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Sugars levels of four sugarcane genotypes in different stem portions during the maturation phase

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ABSTRACT

Maturation is a characteristic of sugarcane plant (*Saccharum* spp.) and even when grown under the same soil and climate conditions the varieties differ on the maturation curve. Thus, studies that allow establishing maturation curves of different sugarcane genotypes in the local soil and climate may indicate the proper harvesting period to ensure better quality of the raw material. This study aimed to analyze the levels of soluble sugars during the maturation phase and assess the technological and productivity indexes of four irrigated sugarcane genotypes in the region of Rio Largo, Alagoas. The experiment was conducted in randomized blocks in a 4 x 2 x 5 factorial: four genotypes (RB92579, RB98710, RB99395 and RB961003), two stem portions (internodes 1-4 and internodes 5-8) and five seasons (82, 49, 25, 13 and 3 days before harvesting), each treatment with three replications. Internodes 1-4 showed the highest levels of reducing sugars, while the largest accumulation of sucrose and total soluble solids occurred in internodes 5-8. RB99395 genotype showed more stability in the sugar levels during sugarcane maturation, which can indicate early maturation and high agricultural yield.

Key words: fructose, glucose, sucrose, total soluble solid, yield.

INTRODUCTION

Sugarcane (*Saccharum* spp.) is a very important crop in the world's economy, especially as raw material for production of sugar, alcohol, yeast and other derivatives (Cesnik and Miocque 2004). The economic yield of this culture is given by the sucrose production, reducing sugars that are used in the formation of molasses and also fiber, which can be used as source of energy in the plant itself (Toppa et al. 2010).

A succession of internodes at different physiological stages comprises the sugarcane stem, i.e., immature (top), at maturation (middle) and mature internodes (bottom). Immature internodes are fibrous, have high concentration of reducing sugars and low sucrose concentration. As these internodes develop, their growth rate progressively decreases until it is null when the stem reaches maturation (Machado 1987). During this process,

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the basal third of the stem has higher sugar content in relation to the middle third, which in turn has higher sugar content compared to the apical third. With advancing maturation, the sucrose content tends to become homogeneous in the various stem portions (Fernandes 1982).

In commercial production, maturation is a continuous process that maybe improved by environmental factors or management practices that paralyze plant growth. These include low temperatures, irrigation suspension, limitation of nitrogen fertilization and application of chemical ripeners (Lingle and Smith 1991, Rodrigues 2013). One way often used to monitor sugarcane maturation is through technological indexes that are evaluated in industry and determine the quality of the raw material.

Given the above, this study aimed to analyze the levels of soluble sugars during the maturation phase and assess technological and productivity indexes of four irrigated sugarcane genotypes in the region of Rio Largo, state of Alagoas, in the 2012/2013 cropping season.

MATERIALS AND METHODS

DESCRIPTION OF THE EXPERIMENTAL AREA

The research was conducted in the experimental area located at Rio Largo (09º 28’ S, 35° 49’ W and 127 m above sea level), state of Alagoas, Brazil. The soil of the area was classified as Cohesive Argisolic Yellow Latosol of medium-clayey texture with declivity less than 3%. The climate is hot and semi-humid type with average annual rainfall of 1,818 mm (Souza et al. 2004).

Prior to the experiment installation, chemical analysis of soil in layers 0-20 and 20-40 cm deep in the profile was carried out. Based on these results, 500 kg ha^{-1} of dolomitic lime were applied to increase the soil saturation to 60% (Oliveira et al. 2007), following harrowing, plowing and foundation fertilization using 150 kg ha^{-1} N, 195 kg ha^{-1} P_2O_5 and 230 kg ha^{-1} K_2O.

CULTURE INSTALLATION

The experimental design was a randomized block in a 4 (genotypes) x 2 (stem portion) x 5 (seasons) factorial scheme with three replications. Sugarcane genotypes were represented by RB92579, RB98710, RB99395 varieties and RB961003 clone, being selected by high agricultural productivity, sugar content and maturation time. Two stem portions were assessed: internodes 1-4 and internodes 5-8, considering that internode 1 is that located immediately below leaf +1, which is the first leaf from apex to bottom presenting visible atrium. Evaluations were performed at five seasons 82, 49, 25, 13 and 3 days before harvest (DBH).

The experimental plot consisted of two double lines of 10 m in length. Planting furrows were opened with spacing of 1.4 m between double rows and 0.4 m within the double row. Planting was held on 22 dez. 2011, with density of about 15 buds per linear meter of furrow and depth of 0.25 m. Stems of about 12 months of age were placed in the furrow bottom in order to be “foot with tip” being chopped with a machete, leaving at least three gems per billet. Then, the stems were covered with a soil layer of ± 0.07 m.

