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Sucrose content, lignocellulose accumulation and *in vitro* digestibility of sugarcane internodes depicted in relation to internode maturation stage and *Saccharum* genotypes

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ABSTRACT

Sugarcane hybrids acquired several characteristics through plant breeding, including high sucrose and lignocellulose production. Recently, biomass-based industries designed to produce high-value chemicals and fuels from whole plant biomass encouraged new breeding efforts to develop plants with high sucrose yields and low lignocellulose recalcitrance. The present study utilized four experimental sugarcane hybrids to evaluate the dynamics of sucrose and lignocellulose accumulation, lignocellulose composition, and enzymatic digestibility during internode development. During the internode maturation stages, the sucrose content increased while the lignocellulose fraction presented an increased lignin and decreased glucan content. Enzymatic digestibility and lignin content of the lignocellulose fraction displayed an inversely related pattern, and the first internode was two-fold more digestible than mature internodes, indicating that digestibility decreases significantly with internode maturation and tissue lignification. Some sugarcane hybrids (H89 and H58) combined desirable phenotype characteristics (high sucrose yield and low lignocellulose recalcitrance) that were not detected in H140 and H321. Proper molecular markers discriminating these samples will help to further design breeding steps to produce sugarcane modern hybrids combining high sucrose yields and low lignocellulose recalcitrance.

1. Introduction

A biomass-based economy depends on the cost-effective separation of polysaccharides and lignin from plant cell walls or the direct conversion of these components into fuels and chemicals (Guo and Song, 2019; Holwerda et al., 2019). Most of the plant biomass available for bioprocessing is contained in the secondary cell walls of vascular plants (Yang et al., 2013). These cell walls are recalcitrant to biodegradation or *in vitro* enzymatic digestion due to a complex ultrastructure (Himmel et al., 2007; Ding et al., 2013). Many studies indicate that *in vitro* polysaccharide solubilization requires combined steps for plant cell wall deconstruction, including pretreatment and enzymatic digestion (Petridis and Smith, 2018; Holwerda et al., 2019). The deconstruction of plant cell walls is facilitated by low original lignin and xylan contents (Jung et al., 2012; Costa et al., 2013; Ding et al., 2013; Santos et al., 2018), whereas high contents of cellulose and mixed linkage glucan (ß-1, 4 and ß-1, 3 glucan), and low crystallinity of cellulose improve digestibility (Vega-Sánchez et al., 2015; Costa et al., 2016).

In grasses, cell wall recalcitrance increases during plant development and tissue maturation, with a significant contribution assigned to the lignification process (Jung and Casler, 2006; Crowe et al., 2017). Some crop grasses, such as sugarcane and sweet sorghum, also accumulate nonstructural carbohydrates (especially sucrose) at high concentrations during plant development (Lingle and Thomson, 2012; Mckinley et al., 2018). The accumulation of sucrose is a desired characteristic for fuel and chemical production from biomass because it represents a less expensive source of carbohydrates promptly suitable for bioprocessing (Santos et al., 2016; Mendes et al., 2017; Mullet, 2017). However, the sucrose accumulation and lignification of secondary cell walls are time-related processes. Sucrose accumulates in parenchyma cells as a plant storage product, whereas most of the lignin accumulates in secondary cell walls of fibers and vessels. Both processes occur during internode maturation (Lingle and Thomson, 2012; Li et al., 2018). For example, genes associated with lignin synthesis are highly expressed during maturation, especially in sugarcane hybrids with high-fiber (biomass) contents (Bottcher et al., 2013; Kasirajan et al., 2018).

Sugarcane serves as a proper plant model to evaluate sucrose accumulation in conjunction with recalcitrance development in plant cell walls given that a range of hybrids with contrasting phenotypes are

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available for insightful evaluation (Waclawovsky et al., 2010; Loureiro et al., 2011; Masarin et al., 2011; Silva et al., 2017). The majority of the available commercial sugarcane hybrids displays high accumulation of sucrose, high field productivity, and resistance to pests and drought (Lakshmanan et al., 2005; Waclawovsky et al., 2010; Loureiro et al., 2011; Tavares et al., 2018). Recent efforts have increased the development of sugarcane hybrids with characteristics oriented to address traditional and novel sugarcane industrial needs, such as the conversion of sugarcane lignocellulose into high value-added chemicals and cellulosic ethanol (Masarin et al., 2011; Laurito-Friend et al., 2015; Silva et al., 2017; Mendes et al., 2018). However, to date, we lack commercial exploitation of plants associated with good fitness for sucrose production and simultaneous accumulation of biomass with low recalcitrance. One of the reasons for such limitation reside in the lack of proper information concerning simultaneous determination of sucrose accumulation, cell wall formation and cell wall digestibility.

One of the earliest studies associating sugarcane development stages with *in vitro* digestibility of the lignocellulose fraction compared ancient sugarcane species (*Saccharum spontaneum*) with modern hybrids developed to accumulate high sucrose concentrations (Poelking et al., 2015). Samples pretreated with diluted acid were digested with commercial enzymes. Maximal digestibility was found in the intermediate internodes in contrast to results reported for other C4 plants, such as untreated maize (Jung and Casler, 2006) and untreated and acid-pretreated switchgrass (Crowe et al., 2017), which presented the highest digestibility in the younger internodes.

