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Quantitative and qualitative evaluation of novel energy cane accessions for sugar, bioenergy, 1 G, and 2 G ethanol production

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ABSTRACT

Traditionally, the sugarcane breeding programs are focused on increasing sugar content, often at the expense of fiber content and biomass yield. However, with the growing interest in 2 G (cellulosic) ethanol and bioenergy production, there has been a paradigm shift toward quantitative parameters. This study investigated the qualitative and quantitative parameters of energy cane clones throughout the harvest season. It hypothesizes that juice composition, fiber content, and maturation curve will vary, reaching a point where these characteristics become more desirable for industrial processes. Three energy cane clones (C33, C34, and C35) derived from a breeding program at the Agronomic Institute of Campinas (IAC) were selected for evaluation alongside a commercial reference cultivar (IAC-942094). The results indicate that energy cane possesses significant potential for biofuel and energy production. Additionally, energy cane clones exhibit higher agricultural yields and greater production of sugars per unit area than traditional sugarcane. The high fiber content in energy cane clones, coupled with their agricultural productivity, makes them excellent sources of lignocellulosic material for both 1 G and 2 G ethanol production and cogeneration of electric energy. Energy cane clones C34 and C35, in particular, demonstrate the potential to increase 2 G ethanol production and the supply of electrical and thermal energy by up to 300% and 250%, respectively, compared to conventional sugarcane. These findings highlight the promising role of energy cane as a sustainable bioenergy production alternative, contributing to improved sustainability indices in the biofuel and biomass energy sectors.

1. Introduction

Brazil is the largest sugarcane producer and represents about 45% of the total sugar exports, with a forecast of 26 million tons of exported sugar in 2022/2023 (USDA, 2022). The sugarcane industry is a representative sector of the Brazilian economy, accounting for 2% of the national gross domestic product (CEPEA, 2022). The sugarcane industry also plays an important role in the domestic renewable energy matrix. In 2020, sugarcane bagasse (12.6%) and ethanol (6.3%) represented 18.9% of the total energy consumed in Brazil (Ministry of Mines and Energy, 2021). In other countries (i.e. India, Thailand, China, Australia), sugarcane is primarily grown as a raw material for sugar production, representing 70% of the world sugar production (Hofer, 2015). For these reasons, traditionally, the focus of sugarcane breeding programs worldwide was to increase the sugar content, reducing fiber content and biomass yield as a consequence of that (Sica, 2021).

In the 1970 s/1980 s, Alexander (1985) drew attention to a paradigm shift in sugarcane breeding programs, from 'qualitative' features of sugarcane, as sugar yield, to quantitative parameters, as 'green yield'. This concept has been further explored in the last decades, as there is a growing interest in 2 G (cellulosic) ethanol and bioenergy production. Kim and Day (2011) proposed the use of energy cane in Louisiana as an alternative feedstock for ethanol production, extending the operation period of sugar mills, and increasing 2 G ethanol production per hectare by three times compared to traditional cane. Sica et al. (2021) proposed the integration of energy cane in corn ethanol industries in Brazil,

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providing fiber for cogeneration. In addition, they found that the use of energy cane juice to replace water in the dilution of corn provided nutrients that increased yeast ethanol production efficiency by 5%. Energy cane can also be integrated into traditional sugar/ethanol industries not only as raw material for 2 G ethanol production but also to increase bioenergy production for cogeneration (Matsuoka et al., 2014).

In 2017, the Brazilian Federal government launched a pioneer program (Law 13.576, December 2017), the National Policy of Biofuels (RenovaBIO), aiming to increase the production and use of biofuels in the domestic energetic matrix by 2030 (Ministry of Mines and Energy, 2017). In this program, biofuel producers receive financial titles equivalent to carbon credits, called CBIO. One CBIO is equivalent to one ton of CO_2 that was not emitted due to the biofuel use and, therefore, varies according to the practices adopted and production systems, from the field to the commercialization of the product (Grassi and Pereira, 2019). Fuels distributors are obliged to buy these CBIOs, which are also available for interested investors. In this context, it is expected that the 2 G ethanol production in Brazil will increase almost 20 times by 2030, reaching about 2 billion liters per year (MME, 2018). This creates an enormous opportunity for using and exploiting sugarcane genetic resources, focusing on increasing yield and fiber contents.

In Brazil, Canavialis started an introgression program, backcrossing a commercial sugarcane hybrid with *Saccharum spontaneum* and found significant increases in fiber content and yield in the F1 clones compared to the commercial hybrid. However, they also found a decrease in the sucrose % cane (%Pol) (Matsuoka et al., 2015). Although energy cane tends to have lower Pol and fermentable sugar contents than traditional sugarcane commercial hybrids it can have similar sugar yields due to higher stem yields (Leal, 2007; Sica, 2021).

Nevertheless, many factors still limit the adoption of energy cane on a large scale. Based on the pre-commercial cultivars studied so far, the juice should not be used for sugar production due to its higher reducing sugar contents (> 1.5% vs < 0.4% for sugarcane: Bull and Glasziou, 1963), lower pol (~5–9% vs 14.6% for sugarcane: Matsuoka et al., 2015) and, consequently, lower juice purity (~70% vs > 90% for sugarcane: Rao et al., 2007). In addition to that, energy cane has high pol: fiber ratios, which hinders the sugar extraction process, increasing the requirement of water and energy in the imbibition process, reducing the extraction efficiency (Leal, 2007) and the distillery global efficiency from 90% to 85% (Leal et al., 2013). Therefore, to reduce these negative effects on the industrial processes, breeders should focus on improving quantitative traits but also qualitative traits. However, sugarcane breeding programs can take from 11 to 13 years from the first cross-breeding until the release of a cultivar, as many studies are necessary to assess resistance/susceptibility to pests and diseases, and the interaction with different environments (Barbosa and da Silveira, 2015).

The Agronomic Institute of Campinas (IAC) has the oldest ongoing sugarcane breeding program in Brazil (Cursi et al., 2022), starting in 1934 and currently having one of the world's largest sugarcane germplasm. In 2005 the IAC Sugarcane Center was inaugurated in Ribeirão Preto (São Paulo, Brazil) (IAC, 2016). In 2007, IAC started to focus not only on the capacity of different cultivars for sugar production, but also considering their potential for primary energy production, which accounts for all the energy that can be produced from sugarcane stem, fibers, and straw (Landell et al., 2013). Later, they started a breeding program to generate genotypes with high fiber contents in the project: *Sustainable bioenergy sugarcane breeding and cultivar development* (FAPESP: 08/56146–5, 2008). In this project, IAC backcrossed commercial sugarcane hybrids with *Saccharum spontaneum*, generating three families and hundreds of progenies. Alvim (2015) assessed these families and clones for different qualitative (i.e. sugar content, sugar yield) and agronomic parameters (i.e. yield, stem per linear meter) and Ogata (2013) characterized the fiber composition of these progenies.

