

## Biometrics, productivity and technological quality of 23 energy sugarcane hybrid clones with higher lignocellulosic biomass

João Paulo de Lima Silva<sup>1\*</sup>, Gisele Silva de Aquino<sup>1</sup>, Deisi Navroski<sup>1</sup>, Ítalo Francisco de Souza<sup>2</sup>, Hugo Lyra Soriano<sup>3</sup>, José Antônio Bressiani<sup>3</sup>, Ricardo Ralisch<sup>1</sup>

<sup>1</sup>State University of Londrina (UEL), Department of Agronomy, Celso Garcia Cid Highway, s/n, Zip Code 10011 – CEP 86057-970, Londrina – PR, Brazil

<sup>2</sup>Usina Caeté – Unidade Paulicéia, Estrada Municipal de Paulicéia a São João do Pau D'Alho, km 7, 17.900-000, Paulicéia, São Paulo, Brazil

<sup>3</sup>GranBio Investimentos AS, Av. Brig. Faria Lima, 2277, cj. 1503, Alto de Pinheiros, São Paulo 01452-000, SP, Brazil

\*Corresponding author: joadelima25@hotmail.com

### Abstract

The development and identification of sugarcane clones with higher amount of lignocellulosic biomass are essential to increase the potential for generation of electrical energy or biofuel. Considering the potential for increased portfolio of products to be explored from the cane energy, the objective of this study was to compare the average results of biometrics, productivity and technological quality of 23 hybrids of cane energy with the commercial variety RB92579 (as a standard). Finally, we will identify energy cane clones of types 1 and/or 2 to become commercial cultivars. The experimental design was randomized blocks with three replications of 24 plots, where each plot consisted of 6 furrows of 10 linear meters with double spacing cycled from 0.9 x 1.5 meters. The evaluated variables were the number of stems per linear meter, stem diameter, dry and fresh matter and industrial quality. The results were submitted to statistical analysis using the Scott-Knott test for comparison of means. The diameter of stem in energy cane was lower (1.172 to 1.772 cm) than the average of sugarcane (2.302 cm). On the other hand, a greater number of stems per linear meter were found for 15 clones evaluated (average 24.19) compared to traditional sugarcane (12.5). All clones had low juice sugars concentration but nine clones reached fiber contents higher than 23.6%, representing a 42% increase compared to the fiber content of cultivar RB92579. The hybrids 1, 9, 11, 13, 16, 18 and 23 showed a better development potential with high tillering and high fiber content.

**Keywords:** bioenergy, cellulose, fiber, cogeneration, ethanol second generation, *Sugarcane* spp.

**Abbreviations:** ADS\_average diameter of stem; CV\_coefficient of variation; NSM\_number of stalks per linear meter; PCC\_sucrose per cent of cane; RB\_República Brasileira; RIDESA\_inter-university network for the development of the sugar-energy sector; TCH= ton of cane per hectare; TFH\_ton of fiber per hectare; TFMH\_ton of fresh matter per hectare; TSR\_total reducing sugar; TPH\_ton of sucrose per hectare; UFAL\_Federal University of Alagoas.

### Introduction

Since the implementation of the Kyoto Protocol in 2005, targets for reduction of greenhouse gases emissions for developed countries have been defined (MMA 2016). The search for renewable sources of energy has begun to be strategically discussed worldwide once fossil fuels represent approximately 87% of the energy production in the world. According to the IPCC (2013), to limit the impacts of global warming, emissions of gases which cause the greenhouse effect are required to have a significant reduction in the upcoming decades. The report of the Ethanol Project, made by the Center for Strategic Studies and Management (CGEE/MCT), showed that Brazil could produce enough ethanol to replace 10% of the entire gasoline consumed in the world in 2025. To do so, a production of 205 billion liters of ethanol per year would be needed. To achieve such a magnitude of increase in ethanol production, it is necessary to invest in science and technology throughout the cycle of

the sugar cane. A significant increase in ethanol production implies a substantial increase in the productivity of the sugarcane field, in terms of liters per hectare (CTBE 2016). There are several factors that can influence the increase of productivity, in which perhaps the most expressive one, is the possibility of using all the biomass of sugarcane as raw material for the production of ethanol, and not only the juice (Yang et al. 2006). It is estimated that this would mean a considerable increase in production without increasing the planted area (Lima and Natalense 2010). The use of ethanol fuel and electrical energy from the burning of bagasse and sugarcane straw was fundamental for Brazil to get a prominent position in the global context. Only these two products obtained from the sugar and biomass of the sugarcane were responsible for 16.1% of the entire energy supply in the country in the year 2013 (EPE 2014), which was enough to position the United States above the world