The current work considered the hypothesis that some selected sugarcane hybrids could achieve the maximal levels of sucrose accumulation before secondary cell walls were completely developed and lignified. The natural occurrence of these characteristics will open new perspectives for plant breeding for high sucrose yield and low lignocellulose recalcitrance. Given that a single culm in grasses is composed of successive internodes at different maturation stages, we followed sucrose accumulation, lignocellulose formation and enzymatic digestibility of the lignocellulosic fraction along the culm of selected sugarcane hybrids. The data showed that during internode maturation stages, sucrose contents increased, while the lignocellulose fraction exhibited increased lignin and decreased glucan content. Enzymatic digestibility and lignin content displayed inversely related patterns. Some of the sugarcane hybrids combined the desirable phenotype characteristics with high sucrose yield and low lignocellulose recalcitrance.

2. Materials and methods

2.1. Sugar cane hybrids and sampling procedures

Four sugarcane hybrids were sampled from an experimental field located at Lorena, SP, Brazil (22°43′51″ S, 45°07′29″ W), which contained a total of 15 different genotypes planted in 0.60 m × 1.0 m rows with 3.0 m × 2.0 m spacing between hybrids. Samples correspond to 1-year-old culms collected in May 2016 from the first ratoon crop of 4 genotypes selected because they present contrasting lignocellulose chemical composition. In brief, two of the genotypes were selected based on their low lignin contents (H89 and H58), contrasting with H140 with the highest lignin content in the group. H321 was selected based on the highest hemicellulose content in the hybrids group (Masarin et al., 2011). Three samples from each genotype were selected to ensure culms of similar height and diameter. After field cutting, leaves were detached to reach the immature region of the culm as indicated in Fig. 1. The internode numbered as one (I_1) corresponded to the first internode in which superior and inferior nodes were clearly identified (Fig. 1). Each odd internode from the top to the base of the culm was sampled. The number of sampled internodes was 13, 15, 19, and 19 for H89, H58, H321, and H140, respectively; however, some culms present up to 24 internodes. After sampling, two 6 mm-long slices were cut from the mid region of the internode and separated for microscopic analysis. The remaining internode material was used for the determination of sucrose and lignocellulosic biomass, chemical composition, and enzymatic digestibility. All samples were stored in plastic bags at −18 °C until use in the described experiments.

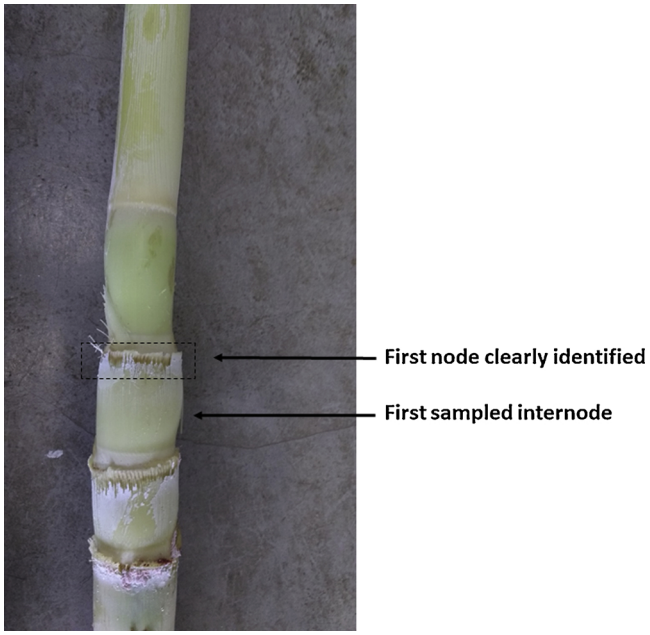

Fig. 1. First internode identification after leaf removal. The first internode corresponded to the culm region in which superior and inferior nodes were clearly identified.

2.2. Sucrose and lignocellulosic biomass determinations

Internodes were thawed at room temperature. Excess of condensed humidity was removed from outside surfaces, and the internodes were weighed (sugarcane fresh weight). The whole fragments were blended with 400 mL water for 5 min. Solids were retained in Miracloth, and the liquid fraction was reserved. This procedure was repeated 3 times, and the liquid fractions were combined. Sucrose was determined in the combined liquid fraction by HPLC using a HPX87C column (BioRad, Hercules, CA) at 85 °C eluted at 0.6 mL/min with water. Lignocellulosic biomass corresponded to the total solids retained after sucrose extraction and was also referred as fiber fraction. These solids were air dried and weighed. A sample was used for humidity determination using Shimadzu MOC-120H moisture balance (Shimadzu, Japan). Dry weight was calculated, and lignocellulosic biomass was expressed as g of dry solids/100 g of fresh sugarcane. Air-dried lignocellulosic biomass was milled to pass a 0.84-mm screen and used for the determination of the chemical composition and *in vitro* enzymatic hydrolysis experiments. Extractives and major cell wall components were determined as described before (Masarin et al., 2011).