Despite the growing interest in energy cane and its potential for 2 G ethanol and bioenergy production, to the best of our knowledge, no studies have been carried out assessing how qualitative parameters and the maturation of energy cane accessions change through the harvest period (from March to November in Brazil). In addition to that, there is a lack of detailed assessment on the potential of using the clones from IAC breeding program as a source of sugar and fiber for the industry. Based on this background, this study was divided into two parts with the following hypotheses:

Part 1: subsamples were collected at different time points during the harvest season and qualitative parameters were analyzed.

Hypothesis 1. the juice composition and fiber contents of different energy cane clones will vary during the harvest season, so will the maturation curve, reaching a point at which these characteristics will be more desirable for industrial processes, including extraction and sugar production.

Part 2: towards the end of the harvest season, plots were harvested and quantitative and qualitative parameters were analyzed. Based on the results, two scenarios were assessed:

1) juice used for 1 G ethanol production and bagasse for cogeneration;.

2) juice used for 1 G ethanol production, cellulose and hemicellulose from bagasse for 2 G ethanol production and remaining lignin for cogeneration.

Hypothesis 2. energy cane clones will have the potential to produce as much 1 G ethanol as the commercial hybrid; however, energy cane may have a greater potential for 2 G ethanol and/or bioenergy cogeneration, being more suitable to the context of the RenovaBio.

For that, based on Alvim (2015) and Ogata (2013) findings, we selected three clones (C33, C34, and C35) due to their potential in terms of agronomic traits and fiber composition. These clones are progenies from the family 5002, which derived from the biparental crossing between the commercial hybrid RB855465 (female parent) and the GLAGAH (male parent) as the *Saccharum spontaneum*. The IAC-942094 was selected as a commercial sugarcane reference cultivar.

2. Materials and methods

2.1. Plant material and experimental site

In the Center of Cane from IAC (Agronomic Institute of Campinas), crosses between *Saccharum spontaneum* and sugarcane cultivars have been performed aiming to develop new varieties for bioenergy production and more than 200 accessions were derived from these crosses. More information about the IAC breeding program for bioenergy and the characterization of these accessions can be found in Ogata (2013) and Alvim (2015). Based on these results, this study selected the clones C33, C34 and C35. The variety IAC-942094 (developed by IAC), was used as a reference for commercial sugarcane.

Field experiments were conducted in 2018 and 2019 in the Sugarcane Center of the Agronomic Institute of Campinas *Saccharum spp.* germplasm, in Ribeirão Preto, in the Center-North of the state of São Paulo (21°12'42'' S; 47°48'24'' W; altitude: 600 m). The soil was classified as purple dystrophic latosol (oxisol), well representative of the region. According to Köppen and Geiger, the climate classification is Aw, with 22.7 °C as the annual average temperature and 1384 mm of precipitation, consisting of hot and humid summer (most of the precipitation) and dry winter. Temperature and precipitation data during the period of the experiments were collected from a meteorological station located approximately 956 m from the field experiments and can be found in Fig. 1 (top). The soil water balance and precipitation can be found in Fig. 1 (bottom).

2.2. Plots and sampling

The plots used in this study were established in 2012 and were harvested manually every year, with the first harvest occurring in 2013.

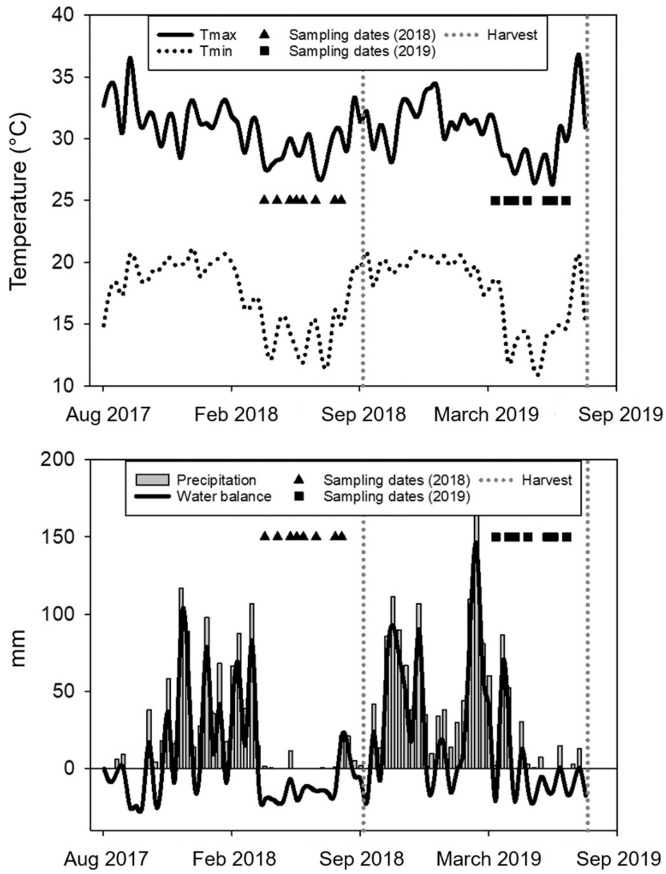

Fig. 1. Maximum and minimum temperatures (top), precipitation, and water balance (bottom) from IAC meteorological station during the period of these experiments.

Thus, in 2018 and 2019 (years of the data collection for this experiment) plants were in the sixth and seventh harvest, respectively. Every year, cultivation operations were carried out primarily in the same way in all the plots, ensuring adequate fertilization and control of weeds, pests, and diseases.

In the experiment layout of this study, each plot with the specific plant material was delimited as 36 m^2 (6 m x 6 m), with four 6 m long rows (1.5 m interrow) and was surrounded on both sides by four rows of the IAC-942094. In each sampling, three subsamples from different rows were collected in each plot. All the subsamples were mixed and processed together.

The fifth harvest was on September 1st, 2017. In 2018, samples were collected on May 14th and 30th, June 14th and 28th, July 5th, August 2nd and 24th, September 6th (255, 271, 286, 300, 307, 335, 357, 370 days after the previous harvest, respectively). All the plants were harvested on the 28th of September (392 days after the previous harvest). In 2019, samples were collected on May 9th and 22nd, June 6th and 18th, July 19th, August 8th and 21st (223, 236, 251, 263, 294, 314, 327 days after the previous harvest). All the plants were harvested on the 5th of September (342 days after the previous harvest).

2.3. Analyses

After sampling and harvesting, all the leaves, including fresh leaves at the top, and dry leaves were removed manually from the stem. All three subsamples were mixed and passed together in a shredder (DM40, IRBI machinery and equipment). After that, each sample was carefully mixed and homogenized. A subsample of 500 g was collected and pressed in a hydraulic press (PHS 250, HIDRASEME), according to Consecana (2006).

All the processing and analysis of the samples were carried out in the IAC laboratory, where all the equipment and methods follow the guidelines from the manual of the Sugarcane, Sugar, and Alcohol Producer Council from the State of Sao Paulo (CONSECANA, 2006). This is the same system used in the sugar and ethanol industries to determine the quality and price of the sugarcane arriving in the industry.