average in the use of renewable energies. In this context, the UFAL/RIDESA initiated a program of obtaining sugarcane energy originated from crossing hybrids with wild accessions of *S. spontaneum* in 2011. The preliminary results led to obtain a number of promising clones with traits of development of: total biomass, number of stems per plant, plant health and vigor. With these results, it is expected to achieve the RB sugarcane energy cultivars in the upcoming years that can meet the ascendant demand of companies dedicated to the production of cellulosic ethanol, bioelectricity and biochemicals (Santos 2013). This type of cane has been conceived to have characteristics of high fiber content, low content of sucrose and a high productivity of biomass (Kim and Day 2011).

With the advent of technologies that enable the production of energy through the fiber of biomass, Matsuoka et al. (2010) identified four basic platforms of the use of this material for energy purposes: i) direct combustion to produce thermal energy (steam) and cogeneration of electricity; ii) chemical or enzymatic hydrolysis of the fiber (cellulose and hemicellulose) to obtain fermentable sugars and production of liquid fuels; (iii) the gasification for the production of synthesis gas (carbon monoxide and hydrogen) or generation of biogas; and (iv) pyrolysis to obtain bio-oil and/or coal/coke.

In view of the possibilities of industrialization of the cane fiber, you can glimpse the full use of sugar cane juice, straw and bagasse), where the latter could significantly increase the production of ethanol per hectare, going from the current 7,000 L to approximately 14,000 L, without needing to expand the cultivated area (Santos et al., 2012a).

The researches around the second generation ethanol, or cellulosic ethanol, are evolving rapidly in Brazil (Brumley et al. 2007). The Brazilian Company of Industrial Biotechnology (GranBio) built the first Brazilian plant of ethanol 2G, located in Queensland, with a capacity for 82 million gallons of ethanol per year. In addition, it has the prospect of installing over 10 industrial plants in the country over the next few years (Segundo et al., 2014), indicating the growing demand for varieties with greater biomass production.

Regarding the research of genetic improvement, the goals will be resized to: (i) the collection of clones of the traditional type "sugar cane", with a greater yield of sugar present in the juice per hectare, and to keep supplying the current companies in the alcohol industry which use industrial processes on agreement; (ii) obtaining clones of the "Biomass Sugarcane energy type I", with a greater yield of biomass (stalks, dry leaves and needles), a higher yield of sugar per hectare and greater fiber content to meet the new ventures of biorefineries for the production of current products, in addition to new products that require more advanced technologies; and (iii) obtaining clones of "Sugarcane energy type II", with high yield in biomass, low sugar content and high fiber content, to meet the biorefineries for the production of cellulosic ethanol, as well as to other industries that need to replace fossil fuels for a cleaner and renewable energy based on the biomass of sugarcane (Ming et al., 2006; Tew; Cobill 2008; Loureiro et al., 2011; Barbosa, 2014).

Considering the need to increase the portfolio of products to be explored from the sugarcane energy, the objective of this work was to evaluate the production potential and

technological quality in the first cut of 23 sugarcane clones with the commercial cultivar RB92579 used as a standard to identify sugarcane clones' energy of types 1 and/or 2 to become commercial cultivars.

## Results and Discussion

### *Biometrics and productivity*

In relation to the biometric data, the 23 evaluated clones of sugarcane energy showed a lower stem diameter (1.172 and 1.772 cm) when compared with the commercial cultivar of sugarcane, RB92579 (2.302 cm). The morphological characters and also production components most relevant to the characterization of genetic materials are the mean diameter of stems (DCM) and the average number of stalks per meter (NCM). This is due to the fact that with the introgression of genes from the wild species *S. Spontaneum*, there is a tendency of the genetic material of the species *Saccharum sp.*, selected for production of biomass, to present a minor diameter and a greater tillering capacity in relation to the commercial hybrids of sugarcane. In a study conducted by Vitti and Prado (2012) values between 1.195 and 2.030 cm in clones sugarcane energy and 2.28 to 2.46 cm in commercial cultivars of sugarcane (RB72454 and RB867515) were found, which is consistent with the results of this research.

