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Poster paper

Cell-wall chemical-composition analysis of a *Saccharum* spp. progeny for increased bagasse digestibility

JM Garcia[1,2], A Acevedo[3], MP Silva[4], L Gomez[4] and LE Erazzú[1]

[1]*EEA Famaillá INTA, Ruta Provincial Nº 301, km 32, 4132, Tucumán, Argentina;* garcia.josemaria@inta.gob.ar
[2] *CONICET, Buenos Aires, Argentina*
[3]*Instituto de Suelos, CIRN-INTA, 1712 Castelar, Buenos Aires, Argentina*
[4]*CNAP, Department of Biology, University of York, Heslington, York YO10 5YW, UK*

Abstract Knowledge of cell-wall composition in sugarcane hybrids could be used for the selection of cultivars with high bagasse digestibility in order to improve sugar release in 2G ethanol production. Cell-wall components of 76 hybrids of a sugarcane cross, their parents and two high-fibre genotypes were analyzed. The Van Soest method was used to determine cellulose, hemicellulose and acid detergent lignin contents (ADL). Spectroscopic acetyl bromide lignin (ABS) was also determined. A correlation analysis was performed with major cell-wall polymers and agronomic traits. Despite the minor differences observed between parental lines' cell-wall composition, genotypes with high cellulose and low lignin content were identified in the progeny. Differences among them depended on the method used for lignin measurement. The ADL mean was 43% lower than the ABS mean, in agreement with lignin losses during acid detergent treatment previously reported. There was no correlation between ADL and ABS assays. Bagasse of genotypes with higher stalk weights was associated with higher cellulose content and lower ADL content. Saccharification studies are needed to validate lignin assessment methodologies to be used as bioenergy cultivar selection tools.

Key words Lignin, cell wall, sugarcane, bagasse

INTRODUCTION

Sugarcane (*Saccharum* spp.) bagasse is a relevant lignocellulosic substrate for second-generation (2G) ethanol production (Masarin *et al.* 2011). Large quantities of bagasse are obtained in the sugarcane industry that could be used for this purpose, and this shares part of the infrastructure of first-generation (1G) ethanol production (Dias *et al*. 2012).

Lignocellulose is the plant biomass formed mainly by cellulose, hemicellulosic polysaccharides and lignin (Vega-Sanchez and Ronald 2010). In cellulosic ethanol production, costly pre-treatments are required to access to monosaccharides (Vanholme *et al.* 2010) and one of the main hindrances in this process is the difficulties caused by lignin (Siqueira *et al.* 2011).

Saccharification efficiency of sugarcane could be enhanced by improving the biomass quality through genotypes with more cellulose and less lignin (Hoang *et al.* 2016). Despite this, the variability in the cell-wall composition of sugarcane bagasse remains unexplored, and its study could provide valuable information for screening in breeding programs.

The objectives of our study were: a) to analyze the bagasse cell-wall composition of a sugarcane progeny and corresponding parents, and of two promising high-fibre genotypes; b) to compare two lignin determination methodologies; and c) to assess bagasse cell-wall composition with agronomic breeding traits.

MATERIALS AND METHODS

A trial was conducted at the experimental field of INTA Famaillá Experimental Station (27°03'S, 65°25'W, 363 masl.), Tucumán, Argentina. A set of 76 sugarcane siblings, their parents (NA 78-724 and LCP 85-384), and two genotypes (INTA 05-3116 and INTA 05-3118) with high-fibre content were planted in a completely randomised design without replicates. Each plot consisted of one row of 3 m length and 1.6 m inter-row spacing.

At the first ratoon, 10 stalks/plot were manually harvested, and tops and leaves were removed. Stalk diameter, height and weight of each sample were recorded and internodes 5 to 7 (from the bottom of the stalk) were separated and shredded. The sugar content was washed with water and stalks were dried in an oven at 100ºC until constant weight. Two replicates of neutral detergent fibre (NDF), acid detergent fibre (ADF) (Van Soest *et al*. 1991) and acid detergent lignin (ADL) (Van Soest 1973) were determined. Cellulose content was calculated as the difference between ADF and ALD, and hemicellulose content as the difference between NDF and ADF. Three replicates of spectroscopic acetyl bromide lignin (ABS) were determined according to Fukushima and Hatfield (2001). Total ash content was determined (AOAC 1990).

