BIOMASS AND BIOENERGY XXX (2012) 1–8

Available online at www.sciencedirect.com

SciVerse ScienceDirect

http://www.elsevier.com/locate/biombioe

# Cultivar variation and selection potential relevant to the production of cellulosic ethanol from wheat straw

J. Lindedam [a,*], S.B. Andersen [b], J. DeMartini [c], S. Bruun [b], H. Jørgensen [a], C. Felby [a], J. Magid [b], B. Yang [d], C.E. Wyman [c]

[a] Forestry and Wood Products, Forest & Landscape, Faculty of Life Sciences, University of Copenhagen, Rolighedsvej 23, DK-1958 Frederiksberg C, Denmark

[b] Plant and Soil Science Laboratory, Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark

[c] Center for Environmental Research and Technology, Bourns College of Engineering, University of California Riverside, 1084 Columbia Avenue, Riverside, CA 92507, USA

[d] Center for Bioproducts and Bioenergy, Washington State University, 2710 University Drive, Richland, WA 99354, USA

ARTICLE INFO

*Article history:*
Received 23 May 2011
Received in revised form
8 November 2011
Accepted 9 December 2011
Available online xxx

*Keywords:*
Bioethanol
Sugar
Wheat straw
Variation
Cultivar

ABSTRACT

Optimizing cellulosic ethanol yield depends strongly on understanding the biological variation of feedstocks. Our objective was to study variation in capacity for producing fermentable sugars from straw of winter wheat cultivars with a high-throughput pretreatment and hydrolysis well-plate technique. This technique enabled us to estimate cultivar-related and environmental correlations between sugar yield, chemical composition, agronomic qualities, and distribution of botanical plant parts of wheat straw cultivars. Straws from 20 cultivars were collected in duplicates on two sites in Denmark. Following hydrothermal pretreatment (180 °C for 17.6 min) and co-hydrolysis, sugar release and sugar conversion were measured. Up to 26% difference in sugar release between cultivars was observed. Sugar release showed negative cultivar correlation with lignin and ash content, whereas sugar release showed positive cultivar correlation with content of carbohydrates and plant height. Accessibility to cellulose can impede the sugar conversion rate, and convertibility of each botanical fraction might be more important to overall sugar conversion than the relative proportions of botanical fractions. Our results suggest that selection of cultivars for improved biofuel feedstock of wheat straw is possible, because heritability of sugar release is 57% and there are few adverse correlations to other agronomic traits.



## 1. Introduction

Decreasing the cost of producing cellulosic ethanol to be competitive with gasoline and grain-based ethanol needs to be achieved for cellulosic ethanol to be successfully used in large amounts. While optimizing processing tools and techniques will initially pave the path to reduced production costs and increased ethanol yield per unit mass of feedstock, further enhancement of the economics may be attained through improving feedstock quality [1]. As wheat (*Triticum*

*Abbreviations:* dm, Dry matter; HTPH, High-throughput pretreatment and hydrolysis system; TS, Total sugar.

* *Corresponding author*. Tel.: +45 232 867 40.

E-mail addresses: lindedam@life.ku.dk (J. Lindedam), sba@life.ku.dk (S.B. Andersen), jdemartini@engr.ucr.edu (J. DeMartini), sab@life.ku.dk (S. Bruun), hnj@life.ku.dk (H. Jørgensen), cf@life.ku.dk (C. Felby), jma@life.ku.dk (J. Magid), binyang@tricity.wsu.edu (B. Yang), charles.wyman@ucr.edu (C.E. Wyman).

0961-9534/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biombioe.2011.12.009

*aestivum* L.) is one of the major crops in the world, the unused straw constitutes a large residual biomass with a vast potential as feedstock for cellulosic ethanol production.