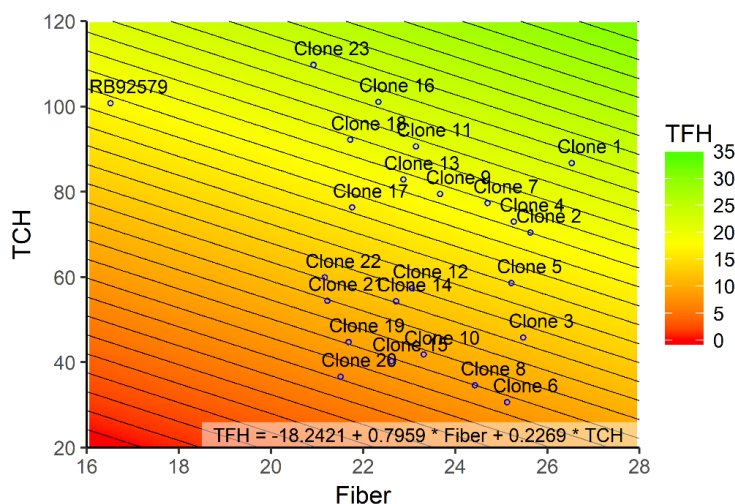
The clones studied presented a greater number of stalks per linear meter (average between 17 and 27) compared to the sugarcane trade (average of 12 stems m<sup>-1</sup>) (Table 2). Matsuoka et al. (2012) also found higher values of NCM in sugarcane energy, where the values ranged between 35 and 40 stems per linear meter, higher than those obtained in this study, achieving NCM between 15 and 27. According to Castro and Christofolletti (2005), Silva et al. (2007) and Landell and Silva (2004), the tillering has direct influence and is highly correlated with the production of sugar cane, being one of the most important morphological characteristics in the components of production of this crop.

Most of the clones had a lower productivity of stalks (TCH) when compared to RM92579, with the exception of the clones 11, 16, 18 and 23 which have attained equal means to the pattern, not differing statistically through the Scott and Konott test, at the level of 5% probability (Table 2). The coefficient of variation of 66.6% for the variable TCH, indicates the great variability among the studied materials. The values achieved in TMFH of hybrids 1, 7, 9, 11, 13, 16, 17, 18 and 23 were between 106.1 and 132.8 t.ha<sup>-1</sup>, and did not differ statistically from the commercial variety, while the other reached an average production of TMFH below RM92579 (124.9 t.ha<sup>-1</sup>) (Table 2). Violante (2012) evaluated 23 hybrids of sugarcane energy in a cycle of planted cane and found the values of TMFH between 69.88 and 143.96.t.ha<sup>-1</sup>.

Sierra et al. (2008), obtained a yield of 200 t.ha<sup>-1</sup> in energy sugarcane, higher values than the hybrids of this study. However, Morais et al. (2015) observed that the influence of the environment is a complicating factor. Since, an individual productivity is observed, it is necessary to ensure that this superiority is due to the genetic potential and not due to the action of environmental factors.

**Table 1.** Technological indicators evaluated 23 hybrids of energy sugarcane and variety RB92579 and their definitions 100 as CONSECAN (2006). <sup>a</sup>PCC=POL (juice sucrose) per cent of the broth, <sup>b</sup>TSR = total sugar reductor.

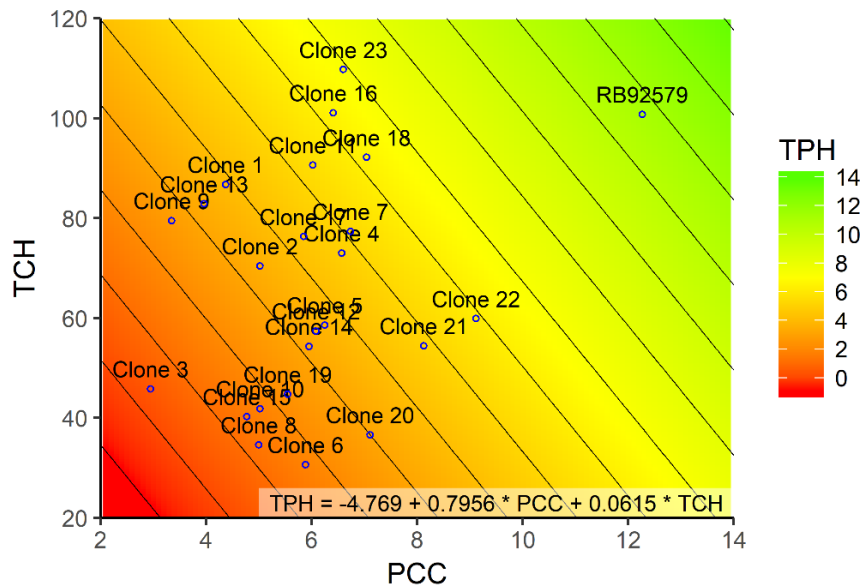
Variables	Definition
<sup>a</sup> PCC	Absolute sucrose content in broth per cent of cane
Fiber	Woody part of cane insoluble in water
<sup>b</sup> TSR	A sum of the various forms of sugar (sucrose, fructose and glucose) recoverable by industry