CROP MANAGEMENT

In the first 15 days after planting, the crop was irrigated by low-pressure conventional sprinkler, applying a 70 mm water slide and split with two-day irrigation interval to ensure uniform germination. Subsequently, the culture was irrigated by dripping with drip tapes of 22 mm and self-compensating emitters every 0.5 m with average flow rate of 1 L ha^{-1} and pressure of 1.4 kg cm^{-2}. In the rainy season (17 may to 10 sep. 2012), irrigation was suspended and resumed in the dry season (from 20 sep. 2012).

The amount of water applied was determined as a function of the crop evapotranspiration (ETc), which was calculated by multiplying the reference evapotranspiration (ETo) by the crop coefficient (Kc) in the initial, intermediate and final phase of sugarcane, according to method proposed by Allen et al. (1998). Weather conditions such as rainfall, air temperature and relative humidity were daily recorded in automatic data acquisition weather station micrologger located 400 m from the experiment. Based on temperature and relative humidity data, the air vapor pressure deficit (VPD) was also calculated according to FAO (1991).

Throughout the culture development, control of native plants was performed through chemical treatment and hand hoeing whenever necessary. At 60 days after planting, topdressing was held with 20 kg ha^{-1} N, using ammonium sulfate as nitrogen source. In the final stage of the crop cycle, at 25 DBH, irrigation was suspended to optimize maturation and increase the sucrose content of stems.

DETERMINATION OF SUGARS AND TOTAL SOLUBLE SOLIDS

After 82, 49, 25, 13 and 3 DBH (from 14 nov. 2012 to 01 feb. 2013), three stems of each sugarcane genotype per experimental plot were randomly collected and immediately taken to the laboratory. Stems were cut into two portions, internodes 1-4 cm and 5-8 cm. These portions were chosen because they are the regions of highest growth, with internodes still immature and possibly with greater differences in concentrations of soluble sugars.

Then, stems were passed in electric milling and filtered to obtain the juice. After juice extraction, a few drops were placed on the prism of the portable digital refractometer to obtain the contents of total soluble solids, expressed in ºBrix. About 2 ml of juice samples were also collected using an automatic pipette, keeping them in microtubes arranged in a Styrofoam box with ice to reduce chemical changes. Subsequently, juice samples were centrifuged at 14,000 x g for 10 minutes at 4 °C and supernatants were transferred to 1.5 ml microtubes, being stored in freezer at -20 °C for subsequent chemical analysis and precipitates were discarded.

The fructose, glucose and sucrose contents were determined by High-Performance liquid chromatography (HPLC) (Hunt et al. 1977) using Shimadzu U-20 chromatograph equipped with an oven (CTO-20A), automatic sample injector (SIL-10Ai), refractive index detector (RID10A), a 25 cm x 4.6 mm column (Shim-pack CLC-NH_2 (M)), stationary phase (Aminopropyl group), separation mode (Reversed, normal, ion, exchange) and pre column (G NH_2, 8 mm). Fructose, glucose and sucrose solutions were used as calibration standard and acetonitrile and ultrapure water at a ratio of 75:25 v/v were used as eluent.

The oven temperature used was 40 °C. Sugarcane juice samples from the four sugarcane genotypes were thawed in a water bath with ice and filtered with Millipore disposable filter (Hidrophilic PVDF) with membranes 0.45 µm of pore and 33 mm in diameter, with the aid of 5 ml syringes. Then, samples were diluted in ultrapure water at a ratio of 1:5. Shortly after, 1 ml fractions were transferred to 1.5 ml glass vials with screw cap and PTFE/Silicone septum, starting the readings on the HPLC. The injection volume was 20 µl.

HARVEST, EVALUATION OF TECHNOLOGICAL AND YIELD INDEXES

Sugarcane was manually harvested without burning on 04 feb. 2013. Stems produced in a double line of 5 m in length of the sample area were collected, which were weighed and sent to the laboratory located at Rio Largo, AL, for the characterization of technological indexes by analyzing the juice extracted by the hydraulic press method described by Fernandes (2000). The following parameters were determined: content of total soluble solids (ºBrix); sugarcane Pol (%); Juice Purity (%); Industrial Fiber (%); Reducing Sugars (%); Total reducing sugars (%), Total Recoverable Sugars (kg t^{-1}) and Agricultural yield (t ha^{-1}), according to Consecana (2006).