Experiments evaluating feedstock quality often generate large numbers of samples. For rapidly screening large sample sets, high-throughput pretreatment and enzymatic hydrolysis (HTPH) well-plate techniques have been developed [2,3]. By screening for wheat cultivars that are less recalcitrant to enzymatic hydrolysis following pretreatment, we may be able to identify gene variants and biochemical characteristics which promote sugar yield and could provide a foundation for selection or engineering of superior feedstocks. However, this would require that selection for sugar enhancement has good heritability without negative consequences on other agronomic qualities such as grain yield, straw height and lodging.

Cultivar variation of ethanol yield relative to maximum theoretical yield have been studied in corn stover samples and ranged between 45% and 73%, showing a strong negative correlation with lignin content, and a low negative correlation with cellulose content [4]. Cultivar variation in ethanol yield from wheat straw is not yet well described, but Jensen at al. [5] found large variation in enzymatic digestibility when examining 106 wheat cultivars from two sites, with dissolved organic matter ranging from 258 to 407 g $kg^{-1}$ of input dry matter (dm). Whether this would correlate with the wheat straw's ability to be enzymatically hydrolyzed after pretreatment is unknown. Research on glucose release and stover quality for cellulosic ethanol from maize have been published, stating that breeding programs should be able to incorporate traits for cellulosic ethanol without adverse effects of genetic gains for grain yield and agronomic traits [6,7].

Over the years, evidence has indicated that morphology and distribution of botanical fractions, such as leaf, nodes, internodes, and flower spike affects digestibility. Capper [8] identified wheat leaf blades to be more digestible in vitro than either leaf sheath or stem, rendering shorter wheat cultivars more digestible than taller cultivars due to a higher proportion of leaves in the biomass. Tolera et al. [9] assessed different wheat cultivars for chemical composition and ruminal digestibility, and found significant variation in digestibility related to differences in the leaf-to-stem ratio. With botanical fractions responding differently to pretreatment [8,10], the influence of botanical fractions on the sugar yield from wheat cultivars is still unclear, and was therefore also examined in the present study.

The aim of this study was to investigate the variation in sugar yield from wheat straw cultivars through an HTPH well-plate technique and to estimate correlations of sugar yield with chemical composition, agronomic qualities, and distribution of botanical fractions.

## 2. Materials and methods

### 2.1. Wheat cultivars, harvest and fractionation

Winter wheat straw was sampled at maturity in 2007 from two completely randomized blocks at each of two sites near the towns of Sejet (55°49′12.43″ N and 9°55′21.82″ E) and Abed (54°49′40.05″ N and 11°19′30.62″ E) in Denmark, where statutory field experiments comparing cultivars were conducted. Crop management (fertilizers, herbicides etc) were similar at the two sites, which were both sandy clayey soil, thus straws represented the natural variation in biomass feedstock in Danish climate. Annual rainfall in the harvest year was 867 mm.

Plant height, measured as the distance from soil surface to basis of the spike, and percentage of lodging in the block were scored before harvest. Lodging was scored on a scale from 0 to 10, where 0 corresponded to 0% lodging in the block and 10 corresponded to 100% lodging. Collecting material was done at the same day at the two sites just after normal grain harvest. Grain yield from every block was recorded automatically by an experimental harvester, which cut the plants approximately 5 cm from the soil surface and discharged the plants free of grain. After harvest, approximately 80 g dm of wheat plants (free of grain) was sampled from each block by taking four to five handfuls from different places in the block. Each sample was then fractionated into botanical components of ears (flower spike free of grain), leaves (leaves without the leaf sheath), and stem (remaining part). After weighing, anatomical parts were mixed together, milled to <1 mm on a cyclone mill (President, Holbæk, Denmark), and stored at ambient temperature until analysis in 2009.

Cultivars were Northern European breeds: Abika, Ambition, Audi, Dinosor, Flair, Florett, Glasgow, Hattrick, Inspiration, Jenga, Oakley, Opus, Penso, Potenzial, Robigus, Samyl, Skalmeje, Smuggler, Tommi, and Tuscan. One sample was lost during harvest; thus total sample set was 79 air-dried samples.