2.3. In vitro enzymatic digestion of lignocellulosic biomass

Lignocellulosic biomass fractions were digested with a commercial cellulolytic cocktail (Cellic Ctec 2 - SAE0020, Sigma-Aldrich, Brazil). Approximately 20 mg of milled samples were immersed overnight in 900 µL of 50 mM acetate buffer at pH 4.8 containing 0.01% sodium azide inside 2-mL cryogenic tubes. After biomass impregnation with the buffer solution, 100 µL of an appropriated dilution of the enzyme preparation was added. The enzyme to biomass ratio corresponded to 10 FPU/g of substrate. The flasks were incubated at 45 °C under 120 rpm agitation for up to 72 h. Samples withdrawn from the reaction mixtures

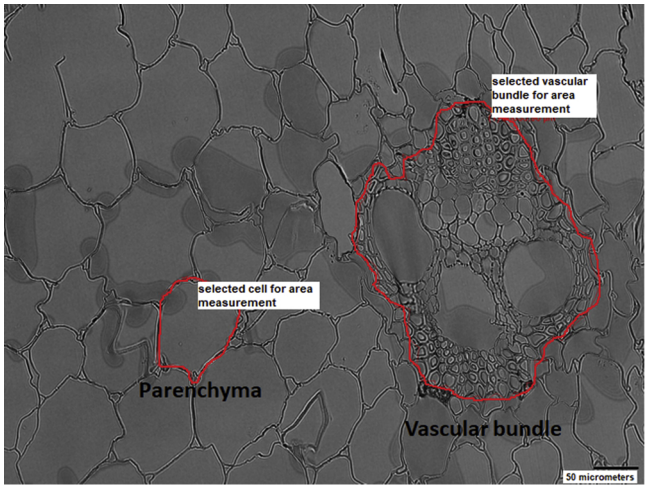

Fig. 2. Typical image from the rind region of the sugarcane internode and the software-aided determination of parenchyma and vascular bundle areas.

were diluted and analyzed for glucose and xylose contents by HPLC using a HPX-87H column (BioRad, Hercules, CA) at 45 °C eluted at 0.6 mL/min with 5 mM H_2SO_4 solution. Sugars were detected in a 35 °C-thermocontrolled RI detector (Waters, Milford, MA) (Masarin et al., 2011).

2.4. Microscopic evaluation of sugarcane samples

Longitudinal fragments measuring approximately 6-mm long and 1-mm in diameter were cut with a razor blade from each internode sample. These fragments were sampled at 8 mm from the outermost region of the internode toward the center, representing a typical rind area (Costa et al., 2013). Each fragment was fixed in epoxy resin and cut transversally in 1-μm slices with the aid of a LEICA EM-UC7 ultramicrotome (Leica Biosystems, Germany) fitted with a diamond knife (4 mm-Histo, Diatome, Hatfield, PA) as previously described (Costa et al., 2013). Sections were observed with an OLYMPUS BX53 microscope (Olympus, Japan). Cell dimensions were recorded with aid of the CellSens software (Olympus, Japan). The areas of the parenchyma cells and the vascular bundle tissue were determined as illustrated in Fig. 2. The thickness of fiber cell walls was recorded from direct measurement using the CellSens software. At least 15 parenchyma and fiber cells were evaluated in each sample and used to calculate average values. In the case of the vascular bundles, only 2 or 3 bundles were visualized in each sample and used to determine average values.

2.5. Statistical analysis

Data for sucrose and biomass contents, tissue and cell dimensions, lignocellulosic biomass composition, and *in vitro* digestibility were recorded for 3 individual culms (biological replicates) from each sugarcane genotype. Data sets from 4 genotypes, 3 individual culms from each genotype, and 3 (or 4) different internodes were evaluated. Analytical data were obtained from triplicate analysis. Each data set passed a normal distribution test, and standard deviations were determined. Averages were compared with basis on Tukey test and corresponding p-values. Two types of data comparison were performed: a) plant characteristics in different internodes from a single sugarcane hybrid, and b) plant characteristics at the same internode from different sugarcane hybrids. In both cases, Tukey test was performed based on two-tailed distribution and paired means (Haynes, 2013). Data pairs presenting $p < 0.05$ were considered to pass the null hypothesis, meaning they differ significantly. A few data set comparisons indicated $0.05 < p < 0.1$ but were also considered to differ significantly. These cases were highlighted in the figures with blue bold letters.

3. Results

3.1. Sucrose and lignocellulosic biomass accumulation as a function of internode maturation stage

Phenotype macrocharacteristics, sucrose and biomass accumulation, tissue anatomy, cell wall characteristics and composition, and *in vitro* digestibility of the four sugarcane hybrids were evaluated as a function of internode maturation stages. Sampling internodes progressively from the top to the base of sugarcane culm provided representative maturation stages in a single cultivation period (Lingle and Thomson, 2012; Bottcher et al., 2013; Poelking et al., 2015). Plant phenotype macrocharacteristics are summarized in Table S1. In general, plants presented culm heights varying from 1.9 to 2.5 m, containing 18 to 24 internodes. Immature internodes collected at the top of the culm (number 1, I_1) were shorter (4.2–7.6 cm) and lighter (19–33 g of fresh weight) than mature internodes collected from the middle to the base of the plant (number 11, I_{11}), which presented 10.4–13.3 cm and 72–80 g of fresh weight. Sugarcane hybrids identified as H89, H58, H321, and H140 presented average culm diameters of 2.4, 2.6, 3.3, and 2.8 cm, respectively.