Brix and pol in juice (%) were obtained from the remainder of the stalk internodes and pol in cane (%), purity (%) and sugar content (%) were calculated as described by Acreche *et al*. (2015).

RESULTS AND DISCUSSION

Cultivar LCP 85-384 had slightly higher mean values of cellulose (+1.7%) and ABS (+ 0.9%) contents and lower hemicellulose (-1.2%) and ADL (-0.6%) contents than NA 78-724 (Table 1). The main component of the NDF mean in the progeny was cellulose (51.4%), followed by hemicellulose (+35.2%) and ADL (+13.4%). Hemicellulose was the cell-wall component with the lowest percentage of variability in the progeny with a range of 9.4%. Higher ranges for cellulose (+19.9%), ADL (+19.7%) and ABS (+17.1%) were observed (Table 1).

Even though small differences were obtained when comparing cell-wall composition of both parents these results demonstrate a significant variability for cell-wall components in the progeny. Genotypes with low ADL and high cellulose contents were identified in the progeny as potential candidates for better bagasse digestibility.

High-fibre genotypes showed higher cellulose and lower hemicellulose and ADL content than the mean of parents and progeny. Interestingly, progeny maximum values were the highest for all the fibre components.

Table 1. Chemical composition of bagasse of sugarcane parental lines, progeny (N= 76), and two high-fibre sugarcane genotypes.

Component	Parents		Progeny				High-fibre genotypes	
	NA 78-724	LCP 85-384	Mean	Min.	Max.	Range	INTA 05-3116	INTA 05-3118
Cellulose (%NDF[a])	52.0±0.8	53.7±0.1	51.4	41.6	61.5	19.9	58.2±2.0	58.3±1.7
Hemicellulose (%NDF [a])	33.0±0.3	31.8±1.1	35.2	31.0	40.4	9.4	29.0±1.2	29.6±1.3
ADL(%NDF [b])	15.0±1.1	14.4±1.0	13.4	4.8	24.5	19.7	12.8±0.9	12.0±0.4
ABS (%DM [c])	23.1±0.2	24.0±1.2	23.5	16.1	33.2	17.1	23.2±1.0	24.1±0.0
Ash (%DM)	2.1±0.0	2.0±0.1	2.4	1.8	3.1	1.3	2.7±0.0	3.0±0.2

[a]Van Soest (1991), [b]Van Soest (1973), [c] Fukushima and Hatfield (2001). NDF= Neutral Detergent Fibre, ADL= Acid Detergent Lignin, ABS= Acetyl Bromide Lignin, DM= Dry Matter.

ADL mean value of parents, progenies and high fibre genotypes was 43% lower than ABS mean. This difference could be attributed to the acid detergent action that dissolves more than 50% of the lignin content in grasses (Hatfield and Fukushima 2005).

Cellulose content in the progeny was positively correlated with stalk weight ($r=0.23$, $p<0.05$) and negatively correlated with Brix ($r=-0.35$, $p<0.01$). Nevertheless, hemicellulose content was negatively correlated with stalk height ($r=-0.26$, $p<0.05$) and though low Brix ($r=0.28$, $p<0.01$), while ADL was negatively correlated with stalk weight ($r=-0.23$, $p<0.05$). These results suggest that bagasse of genotypes with greater stalk weight were associated with

a higher cellulose content and lower ADL content. Although cellulose was negatively correlated with Brix, no correlation with quality sugar parameters (pol in juice%, pol in cane%, purity% and sugar content %) was observed.

Spectroscopic acetyl bromide lignin showed no correlation with any agronomic trait evaluated. Similar results were obtained by Baffa *et al.* (2014) for lignin content determined by Klason's method. It is worth noting, that no correlation between ADL and ABS was found (r=0.04, p=0.7).

