

Original Article

## Calcium pyruvate in the attenuation of the water deficit on the agro-industrial quality of ratoon sugarcane

Piruvato de cálcio na atenuação do déficit hídrico sobre a qualidade agroindustrial de cana soca

M. S. Dias<sup>a</sup> , F. A. Silva<sup>a</sup> , P. D. Fernandes<sup>a</sup> , C. H. A. Farias<sup>a</sup> , I. J. Silva<sup>a</sup> , M. F. C. Silva<sup>a</sup> , R. F. Lima<sup>a</sup> , C. N. Lacerda<sup>a</sup> , A. M. Lima<sup>a</sup> , V. R. N. Lima<sup>a</sup> , A. A. R. Silva<sup>a</sup>  and L. S. Reis<sup>b</sup> 

<sup>a</sup>Universidade Federal de Campina Grande – UFCG, Unidade Acadêmica de Engenharia Agrícola – UAEA, Centro de Tecnologia e Recursos Naturais – CTRN, Campina Grande, PB, Brasil

<sup>b</sup>Universidade Federal de Alagoas – UFAL, Campus de Engenharias e Ciências Agrárias – CECA, Rio Largo, AL, Brasil

### Abstract

Sugarcane is one of the largest agricultural commodities when considering the export volume and the number of jobs generated. Sugarcane production in the Brazilian Northeast region is generally low due to several factors, including the irregular rainfall distribution, which highlights the importance of studies aimed at mitigating the deleterious effects of water stress. In this scenario, this study aimed to evaluate calcium pyruvate as a water deficit attenuator on the agro-industrial quality of sugarcane in the second cycle of cultivation. The experiment was conducted out under greenhouse conditions of the Federal University of Campina Grande, where five sugarcane commercial genotypes tested (G1- RB863129, G2- RB92579, G3- RB962962, G4- RB021754, and G5- RB041443) and three irrigation management strategies (E1- full irrigation, E2- water deficit with application of 30 mM of calcium pyruvate, and E3- water deficit without calcium pyruvate application), distributed in randomized blocks in 5 × 3 factorial arrangement with three replications. The RB021754 genotype under water deficit and without foliar application of calcium pyruvate increased the fiber content (13.2%) and the sugarcane moist cake weight (143.5 g). The effects of water deficit in sugarcane genotypes are attenuated by the exogenous application of 30 mM of calcium pyruvate, with benefits on the polarized sucrose content, apparent sucrose content of the juice, soluble solids content, purity, corrected cane POL, total recoverable sugars, and stem mass in relation to plants under water deficit without calcium pyruvate application.

**Keywords:** *Saccharum officinarum* L., water stress, attenuator, biotechnology.

### Resumo

A cana-de-açúcar é uma das maiores commodities agrícolas, quando se considera o volume de exportação e o número de empregos gerados. A produção no Nordeste brasileiro, em geral, é baixa, decorrente de diversos fatores, entre eles, a irregularidade de distribuição das chuvas, o que realça a importância de estudos com vistas a minorar os efeitos deletérios do estresse hídrico. Nesse cenário, objetivou-se avaliar o piruvato de cálcio como atenuante do déficit hídrico sobre a qualidade agroindustrial da cana-de-açúcar no segundo ciclo de cultivo. O experimento foi conduzido em casa de vegetação da Universidade Federal de Campina Grande, onde foram testados cinco genótipos de cana-de-açúcar (G1- RB863129, G2- RB92579, G3- RB962962, G4- RB021754 e G5- RB041443) submetidos a três estratégias de manejo da irrigação (E1- Irrigação plena, E2- déficit hídrico com aplicação de 30 mM de piruvato de cálcio e E3- déficit hídrico sem aplicação de piruvato de cálcio), distribuídos em blocos casualizados, em esquema fatorial 5 × 3, com três repetições. O genótipo RB021754 sob déficit hídrico e sem aplicação foliar do piruvato de cálcio aumentou o teor de fibra (13,2%) e o peso do bolo úmido da cana (143,5 g). Os efeitos do déficit hídrico em genótipos de cana-de-açúcar são atenuados com aplicação exógena de 30 mM de piruvato de cálcio, com benefícios sobre o teor sacarose polarizada, teor de sacarose aparente do caldo, teor de sólidos solúveis, pureza, pol da cana corrigida, açúcares totais recuperáveis e massa de colmos, em relação as plantas sob déficit hídrico sem aplicação de piruvato de cálcio.

**Palavras-chave:** *Saccharum officinarum* L., estresse hídrico, atenuador, biotecnologia.

\*e-mail: mirandysd@gmail.com

Received: May 23, 2023 – Accepted: August 14, 2023



This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

## 1. Introduction

Sugarcane is one of the largest agricultural commodities when considering the export volume and the number of associated jobs generated worldwide. In addition to sugar and ethanol production, this plant is used for a variety of purposes, e.g., vinasse, filter cake, and bagasse production, which are used as animal food, fertilizers, and in bioelectricity generation (Elsheery et al., 2020; Aquino and Carlos, 2022). The Brazilian sugarcane production is approximately 579 million tons in an area of 8.35 million ha<sup>-1</sup>, corresponding to a mean yield of 69.36 t ha<sup>-1</sup> (CONAB, 2022).

Faced with the need to increase agricultural production in order to meet the growing food demand, sugarcane plantations are expanding into arid and semi-arid regions (Leanasawat et al., 2021). Morais et al. (2022) mentioned that approximately 80% of the sugarcane growing areas in Brazil are in regions with water scarcity. In such regions, the occurrence of dry periods, as a result of rainfall irregularity, is the environmental factor that most limits the uplift of agricultural crops, which also occurs in sugarcane cultivation (Pereira et al., 2021). The species requires 1,850 to 2,500 mm year<sup>-1</sup> (Mohanraj et al., 2021), and the most sensitive phase to water deficit begins at tillering and intensifies during elongation (Hoang et al., 2019).

The adequate water supply during these phases, in addition to favoring plant growth and development, increases the yield and technological indices of sugarcane culms (Farias et al., 2009; Moura et al., 2014). On the other hand, low water availability reduces the capture of solar radiation and interferes with the partition of photoassimilates to different plant organs, negatively affecting culm formation, sucrose production, and technological quality as a whole (Ramesh and Mahadevaswamy, 2000; Inman-Bamber and Smith, 2004; Leanasawat et al., 2021).