STATISTICAL ANALYSIS

Results were submitted to analysis of variance by the F test with subsequent comparison of means using the Tukey test ($p < 0.05$) with the statistics program SISVAR (Variance Analysis System, version 5.3). Data of sugars and total soluble solids were analyzed in a split plot arrangement in time.

RESULTS

METEOROLOGICAL CONDITIONS

The amount of water supplied to plants considering the sum of rainfall and irrigation for 90 DBH was 371 mm. The highest crop water demand occurred between 90 and 70 DBH, which was around 5.9 mm day^{-1}, and from that period, the crop evapotranspiration (ETc) remained constant, averaging 3.4 mm day^{-1} (Figure 1a).

During the experimental period, the air temperature ranged from 25.9 to 27.5 °C, averaging 26.4 °C (Figure 1b). The relative humidity averaged 73%; however, at 60 DBH, moisture was more pronounced (82%) (Figure 1b). In the period from 30 to 10 DBH, there was a decrease of moisture due to the increase in temperature in the region, which directly contributed to the increase in the vapor pressure deficit in the same period, ranging from 1.0 to 1.2 kPa (Figure 1c).

CONTENT OF SUGARS AND TOTAL SOLUBLE SOLIDS

Internodes 1-4 showed fructose and glucose levels significantly higher than internodes 5-8 in most assessment periods (Figure 2). RB92579 genotype at 49 DBH (Figures 2a, 2e) and RB961003 genotype at 13 DBH (Figures 2d, 2h) showed an increase in fructose and glucose levels in internodes 5-8. RB99395 was the only genotype that maintained fructose and glucose contents practically constant in both stem portions throughout the maturation period, with an increase of 0.5% fructose at 25 DBH (Figure 2c) and an increase of 0.6% glucose at 3 DBH (Figure 2g), both in internodes 1-4. Except for RB92579 genotype, there were reductions in fructose and glucose levels in internodes 5-8 at 3 DBH (Figure 2).

The sucrose content of sugarcane juice was higher in internodes 5-8 in all genotypes and periods analyzed (Figure 3). The sucrose levels in RB99395 genotype remained constant throughout the assessment period, with average value of 15.1% sucrose in sugarcane juice in internodes 5-8, standing out among the other genotypes (Figure 3c). On the other hand, RB961003 clone was the genotype that showed the lowest sucrose values, averaging only 6.4% sucrose in the same stem portion (Figure 3d).

In summary, internodes 5-8 showed high levels of soluble solids in all genotypes (Figure 3). At 3 DBH, RB99395 and RB961003 genotypes showed increments of 3 and 2 ºBrix (Figures 3g, 3h), respectively, in the same stem portion in relation to the previous period.

In general, during the maturation process, internodes 1-4 showed the highest fructose and glucose concentrations, and RB92579 was the genotype with the lowest levels of reducing sugars in this stem portion, 1.2% fructose and 1.5% glucose. RB961003 showed high glucose levels in internodes 5-8, and when compared with the other genotypes, it showed an average of 1.8% glucose in the sugarcane juice (Table I).

Sucrose and ºBrix levels were higher in internodes 5-8 in all genotypes analyzed, with differences among genotypes. RB99395 showed the highest sucrose concentrations in the juice in both stem portions analyzed, especially in internodes 5-8, which showed, on average, 15.1% sucrose.