2.3.1. Juice BRIX, POL and purity

The juice soluble solids content (BRIX %) was determined by using a bench refractometer at 20 °C. The juice's apparent sucrose content (pol % juice) by using octapol as a clarifying agent and measuring the content in an automatic polarimeter, according to the CONSECANA (2006) manual for sugarcane quality analysis. The juice purity was calculated as:

$$Juice \quad purity \quad (\%) = 100x(\frac{pol\%}{brix\%}) \tag{1}$$

2.3.2. Fiber

The fiber contents were determined using the calculations suggested by the CONSECANA (2006) manual for sugarcane quality analysis, according to Eq. 2:

$$Fiber(\%) = (0.08x \quad WWP) + 0.876 \tag{2}$$

Where PWW, is the wet weight of the sample after being pressed in the hydraulic press.

The wet fiber sample was dried at 65 °C for 48 h and ground. The fiber's inferior calorific power was determined by a combustion calorimeter (IKA C200).

2.3.3. Sugars

Reducing sugars and total fermentable sugars were determined by hot titration, following the method described by Lane and Eynon (1934), using Fehling solution (an alkaline solution composed of copper sulfate and a buffer solution of potassium sodium tartrate). Reducing sugars were determined by properly diluting the samples and titration. For the determination of total fermentable sugars, acidic hydrolysis was performed and the total content was determined after proper dilution and titration. More information about the method can be found in Silva et al. (2003).

2.3.4. Yield

In both years, the harvests were done manually. In each plot, three lines were randomly assigned in different rows. All the stems were cut in the bottom, and cleaned, with all the leaves, top and straw being removed. The total stem weight in each line was determined and the results were extrapolated to one hectare.

2.4. Calculations and conversion factors

2.4.1. Conversion of cellulose and hemicellulose to fermentable sugars

To convert the amount of glucose that could be theoretically hydrolyzed from cellulose, the following equation was used:

$$Glucose = glucan \quad x1.11 \tag{3}$$

Where the glucan content is based on the cellulose content and 1.11 is a conversion factor considering water addition during hydrolysis (Kim and Day, 2011).

To convert the amount of xylose that could be theoretically hydrolyzed from hemicellulose, the following equation was used:

$$Xylose = xylan \quad x1.14 \tag{4}$$

Where xylan content is based on the hemicellulose content and 1.14 is a conversion factor considering the water addition during hydrolysis (Kim and Day, 2011).

2.4.2. Potential ethanol production from 5 and 6 carbon fermentable sugars

Potential ethanol production was calculated based on the stoichiometric biochemistry of yeast, as indicated by Kim and Day (2011). For five (i.e. xylose) and six (i.e. glucose and fructose) carbon fermentable sugars:

$C_6H_{12}O_6$ (6 carbon sugar: 180 g) → 2 C_2H_5OH (ethanol: 2 ×46 g) + 2 CO_2 (2×44 g)

$C_5H_{10}O_5$ (5 carbon sugar: 3 ×150 g) → 5 C_2H_5OH (ethanol: 5 ×46 g) + 5 CO_2 (5×44 g)

Thus, theoretically, 180 g of six carbon fermentable sugar produces 92 g of ethanol, then, 100 g would produce 51.1 g of ethanol. For five carbon fermentable sugar, 450 g would produce 230 g of ethanol, then, 100 g would produce 51.1 g of ethanol. Considering ethanol density as 0.78932 g mL^{-1} at 20 °C:

$$100kg \quad of 5 or 6 carbon \quad fermentable \quad sugars = 64.75 liters \quad of \quad ethanol \tag{5}$$

The potential 1 G ethanol production was calculated as:

$$Potential 1G = \frac{Total \quad fermentable \quad sugars \quad content \quad x64.75}{100} \tag{6}$$

Where, potential 1 G ethanol production is in liters of ethanol per ton of stem, and total fermentable sugars is the values obtained from the analysis described in the previous section.

The potential 2 G ethanol production was calculated as:

$$Potential 2G = (xylose + glucose) \quad x\frac{64.75}{100} \tag{7}$$

Where potential 2 G ethanol production is in liters of ethanol per ton of stem, and xylose and glucose were calculated by converting the hemicellulose and cellulose contents from the fibers.

$$Potential \quad total \quad ethanol \quad production = potential 1G + potential 2G \tag{7}$$

Relative productions were calculated using the IAC results as reference, as:

$$Relative \quad production = 100x(\frac{energy \quad cane}{IAC}) \tag{8}$$

2.5. Statistics

The data from the chemical analyses performed in IAC was organized and statistical analysis was performed using IBM SPSS Statistics 28.0. For both years, an analysis of variance (ANOVA) was performed to assess the effects of accessions, sampling time and their interaction. Means were compared by Tukey HSD test ($p < 0.05$). ANOVA was also used to assess the effects of the year (2018 vs. 2019), and a t-test was used to compare the averages. All the graphs were prepared using SigmaPlot 14.0.

3. Results

3.1. Maturation curves

3.1.1. Juice pol % and purity

Accessions, sampling time, and their interaction showed a significant effect on juice pol %. There was no significant difference on pol % between 2018 and 2019 (Table 1).

In both years, the IAC showed the highest pol % in all sampling time. In 2018, C34 had the highest pol% in all sampling times among the energy cane accessions. In 2019, C33 had highest pol% in the first 6 sampling times, however, in the last sampling time it had almost same pol% as C34 (Fig. 2).

The effects of accessions, sampling time, and their interaction on juice purity were found to be statistically significant. Notably, there was a significant difference in juice purity between the years 2018 and 2019 (Table 1), increasing from 66.1% in 2018 to 71.8% in 2019 (Fig. 3).

For both years, IAC had the highest juice purity, usually above 90% in all sampling times. Among the energy cane accessions, in 2018, C34

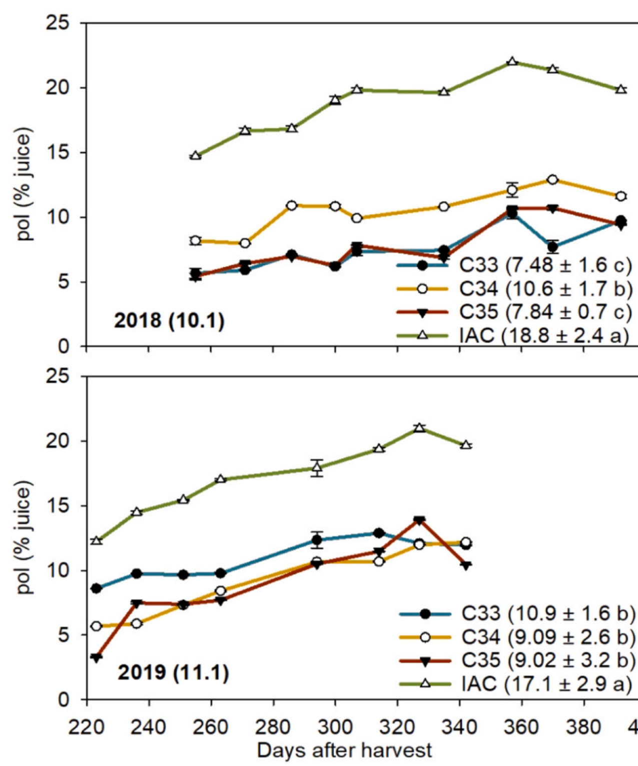

Fig. 2. Pol (% juice) of the four accessions assessed in this study at different sampling dates in 2018 and 2019. The yearly average of all accessions is represented in parenthesis after the year. The yearly average of each accession is represented in parenthesis after accession in the legend, followed by ± standard error. Different letters indicate a significant difference between accessions in the same year (Tukey HSD; $p < 0.05$).