The efficiency of sugar recovery depends on the quality of the cane delivered to the industrial unit and is measured by technological parameters such as the moist cake weight, soluble solids, and sucrose content, which are used to determine the percentage of fibers, purity, reducing sugars, and total recoverable sugars, important variables to evaluate the richness and quality of sugarcane for sugar recovery and estimate the value of the ton produced (CONSECANA, 2006).

Although the negative effects of water stress on sugarcane are well reported, there are few studies on the exogenous application of organic substances while investigating their effects on plant metabolism as an alternative to maximize production and improve the technological quality of plants under water stress. Pyruvate is one of the products that can be used in such studies since, in the metabolism of C4 plants, which includes sugarcane, it is responsible for regenerating phosphoenolpyruvate (an HCO<sub>3</sub><sup>-</sup> acceptor) in mesophyll cells (Taiz et al., 2017).

It is well-established that the exogenous application of pyruvate in plants reduces the deleterious effects of abiotic stresses. Shen et al. (2017) incubated *Arabidopsis* leaves for 2.5 h in pyruvate at concentrations of 10, 100, and 1000 μM and found that, at 100 μM, pyruvate increased the anionic current in guard cells, inducing stomatal closure.

Barbosa et al. (2021) evaluated the effects of exogenous pyruvate application in two peanut cultivars under water restriction and found that the pyruvate concentration of 50 mM mitigated the effects of water stress in the cultivar IAC Caiapó, a water-demanding genotype, and restored antioxidant enzymes in cv. BR1, which is drought tolerant. Silva et al. (2023) evaluated the benefits of exogenous pyruvic acid application (100 μM) in cotton plants under water deficit and observed that the exogenous pyruvate supply attenuated the effects of water suppression on boll production 31% in the cv. BRS Seridó and 34% in the cultivars CNPA 7MH and FM 966.

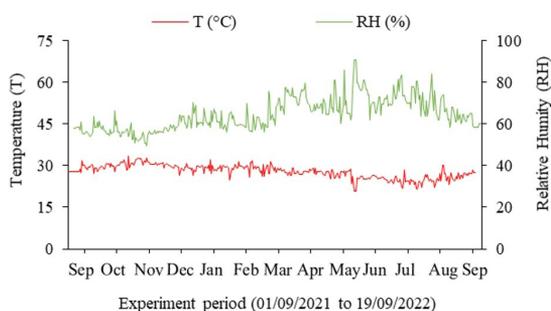
However, studies involving production and industrial quality under the association between pyruvate and water deficit in plants with C4 metabolism, especially sugarcane, are not available in the literature. In this scenario, due to the socioeconomic importance of sugarcane in the different producing regions of Brazil and the effects caused by water deficit, especially in the Northeast region of the country, studies on the exogenous application of pyruvate become highly relevant as they may clarify whether pyruvate supplementation can increase sugarcane production and industrial quality.

From this perspective, this study aimed to evaluate the role of calcium pyruvate as a water deficit attenuator on the agro-industrial quality of sugarcane in the second cultivation cycle.

## 2. Material and Methods

The experiment was conducted out between September 2021 to September 2022 under greenhouse conditions in the experimental area of the Agricultural Engineering Academic Unit of the Federal University of Campina Grande (UFCG), located in the municipality of Campina Grande, Paraíba, Brazil, at the geographic coordinates 07° 15' 18" S, 35° 52' 28" W, and at a mean elevation of 550 m. The mean temperature and relative humidity data for the internal area of the greenhouse during the experimental period are available in Figure 1.

The treatments consisted of a combination of two factors: five sugarcane commercial genotypes (G1- RB863129, G2- RB92579, G3- RB962962, G4- RB021754, and G5- RB041443) and three irrigation



**Figure 1.** Air temperature and mean relative air humidity observed in the internal area of the greenhouse during the experimental period.

management strategies (E1- full irrigation, E2- water deficit with application of 30 mM of calcium pyruvate, and E3- water deficit without calcium pyruvate application). The experimental design was in randomized blocks in a 5 × 3 factorial arrangement with three replicates, totaling 45 experimental plots.

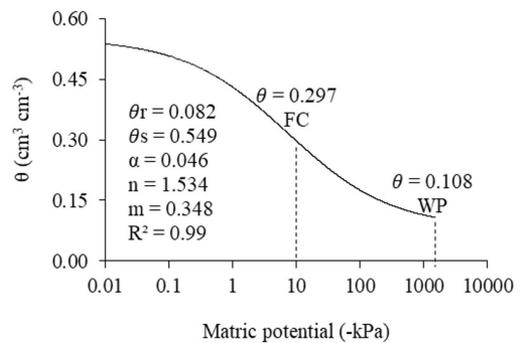
The pyruvate concentration was established based on a study developed by Shen et al. (2017) with *Arabidopsis thaliana*, in which the authors used exogenous pyruvate application at the concentrations of 10, 100, and 1000 μM. Adjustments were made in the pyruvate concentration since the original research was carried out with a sample of incubated leaves of a non-cultivated species (*Arabidopsis*). The adjustment was also based on the study developed by Barbosa et al. (2021), who used 100 μM and 50 mM of pyruvate in peanut plants under water stress.

The plants were grown in polyethylene pots serving as drainage lysimeters, which were distributed in a 1.0 x 1.5 m spatial arrangement and received a 2.0 cm layer of gravel and a geotextile layer at the bottom of the container. A 10 mm wide transparent hose coupled to two 2 L bottles was connected to each lysimeter to collect the drained water. Then, the pots were filled with soil with a sandy loam texture (45 dm<sup>3</sup>), classified as Entisol, from the municipality of Lagoa Seca, Paraíba, Brazil, whose physical and chemical attributes were determined according to the methodology described by Teixeira et al. (2017) (Table 1).

The soil water retention curve (WCR) was determined with soil moisture values at pressures of -10, -33, -100, -500, -1000, and -1500 kPa (Figure 2). To obtain the adjustment parameters, the volumetric moisture values (θ) corresponding to the matric potentials applied (Ψ<sub>m</sub>) were modeled using the RETC not-linear model software proposed by van Genuchten (1980).

After harvesting the plant cane (first culm production after planting), the second cycle of cultivation (ratoon cane) began. The pots were irrigated regularly by maintaining soil moisture close to the maximum retention water capacity in all experimental plots until the water treatments were applied.