Sugarcane culms contains mainly water, sucrose and lignocellulosic biomass. The water proportion in the plant decreases with maturing stages (Lingle and Thomson, 2012) because sucrose and lignocellulosic biomass accumulates along plant maturation. For all evaluated sugarcane hybrids, sucrose accumulation in the internodes followed a similar pattern, increasing rapidly from I_1 to I_5, with smaller increments up to I_{11} (Figure S1 and Fig. 3). When available, internodes I_{13} to I_{19} were also evaluated for sucrose and lignocellulosic biomass contents; however, the mean values did not differ significantly from I_{11}, suggesting that, from I_{11}, all subsequent internodes represent the mature region of the culm. Sucrose contents detected at I_{11} varied slightly within different hybrids (from 16.1% in H89 to 18.7% in H58) and followed the same pattern previously reported for these hybrids when entire mature plants were evaluated (Masarin et al., 2011). Sucrose contents increased from immature to mature internodes independently on the measurements expressed on sugarcane fresh or dry weight (Figure S1), indicating that sucrose accumulation occurs at expenses of water depletion during maturation, which corroborates previous studies by Lingle and Thomson (2012). In contrast with sucrose accumulation, lignocellulosic biomass contents (expressed on fresh sugarcane weight) steadily increased along internodes 1 to 11, and H140 and H321 displayed the highest and the lowest contents, respectively. However, owing to massive sucrose accumulation in the plant, the lignocellulosic biomass measured as sugarcane dry weight basis decreases along maturation (Figure S1).

Fig. 3 illustrates sucrose and lignocellulosic biomass averages pooled from all hybrids, whereas doted lines indicate maximal and minimal values measured in the dataset. The diverse pattern for sucrose and lignocellulosic biomass accumulation in sugarcane culms suggests that sucrose accumulation in parenchyma cells precedes the complete development of secondary cell walls in vascular bundles.

The microscopic evaluation of samples from the rind region of the internodes was used to determine the average areas of parenchyma cells and vascular bundles and to estimate the thickness of secondary cell walls from fiber cells (Fig. 4). Parenchyma cell area increased significantly from I_1 to I_{11} in the hybrids H58 and H321. In contrast, the vascular bundle area did not change significantly during the progressive maturation stages. Fiber cell wall thickness increased from internode I_1 to I_{11}, agreeing with the progressive accumulation of lignocellulosic biomass. Most of the lignocellulosic biomass dry weight observed in the sugarcane internodes is assigned to fiber cell walls occurring in the vascular bundles. Despite being large and numerous, parenchyma cells present very thin cell walls, representing a minimal portion the overall lignocellulosic biomass dry weight (Moore, 1987; Brienzo et al., 2016).

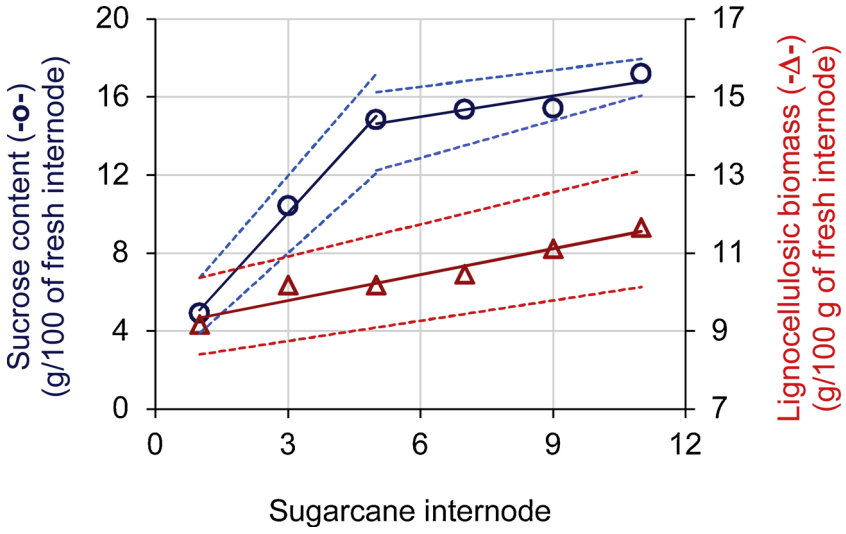

Fig. 3. Sucrose and lignocellulosic biomass contents related to the internode position in the culm. (Blue circles) Pooled sucrose contents calculated from H89, H58, H321 and H140 sugarcane hybrids. (Red triangles) Pooled lignocellulosic biomass contents for the same group of samples.

3.2. Chemical composition of lignocellulosic biomass

The data for the main cell wall constituents from each hybrid are shown in Fig. 5 (for regression models see Figure S2). In general, the hybrids exhibited a contrasting pattern of lignin and polysaccharides accumulation. H89 and H58, with the lowest overall lignin contents, rapidly accumulated lignin from I_1 to I_5. In these hybrids, lignin accumulated at the ratio of 1.5% per internode, starting with basal levels of 11.9–12.3% in I_1 up to 19.6–20.5% in I_5. In contrast, H321 and H140 steadily increased lignin contents at a lower ratio of 0.5-0.6% per internode from the higher initial levels of 15.8–16.6%, reaching maximal values at I_{11} in the range of 20.9–23.5%. Polysaccharides followed an inverse behavior, decreasing with increasing maturation stage of the internodes (Fig. 5). The decreases in the proportion of polysaccharides indicate that developed cell walls lacking lignin become lignified with plant development, increasing cell wall density. The overall effect is a decreased proportion of the polysaccharides as related to lignin. The comparison of contrasting internodes clearly demonstrates that I_1 contains fewer lignified tissues, while I_{11} represents a completely mature, lignified internode.