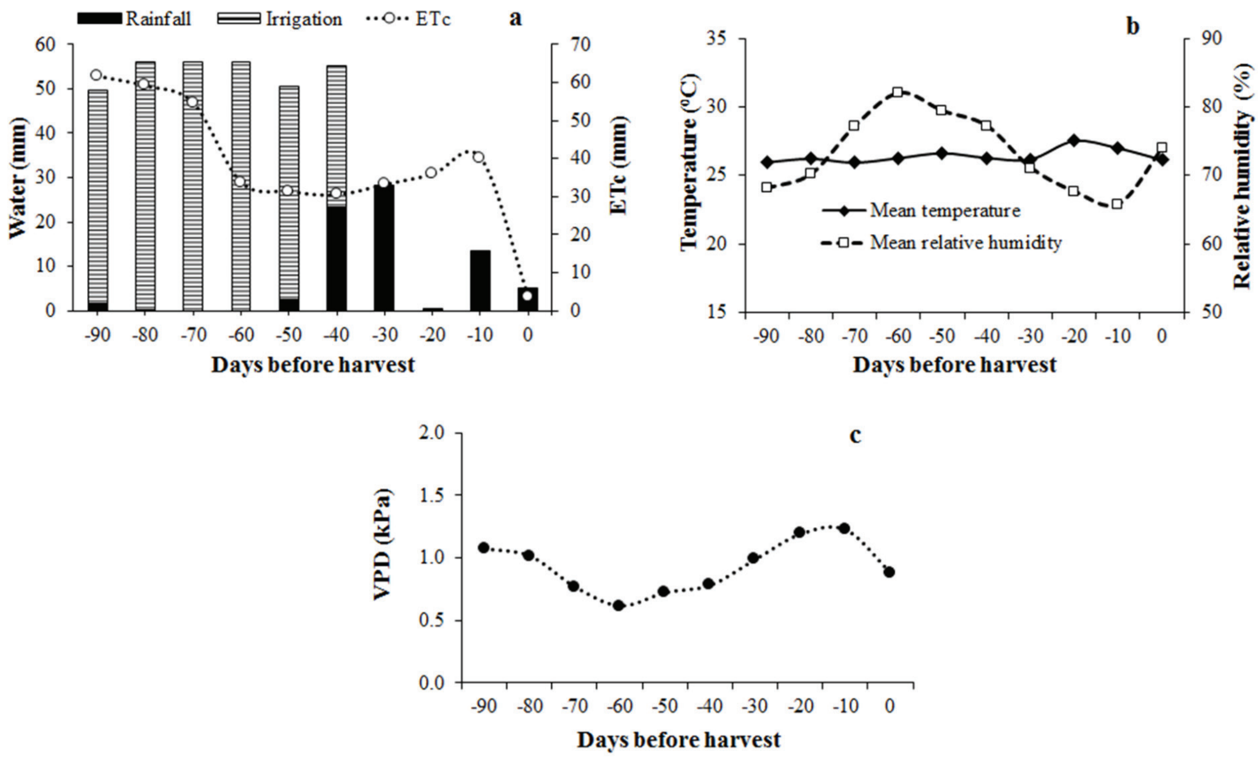

Figure 1 - Rainfall, irrigation and evapotranspiration (ETc) of sugarcane (**a**), temperature and relative humidity (**b**) and vapor pressure deficit (VPD) of air (**c**) in the region of Rio Largo, AL, from 06 nov. 2012 to 04 feb. 2013.

TABLE I
Mean levels of fructose, glucose, sucrose and soluble solids (Brix) in the juice of four sugarcane genotypes in internodes 1-4 and 5-8 at 90 days before harvest in the region Rio Largo, AL.

Genotypes	Fructose (%)		Glucose (%)	
	Internodes 1 to 4	Internodes 5 to 8	Internodes 1 to 4	Internodes 5 to 8
RB92579	1.2 Ac	0.9 Bb	1.5 Ac	1.1 Bc
RB98710	1.7 Aa	1.0 Ba	2.1 Aa	1.4 Bb
RB99395	1.4 Ab	0.5 Bc	2.0 Aa	0.7 Bd
RB961003	1.4 Ab	1.1 Ba	1.8 Ab	1.8 Ba
VC1 (%)	7.3		5.5	
VC2 (%)	7.9		8.1	

Genotypes	Sucrose (%)		ºBrix	
	Internodes 1 to 4	Internodes 5 to 8	Internodes 1 to 4	Internodes 5 to 8
RB92579	2.2 Bb	8.9 Ac	7.2 Bb	13.5 Ab
RB98710	1.4 Bc	10.2 Ab	7.2 Bb	13.7 Ab
RB99395	4.5 Ba	15.1 Aa	9.7 Ba	16.5 Aa
RB961003	1.0 Bc	6.4 Ad	6.4 Bb	11.3 Ac
VC1 (%)	7.8		5.4	
VC2 (%)	10.2		8.4	

Means followed by the same capital letter in line and small letter in column do not differ at Tukey test ($p < 0.05$). VC1 = variation coefficient of the plot. VC2 = variation coefficient of the subplot.