Fertilization with nitrogen (N), potassium (K), and phosphorus was performed weekly, via irrigation water and adjusted according to the crop's absorption rate for each phenological phase (Marin et al., 2009; Souza et al., 2016). Throughout the cultivation cycle, a total of 68 g of urea (45%N), 64 g of monoammonium phosphate (51% P<sub>2</sub>O<sub>5</sub>, 11% N), and 110 g of potassium chloride (60% K<sub>2</sub>O) was applied per pot. The micronutrients were applied at 15-day intervals to avoid nutrient deficiency, consequently limiting crop development, with 1.0 g L<sup>-1</sup> of Quimifol® applied in



**Figure 2.** Soil water retention curve according to the van Genuchten model. θ<sub>r</sub>: residual moisture, θ<sub>s</sub>: saturation moisture, and α, n, and m are tuning parameters. FC: field capacity and WP: wilting point.

**Table 1.** Chemical and physical attributes of the soil used in the experiment.

Chemical attributes				Physical attributes		
	a	b		Sand	63.48%	Loamy sand
pH	6.50	6.46	-	Silt	25.14%	
P	79.0	50.64	mg dm <sup>-3</sup>	Clay	11.38%	
K <sup>+</sup>	0.24	0.20	cmolc dm <sup>-3</sup>	Soil density		1.13 g cm <sup>-3</sup>
Ca <sup>2+</sup>	9.50	6.12	cmolc dm <sup>-3</sup>	Particle density		2.72 g cm <sup>-3</sup>
Na <sup>+</sup>	0.51	2.54	cmolc dm <sup>-3</sup>	Porosity		58.45%
Mg <sup>2+</sup>	5.40	8.00	cmolc dm <sup>-3</sup>	Matric potential (-kPa)		Soil moisture (%)
Al <sup>3+</sup>	0.00	0.20	cmolc dm <sup>-3</sup>	Natural		0.55
H <sup>+</sup>	0.90	1.32	cmolc dm <sup>-3</sup>	10		24.86
SB	15.65	16.86	cmolc dm <sup>-3</sup>	33		17.05
CEC	16.55	18.38	cmolc dm <sup>-3</sup>	100		12.57
BS	94.56	91.73	%	500		9.01
AS	0.00	1.17	%	1000		8.91
OM	8.10	21.00	g dm <sup>-3</sup>	1500		8.84

pH (H<sub>2</sub>O): potential of hydrogen; SB: sum of bases; OM: organic matter; extraction: water (pH); Mehlich (P, K, Na); KCl 1N (Ca, Mg, and Al); calcium acetate pH 7.0 (H + Al); CEC: cation exchange capacity at pH 7; BS: base saturation; AS: aluminum saturation; a: before the culture is implanted; b: at the end of the second cycle.

each event (composition: Mg (5.0%), S (11.0%); B (3.5%); Cu (0.10%); Fe (0.20%); Mn (1.0%); Mo (0.10%); and Zn (6.0%)) using a knapsack sprayer.

Calcium pyruvate was purchased from Natusvita®. The pyruvate solution was prepared by dissolving calcium pyruvate in distilled water, minutes before spraying, forming a solution that was applied using a Jacto XP sprayer with a capacity of 12 L, a maximum working pressure of 88 psi (6 bar), and a JD 12P nozzle. Spraying occurred at 5:00 p.m. on all plant leaves. The solution volume applied ranged from 100 to 200 mL per experimental plot, according to plant growth. The treatments applied during the experimental period are described in Table 2.

For better adhesion and absorption during spraying, an adhesive spreader was added to the solutions. In addition, the plants in each pot were protected with plastic to prevent wind drift during spraying, and the soil was covered with an impermeable blanket to prevent runoff.

Irrigation was conducted daily at 5:00 p.m. and applied to each pot, with the water volume applied corresponding

to the demand of the plant subjected to the respective treatment. The volume applied in each irrigation event was estimated using the water balance based on Equation 1. A monthly 20% leaching fraction was used to remove the excess salt from the soil (Soares et al., 2021).

$$VI = Va - Vd \tag{1}$$

where:

VI: water volume to be applied in the irrigation event (mL);  
 Va: water volume applied on the previous day (mL);  
 Vd: drained volume, quantified on the next morning (mL).

At the end of the water deficit period in each phase (64 and 211 days after regrowth), soil samples were taken with the aid of a mini-auger, and the soil moisture content was determined using the standard greenhouse method. They were then placed in aluminum cans and weighed to obtain the wet mass. Next, the soil samples were oven-dried at 105 °C for 72 h to obtain the dry mass, from which the soil moisture content was determined in relation to the soil matric potential (Table 3).

**Table 2.** Application of treatments in ratoon cane plants.

	Water deficit		Applications		Total
	Period (DAR)	Total (Days)	Calcium pyruvate	Water	
			Period (DAR)		
E1	-	-	-	-	-
E2	24 to 64 and 182 to 211	71	39 to 63 and 192 to 210	-	23
E3	24 to 64 and 182 to 211	71	-	39 to 63 and 192 to 210*	23

\*Plants with water deficit that did not receive calcium pyruvate (E3) were sprayed with distilled water plus adhesive spreader. E1: irrigation throughout the crop cycle; E2: water deficit with application of 30 mM of calcium pyruvate; E3: water deficit without application of calcium pyruvate; DAR: days after regrowth. Applications were performed at two-day intervals between each application.

**Table 3.** Soil moisture and matric potential for treatments.

Genotypes	64 DAR					
	E1		E2		E3	
	Soil moisture (cm³ cm⁻³)	ψm (-kPa)	Soil moisture (cm³ cm⁻³)	ψm (-kPa)	Soil moisture (cm³ cm⁻³)	ψm (-kPa)
RB863129	0.259	18.61	0.149	207.9	0.138	305.8
RB92579	0.240	25.93	0.143	254.6	0.145	237.5
RB962962	0.240	25.93	0.142	263.7	0.141	273.3
RB021754	0.266	16.55	0.145	237.5	0.139	294.1
RB041443	0.244	24.14	0.149	207.9	0.148	214.8
Genotypes	211 DAR					
	E1		E2		E3	
	Soil moisture (cm³ cm⁻³)	ψm (-kPa)	Soil moisture (cm³ cm⁻³)	ψm (-kPa)	Soil moisture (cm³ cm⁻³)	ψm (-kPa)
RB863129	0.242	25.01	0.126	504.4	0.135	342.9
RB92579	0.257	19.27	0.133	371.6	0.131	403.9
RB962962	0.240	25.93	0.133	371.6	0.133	371.6
RB021754	0.234	28.88	0.127	481.6	0.129	440.9
RB041443	0.256	19.60	0.130	421.6	0.131	403.9

ψm: matric potential; E1: irrigation throughout the crop cycle; E2: water deficit with application of 30 mM of calcium pyruvate; E3: water deficit without application of calcium pyruvate.