Table 1
Analysis of variance (ANOVA) table with the effects of year, accession, sampling date and of the interaction between accession and sampling dates on different qualitative parameters.

Year	2018			2019			
Parameter	Accesion	Sampling date	Sampling date x Accesion	Accesion	Sampling date	Sampling date x Accesion	Year
pol % juice	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.390
Juice purity	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.021
Reducing sugars	< 0.001	< 0.001	< 0.001	< 0.001	0.02	< 0.001	0.002
Fermentable sugars	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.021
Fiber	< 0.001	< 0.001	< 0.001	< 0.001	0.02	< 0.001	0.909

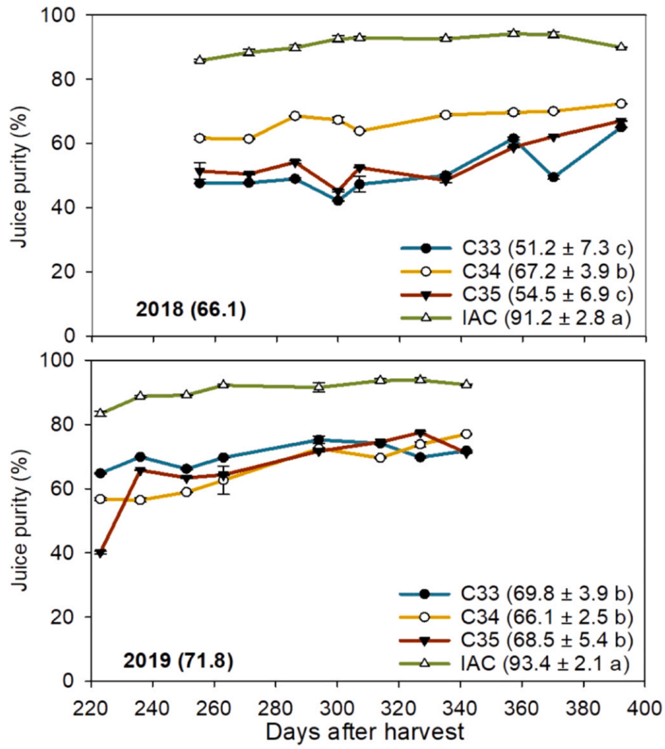

Fig. 3. Juice purity (%) of the four accessions assessed in this study at different sampling dates in 2018 and 2019. The yearly average of all accessions is represented in parenthesis after the year. The yearly average of each accession is represented in parenthesis after accession in the legend, followed by ± standard error. Different letters indicate a significant difference between accessions in the same year (Tukey HSD; $p < 0.05$).

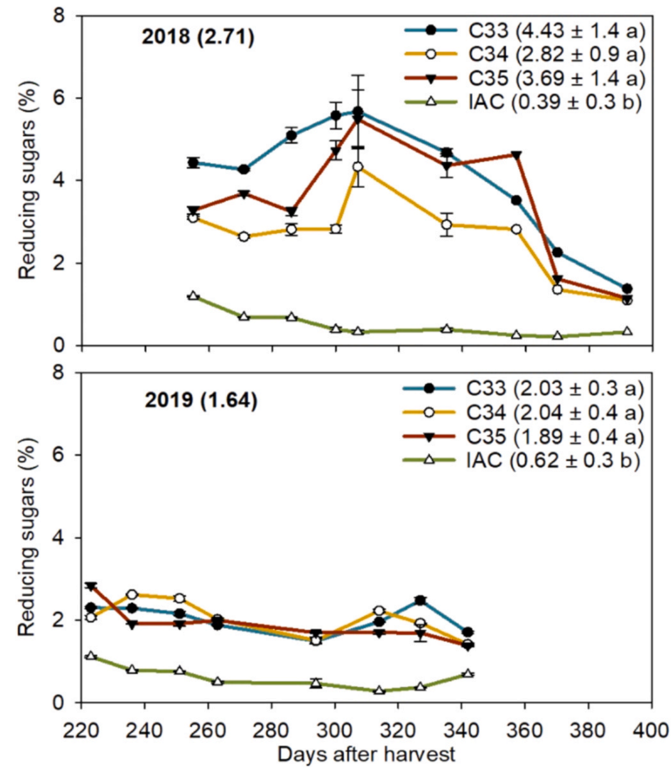

Fig. 4. Reducing sugars (%) of the four accessions assessed in this study at different sampling dates in 2018 and 2019. The yearly average of all accessions is represented in parenthesis after the year. The yearly average of each accession is represented in parenthesis after accession in the legend, followed by ± standard error. Different letters indicate a significant difference between accessions in the same year (Tukey HSD; $p < 0.05$). Accessions, sampling time, and their interaction showed a significant effect on juice reducing sugars and total fermentable sugars. There was a significant difference between 2018 and 2019 for both parameters (Table 1).

had the highest juice purity in all sampling times, reaching a maximum of 72.4% in the last sampling date (harvest). In 2019, there were slightly differences among the energy cane accessions and all the three accessions reached a maximum juice purity of around 75% in the last sampling dates (Fig. 3).

3.1.2. Sugars

The reducing sugars contents of all three energy cane accessions were higher than the IAC in all the sampling times in both years. In 2018, in the fifth sampling date all the three energy cane accessions had an increase in reducing sugar, reaching around 5.7% (C33), 5.5% (C35), and 4.4% (C34). In the last sampling times (harvest) the reducing sugars of all three energy cane accessions were below 2%. In 2019, the reducing sugar contents of all three energy cane accessions were below 3% in all sampling times (Fig. 4).

In all the sampling times in both years, the total fermentable sugar contents of IAC were greater than all the three energy cane accessions (Fig. 5).

3.1.3. Fibers

Accessions, sampling time, and their interaction showed a significant effect on fiber content. There was no significant difference on fiber content between 2018 and 2019 (Table 1).

In both years, all the three energy cane accessions showed higher fiber contents in all sampling times when compared to IAC. In 2018, there was no significant difference in fiber contents among the energy cane accessions. However, in the harvest the C33 had fiber content above 20%, the highest among all the accessions. In 2019, the IAC had the lowest fiber contents in all sampling times. In all the sampling times, C33 showed the highest fiber content, always above 20% (Fig. 6).

The calorific power was measured at harvest. In 2018, there were no considerable differences among accessions, ranging from 4.19 to 4.21 Gcal ton^{-1}. In 2019, the calorific power of all three energy cane accessions increased compared to 2018, ranging from 4.34 to 4.40 Gcal ton^{-1} (Table 2).

3.2. Yield – stem, fiber and sugars

All the accessions had higher stem yields in 2018 than in 2019 and all the three energy cane accessions produced more stem biomass per hectare than the IAC in both years. In terms of fiber per hectare, all the accessions produced significantly higher amounts of fiber per hectare in 2018 than in 2019. In both years, IAC had the significantly lowest fiber production per hectare. Among the energy cane accessions, C35 produced 66 tons of fiber per hectare in 2018 and C34 produced the lowest fibers amounts in both years, 44.4 in 2018, and 36.6 in 2019 (Table 2).