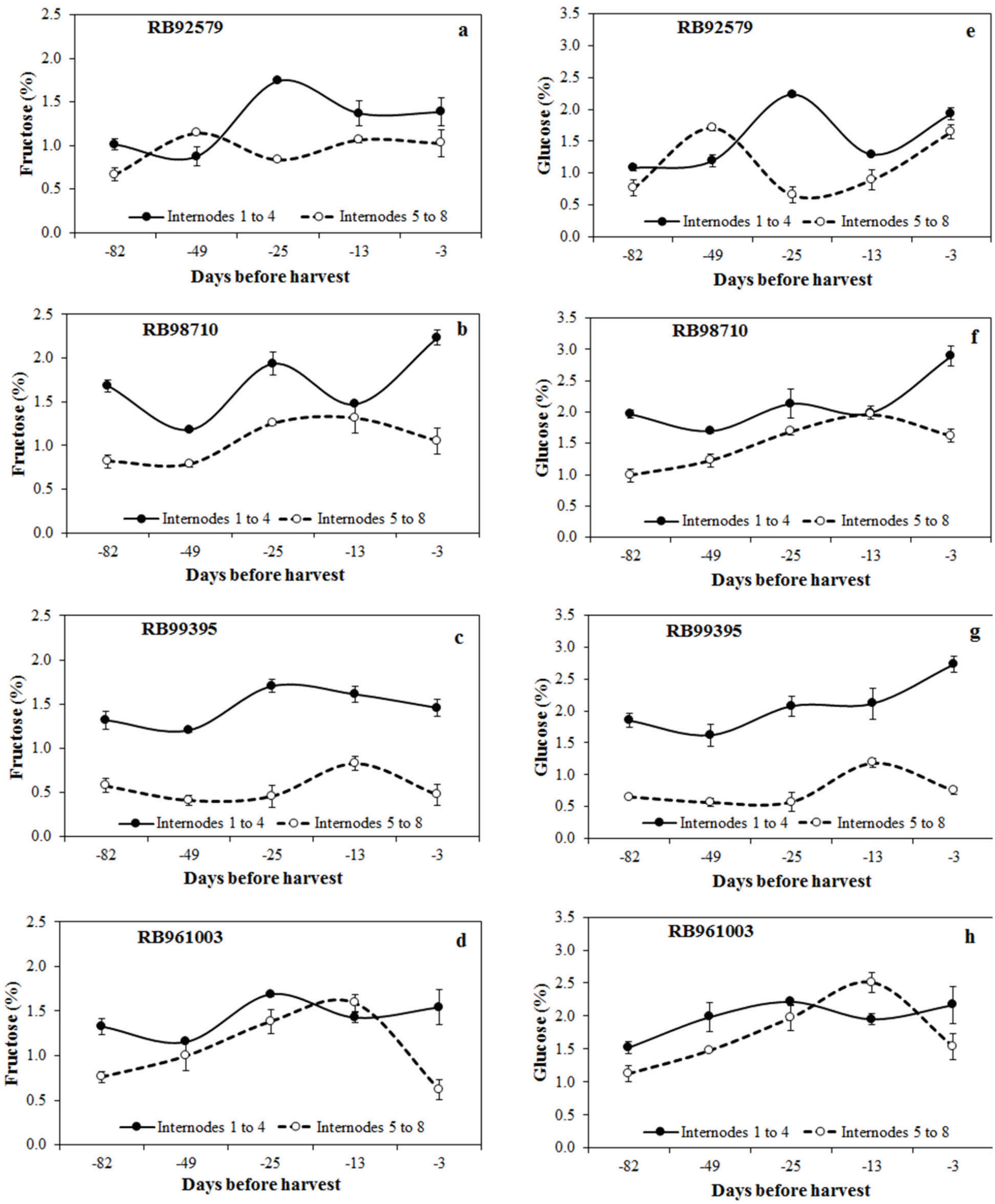

Figure 2 - Fructose (**a, b, c** and **d**) and glucose levels (**e, f, g** and **h**) in the juice of four sugarcane genotypes in internodes 1-4 and 5-8 as a function of days before harvest in the region of Rio Largo, AL. Bars indicate the mean standard deviation of three observations.