At the end of soil sampling in all treatments, soil moisture was restored to a level equivalent to that of the maximum water retention capacity. However, irrigation was suspended before harvest to enhance sucrose production (Inman-Bamber and Smith, 2005).

The culm mass per plot (CMP - kg) was evaluated at the end of the cycle by cutting the stalks close to the ground, removing the dry leaves, and eliminating the apical meristem using a portable digital dynamometer. After the culms were weighed, three of them (the most developed) were selected from each experimental plot, identified, and sent to the laboratory at the Japungu Plant to determine the moist cake weight (WMC - g), the polarized sucrose content (POL - %), the apparent sucrose content in the juice (POLj - %), the soluble solids content (SS - °Brix), the purity (PUR - %), the corrected cane POL (POLc - %), the percentage of reducing sugars in the juice (RS - %), the percentage of reducing sugars in sugarcane (RSC - %), the fiber content (Fiber - %), and the total recoverable sugars (TRS - kg t<sup>-1</sup>), according to the methodology described by CONSECANA (2006).

The data were normalized to zero mean (0.0) and unitary variance ( $S^2 = 1.0$ ) and evaluated by multivariate analysis through principal component analysis (PCA), which summarizes the amount of relevant information contained in the original dataset into fewer dimensions resulting from linear combinations of the original variables generated from the eigenvalues ( $\lambda > 1.0$ ) in the covariance matrix, explaining a percentage greater than 10% of the total variance (Govaerts et al., 2007). Only the variables with correlation coefficients above 0.5 were maintained in the composition of each Principal Component (PC) (Hair Junior et al., 2009).

Based on the reduction of dimensions, the original data of the variables each component were subjected to multivariate analysis of variance (MANOVA) by the Hotelling test (Hotelling et al., 1947) at 0.05 of probability for the genotypes and irrigation management strategies. The analyses were performed using the software Statistica, version 7.0 (StatSoft, 2004).

### 3. Results and Discussion

The multidimensional space of the original variables was reduced to two dimensions represented by the first two principal components (PC<sub>1</sub> and PC<sub>2</sub>) with eigenvalues greater than  $\lambda > 1.0$ , according to Kaiser (1960). Based on these results, the respective eigenvalues and percentages of variation explained for each component can be observed in Table 4. Together, these components explained 91.13% of the total variance. PC<sub>1</sub> explained 71.90% of the total variance, formed by the linear combination of the attributes of polarized sucrose (POL), soluble solids content (SS), apparent sucrose content in the juice (POLj), purity (PUR), corrected cane POL (POLc), percentage of reducing sugars in the juice (RS), total recoverable sugars (TRS), percentage of reducing sugars in sugarcane (RSC), and culm mass per plot (CMP). PC<sub>2</sub> represented 19.24% of the remaining variance, formed by the sugarcane moist cake weight (WMC) and fiber percentage (Fiber) (Table 4).

According to the MANOVA (Table 4), there was a significant effect ( $p \leq 0.01$ ) for the interaction between genotypes (G) and management strategies (E) only on the constituent variables of PC<sub>1</sub>. However, a significant difference was observed in isolation for the genotypes and between the management strategies in the two PCs.

When analyzing the correlation between the variables studied (Table 4), a positive correlation was observed in PC<sub>1</sub> between POL (0.94), SS (0.94), POLj (0.98), PUR (0.96), POLc (0.99), TRS (0.95), and CMP (0.64). However, these variables were inversely correlated with RS (-0.77) and RSC (-0.94), i.e., as the RS and RSC increased, the other technological quality attributes grouped in PC<sub>1</sub> decreased. In PC<sub>2</sub>, the WMC and Fiber attributes are proportionally correlated since, as the fiber content increased, the sugarcane moist cake weight also increased.

The two-dimensional projections of the effects of treatments and technological attributes of sugarcane on the first and second principal components (PC<sub>1</sub> and PC<sub>2</sub>) are shown in Figure 3A and 3B. The two principal components built from the original characteristics accurately described differences between varieties and irrigation management strategies. Attributes with positive correlations are responsible for the discrimination of treatments located to the right of PC<sub>1</sub>, whereas attributes with negative correlations are responsible for treatments to the left of PC<sub>1</sub> and below PC<sub>2</sub>.

Based on the technological quality parameters of sugarcane, grouped in PC<sub>1</sub>, the most expressive values of total recoverable sugars (148.2 and 154.9 kg t<sup>-1</sup>), corrected cane POL (15.9 and 16.8%), purity (82.6 and 84.2%), apparent sucrose content of the juice (18.7 and 19.6%), soluble solids (22.2 and 23.2 °Brix), polarized sucrose (77.7 and 77.2%), and culm mass per plot (14.1 and 13.0 kg) were obtained by genotypes RB92579 (G<sub>2</sub>) and RB041443 (G<sub>5</sub>), respectively, under full irrigation (E<sub>1</sub>) in relation to the other genotypes studied (Figure 3A and 3B).

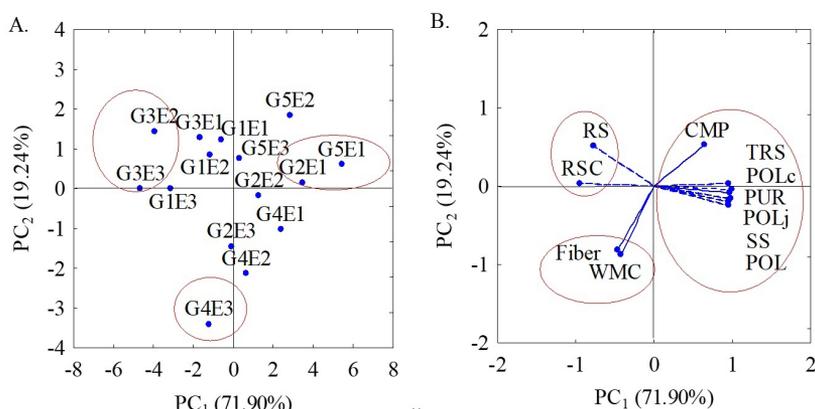
The adequate supply of water during the sugarcane cycle, in addition to favoring growth and development, increases the production and content of total recoverable sugars (TRS) (Farias et al., 2009; Sugiharto, 2018). Furthermore, the divergence of technological attributes between varieties may be related to the genetic characteristics of the materials.