CONCLUSIONS

Genotypes with cell-wall composition with improved biomass digestibility (high cellulose and low lignin contents) in a sugarcane biparental population were identified. Because ADL and ABS methods showed no correlation, different genotypes could be selected according to the lignin method considered. These divergences indicate that further research is needed to validate lignin assessment as a cultivar selection tool, e.g. complementary saccharification analyses.

Few significant correlations between cell-wall components and agronomic traits were found. Stalk weight was associated with higher cellulose and lower ADL content.

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Analyse de la composition chimique de la paroi cellulaire d'une progéniture de *Saccharum* spp. pour une digestibilité accrue de la bagasse

Résumé. La connaissance sur la composition des parois cellulaires des hybrides de canne à sucre pourrait être utilisées dans la sélection de variété à haute digestibilité de la bagasse afin d'améliorer la libération de sucre dans la production d'éthanol 2G. Dans cette étude, les composantes de la paroi cellulaire de 76 hybrides d'une progéniture de canne, leurs progéniteurs et deux génotypes à haute teneur en fibre ont été analysés. La méthode Van Soest a été utilisée pour déterminer la teneur en cellulose, en hémicellulose et en lignine au détergent acide (ADL). Le pourcentage de lignine soluble dans le bromure d'acétyle (ABS) fut déterminé. Les corrélations entre les principaux polymères des parois cellulaires et les caractères agronomiques ont été effectuées. Malgré les différences mineures observées entre la composition de la paroi cellulaire des géniteurs, des génotypes à forte teneur en cellulose et à faible teneur en lignine ont été identifiés dans la progéniture. Les différences entre eux dépendaient de la méthode utilisée pour déterminer la teneur en lignine. La moyenne de l'ADL était inférieure de 43% à la moyenne de l'ABS, en accord avec les pertes en lignine pendant le traitement aux détergents acides. Aucune corrélation n'a été observée entre les dosages ADL et ABS. La bagasse de génotypes ayant un poids de tiges plus élevé était associée à une

teneur plus élevée en cellulose et à une teneur plus faible en ADL. D'autres analyses de saccharification sont nécessaires afin d'évaluer la libération de sucre de la biomasse et améliorer la digestibilité de la bagasse dans des génotypes de canne à sucre.

Mots-clés: Lignine, paroi cellulaire, bagasse de canne à sucre

Análisis de la composición química de la pared celular de una progenie de *Saccharum* spp. para el incremento de la digestibilidad del bagazo

Resumen. El conocimiento de la composición de la pared celular en híbridos de caña de azúcar puede ser utilizado en la selección de cultivares de elevada digestibilidad del bagazo, orientados a incrementar la liberación de azucares en la producción de etanol 2G. Se analizaron los componentes de la pared celular de 76 híbridos obtenidos de un cruzamiento de caña de azúcar, sus progenitores y dos genotipos de alta fibra. Se utilizó el método de Van Soest para determinar los contenidos de celulosa, hemicelulosa y lignina detergente ácido (LDA); además se determinó el contenido de lignina mediante espectroscopía utilizando brumuro de acetilo (EBA). Se realizó un análisis de correlaciones con los componentes de la pared celular y características agronómicas. A pesar de las diferencias mínimas en la composición de la pared celular observadas entre progenitores, fueron identificados en la progenie genotipos con contenido elevado de celulosa y bajo de lignina. Sin embargo, estos genotipos fueron distintos dependiendo del método empleado en la determinación de lignina. El promedio calculado para LDA fue un 43% más bajo que el promedio de EBA, en coincidencia con pérdidas previamente reportadas en la bibliografía debido al uso del detergente ácido. Se observó una ausencia de correlación entre LDA y EBA, mientras que el peso de tallos estuvo asociado a un contenido mayor de celulosa y menor de LDA. Futuros análisis de sacarificación son necesarios para evaluar la liberación de azúcares de la biomasa y validar los métodos de determinación de lignina como herramientas de selección.

Palabras clave: Lignina, pared celular, bagazo, caña de azúcar