**Fig 1.** Isoquant of TFH 23 clones of sugarcane energy and a commercial cultivar of sugar cane (RB92579). Clones in the same color range have the same TFH. Subtitle: TCH = tons of cane per hectare, Fiber = fiber content of cane, TFH = Tons of fiber per hectare

**Table 2.** Biometric indexes, yield of fresh matter and yield of stems of 23 hybrids of sugarcane energy and a variety of sugar cane RB92579. <sup>a</sup>ADS = average diameter of stem, <sup>b</sup>NSM = Average number of stalks per linear meter, <sup>c</sup>TFMH = ton of fresh matter per hectare, <sup>d</sup>TCH = ton of cane per hectare. \*Means followed by equal letters in the same column, for each attribute, do not differ among themselves by the Scott-Konott test, significant at the probability level of 5%.

Plant material	<sup>a</sup> ADS	<sup>b</sup> NSM	<sup>c</sup> TFMH	<sup>d</sup> TCH
1	1.266c	27.100a	116.106a	86.730b
2	1.455b	24.450a	97.264b	70.427b
3	1.322c	24.250a	96.894b	45.833d
4	1.572b	22.150a	101.708b	73.018b
5	1.636b	18.100b	100.829b	58.610c
6	1.772b	17.050b	84.209b	30.649d
7	1.644b	16.000c	106.523a	77.371b
8	1.516b	24.450a	75.043b	34.626d
9	1.500b	26.550a	122.541a	79.476b
10	1.377c	26.650a	88.607b	41.866d
11	1.269c	23.650a	111.893a	90.618a
12	1.594b	24.200a	92.172b	57.412c
13	1.372c	21.200a	110.875a	82.874b
14	1.350c	22.950a	98.607b	54.309c
15	1.511b	23.650a	97.310b	40.278d
16	1.508b	18.400b	132.818a	101.074a
17	1.486b	20.150b	114.578a	76.321b
18	1.383c	21.600a	116.615a	92.163a
19	1.172c	15.000c	87.635b	44.749d
20	1.558b	18.150b	75.598b	36.627d
21	1.511b	19.050b	85.413b	54.433c
22	1.527b	24.800a	92.496b	59.878c
23	1.733b	25.200a	122.217a	109.726a
RB92579	2.302a	12.500c	124.208a	100.766a
Mean	1.514	21.552	6.300	14.540
CV (%)	10.23	11.48	23.04	66.66



**Fig 2.** Isoquanta of TPH 23 sugarcane clones energy and a commercial cultivar of sugar cane 209 (RB92579). Clones in the same color range have the same TPH. Subtitle: TCH = tons of cane per hectare, PCC = sucrose of cane, TPH = Tons of sucrose per hectare.

**Table 3.** Analysis of 23 sugarcane clones and RB92579. <sup>a</sup> PCC = Absolute sucrose content in broth per cent of cane, <sup>b</sup> TSR = Sugar total recoverable, <sup>c</sup> 173 Fiber = Fiber content in sugar cane (the woody plant of the sugarcane, insoluble in water). \*Means accompanied by the same letter in the same column do not differ at the level of 5% probability by Scott-Knott.