For the percentage of reducing sugars in the juice (69.1 and 67.7%) and the percentage of reducing sugars in the cane (1.14 and 1.25%), the highest values were observed in treatments G3E2 and G3E3, respectively, indicating that increases in these attributes directly influenced low TRS contents (Figure 3A and 3B). The reducing sugars parameter is used to designate the percentage of glucose and fructose, which are precursor products of color in the sugar industrial process, i.e., they intensify the color of the sugar, and their increase promotes less efficiency in sucrose recovery, depreciating the product's quality (Prado et al., 2017; Morais et al., 2022). When evaluating the effects of water deficit in different phenophases of six sugarcane genotypes (SP79-1011, RB855113, RB92579, RB867515, RB72454, and RB855536), Endres et al. (2018) found low industrial quality of sugarcane and consequently lower TRS values in RB855113.

**Table 4.** Eigenvalues, percentage of total variance explained in the multivariate analysis of variance (MANOVA) and coefficient of correlations (r) between original variables and the principal components.

PCs	Principal components										
	PC <sub>1</sub>		PC <sub>2</sub>								
Eigenvalues ( $\lambda$ )	7.90		2.11								
Percentage of total variance ( $S^{2\%}$ )	71.90		19.24								
Hotelling test ( $T^2$ ) for genotypes (G)	0.000		0.000								
Hotelling test ( $T^2$ ) for irrigation management strategies (E)	0.000		0.023								
Hotelling test ( $T^2$ ) for interaction (G $\times$ E)	0.000		0.999								
PCs	Correlation coefficient (r)										
	WMC	POL	SS	POLj	PUR	POLc	RS	Fiber	TRS	RSC	CMP
PC <sub>1</sub>	-0.41	0.94	0.94	0.98	0.96	0.99	-0.77	-0.47	0.95	-0.94	0.64
PC <sub>2</sub>	-0.88	-0.24	-0.20	-0.15	-0.08	-0.03	0.51	-0.82	0.03	0.03	0.52
Means											
	WMC (g)	POL (%)	SS ( $^{\circ}$ Brix)	POLj (%)	PUR (%)	POLc (%)	RS (%)	Fiber (%)	TRS (kg t <sup>-1</sup> )	RSC (%)	CMP (kg)
G1E1	131.47	69.62	20.73	16.72	80.65	14.25	67.18	11.61	136.17	0.96	11.83
G1E2	131.67	68.73	20.70	16.57	80.03	14.13	67.06	11.62	136.13	0.99	9.74
G1E3	134.83	66.98	20.40	15.86	77.75	13.40	67.18	12.13	131.03	1.12	8.81
G2E1	131.90	77.77	22.67	18.74	82.69	15.95	65.07	11.72	148.20	0.86	14.12
G2E2	134.87	73.78	21.65	17.88	82.57	15.10	66.63	12.14	144.55	0.93	12.72
G2E3	138.27	71.65	21.47	17.45	81.28	14.58	65.96	12.75	138.81	0.98	11.48
G3E1	132.93	67.54	20.33	16.31	80.20	13.84	67.75	11.87	129.06	1.00	12.92
G3E2	133.47	64.24	20.20	15.54	76.93	13.17	69.14	11.95	124.83	1.14	11.16
G3E3	136.00	63.99	20.20	15.46	76.53	13.03	67.74	12.27	124.61	1.25	8.47
G4E1	136.57	76.02	21.93	18.25	83.19	15.50	64.26	11.83	141.83	0.84	11.72
G4E2	138.53	75.60	21.67	17.75	81.91	14.91	65.16	12.47	137.29	0.89	8.79
G4E3	143.53	71.87	21.60	17.22	79.94	14.30	65.43	13.28	136.67	1.02	8.18
G5E1	127.50	77.21	23.25	19.60	84.28	16.87	63.31	11.06	154.91	0.78	13.04
G5E2	125.57	76.33	22.10	18.31	82.85	15.72	67.15	11.22	151.34	0.88	12.35
G5E3	131.17	70.88	21.47	17.24	80.39	14.62	67.22	11.90	144.06	0.91	11.67

Weight of moist cake (WMC), polarized sucrose (POL), apparent sucrose content in the juice (POLj), soluble solids content (SS), purity (PUR), corrected cane POL (POLc), percentage of reducing sugars in the juice (RS), percentage of reducing sugars in sugarcane (RSC), fiber content (Fiber), total recoverable sugar (TRS), and culm mass per plot (CMP).



**Figure 3.** Two-dimensional projection of the scores of the principal components for the studied factors (genotypes and irrigation management strategies) (A) and industrial quality attributes of sugarcane (B) in the two principal components (PC1) and (PC2). Weight of moist cake (WMC), polarized sucrose (POL), apparent sucrose content in the juice (POLj), soluble solids content ( $^{\circ}$ Brix), purity (PUR), corrected cane POL (POLc), percentage of reducing sugars in the juice (RS), percentage of reducing sugars in sugarcane (RSC), fiber content (Fiber), total recoverable sugar (TRS), and culm mass per plot (CMP).

In PC<sub>2</sub>, the G4E3 treatment (water stress without foliar application of calcium pyruvate) was responsible for the highest fiber content (Fiber - 13.2%) and sugarcane moist cake weight values (WMC - 143.5 g) (Figure 3A and 3B).

From an industrial point of view, the fiber content is important for the energy balance of the industry as fibers are used to generate electricity and for surplus sale (Simões et al., 2018). High fiber contents increase the resistance to juice extraction by the industry, while plants with low fiber contents show less resistance to damping-off and are generally more resistant to the penetration of pests into the culm (Viana et al., 2019; Morais et al., 2022). However, Prado et al. (2017) stressed that, for the production of ethanol and sugar, the fiber increase is undesirable as these components limit sucrose extraction and result in low ethanol yield. Therefore, the sugar-energy industry requires materials with fiber contents from 10.5 to 12.5% (Oliveira et al., 2009). According to Simões et al. (2018), each 0.5% increase in the fiber content reduces the milling yield by 10% to 20%, reducing approximately 1 kg of sugar per ton.

When analyzing the irrigation management strategies (E1 and E3) (Table 4), it is seen that the sugarcane plants under these treatments obtained lower values of POL (69.0%), POLj (16.6%), PUR (79.12%), POLc (13.96%), TRS (135.0 kg t<sup>-1</sup>), and CMP (9.66 kg), consequently showing higher values for Fiber (12.42%) and RSC (1.06%) when subjected to the E3 strategy in relation to the full irrigation treatment (E1) (POL (73.6%), POLj (16.60%), (82.14%), POLc (15.24%), Fiber (11.58%), TRS (142.0 kg t<sup>-1</sup>), RSC (0.885), and CMP (12.7 kg)).