3.3. Enzymatic hydrolysis of the polysaccharide fraction

A time course for *in vitro* enzymatic digestion of the samples (Figure S3) was evaluated to estimate the initial hydrolysis rates as well as the glucan and xylan conversion levels after a 72-h reaction (Fig. 6). The initial glucan hydrolysis rates in the I_1 ranged from 4.7 ± 0.4%.h^{-1} for H140 to 7.6 ± 0.4%.h^{-1} for H89. For the same hybrids, the initial glucan hydrolysis rates observed for I_{11} were 3.5 ± 0.1%.h^{-1} and 6.1 ± 0.5%.h^{-1}, respectively. The xylan hydrolysis kinetics followed similar behaviors of glucan hydrolysis (Figure S3).

The conversion of glucan and xylan detected after 72 h of enzymatic hydrolysis (Fig. 6) indicated that the highest glucan digestibility (Fig. 6A) was observed in the first internodes and decreased up to I_{11}. However, pronounced differences were detected among the hybrids. For example, I_1 from H140 presented almost the same digestibility as I_{11} from H89. The less recalcitrant hybrids were H89 and H58, for which internode I_1 reached values of 45% glucan conversion without any pretreatment of the lignocellulosic fraction. The conversion levels for xylan were slightly lower than glucan but followed a similar behavior as observed for glucan conversion (Fig. 6B).

The digestibility levels of the internodes seem to be a direct consequence of low lignin and high glucan contents. A simple linear regression model was developed to describe sugarcane internode digestibility as a function of chemical composition. The model considers the widely accepted ultrastructure of secondary cell walls where hemicellulose and lignin encapsulate cellulose microfibrils, hindering enzyme access to the cellulose backbone (Loque et al., 2015; Petridis and Smith, 2018). Fig. 7A relates internode digestibility with a variable calculated as the glucan content divided by sum of lignin plus xylan detected in each internode. Direct and linear correlations can be observed in this graph, suggesting that digestibility can be easily estimated based on the chemical composition of the samples. The same model was applied to a larger data set, including samples from mature internodes of 6 sugarcane hybrids sampled from the rind to the pith region (Costa et al., 2016), and resulted in a broader range for digestibility prediction as a function of the internodes chemical compositions (Fig. 7B). Both data groups show direct correlations for digestibility as a function of the high content of glucan and the low content of the sum of lignin plus xylan.

4. Discussion

The current work joined, for the first time, information on sucrose and lignocellulose accumulation in sugarcane as related to the lignocellulose digestibility. Four different sugarcane hybrids were used to provide broad evaluation of sugarcane genotypes. Lingle and Thomson (2012) pioneered to describe sugarcane maturation process as related to sucrose accumulation and lignocellulose composition, showing that late harvest of sugarcane can favors sucrose accumulation; however, simultaneous increases of lignin content into cell walls suggested formation of a more recalcitrant lignocellulose. Here, simultaneous determination of *in vitro* lignocellulose digestibility, sucrose accumulation and cell wall characteristics indicated that sugarcane digestibility decreases significantly with plant maturation (Fig. 6). Most variable characteristics of cell walls along maturation were increases in fiber cell wall thickness (Fig. 4) and in lignin content, with simultaneous decreases in glucan content (Fig. 5).

The results described for sugarcane were in close agreement with other observations already reported for other C4 species such as untreated maize (Jung and Casler, 2006) and untreated and acid-pretreated switchgrass (Crowe et al., 2017) but differ from data reported for acid-pretreated sugarcane (Poelking et al., 2015). In the last work, sugarcane internodes were sampled from an ancient species (*S. spontaneum*) and a modern hybrid. Digestibility was evaluated in acid-pretreated samples. Acid pretreatment can induce severe alterations in the