Plant material	<sup>a</sup> PCC	<sup>b</sup> ATR	<sup>c</sup> Fiber
1	4.372d	52.660d	26.530 <sup>a</sup>
2	5.021d	57.992d	25.636 <sup>a</sup>
3	2.950d	41.036d	25.476 <sup>a</sup>
4	6.570c	70.610c	25.276 <sup>a</sup>
5	6.248c	68.525c	25.220 <sup>a</sup>
6	5.888c	65.001c	25.130 <sup>a</sup>
7	6.735c	72.451c	24.706 <sup>a</sup>
8	4.996d	57.942d	24.433 <sup>a</sup>
9	3.347d	45.294d	23.673 <sup>a</sup>
10	5.021d	57.697d	23.316 <sup>b</sup>
11	6.024c	67.292c	23.150 <sup>b</sup>
12	6.081c	67.177c	23.063 <sup>b</sup>
13	3.963d	49.001d	22.876 <sup>b</sup>
14	5.948c	66.767c	22.720 <sup>b</sup>
15	4.772d	56.135d	22.620 <sup>b</sup>
16	6.411c	70.259c	22.336 <sup>b</sup>
17	5.847c	65.915c	21.766 <sup>b</sup>
18	7.040c	76.211c	21.726 <sup>b</sup>
19	5.545c	63.144c	21.686 <sup>b</sup>
20	7.104c	76.373c	21.513 <sup>b</sup>
21	8.128b	86.275b	21.230 <sup>b</sup>
22	9.114b	94.468b	21.173 <sup>b</sup>
23	6.601c	72.655c	20.923 <sup>b</sup>
RB92579	12.266 <sup>a</sup>	123.865 <sup>a</sup>	16.516 <sup>c</sup>
Mean	6.083	67.698	23.035
CV (%)	21.71	16.82	6.3

Pereira et al. (2016) noted that in the system of sugarcane planting that occurs from September to November, there are favorable conditions for germination and growth, but they have unfavorable conditions to the vegetative development, presenting low yields and lower longevity of tamping. This indicates that the clones 11, 16, 18 and 23 must be re-evaluated in planting times and different environments.

It is also important to note that the data collected and analyzed so far is from the first cut (sugarcane plant), and the sugarcane-energy tends wisely to increase productivity along the cuts due to an increase in the number of stems per linear meter while the sugarcane tends to decrease the number of stalks and consequently the productivity along the cuts (Carvalho-Neto et al., 2014; Bischoff et al., 2008).

### Technological quality

All clones had a lower sugar concentration compared to the commercial cultivar but all have achieved high levels of fiber (21 to 26%), significantly higher than the RB92579 (16%). Clones 1, 2, 3, 4, 5, 6, 7, 8 and 9 reached the highest levels of fiber with means between 23.7 and 26.5%, not differing statistically among one another (Table 3). These results indicate the great potential of sugarcane for the production of energy and other products with high aggregated value (Santos et al., 2012b; Santchurn et al., 2012; Rocha et al., 2015). Tew and Cobill (2008) emphasized that in order to meet the new demands of Bt 11 corn, the focus on obtaining of future cultivars should be concentrated in the substantial increase in the fiber content, coupled with increased productivity. Ramos (2015) reported levels of fiber between 13.0 and 20.3% for genotypes derived from *S. Spontaneum* and *S. Robustum*, while Dennedy (2008) obtained clones with 42% of fiber from a long recurrent selection program.

Clones 1, 2, 4, 7, 9, 11, 13, 16, 18 and 23 showed a production of fiber per hectare (TFH) larger than the commercial variety, where clones reached averages of TFH above 20 t.ha<sup>-1</sup> (Fig. 1), being higher than those obtained by Samuels et al. (1984) which reached an average of 10.7 to 18.0 t ha<sup>-1</sup>.

The number and weight of stems are important components to increase the production of biomass and, consequently, the productivity of fiber (Silveira, 2014). Therefore, clones that presented higher means of these components can be added to the selection process applicant or move forward in the program of genetic improvement of sugarcane to obtain cultivars of sugarcane energy. For this reason, the agronomic characterization and technology of clones is important for the selection of sugarcane energy.

Matsuoka et al. (2014) reported some characteristics of clones which are candidates to energy sugarcane such as rusticity, high tillering, vigorous rhizome, resistance to pests and diseases, in addition to a high content of fiber.

Therefore, we can highlight the hybrids 1, 9, 11, 13, 16, 18 and 23 that showed high tillering, biomass production per hectare and high fiber content, fulfilling the characteristics required. It is noteworthy that the soil, in which they were assessed, had an average sandy texture with low productivity potential, according to the classification of Prado (2008). In the culture of sugarcane, the genotype is greatly influenced by the environment and usually presents a decrease in yields of cane per hectare (TCH) as the

production environment worsens (Vitti and Prado, 2012). Therefore, evaluation of clones also in environments with best productive potential is recommended.

Fig. 2 shows the isoline of combination between TCH and PC, where they compare the amount of inches per unit of area (TPH) of 23 hybrids of sugarcane energy with the commercial cultivar of sugarcane (RB92579). The lower sucrose content was expected for the energy sugarcane clones. The clones 16, 18, 22 and 23 showed the greatest results of TPH with values between 5.45 and 7.24 t.ha<sup>-1</sup>, when compared to the other clones, but with a production less than RB92579, which reached the highest value with 12.27 t.ha<sup>-1</sup>. Ramos (2015) obtained values of TPH between 12.4 and 19.9 t ha<sup>-1</sup> for genotypes derived from *S. Spontaneum* and *S. Robustum*.