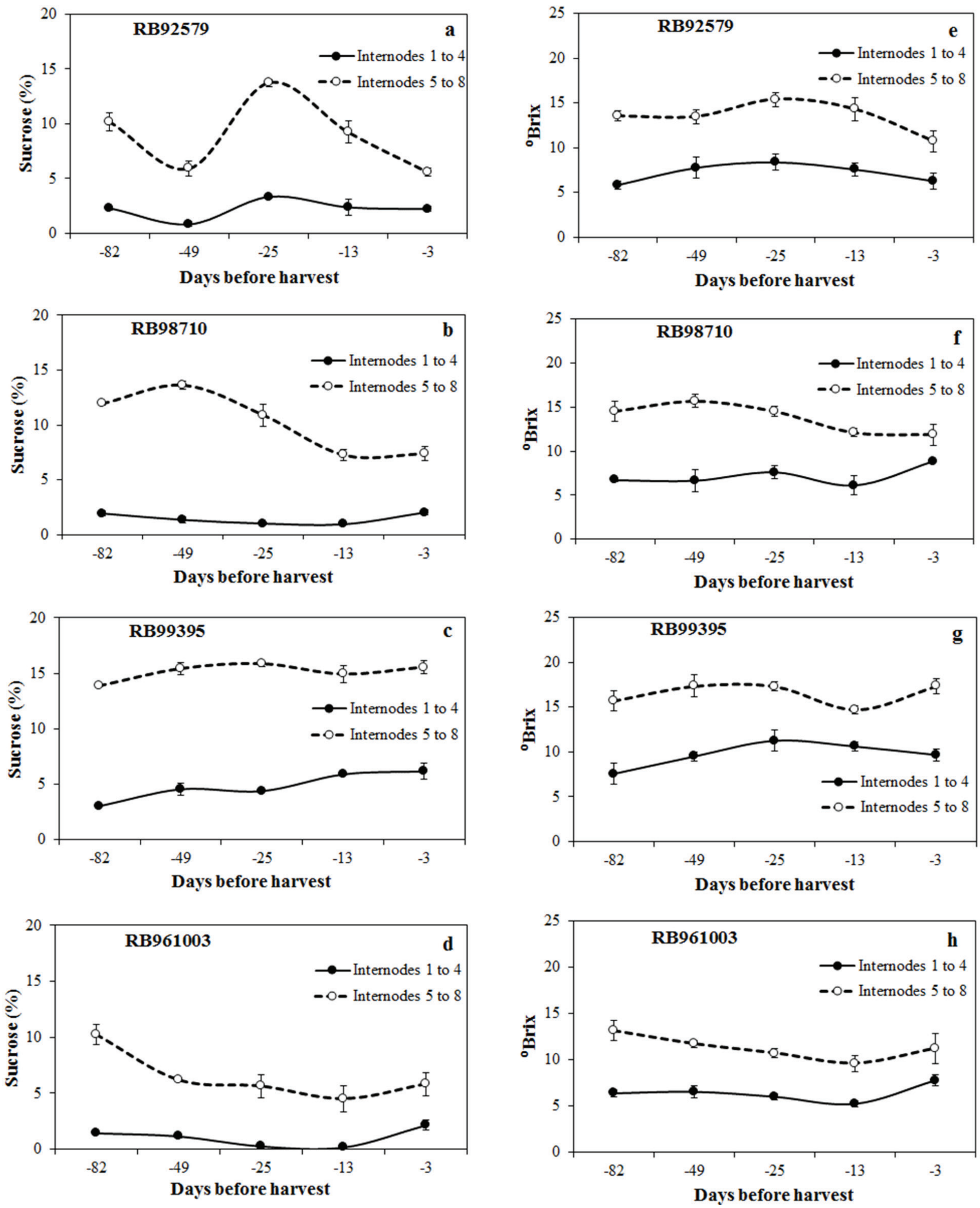

Figure 3 - Levels of sucrose (**a, b, c** and **d**) and total soluble solids (ºBrix) (**e, f, g** and **h**) in the juice of four sugarcane genotypes in internodes 1-4 and 5-8 as a function of days before harvest in the region of Rio Largo, AL. Bars indicate the mean standard deviation of three observations.

RB961003 genotype showed the lowest levels of this sugar in internodes 5-8, with average value of 6.4%, since it showed high levels of reducing sugars in the same stem portion. RB99395 was the genotype that accumulated ºBrix in both stem portions during the maturation period (Table I).

TECHNOLOGICAL INDEXES AND SUGARCANE YIELD

In the analysis of juice extracted from the entire sugarcane, including top, middle and bottom internodes at harvest, the ºBrix levels did not differ among genotypes, with average values from 20.4 to 21.3º, and these levels were higher than those obtained at 82, 49, 25, 13 and 3 DBH (Table II).

Genotypes showed sugarcane Pol values between 14.6 and 15.1% and the fiber contents remained between 12.8 and 13.5%. Despite the fluctuation in the glucose and fructose levels in the different stem portions at 90 DBH, there were no differences among genotypes studied for reducing sugars (RS) in the final stem evaluation, ranging from 0.6 to 0.7% (Table II).

In this work, the different genotypes showed average value of 16.4% for total reducing sugars (TRS). There was no difference among genotypes for total recoverable sugars (TR), with average yield of 146.0 kg sugar t^{-1}. Evaluating agricultural productivity (TCH), RB99395 stood out among the others with average yield of 152.9 tons of stems per hectare; however, it did not differ from RB92579 and RB98710. RB961003 clone showed the lowest average agricultural productivity (Table II).

