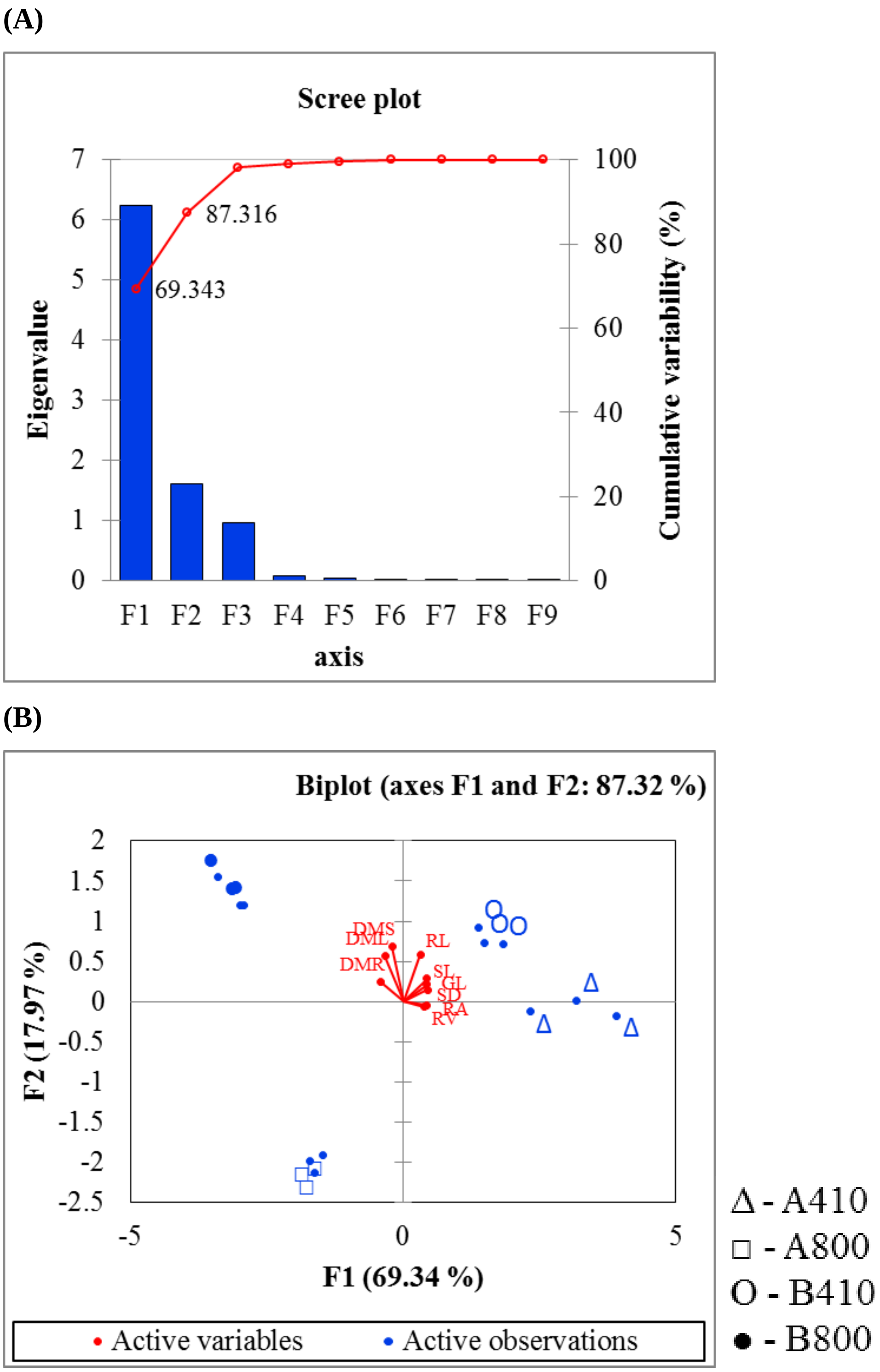
Table 1. Distribution of the cluster centroids.

Class	1*	2*	3*
Objects	3	5	4
Within-class variance	32362914.902	21026102.545	16562951.450
Average distance to centroid	4531.166	3411.634	2977.588
	Δ	□	□
	Δ	□	O
	Δ	●	O
		●	O
		●	

*Δ A410; □ A800; O B410; ● B800. A410 (genotype A control); A800 (genotype A under stress); B410 (genotype B control); and B800 (genotype B under stress). Source: Authors.

Figure 4. Principal component analysis. (A) Scree plot showing eigenvalue and cumulative variability of the nine traits evaluated; (B) Biplot showing the dispersion of the 12 treatments,

for the principal components F1 x F2. A410 (genotype A control); A800 (genotype A under stress); B410 (genotype B control); and B800 (genotype B under stress).



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Therefore, the results show that the correlation coefficients, exploratory dissimilarity analyses and principal components are a good strategy to compare and to classify the treatments tested based on the multiple characteristics. This type of analysis is an exploratory method that characterizes the interaction of data in understanding the mutual influence of plants responses to adverse environmental conditions (Queiroz et al., 2011; Silva et al., 2012).

This approach can be useful in the study of the several morphophysiological and molecular characteristics responsive to climate changes expected for the coming years, evaluating, for example, a wider group of sugarcane genotypes and other C_4 crops.

4. Final Considerations

Correlation coefficients between the biometric parameters were positively significant, and these establish a negative correlation with the dry mass measurements. Multivariate analyses successively discriminate the treatments tested. The sugarcane genotypes (IACSP94-2094 and IACSP94-2101) present divergent performances in the experimental conditions evaluated. As a suggestion for the next researches, we recommend the evaluation of a greater number of sugarcane genotypes. In addition, the determination of physiological and molecular responses may be an important tool for understanding the different biological mechanisms (tolerance) that are regulated in sugarcane under associated abiotic stress.

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Conflict of interest

The authors declare no conflicts of interest.

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Sonia Marli Zingaretti – 15%