The fermentable sugars production per hectare were significantly greater for all the four accessions in 2018 when compared to 2019. In both years, C34 (44.4 and 19.7 tons, respectively) and C35 (28 and 20.1 tons, respectively) produced significantly more fermentable sugars per hectare than C33 and IAC. In 2018, C33 and C35 had significantly higher fiber/pol ratios compared to 2019, and the highest values in both years (Table 2).

3.3. Scenario 1 –potential 1 G ethanol production and calorific power

IAC showed the highest potential 1 G ethanol production per ton of stalk processed. In terms of ethanol production per hectare, C35 showed the highest potential in 2018 and 2019, 18.1 and 13 m^3, respectively,

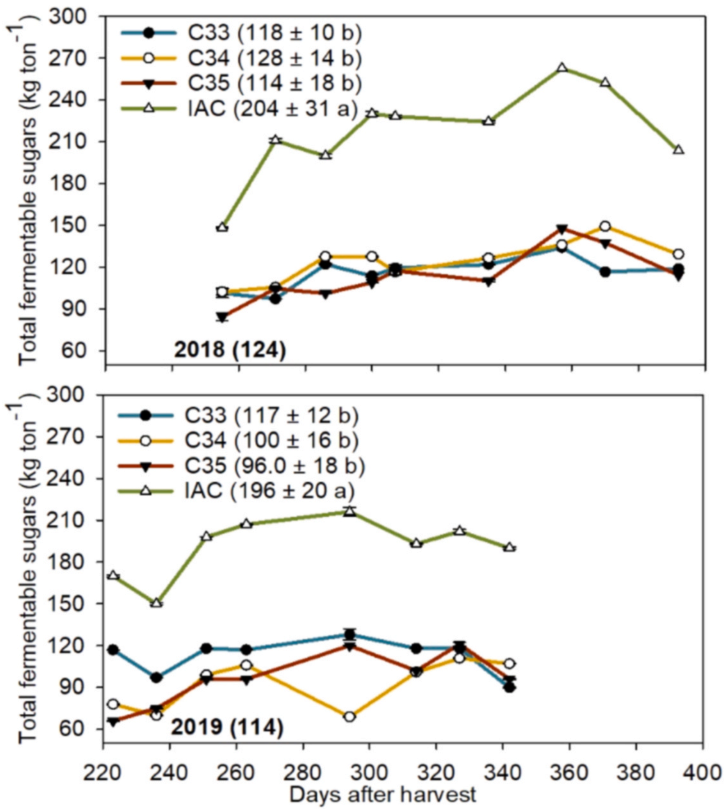

Fig. 5. Stem total fermentable sugars (kg ton^{-1}) of the four accessions assessed in this study at different sampling dates in 2018 and 2019. The yearly average of all accessions is represented in parenthesis after the year. The yearly average of each accession is represented in parenthesis after accession in the legend, followed by ± standard error. Different letters indicate a significant difference between accessions in the same year (Tukey HSD; $p < 0.05$).

which is equivalent to 133% and 123% compared to IAC commercial sugarcane in both years, respectively. C35 also showed the highest calorific power generation in 2018, 352% compared to IAC. In 2019, both C33 and C35 had the highest calorific power potential per hectare, 335–337% in relation to IAC (Table 3).

3.4. Scenario 2 – bagasse for 2 G ethanol production

For all the three energy cane accessions, the potential 2 G ethanol from cellulose and hemicellulose were considerably higher compared to the IAC. In both years, C35 was the accession with highest 2 G ethanol potential production, 31.6 and 25.5 m^3 per hectare in 2018 and 2019, respectively. C35 was also the accession with the highest total ethanol potential production in both years, potentially producing more than two times than IAC in both years. All three energy cane accessions also showed potentially greater calorific power generation from the remaining lignin, ranging from 20.9 Gcal ha^{-1} for the C34 in 2018–25.3 for the C33 in 2019, considerably higher than the IAC, 13.6 and 14.1 Gcal ha^{-1} in 2018 and 2019, respectively (Table 4).

4. Discussion

4.1. Energy cane classifications and aptitudes

According to Tew and Cobill (2008) energy cane can be classified based on their characteristics and aim of the breeding program as: i) Type I: aiming to maximize sugar and fiber components; ii) Type II: aiming mainly or only for its fiber contents. The energy cane accessions assessed in this study were bred for enhanced fiber, yet keeping a relatively high sugar content, thus, can be classified as type I.

4.1.1. Effects of sampling dates on qualitative parameters

In both years the sampling dates were from May to September, in late fall and winter, as these are periods with lower temperatures and precipitation in the region of Ribeirão Preto (Fig. 1) and in the middle of the sugarcane harvest season in the Brazilian center-south regions (UNICA (Brazilian Sugarcane Industry Association), n.d.). Water and/or heat stress during the maturation period of sugarcane can increase qualitative yields (Noorghadami et al., 2022), thus, drought events before maturation and harvesting can have significant effects on sugar and fiber contents, as it reduced the stem moisture (Araújo et al., 2016) and increase the solids concentrations. Knoll et al. (2013) assessed the effects of harvest dates on potential ethanol production from nine different energy canes and recommended that sugarcane and energy cane type I (case of the ones assessed in this study) should be harvested earlier in the season, reducing loss of fermentable sugars. This was not the case for type II energy canes. In this study, the total fermentable sugar contents of all the four accessions significantly changed over sampling times (Table 1). For the IAC, however, higher variabilities were observed specially in the first sampling times of both years and in the two last sampling dates of 2018 (Fig. 5). Precipitation events were recorded around the dates of the last two sampling (Fig. 1, bottom), indicating that the increasing on water available in the soil diluted the total fermentable sugars (Fig. 5) and the pol % juice (Fig. 2) and no differences were observed in the fiber contents (Fig. 6). However, interestingly, in the last two sampling dates of 2018 the same dilution effect was not observed in terms of pol % juice nor total fermentable sugars. In fact, there was a decreasing on fiber contents. This can be occurred because in the end of the sampling period sugarcane plants started to issue tillers, in functions of the rains in the period.