When comparing E3 with E1, there is a reduction in the POL (6.25%), POLj (7.26%), PUR (3.68%), POLc (8.40%), TRS (4.93%), and CMP (23.94%) values. However, there was an increase in the fiber content (7.25%) and in the percentage of reducing sugars (20.45%). Such reductions may have occurred because, under water stress, there is a reduction in the activity of carbohydrate metabolism enzymes, interfering with the partition of photoassimilates to different plant organs (Venkataramana et al., 1986). Consequently, there is a reduction in sucrose content, culm formation, and industrial quality attributes of culms (Marin et al., 2009; Begum et al., 2012).

Reductions in these attributes due to water stress were also observed by Wiedenfeld (2000) when subjecting plants to six weeks of water stress during the stem elongation phase, with a 4.7% reduction in the sucrose content and 19.1% in the cane yield. In another study, Begum et al. (2012) evaluated the effects of water deficit on the juice quality of six sugarcane genotypes and found that the content of reducing sugars (RS), °Brix, and POL decreased in all genotypes. Endres et al. (2018) evaluated the water deficit in different sugarcane phenophases (Genotypes: SP791011, RB855113, RB92579, RB867515, RB72454, and RB855536) and found that water stress significantly affected the °Brix and TRS, with a mean reduction of 31.6 and 2.83%, respectively, in relation to plants grown under irrigation.

Although the present research focuses only on the deleterious effects of water deficit, there is evidence that substances from organic products can lessen the deleterious effects of drought on plants, increasing their ability to

adjust to such conditions. In the present study, a beneficial effect of the application of calcium pyruvate was verified in sugarcane plants cultivated under water stress (E2), with values of POL, POLj, PUR, POLc, Fiber, TRS, and CMP of 71.7%, 17.16%, 80.92%, 14.58%, 11.84%, 138.6 kg t<sup>-1</sup>, and 10.90 kg, respectively, highlighting respective reductions of only 2.58, 4.13, 1.60, 4.33, 2.18, and 14.17% for these attributes, and an increase of only 2.24 and 8.78% in the fiber content and RSC in relation to irrigated plants (E1).

The beneficial effect of pyruvate observed in the treatment with water stress and pyruvate application may have occurred due to pyruvate being a crucial molecule in the metabolism of C<sub>4</sub> plants, being responsible for the regeneration of phosphoenolpyruvate (PEP), an acceptor of initial CO<sub>2</sub> concentration in mesophyll cells (Taiz et al., 2017). However, there is no record in the available literature of studies on the industrial quality of sugarcane in which the effects of the association between calcium pyruvate and water deficit in C<sub>4</sub> plants are reported.

In Brazil, Barbosa et al. (2021) evaluated the effects of exogenous pyruvate application in two peanut cultivars (IAC Caiapó and BR1) subjected to water restriction. The authors found that the pyruvate concentration of 50 mM mitigated the effects of water stress in the cultivar IAC Caiapó, a water-demanding genotype, and restored the antioxidant enzymes SOD (45%), CAT (129%), and APX (60%) in the drought-tolerant cv. BR1. In another study, Silva et al. (2023) evaluated the benefits of exogenous pyruvic acid (100 µM) in cotton plants under water deficit and observed that the pyruvate attenuated the effects of water suppression on boll production by 31% in the cv. BRS Seridó and by 34% in the cultivars CNPA 7MH and FM 966. In another study, Silva et al. (2022) observed an attenuation in the gas exchange and root dry mass parameters of the passion fruit cv. Redondo Amarelo cultivated under salt stress with the exogenous application of 50 mM of pyruvate.

Across Brazil, sugarcane has been valued based on its qualitative indices, so that the better the quality of the raw material, the higher the price paid per ton of stalks (Farias et al., 2009). All sugarcane technological indices discussed in this study are used as a calculation basis to determine the amount of total recoverable sugars, expressed in kg of TRS t<sup>-1</sup> of sugarcane (Fernandes, 2000). This attribute is one of the most important in the production of sugarcane for industry, depending on the percentage of reducing sugars in the cane (RSC). Based on the results of this research, which found a lower mean RSC for E2 (0.96%) compared to E3 (1.05%), it can be concluded that the quality of the raw material can be improved with the application of calcium pyruvate.

The effects of water deficit on sugarcane genotypes are attenuated by the exogenous application of 30 mM of calcium pyruvate, with benefits on the polarized sucrose content, apparent sucrose content of the juice, soluble solids content, purity, corrected cane POL, total recoverable sugars, and culm mass per plot in relation to plants under water deficit without calcium pyruvate application. In terms of gain, there were TRS increases of 3.75% (RB863129), 3.95% (RB92579), and 4.82% (RB041443), and culm mass increases of 10.23% (RB863129), 11.40% (RB92579), and 5.9% (RB041443).

Overall, the available data highlight the outstanding beneficial effects of pyruvate as a result of supplementation in the C4 metabolism, with pyruvate being responsible for the regeneration of phosphoenolpyruvate, an  $\text{HCO}_3^-$  acceptor in mesophyll cells (Taiz et al., 2017). With supplementation, a fact considered in the hypothesis of this study, pyruvate molecules are made available to the cells for the regeneration of phosphoenolpyruvate, and the energy that would be spent is available to other plant adaptation mechanisms to water stress. These results confirm that the studied genotypes respond satisfactorily to pyruvate supplementation, achieving satisfactory production results and industrial quality for the raw material produced. Evidently, subsequent validation tests will be necessary to test and validate the adoption of this technology under field conditions.

### Acknowledgements

The authors thank the Graduate Program in Agricultural Engineering at the Federal University of Campina Grande (UFPG), the Coordination for the Improvement of Higher Education Personnel (CAPES), To the National Council for Scientific Development and Technology (CNPq) and the Usina Japungu for all their support in carrying out the technological analyses of the sugarcane.