### Materials and Methods

The experiment was carried out in October 2014 in an area belonging to the Caeté Usine - Unit Paulicéia, located in the west of São Paulo, Brazil (latitude 21°16'46"; longitude 51°46'39" and 365 meters of altitude). The soil of the area is classified as a dystrophic Red Yellow Latosol (Embrapa 2006), with average sandy texture and flat topography. The rainfall during the studied cycle was 1,580 mm, with a monthly precipitation of at least 0 mm in October 2014 and a maximum of 352.2 mm in March 2015. Before planting, the operations of lime and gypsum were conducted with the application of one t. ha<sup>-1</sup> of lime and one t ha<sup>-1</sup> of gypsum. Afterwards the phosphorus was performed with 188 kg.ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>. The operations of soil preparation were composed of a subsoiling, a disk harrow and a harrowing. During the groove, 550 kg.ha<sup>-1</sup> of the NPK 05-25-25 formula was applied.

24 sugarcane clones were evaluated, including 23 clones of sugarcane energy (numbered 1 to 23) and a commercial cultivar of sugarcane RB92579, being all the materials in a cycle of planted sugarcane. The clones of energy sugarcane were obtained from the genetic breeding program of BioVertis, a company of the economic group of GranBio, headquartered in Alagoas. They were obtained through sexual reproduction, in the biparental crossbreeds scheme between commercial hybrids and pre-traded with wild genotypes of sugarcane from the species *Saccharum spontaneum* present in the germplasm bank at the Experimental Station of Flowering and Crossing, located in the municipality of São Miguel dos Campos-AL. The crossbreeds between the genetic materials occur annually in this season between the months of May to July. The materials in this study obtained from crossbreeding campaigns in 2012 and 2013. The seeds from these crossbreeds were sown in plastic boxes with dimensions of 0.45 m x 0.30 m x 0.10 m containing commercial substrate. When these seedlings have reached the ideal size, 15 to 20 days after germination, they were individually transferred to plastic tubes with dimensions of 6.3 cm in diameter and 13.0 cm tall, filled with substrate, where they remained for a period of 90 days and then transferred to the Caeté Usine - Paulicéia Unit, where they were transplanted to the field, on October 23 2014. The planting of small tubes in the line were spaced at 0.5 m, so that each plot consisted of 120 plants. The experimental design was of randomized blocks with three replications and with 24 plots each (23 clones and

a default), where each plot consisted of 6 furrows of 10 linear meters with double spacing cycled from K1 x 1.5m, totaling 72 m<sup>2</sup> per plot.

The biometry was performed 9 months after planting. The industrial quality and productivity were evaluated 11 months after planting. The assessed biometric indicators were: the number of stems per linear meter (NCM) obtained by counting and the stem diameter (DCM) measured by caliper at the median of the internode located at the median of the stem. The biometrics was evaluated in two rows of each plot, totaling 20 linear meters. For the analysis of industrial quality, 10 stems of each parcel were collected randomly, then they were identified and sent to the laboratory of the Caeté Usine - Unit Paulicéia for the analysis of technology to be performed, described in Table 1 (CONSECANA 2006). For the evaluation of productivity of fresh matter (TMFH), all the stems of the plot were cut and then weighed (stems and leaves) being converted proportionally to tons of fresh matter per hectare. To quantify the stalks productivity (TCH), after the weighing of each sample to estimate TMFH, the reeds were dawned on sheet +3 and then were scattered and the stalks were weighed separately, being converted proportionally to tons of stalks per hectare.

The data was tested for homoscedasticity and normality by the tests of Bartlett and Shapiro-Wilk test, respectively. The results were evaluated by the analysis of variance (F test) and when significant ( $P \leq 0.05$ ) were submitted to the Scott-Knott test for a comparison of means.

## Conclusion

The clones of sugarcane energy, when compared to the cultivar RB92579 of sugarcane, showed a lower stem diameter and a greater number of stalks per linear meter. These clones reached high fiber contents and had low concentration of sugars in the juice, exhibiting high potential for biomass production. The hybrids 1, 9, 11, 13, 16, 18 and 23 showed a better development potential with elevated tillering and high fiber content.

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