4.1.2. Aptitude for sugar production

Although the pol (% juice) tended over the sampling periods in both

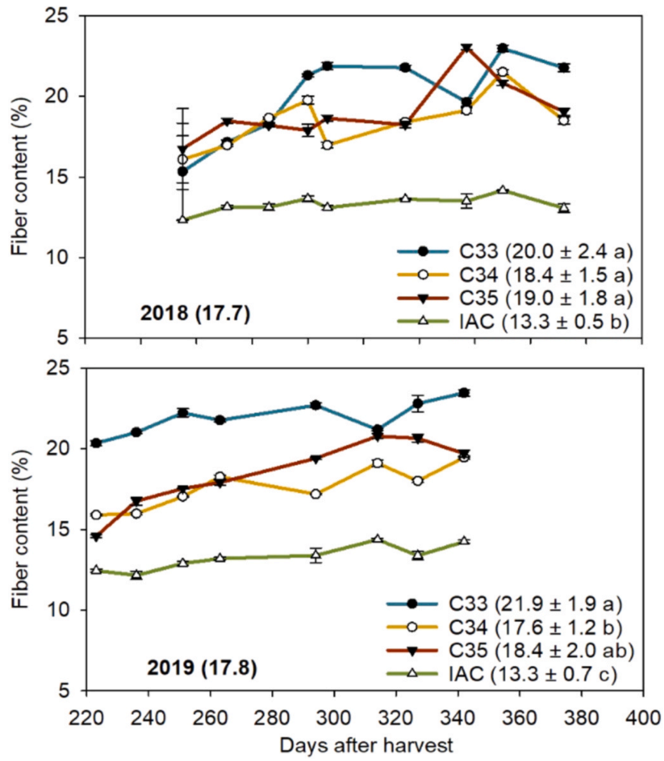

Fig. 6. Stem fiber content (%) of the four accessions assessed in this study at different sampling dates in 2018 and 2019. The yearly average of all accessions is represented in parenthesis after the year. The yearly average of each accession is represented in parenthesis after accession in the legend, followed by ± standard error. Different letters indicate a significant difference between accessions in the same year (Tukey HSD; $p < 0.05$).

years, it was always significantly lower for the energy cane accessions compared to the IAC (Fig. 2). The reducing sugars, showed high variabilities in 2018 at different sampling times and it was always significantly lower than the IAC (Fig. 4). As a consequence of that, the juice purity was always lower for the energy cane accessions juices in all the sampling times, reaching a maximum of 70% and 76% for the C34 accession in 2018, and 2019, respectively, values much lower than 90%, usually found for commercial hybrid sugarcane varieties used for sugar production and for IAC in this study (Fig. 3) (Leal et al., 2013). Thus, the results of reducing sugars, pol % juice, and juice purity demonstrate that none of the energy cane accessions assessed in this study are suitable for sugar production.

4.1.3. Aptitude for 1 G and 2 G ethanol production

The total fermentable sugar contents (kg per ton of stem) was significantly highest for IAC in all the sampling points including at the harvest date. It is known that energy cane total sugar contents are considerably lower compared to commercial sugarcane varieties (Kim and Day, 2011; Matsuoka et al., 2014) and it is expected to have similar values per hectare due to the higher yields (Sica, 2021). However, the last is not necessarily the case some of the studies found in the literature. Samuels et al. (1987) (cited by Matsuoka et al., 2015) found a considerable increase in fiber yield comparing an energy cane from second generation with a commercial sugarcane cultivar (L79–1002), however, they also found lower brix per hectare. Rao et al. (2007) and Matsuoka et al. (2015) (presenting results from CanaVialis breeding program) found lower pol % cane per hectare when comparing three energy cane to a commercial hybrid (B77602 and RB72454, respectively). In this study, due to considerably higher yields, in both years, the C34 and C35 produced significantly higher amounts of fermentable sugar per hectare than the IAC (Table 2). Therefore, these clones could have high potential to be explored for 1 G ethanol production.

Table 2
Yield (stem), fiber, and fermentable sugars production per hectare and the calorific power in (2018 and 2019) and fiber composition of the four cane accessions.

		C33	C34	C35	IAC
Yield (ton ha^{-1})	2018	237	228	335	132
	2019	208	199	250	103
Fiber (ton ha^{-1})	2018	55.6 ± 0.5 Ba	44.4 ± 0.3 Ca	66.0 ± 0.8 Aa	18.8 ± 0.2 Da
	2019	45.3 ± 0.3 Ab	36.6 ± 0.2 Bb	45.6 ± 0.5 Ab	14.0 ± 0.1 Cb
Fiber/Pol ratio	2018	2.93 ± 0.02 Aa	1.70 ± 0.05 Ca	2.66 ± 0.07 Ba	0.70 ± 0.03 Da
	2019	1.96 ± 0.02 Ab	1.60 ± 0.04 Ba	1.89 ± 0.02 Ab	0.72 ± 0.01 Ca
Fiber composition (%)*	Cellulose	43.1 ± 0.9	46.7 ± 0.8	49.3 ± 0.6	41.6
	Hemicellulose	19.4 ± 0.2	21.2 ± 0.4	22.0 ± 0.2	25.1
	Lignin	22.1 ± 0.1	23.7 ± 0.5	23.7 ± 0.2	20.3
Calorific power (Gcal ton^{-1})	2018	4.21	4.19	4.20	4.19
	2019	4.40	4.35	4.34	4.21
Fermentable sugars (ton ha^{-1})	2018	21.3 ± 0.1 Ca	24.4 ± 0.1 Ba	28.0 ± 0.1 Aa	21.0 ± 0.2 Ca
	2019	16.9 ± 0.1 Bb	19.7 ± 0.4 Ab	20.1 ± 0.4 Ab	16.3 ± 0.2 Bb

Different lowercase letters indicate a significant difference for the same accession between years. Different uppercase letters indicate a significant difference between accessions within the same year.
* Fiber compositions were characterized by Ogata (2013).

Table 3
Potential 1 G ethanol production and calorific power generation per ton of stalk, per hectare, and relative production per hectare to the IAC in 2018 and 2019.

		C33	C34	C35	IAC
Potential 1 G ethanol production (liters ton^{-1})*	2018	58.1 ± 0.3	62.4 ± 0.3	54.1 ± 0.1	103.1 ± 0.8
	2019	52.6 ± 0.4	64.0 ± 0.4	53.0 ± 1.1	102.6 ± 0.9
Calorific power (Gcal ton^{-1})*	2018	988 ± 8	815 ± 5	828 ± 10	573 ± 2
	2019	958 ± 7	801 ± 5	792 ± 8	596 ± 5
Potential 1 G ethanol production (m^3 ha^{-1})*	2018	13.8 ± 0.07 Ba	14.2 ± 0.05 Ba	18.1 ± 0.01 Aa	13.6 ± 0.1 Ba
	2019	10.9 ± 0.09 Bb	12.7 ± 0.24 Ab	13.0 ± 0.27 Ab	10.6 ± 0.1 Bb
Calorific power (Gcal ha^{-1})*	2018	234 ± 1.9	186 ± 1.0	277 ± 3.2	78.7 ± 0.6
	2019	199 ± 1.5	159 ± 0.9	198 ± 2.1	59.1 ± 0.2
Relative 1 G ethanol production per hectare*	2018	103	120	133	100
	2019	101	105	123	100
Relative calorifc power per hectare* *	2018	297	236	352	100
	2019	337	269	335	100

* Calculations can be found in the Materials and Methods section.

The 2 G ethanol production is highly dependent on the raw materials cellulose and hemicellulose contents, as the lignin cannot be converted to 5 and 6 carbon sugars and its main use is for producing heat and electricity (Wyman, 2007). Thus, for breeding programs aiming to develop materials suitable for 2 G ethanol production, low lignin

Table 4
Simulation of glucose and xylose extraction from cellulose and hemicellulose, respectively, and potential 2 G and total ethanol production for each of the four accessions assessed in this study.