### References

- AQUINO, G.S. and CARLOS, E.F., 2022. The NPK1 gene increases sugarcane productivity under water deficit and conventional crop management conditions. *Biocatalysis and Agricultural Biotechnology*, vol. 44, p. 102454. <http://dx.doi.org/10.1016/j.bcab.2022.102454>.
- BARBOSA, D.D., FERNANDES, P.D., MARCELINO, A.D.A.L., SILVA, F.A., DIAS, M.S., SILVA, C.R.C. and SANTOS, R.C., 2021. Exogenous pyruvate mitigates the detrimental effects of water stress in contrasting peanut genotypes. *Genetics and Molecular Research*, vol. 20, no. 3, pp. 1-14. <http://dx.doi.org/10.4238/gmr18907>.
- BEGUM, M.K., ALAM, M.R., ISLAM, M.S. and AREFIN, M.S., 2012. Effect of water stress on physiological characters and juice quality of sugarcane. *Sugar Tech*, vol. 14, no. 2, pp. 161-167. <http://dx.doi.org/10.1007/s12355-012-0140-6>.
- COMPANHIA NACIONAL DE ABASTECIMENTO – CONAB, 2022 [viewed 22 January 2023]. *Safra brasileira de cana-de-açúcar* [online]. CONAB. Available from: <https://www.conab.gov.br/info-agro/safras/cana>
- CONSELHO DOS PRODUTORES DE CANA-DE-AÇÚCAR, AÇÚCAR E ETANOL DO ESTADO DE SÃO PAULO – CONSECANA, 2006. *Manual de instruções*. 5th ed. Piracicaba: CONSECANA, 112 p.
- ELSHEERY, N.I., SUNOJ, V.S.J., WEN, Y., ZHU, J.J., MURALIDHARAN, G. and CAO, K.F., 2020. Foliar application of nanoparticles mitigates the chilling effect on photosynthesis and photoprotection in sugarcane. *Plant Physiology and Biochemistry*, vol. 149, pp. 50-60. <http://dx.doi.org/10.1016/j.plaphy.2020.01.035>. PMID:32035252.
- ENDRES, L., SANTOS, C.M., SOUZA, G.V., MENOSSI, M. and SANTOS, J.C.M., 2018. Morphological changes recorded in different phenophases of sugarcane plants subjected to water stress in tropical field conditions. *Australian Journal of Crop Science*, vol. 12, no. 7, pp. 1041-1050. <http://dx.doi.org/10.21475/ajcs.18.12.07.PNE780>.
- FARIAS, C.H.D.A., FERNANDES, P.D., GHEYI, H.R. and DANTAS NETO, J., 2009. Qualidade industrial de cana-de-açúcar sob irrigação e adubação com zinco, em Tabuleiro Costeiro paraibano. *Revista Brasileira de Engenharia Agrícola e Ambiental*, vol. 13, no. 4, pp. 419-428. <http://dx.doi.org/10.1590/S1415-43662009000400008>.
- FERNANDES, A.C., 2000. *Cálculos na agroindústria da cana de açúcar*. Piracicaba: Sociedade dos Técnicos Açucareiros e Alcooleiros do Brasil. 193 p.
- GOVAERTS, B., SAYRE, K.D., LICHTER, K., DENDOOVEN, L. and DECKERS, J., 2007. Influence of permanent raised bed planting and residue management on physical and chemical soil quality in rain fed maize/wheat systems. *Plant and Soil*, vol. 291, no. 1, pp. 39-54. <http://dx.doi.org/10.1007/s11104-006-9172-6>.
- HAIR JUNIOR, J.F., BLACK, W.C., BABIN, B.J., ANDERSON, R.E. and TATHAM, R.L., 2009. *Análise multivariada de dados*. 6th ed. Porto Alegre: Bookman, 688 p.
- HOANG, D.T., HIROO, T. and YOSHINOBU, K., 2019. Nitrogen use efficiency and drought tolerant ability of various sugarcane varieties under drought stress at early growth stage. *Plant Production Science*, vol. 22, no. 2, pp. 250-261. <http://dx.doi.org/10.1080/1343943X.2018.1540277>.
- HOTELLING, H., EISENHART, C., HASTAY, M.W. and WALLIS, W.A., 1947. *Multivariate quality control. Techniques of statistical analysis*. New York: John Wiley & Sons, 73 p.
- INMAN-BAMBER, N.G. and SMITH, D.M., 2004. Sugarcane water stress criteria for irrigation and drying off. *Field Crops Research*, vol. 89, no. 1, pp. 107-122. <http://dx.doi.org/10.1016/j.fcr.2004.01.018>.
- INMAN-BAMBER, N.G. and SMITH, D.M., 2005. Water relations in sugarcane and response to water deficits. *Field Crops Research*, vol. 92, no. 2-3, pp. 185-202. <http://dx.doi.org/10.1016/j.fcr.2005.01.023>.
- KAISER, H.F., 1960. The application of electronic computers to factor analysis. *Educational and Psychological Measurement*, vol. 20, no. 1, pp. 141-151. <http://dx.doi.org/10.1177/001316446002000116>.
- LEANASAWAT, N., KOSITTRAKUN, M., LONTOM, W. and SONGSRI, P., 2021. Physiological and agronomic traits of certain sugarcane genotypes grown under field conditions as influenced by early drought stress. *Agronomy*, vol. 11, no. 11, p. 2319. <http://dx.doi.org/10.3390/agronomy11112319>.
- MARIN, F.R., PELLEGRINO, G.Q., ASSAD, E.D., PINTO, H.S. and ZULLO JUNIOR, J., 2009. Cana-de-açúcar. In: J.E.B.A. MONTEIRO, ed. *Agrometeorologia dos cultivos: o fator meteorológico na produção agrícola*. Brasília: Instituto Nacional de Meteorologia, pp. 109-130.
- MOHANRAJ, K., HEMAPRABHA, G. and VASANTHA, S., 2021. Biomass yield, dry matter partitioning and physiology of commercial and Erianthus introgressed sugarcane clones under contrasting water regimes. *Agricultural Water Management*, vol. 255, p. 107035. <http://dx.doi.org/10.1016/j.agwat.2021.107035>.
- MORAIS, J.E.F., SILVA, Ê.F.F., GODOI NETO, A.H., LIMA, B.L.C., LIRA, R.M., BERTO, S.D.C., JARDIM, A.M.R.F., SIMÕES NETO, D.E., SILVA, T.G.F. and ROLIM, M.M., 2022. Sugarcane (*Saccharum officinarum* L.) under saline stress: growth, productivity, technological quality, and industrial yield. *Industrial Crops and Products*, vol. 188, p. 115642. <http://dx.doi.org/10.1016/j.indcrop.2022.115642>.
- MOURA, L.C., SILVA, N.F., CUNHA, F.N., BASTOS, F.J.C., CÉLIA, J.A. and TEIXEIRA, M.B., 2014. Índice de maturação da cana-de-açúcar fertirrigadas sobre diferentes lâminas. *Revista Brasileira de Agricultura Irrigada*, vol. 8, no. 1, pp. 64-76. <http://dx.doi.org/10.7127/rbai.v8n100199>.
- OLIVEIRA, E.L., ANDRADE, L.A.B., FARIA, M.A., EVANGELISTA, A.W.P. and MORAIS, A.R., 2009. Uso de vinhaça de alambique e nitrogênio em cana-de-açúcar irrigada e não irrigada. *Pesquisa Agropecuária Brasileira*, vol. 44, no. 11, pp. 1398-1403. <http://dx.doi.org/10.1590/S0100-204X2009001100005>.