DISCUSSION

The study area is located in the tableland of the state of Alagoas, characterized by a semi-humid tropical climate with rainy season between March and August and dry season from September to February. Rainfall in the region is influenced by maritime tropical air mass and penetration of cold polar air, with average annual rainfall between 1,500 and 2,000 mm, with minimum total of 41 mm in December and maximum of 294 mm in July. The average monthly relative humidity shows values above 70% and the air temperature ranged from 26.0 to 32.8 °C for maximum temperature and from 18.3 °C to 23.2 °C for the minimum temperature (Souza et al. 2004, 2005).

Abreu et al. (2013) evaluated interactions between meteorological variables and the yield components of six sugarcane varieties in the tableland of Alagoas and found that the rainfall irregularity in the region promotes different responses of varieties, both of growth and productivity, in the different cultivation cycles. Inman-Bamber and Smith (2005) reported that the temporal variation of soil moisture conditions caused by the rainfall irregularity is the most relevant factor in the yield oscillation of this culture.

Sugarcane stem has genetically suitable potential to accumulate sugars, mainly sucrose. Under ideal growing conditions, this potential is optimized with maturation, which takes place at the end of the plant cycle (Machado 1987, Santos et al. 2011). Sugarcane maturation depends on a complex combination of climatic variables, genetic potential and crop management (Cardozo and Sentelhas 2013).

Studies have shown that sucrose accumulation in stems in the maturation phase is favored by relatively low temperatures, which reduce the absorption of nutrients and plant growth, and a period of water restriction, because the dehydration of plant tissues forces the conversion of reducing sugars into sucrose. During this process, sucrose content increases to reach extreme limits from 12 to 18%, as the other sugars such as glucose and fructose have their contents reduced by up to limits of 0.2% (Marafon 2012).

The increase in fructose and glucose levels found in internodes 5-8 in RB92579 and RB961003 genotypes at 49 and 13 DBH, respectively (Figure 2)

TABLE II
Technological (Brix, Pol, fiber, purity, reducing sugars (RS) and total reducing sugars (TRS)) and productivity indexes (total recoverable sugars (TR) and agricultural yield (TCH)) of four irrigated sugarcane genotypes in the region Rio Largo, AL, 2013.

Genotypes	ºBrix	Pol (%)	Fiber (%)	Purity (%)	RS (%)	TRS (%)	TR (kg t^{-1})	TCH (t ha^{-1})
RB92579	20.7	14.9	12.8	83.6	0.7	16.3	145.3	125.9 ab
RB98710	21.3	14.6	13.5	86.1	0.7	16.2	144.4	115.9 ab
RB99395	20.4	15.1	13.1	87.7	0.7	16.6	147.8	152.9 a
RB961003	20.4	15.0	12.9	88.2	0.6	16.4	146.5	108.0 b
	ns	ns	ns	ns	ns	ns	ns	*
VC (%)	3.9	5.2	5.5	3.5	9.6	4.9	4.9	11.6

[ns]not significant by the F test. *Means followed by the same letter in the column do not differ at Tukey test ($p < 0.05$). VC = variation coefficient.

probably occurred by influences of management and climate conditions, as at 49 DBH was preceded by an excessive amount of water in the soil, especially by irrigation, combined with low crop evapotranspiration (Figure 1a), while 13 DBH was marked by an increase in temperature, averaging 27.5 °C (Figure 1b). The application of 150 kg ha^{-1} of N at planting and 20 kg ha^{-1} N at topdressing may have favored vegetative growth, delayed maturation and reduced percentage of sucrose by increasing the content of reducing sugar. In contrast, at 3 DBH, all genotypes except for RB92579 showed reductions in the levels of fructose and glucose in internodes 5-8 (Figure 2), which would be justified by the suspension of water supply and reduction in the metabolic activity in the growing apical meristem.

Sucrose is the main form by which carbohydrates are translocated from leaves to the rest of the plant by phloem aiming to provide carbon and energy needed for growth and accumulation of reserve products (Felix et al. 2009). Sugarcane maturation is a physiological process involving the synthesis of sugars in leaves, translocation of formed products and storage of sucrose in the stem (Fernandes 1982).