		C33	C34	C35	IAC
Glucose from cellulose (ton ha^{-1})*	2018	24.7 ± 0.2	21.8 ± 0.1	33.5 ± 0.4	8.3 ± 0.02
	2019	23.4 ± 0.2	20.1 ± 0.1	27.0 ± 0.3	6.8 ± 0.06
Xylose from hemicellulose (ton ha^{-1})*	2018	11.4 ± 0.08	10.2 ± 0.06	15.3 ± 0.21	5.15 ± 0.02
	2019	10.8 ± 0.09	9.4 ± 0.05	12.4 ± 0.14	4.19 ± 0.03
Potential ethanol production from cellulose (m^3 ha^{-1})*	2018	16.0 ± 0.1	14.1 ± 0.1	21.7 ± 0.2	5.4 ± 0.1
	2019	15.1 ± 0.1	13.0 ± 0.1	17.5 ± 0.2	4.4 ± 0.1
Potential ethanol production from hemicellulose (m^3 ha^{-1})*	2018	7.4 ± 0.05	6.6 ± 0.04	9.9 ± 0.11	3.3 ± 0.01
	2019	6.9 ± 0.06	6.1 ± 0.03	8.0 ± 0.09	2.7 ± 0.02
Potential 2 G ethanol production (m^3 ha^{-1})*	2018	23.4 ± 0.17	20.7 ± 0.13	31.6 ± 0.34	8.7 ± 0.02
	2019	22.1 ± 0.19	19.1 ± 0.11	25.5 ± 0.29	7.1 ± 0.06
Potential total ethanol production (m^3 ha^{-1})* *	2018	34.3 ± 0.15	33.4 ± 0.14	44.6 ± 0.19	19.3 ± 0.10
	2019	35.9 ± 0.14	33.3 ± 0.06	43.6 ± 0.30	20.7 ± 0.04
Relative total ethanol production*	2018	178	173	231	100
	2019	173	160	210	100
Remaining lignin (ton ha^{-1})*	2018	4.8 ± 0.04	4.4 ± 0.03	4.3 ± 0.05	2.8 ± 0.01
	2019	5.2 ± 0.04	4.6 ± 0.03	4.7 ± 0.05	2.9 ± 0.02
Calorifc power from remaining lignin (Gcal ha^{-1})* **	2018	23.3	21.4	20.9	13.6
	2019	25.3	22.4	22.8	14.1

* *Calculated as the sum of total 1 G ethanol presented in Tables 3 and 2G ethanol.
* ** Remaining lignin was calculated considering that there is not lignin loss during the 2 G ethanol production process and a calorific power of 4.86 Mcal per kg of lignin (Kim et al., 2017).
* Calculations can be found in the Materials and Methods section.

contents are desirable increase conversion yield of fermentable sugars, improving its viability (Schmatz et al., 2020). Considering that, Ogata (2013) proposed a model to classify energy and sugarcane according to its cellulose, hemicellulose and lignin contents. Based on Ogata's (2013) model, all the four accession assessed in this study can be considered C2 in terms of cellulose (40–50%), indicating relatively high cellulose contents. However, in terms of hemicellulose, only IAC was classified as H3 (25–30%), whereas C34 and C35 were classified and H4 (20–25%), and C33 H5 (< 20%), indicating relatively low hemicellulose contents. All the four accessions can be considered L4 (20–25%), indicating a relatively low lignin content. Based on that, all three accessions assessed in this study demonstrated high potential to be used as raw material for 2 G ethanol production.

4.2. Scenarios

4.2.1. Yields

In 2018, C35 had a considerably higher yield compared to C33 and C34. All the energy cane accessions had a higher yield than the commercial sugarcane hybrid (132 ton per hectare). In 2019, all the accessions assessed in this study showed a reduction on yield, mainly the C35, from 335 tons per hectare in 2018–250 tons per hectare in 2019. However, in 2019 C35 still had the highest yield. Many studies have already demonstrated considerably higher yields for energy cane. According to Waclawovsky et al. (2010), sugarcane theoretical maximum yield is 381 tons per hectare, the experimental maximum is 212 tons per hectare. In 2018, all three energy cane accessions had yields above the experimental maximum considered by Waclawovsky et al. (2010) and C35 had a yield close to the theoretical maximum for sugarcane.

The region of Ribeirão Preto (Sao Paulo) is one of the main producers of sugarcane in Brazil and in the world, with ideal edaphoclimatic conditions for its cultivation and, in addition to that, the Sugarcane Center from IAC has a long term tradition on cultivating sugarcane in experimental conditions, ensuring to perform all the recommended practices for ideal plant growth and reaching high yields. The commercial hybrid (IAC), for example, had yields relatively higher than the average in the state of Sao Paulo, 132 and 103 tons per hectare in 2018 (sixth year) and 2019 (seventh year), respectively, whereas the average yield in Brazil is around 80 ton per hectare (Marin et al., 2021). Thus, of course, the yields obtained in this study are not realistic to be considered for a commercial scale cultivation. Waclawovsky et al. (2010) considered a yield reduction of 25% from sugarcane experimental maximum to the commercial maximum yield. This reduction could be even higher for a commercial average.

4.2.2. Potential 1 G ethanol production

All energy cane accessions had a significantly lower fermentable sugar contents compared to the commercial hybrid IAC, thus, the production of 1 G ethanol per ton of stalk would be lower. However, considering the high biomass yields, the potential 1 G ethanol production per hectare were similar or higher in both year for all the energy cane accessions (relative values: from 101% C33 in 2019–133% C35 in 2018). In these calculations we only considered the stoichiometric biochemistry of yeasts, not taking into account potential differences in the fermentation efficiency (~90%: Sica et al., 2021), which we do not expect do differ considerably between energy cane and sugarcane. One factor that could reduce the total 1 G ethanol produced from energy cane compared to the sugarcane is the sugar extraction efficiency. According to Leal (et al. (2013) sugar extraction from sugarcane is between 85% and 90%, slightly higher than the energy cane (85–86%), as during the extraction the fibers acts like a sponge, reabsorbing part of the sugars and, as can be seen in Table 2, the fiber/pol ratio of energy cane are significantly higher than the commercial hybrid.

4.2.3. Scenario 1: fiber for cogeneration

The use of energy cane fiber for bioelectricity has a high potential in Brazil, as hydropower represents more than two thirds of the Brazilian energetic matrix (Ministry of Mines and Energy, 2021) and the harvest season covers most of the winter, when in the Center-South is a drier season (i.e. Fig. 1), reducing the reservoir storage levels. This has been aggravated due to climate change. In 2014, in the state of Sao Paulo some reservoirs reached 5% of their 1.3 billion m^3 capacity due to warmer summers and lack of rainfall, affecting also the electricity supply and increasing energy prices from 20% to 25% in 2015 (Nobre et al., 2014). These weather conditions had not been observed since 1951 and is likely to become more frequent (Nobre et al., 2014). Currently, sugarcane and its derived products already accounts for a significant part of the Brazilian energetic matrix (Ministry of Mines and Energy, 2021) and has the potential to increase its participation with an increasing bioelectricity cogeneration from energy cane. In the first scenario of this study, we simulated the use of the energy cane accessions fiber exclusively for bioelectricity. Although we found a relatively high variation on the fiber content of these accessions through the harvest season (Fig. 6), the values were usually relatively high, above 15%. At harvest, all three energy cane accessions demonstrated a considerably higher calorific power per hectare relative to commercial hybrid, ranging from 236% (C34 in 2018) to 352% (C35 in 2019). The higher fiber contents and higher yields are core characteristics for new energy cane varieties, assuring a potential higher primary energy per hectare (Leal et al., 2013; Matsuoka et al., 2015, 2014).