- PEREIRA, L.B., GAMBARINI, V.M.O., MENEZES, A.B., OTTOBONI, L.M. and VICENTINI, R., 2021. Responses of the sugarcane rhizosphere microbiota to different levels of water stress. *Applied Soil Ecology*, vol. 159, p. 103817. <http://dx.doi.org/10.1016/j.apsoil.2020.103817>.
- PRADO, E.A.F., VITORINO, A.C.T., MAUAD, M., ENSINAS, S.C. and PAIM, L.R., 2017. Características tecnológicas da cana-de-açúcar sob aplicação de doses de vinhaça em Latossolo Vermelho distroférrico. *Revista de Ciências Agrovetenárias*, vol. 16, no. 4, pp. 386-395. <http://dx.doi.org/10.5965/10.5965/223811711642017386>.
- RAMESH, P. and MAHADEVASWAMY, M., 2000. Effect of formative phase drought on different classes of shoots, shoot mortality, cane attributes, yield and quality of four sugarcane cultivars. *Journal Agronomy & Crop Science*, vol. 185, no. 4, pp. 249-258. <http://dx.doi.org/10.1046/j.1439-037x.2000.00399.x>.
- SHEN, J.L., LI, C.L., WANG, M., HE, L.L., LIN, M.Y., CHEN, D.H. and ZHANG, W., 2017. Mitochondrial pyruvate carrier 1 mediates abscisic acid-regulated stomatal closure and the drought response by affecting cellular pyruvate content in *Arabidopsis thaliana*. *BMC Plant Biology*, vol. 17, no. 1, p. 217. <http://dx.doi.org/10.1186/s12870-017-1175-3>. PMID:29166881.
- SILVA, F.A., DIAS, M.S., FERNANDES, P.D., LACERDA, C.N., SILVA, A.A.R., MARCELINO, A.D.A.L., LIMA, A.M. and BARBOSA, D.D., 2022. Calcium pyruvate as a salt stress mitigator in yellow passion fruit seedlings. *Revista Brasileira de Engenharia Agrícola e Ambiental*, vol. 26, no. 11, pp. 763-770. <http://dx.doi.org/10.1590/1807-1929/agriambi.v26n11p763-770>.
- SILVA, F.A., DIAS, M.S., FERNANDES, P.D., MARCELINO, A.D.A.L., LIMA, A.M., PEREIRA, R.F., BARBOSA, D.D., SILVA, M.F.C., SILVA, A.A.R. and SANTOS, R.C., 2023. Pyruvic acid as attenuator of water deficit in cotton plants varying the phenological stage. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 83, p. e272003. <http://dx.doi.org/10.1590/1519-6984.272003>. PMID:37162072.
- SIMÕES, W.L., CALGARO, M., GUIMARÃES, M.J., OLIVEIRA, A.R. and PINHEIRO, M.P.M.A., 2018. Sugarcane crops with controlled water deficit in the Sub-Middle São Francisco Valley, Brazil. *Revista Caatinga*, vol. 31, no. 4, pp. 963-971. <http://dx.doi.org/10.1590/1983-21252018v31n419rc>.
- SOARES, L.A.A., FERNANDES, P.D., LIMA, G.S., SILVA, S.S., MOREIRA, R.C. and MEDEIROS, T.L., 2021. Phytomass and production components of colored cotton under salt stress in different phenological stages. *Revista Brasileira de Engenharia Agrícola e Ambiental*, vol. 25, no. 2, pp. 132-138. <http://dx.doi.org/10.1590/1807-1929/agriambi.v25n2p132-138>.
- SOUZA, C.A., VITTI, A.C., SANQUETTA, C.R. and GAITAROSSA, E.C., 2016. Produção de biomassa da cana-de-açúcar por meio do uso de organominerais em cana planta e cana soca. *BIOFIX Scientific Journal*, vol. 1, no. 1, pp. 38-43. <http://dx.doi.org/10.5380/biofix.v1i1.49098>.
- STATSOFT, 2004. *Programa computacional Statistica 7.0* [software]. Hamburg: StatSoft Inc.
- SUGIHARTO, B., 2018. Biotechnology of drought-tolerant sugarcane. In: A.B. OLIVEIRA, ed. *Sugarcane - technology and research*. London: IntechOpen, pp. 139-145. <http://dx.doi.org/10.5772/intechopen.72436>.
- TAIZ, L., ZEIGER, E., MOLLER, I.M. and MURPHY, A., 2017. *Fisiologia e desenvolvimento vegetal*. 6th ed. Porto Alegre: Artmed, 858 p.
- TEIXEIRA, P.C., DONAGEMMA, G.K., FONTANA, D. and TEIXEIRA, W.G., 2017. *Manual de métodos de análise de solo*. 3rd ed. Brasília: Embrapa Solos, 573 p.
- VAN GENUCHTEN, M.T., 1980. A closed-form equation for predicting the hydraulic conductivity on unsaturated soil. *Soil Science Society of America Journal*, vol. 44, no. 5, pp. 892-898. <http://dx.doi.org/10.2136/sssaj1980.03615995004400050002x>.
- VENKATARAMANA, S., GURURAJARAO, P.N. and NAIDU, K.M., 1986. The effects of water stress during the formative phase on stomatal resistance and leaf water potential and its relation with yield in ten sugarcane varieties. *Field Crops Research*, vol. 13, pp. 345-353. [http://dx.doi.org/10.1016/0378-4290\(86\)90035-3](http://dx.doi.org/10.1016/0378-4290(86)90035-3).
- VIANA, R.S., MOREIRA, B.R.A., LISBOA, L.A.M., FIGUEIREDO, P.A.M., RAMOS, S.B., RAMOS, S.B., DIAS, G.H.O., MOREIRA, L.C., SPOTONI, M.T. and RECCO, C.R.S.B., 2019. Exogenous spraying of plant's resistance inducers improves yield and sugarcane quality. *Bulgarian Journal of Agricultural Science*, vol. 25, no. 5, pp. 833-844.
- WIEDENFELD, R.P., 2000. Water stress during different sugarcane growth periods on yield and response to N fertilization. *Agricultural Water Management*, vol. 43, no. 2, pp. 173-182. [http://dx.doi.org/10.1016/S0378-3774\(99\)00053-0](http://dx.doi.org/10.1016/S0378-3774(99)00053-0).