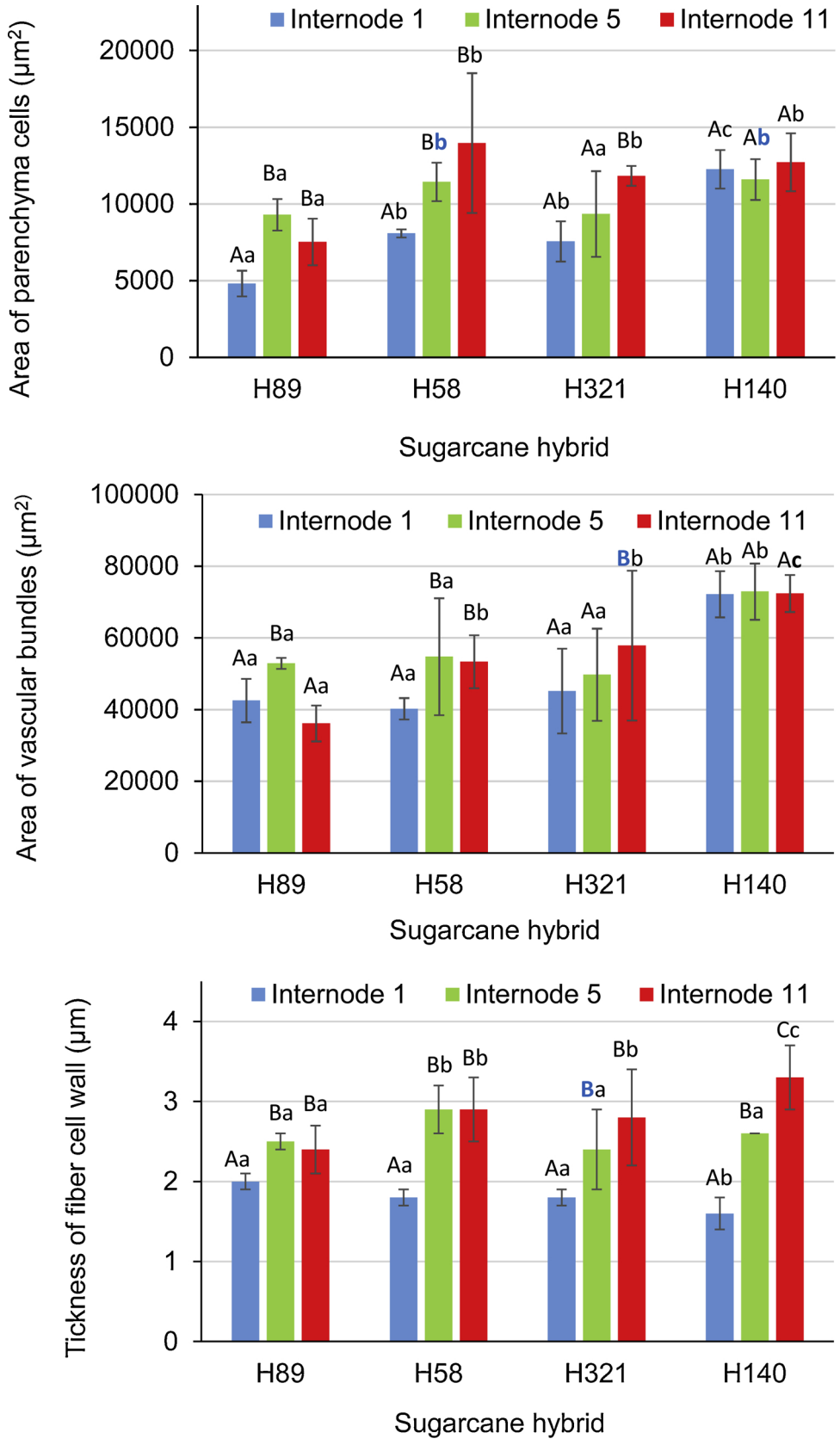

Fig. 4. Cell and tissue dimensions detected from the rind region of 4 different sugarcane hybrids sampled from internodes representing progressive maturation stages. Samples with the same letters did not differ from each other at $p < 0.05$. A few blue bold letter indicates data comparison at $0.1 < p < 0.05$. The first letter (capital letters) compare different internodes in the same hybrid. The second letter compare the same internode in different hybrids.