4.2.4. Scenario 2: cellulose and hemicellulose for 2 G ethanol and lignin for cogeneration

The potential 2 G ethanol production from the energy cane were considerably higher than the IAC, from 19.1 (C34 in 2019) to 31.6 (C35 in 2018) m^3 per hectare, whereas IAC could potentially produce 8.7 and 7.1 m^3 per hectare in 2018 and 2019, respectively. Therefore, the use of energy cane could increase by 2.5–4 times the 2 G ethanol production per hectare, keeping similar 1 G ethanol production values. These estimations are in agreement with Kim and Day (2011) who found that energy cane 2 G ethanol production could be increased from 3.6 m^3 per hectare with an commercial hybrid to 12.9 m^3 per hectare with an energy cane. Leal et al. (2013) suggested that the 2 G ethanol production could be increased from 2.7 m^3 per hectare (sugarcane) to 7 m^3 per hectare (energy cane) considering the current technology available for this process. They expect that with technological advances, these values can be increased to 3.5 m^3 per hectare and 12.4 m^3 per hectare for sugarcane and energy cane, respectively.

The values reported by Kim and Day (2011) and Leal et al. (2013) are considerably lower than the ones we found in this study. In the first study, Kim and Day (2011) also considered the potential 2 G ethanol production, as we did in this study. However, their yields were considerably lower than the ones we got in this study, 100 ton per hectare. This could be due to the fact that they considered the production in Louisiana and due to weather conditions the energy cane would have lower yields. In the later study, Leal et al. (2013) also considered lower yields (111 tons per hectare) and also took into account the process efficiency. In this study, the yields were relatively high probably due to ideal experimental conditions (as discussed before), so it would probably be reduced when applied in a commercial scale. In addition to that, the calculations of potential 2 G ethanol production based on cellulose and hemicellulose contents does not account for the processes efficiency and loses. However, we assume that these values would have little or negligible differences between accessions and we used this as a proxy to compare them.

4.3. Trends and potential adoptions of energy cane

The energy cane accessions assessed in the study show similar potential 1 G ethanol production per hectare as the commercial hybrid. Considering scenario 1, the energy cane could generate much higher total calorific power per area (specially C35), mainly during dry periods, when energy prices are higher in Brazil. Considering scenario 2, in the context of the RenovaBio in Brazil and trend on increasing 2 G ethanol production (Grassi and Pereira, 2019). Our results indicate that the energy cane could be a viable alternative, increasing 2 G ethanol production from 2.5 t to 4 times per area compared to the commercial hybrid. Thus, in both scenarios, the adoption of energy cane could increase total energy generation (Scenario 1) or total 2 G ethanol production (Scenario 2) and keep the same 1 G ethanol production in the same area. The sugarcane industry could then dedicate a small part of the area for energy cane production to increase ethanol and bioelectricity production and in the remaining area cultivate sugarcane commercial hybrids suitable for sugar production.

In the United States, Kim and Day (2011) also proposed the integration of energy cane into sugar production processes in Louisiana, extending the operation season and increasing the biofuel production. This could also be applied in other states producing sugar from sugarcane, as Florida. However, it would probably need to be limited to states in the Southeast and Central East of United States (USDA, 2010), as Knoll et al. (2021) cultivated five different energy cane cultivars in Tifton (Southern Georgia) and Watkinsville (Northern Georgia) and found a significant differences between both localities, mainly due to high number of cold weather events (including freezing) in the north of Georgia. Another option in the United States could be the integration of energy cane into the corn ethanol production processes, proving biomass to supply the plant energetic demands (Sica et al., 2021).

In Europe, energy crops are cultivated to be added to reactors and boost biogas production (Lijó et al., 2017; Weiland, 2003). In Germany, for example, maize is cultivated as an energy crop and energy cane has been proposed to complement/replace it. However, Hoffstadt et al. (2020) drew the attention for some aspects that could limit this adoption: i) energy cane harvest would be seasonal and it's not known if it could be ensiled as maize; ii) high fiber contents could lead to problems as flotation layers in the biogas reactor; iii) high lignin contents could reduce the carbon degradability.

4.4. Potential limitations and alternatives to the adoption of energy cane

There are still some factors to be overcome for the adoption of energy cane in a large scale.

- There is a big gap between experimental yields and yields on commercial scale. Thus, new energy cane commercial varieties should be developed. It is know that takes time for that, but many programs (as IAC in 2008) have already start and it is expected that it will happen in the coming years;
- The lower sugar contents, which may lead to two problems:

1. Transportation costs from the field to the industry are high, with lower sugar contents, higher amounts of energy cane would need to be transported, increasing logistics costs. For that, areas close to the industry would probably be more suitable for energy cane production;
2. In the state of Sao Paulo the prices paid by the industry to cane suppliers are calculated based on an standardized system, which awards higher sugar contents and penalizes high fiber contents. Thus, a specific payment system should be considered in order to account for the potential energetic values of the raw material as well.

- The 2 G ethanol technology still needs to be improved and have cost reduced. In Brazil, currently only Raízen produces 2 G ethanol in a large scale. However, it is expected that with the RenovaBio and technological advances in the coming years, this could be overcome and become more viable.

5. Conclusions

Our findings suggest that energy cane has great potential as a raw material for biofuel and energy production. Despite its lower initial potential for sucrose and total reducing sugar accumulation per unit mass compared to traditional sugarcane, the levels of these sugars increase with water stress, indicating an optimal harvesting period for industrial yield. Additionally, energy cane accessions C33, C34, and C35 exhibit higher agricultural yields and greater production of sucrose and total reducing sugars per unit area than traditional sugarcane. Moreover, the high fiber content in energy cane clones, combined with their agricultural productivity, makes them an excellent source of lignocellulosic material for the production of both 1 G and 2 G ethanol, as well as for cogeneration of electric energy. Moreover, the use of energy cane clones C34 and C35 can significantly increase the production of 2 G ethanol and supply of electrical and thermal energy by up to 300% and 250%, respectively, compared to conventional sugarcane, thus improving the sustainability indices of biofuel and biomass energy production. Overall, these results highlight the potential of energy cane as a promising alternative for sustainable bioenergy production.

CRediT authorship contribution statement

Pietro Sica and Eduardo de Castro Mattos: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Giovanni Módolo Silveira, João Paulo Abdalla, and Victor Kainã Alves:** Methodology, Investigation, Data curation. **Ivo Soares Borges, Marcos Landell, and Mauro Alexandre Xavier:** Conceptualization Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing. **Antonio Sampaio Baptista:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

Data availability

Data will be made available on request.

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