Sucrose contents in internodes 5-8 were higher than those in internodes 1-4 in all genotypes analyzed (Figure 3), as expected, since sucrose is found in increasing concentrations from bottom to top of the stem. Qudsieh et al. (2001) studied the chemical changes of sugarcane juice at different stem portions during development and maturation and found that juice extracted from both middle and bottom stem portions showed the highest levels of sucrose and total soluble solids, whereas fructose and glucose levels were reduced in relation to the top portion of the stem.

Sugarcane quality cannot be analyzed only by its sucrose content, although it is the most important indicator, but rather by a number of other variables responsible for its yield in the industry (Ribeiro et al. 1999). Through studies, sugarcane maturation can be evaluated by technological indexes such as Pol, Brix, Purity and Reducing Sugars (Silva and Segato 2011).

Pol refers to the apparent sucrose content contained in sugarcane juice determined by saccharimetric methods. The higher the content, the more mature sugarcane is. Immature sugarcane has high content of reducing sugars and color precursors compounds, which reduce Pol values, resulting in juice with darker color (Ripoli and Ripoli 2004). According to the authors, Pol greater than 14% is considered adequate in terms of quality to industrialization. This was verified in this study, which showed that sugarcane was ripe at harvest.

Dinardo et al. (2011) evaluated the Pol of several sugarcane cultivars over the 2009/2010 cropping season and found a variation in apparent sucrose content in all cultivars, characterized by an increase with the advancement of phenological stages. According to Rodrigues and Santos (2011), when it comes to mature sugarcane, there is a close relationship between apparent percentage of soluble solids and sucrose content in the solution, and sugarcane is considered mature with minimum Brix of 18º, among other factors.

Overall, the fiber content of genotypes was on average 13.1% (Table II). Similar results were found by Silva et al. (2014), who studied the productive potential of different sugarcane varieties grown in irrigated conditions. According to Dias et al. (2012), the fluctuation in fiber content among varieties is a genetic trait and the higher the sucrose content of varieties, the lower their fiber content.

The supply of raw materials of technological quality in order to provide economic extraction of sugars is one of the greatest needs of the sugar and alcohol industry (Leite et al. 2010). Improvements in sugar yield may be achieved by increases in juice purity, which represents the proportion of sucrose in total sugars (Lingle and Smith 1991). During the harvest process, it is important to obtain values greater than 80% purity, because the higher its value, the lower the amount of impurities in the juice and consequently the greater its economic value (Assis et al. 2004). The high quality of the raw material was evidenced in this work. Similar results were also found by Carvalho et al. (2008).

Sucrose accumulation during the maturation process occurs simultaneously with reductions in the content of reducing sugars and increased purity. Demetrio et al. (2008) analyzed ten RB's clones and found that the content of reducing sugars ranged from 0.4 to 0.6%, indicating that sugarcane was suitable for industrialization, considering that values can be up to 1.5% at the beginning of harvest, and 1% in the course of the cropping season.

Total reducing sugars (TRS) are all sugars in the sugarcane in the form of reducing sugars in which all sucrose was converted into glucose and fructose (Fernandes 2000). Thus, the TRS values must follow the trend of Pol values, increasing during the maturation process (Henrique et al. 2011). The genotypes under study showed similar TRS results, although there were differences in the levels of sugars in stem portions during the maturation period.

Total recoverable sugars (TR) indicate the amount of total sugars in the sugarcane mainly consisting of sucrose and reducing sugars, reduced by 11%, which is the average loss allowed in the industrial process (Consecana 2006). Costa et al. (2011) point out that TR is the most important parameter both for industry and for producers, since industrial plants elaborate the price paid to producers based on it.

The results of this study showed differences among genotypes for agricultural productivity. According to Dantas Neto et al. (2006), the yield of irrigated sugarcane depends on the amount of water applied, irrigation management combined with adequate amount of fertilizer, variety, cutting age, type of soil and climate. Therefore, it is suggested that the occurrence of rainfall in the maturation period combined with irrigation and fertilization management may have influenced the sugarcane yield of the different genotypes.

In conclusion, sugarcane juice extracted from internodes 1-4 show higher glucose and fructose levels, while the greatest accumulation of sucrose and soluble solids occurs in internodes 5-8, showing higher maturation in the bottom portion of stems. RB92579, RB98710, RB99395 and

RB961003 genotypes do not differ in relation to the contents of total soluble solids, Pol, juice purity, industrial fiber, reducing sugars, total reducing sugars and total recoverable sugars when grown under the soil and climatic conditions of Rio Largo, AL. RB99395 shows higher stability in the content of sugars during the maturation process, which may indicate early maturation and high industrial productivity of this variety.

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