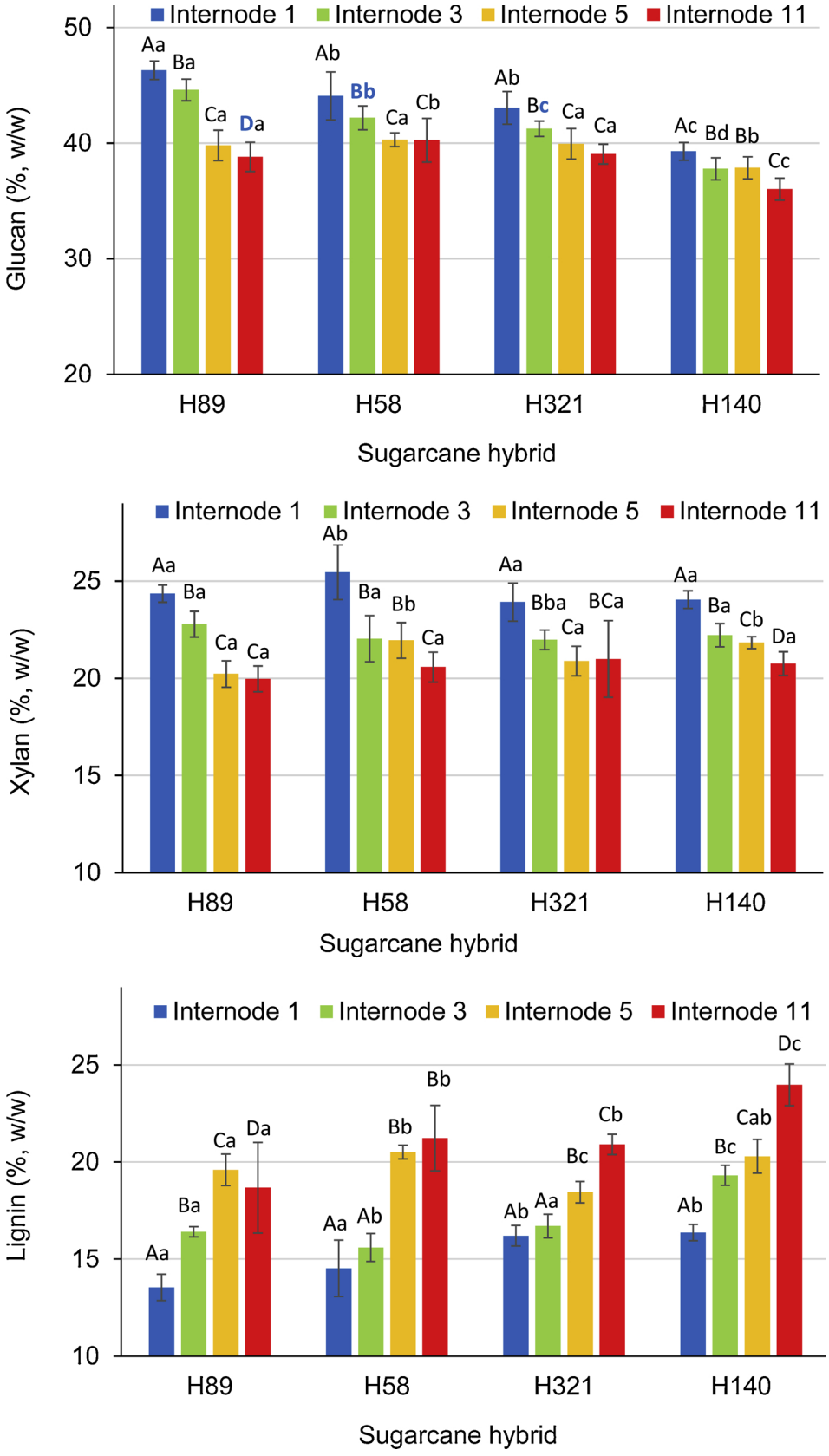

Fig. 5. Lignocellulosic biomass chemical composition of internodes representing progressive maturation stages from 4 different sugarcane hybrids. Samples with the same letters did not differ from each other at $p < 0.05$. A few blue bold letter indicates data comparison at $0.1 < p < 0.05$. The first letter (capital letters) compare different internodes in the same hybrid. The second letter compare the same internode in different hybrids.

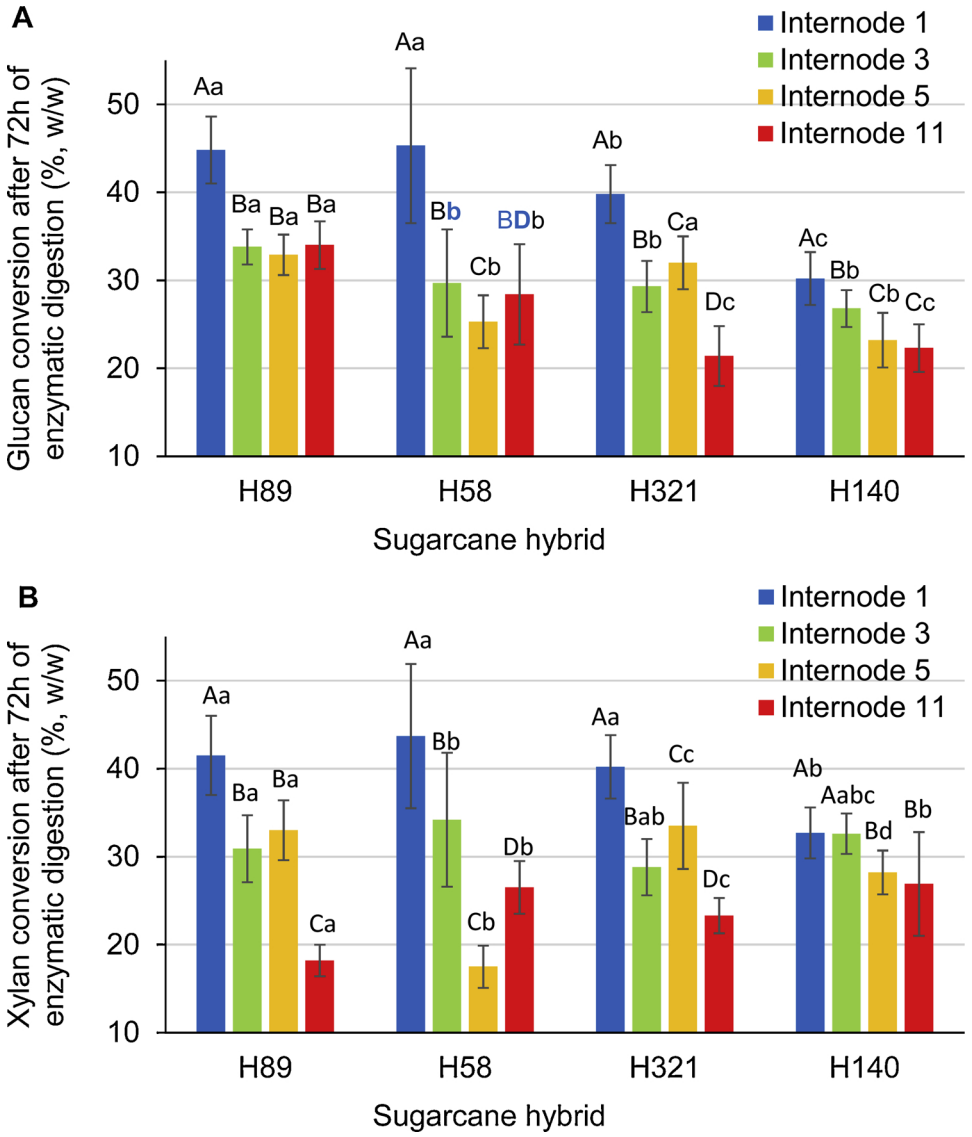

Fig. 6. Glucan (A) and xylan (B) conversion after *in vitro* enzymatic hydrolysis of internodes representing progressive maturation stages from 4 different sugarcane hybrids. Samples with the same letters did not differ from each other at $p < 0.05$. A few blue bold letter indicates data comparison at $0.1 < p < 0.05$. The first letter (capital letters) compare different internodes in the same hybrid. The second letter compare the same internode in different hybrids.

lignocellulosic biomass, such as xylan removal and lignin reallocation (Donohoe et al., 2008). Therefore, it is probable that biomass changes induced by acid pretreatment were more severe compared with results reported for switchgrass (Crowe et al., 2017), causing a biased effect on the lignocellulose digestibility of the sugarcane internodes sampled at progressive maturation stages.

In the current work, we hypothesize that at least one of the selected hybrids could display maximal sucrose accumulation and a low lignification pattern in the culm tissues. These characteristics are favorable for further sugarcane breeding aiming to select hybrids with

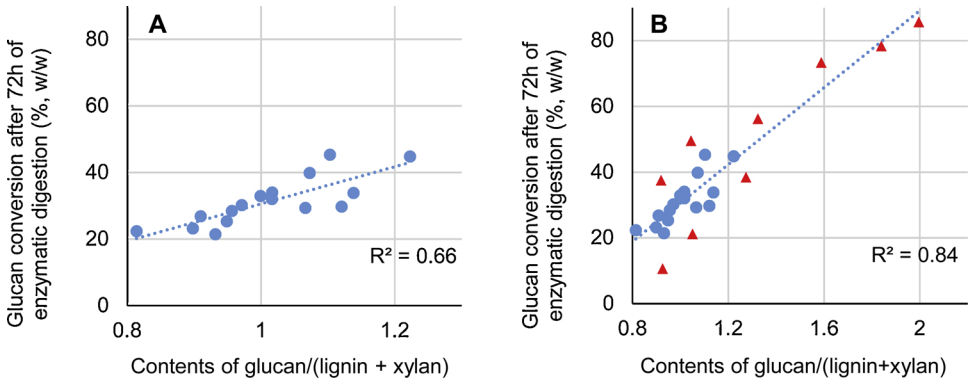

Fig. 7. Simple linear regression model and the correlation coefficient explaining glucan conversion levels as a function of glucan contents divided by the sum of lignin plus xylan contents in sugarcane internodes. (A) Data obtained from internodes I_1 to I_{11} representing progressive maturation stages. (B) Data obtained in current work (blue circles) superposed with data from Costa et al. (2016) (red triangles).

premature sucrose accumulation but incomplete secondary cell wall lignification, resulting in less recalcitrant lignocellulose. These characteristics were not previously described in sugarcane, being detected in H89 and H58 in the current work. Although these hybrids presented a rapid lignification of young internodes (I_1 to I_5), the lignin contents started from low basal levels compared with H321 and H140 (Figure S2). The young internodes of H89 and H58 were highly digestible without any pretreatment, whereas the sucrose contents reached levels close to the maximum at I_5 (Figure S1). These characteristics contrasted with H140, which presented significantly more recalcitrant young internodes despite accumulating sucrose in a similar behavior (Fig. 6). Lignin contents were significantly lower in I_1 and I_3 of H89 and H58 than in H140, whereas glucan contents presented an inverse behavior (Fig. 5). Xylan contents varied to a lower extent in these samples.

The comparison of extreme sample values in the data set indicated that I_1 from H140 (16.4% lignin) was still less digestible than I_{11} from H89 (18.7% lignin), indicating that initial levels of lignification are even more critical for high recalcitrance than the progress of lignification during internode maturation. Data from previous studies corroborate that other cell wall characteristics affect recalcitrance in addition to the lignification (Himmel et al., 2007; Costa et al., 2013; Ding et al., 2013; Loque et al., 2015; Holwerda et al., 2019). A simple model relating the contents of glucan with lignin plus xylan in the samples demonstrated a good ability to predict lignocellulose biomass digestibility since it accounted for the proportional occurrence of more glucan in the cell walls of less recalcitrant samples (Fig. 7A). The glucan contents in sugarcane internodes accounted not only for cellulose but also for mixed-linkage glucans (a non-branched polymer of glucose residues with ß-1, 4 and ß-1, 3 linkages), which are easily digested by commercial cellulase preparations (Vega-Sánchez et al., 2015; Costa et al., 2016). The predicting model was still suitable to estimate digestibility in a broader sample set, including sugarcane samples from another study (Costa et al., 2016) (Fig. 7B).

Recent work on transcriptomics of sugarcane during maturation process has revealed genes involved in the control of culm development (Tavares et al., 2018), transcription factors associated with cell wall formation (Ferreira et al., 2016) and genes associated with cellulose and monolignol biosynthesis as well as key regulators for the secondary cell wall synthesis (Kasirajan et al., 2018). Further association of data obtained in the current work, which identified desirable phenotype characteristics (high sucrose yield and low lignocellulose recalcitrance) in H89 and H58 that were not detected in H140 and H321, with transcriptomic studies for the same hybrids would help to identify contrasting genetic characteristics discriminating these plants. This approach will help design further breeding steps to produce sugarcane that combines high sucrose yields and low lignocellulose recalcitrance.

5. Conclusions

Sugarcane hybrids rapidly accumulated sucrose from internode I_1 to I_5, whereas lignocellulose accumulation steadily increased from I_1 to I_{11}. For all hybrids, the digestibility decreased significantly during the maturation stages. Despite presenting similar behaviors for sucrose and lignocellulose accumulation, some of the hybrids started the maturation process with lower initial lignin contents, which let to highly digestible samples. In contrast, the samples in which lignin deposition started at higher levels provided significantly more recalcitrant sugarcane internodes. When low lignin contents led to high glucan contents, the samples presented low recalcitrance.

We hypothesize that selected sugarcane hybrids could display maximal sucrose accumulation with simultaneous low lignification of the culm tissues. These desirable characteristics occurred in immature internodes of H89 and H58, contrasting with H140 and H321, which qualify these plants for further evaluation of their genetic toolbox aiming straightforward breeding programs focused on high sucrose yield and low lignocellulose recalcitrance.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2019.111543.

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