### 2.2. Biochemical composition

Chemical composition of the raw material was determined by a two-step acid hydrolysis of the carbohydrates according to the procedure published by the National Renewable Energy Laboratory (NREL) [11]. Analyses were done on air-dried samples with an average water content of $w_B = 7.9 \pm 0.9\%$. Dry matter content was determined on a Sartorius MA30 dry weight balance. No extractions were preformed prior to the acid hydrolysis in order to maintain the original composition of the biomass. First, 3 mL 72% $H_2SO_4$ was added to 300 mg dm milled wheat straw and incubated at 30 °C for 1 h. Next, the samples were diluted with 84 mL Millipore water and autoclaved at 121 °C for 1 h (Tuttnauer, 2540 EL). Finally, the hydrolyzates were filtered, neutralized with $CaCO_3$ and diluted with eluent before quantification of monomeric sugar on a Dionex Summit high performance liquid chromatography (HPLC) system. The separation was performed in a Phenomenex Rezex ROA column at 80 °C with 5 mol $m^{-3}$ $H_2SO_4$ as eluent, running at a flow rate of 0.6 mL $min^{-1}$ with a Shimadzu RI-detector. Hemicellulose was calculated as the sum of xylose and arabinose concentrations. Klason lignin content was determined as the weight of the dried filter cake (dried over night at 105 °C) minus the ash content (dried 3 h at 550 °C). Measurements on all samples were performed in triplicates.

### 2.3. Sugar yield analysis

To measure sugar yield from straw, we employed the relatively new HTPH 96-well-plate screening system, developed at

Please cite this article in press as: Lindedam J, et al., Cultivar variation and selection potential relevant to the production of cellulosic ethanol from wheat straw, Biomass and Bioenergy (2012), doi:10.1016/j.biombioe.2011.12.009

the University of California, Riverside [2]. The conditions chosen for the experiment were determined by testing different pretreatment conditions and enzyme loadings on a sample chosen from the data set as a standard (Flair cultivar). All 79 samples were analyzed in triplicates with the HTPH system. Hydrothermal pretreatment was performed at log severity 3.6 [12] and a mass fraction of 1%. This was achieved by loading 2.5 mg dm milled straw to each well and soaking for 4 h in de-ionized water (total reaction mass of 250 mg) before heating with indirect steam for 17.6 min at 180 °C.

Hydrolysis was then performed on the entire pretreated slurry by applying 12.5 μL Na–citrate buffer with an amount of substance concentration of 1 kmol $m^{-3}$, 2.5 μL $NaN_3$ with a concentration of 1 kg $m^{-3}$, and 13 μL diluted enzyme mix to all wells. The enzyme mix was made with a 5:1 weight ratio of cellulase (Celluclast, Novozymes) and cellobiase (Novozyme 188, Novozymes) and diluted 10 times with 50 mol $m^{-3}$ citric acid buffer, pH 4.8. Enzyme loading was fixed at 40 FPU $g^{-1}$ dm and thus ranged for individual cultivars from 58 to 72 FPU $g^{-1}$ glucan + xylan in the raw material (standard deviation 3.1 FPU $g^{-1}$ glucan + xylan). Hydrolysis ran for 72 h in an incubation shaker (Multitron InFors, ATR Biotech, MD) at 50 °C, 150 rpm. The content of each well was then transferred to 2 mL centrifuge tubes and centrifuged for 10 min at 18,200 g-forces (5415 D, Eppendorf, Hamburg, Germany). Sugar concentrations in the supernatant were analyzed using HPLC with an Alliance 2695 system (Waters, Milford, MA), an Aminex HPX-87H column (BioRad, Hercules, CA) heated to 65 °C and using 5 mol $m^{-3}$ $H_2SO_4$ as eluent in an isocratic mode. Detection was performed by a refractive index detector (2414, Waters).

### 2.4. Data analysis

Convertibility of glucan and xylan was calculated as the amount of glucose and xylose released from combined pretreatment and enzymatic hydrolysis as a percentage of the maximum theoretical release (Eq. (1)):

$$\text{Convertibility}(x) = \frac{C_{x\ \text{enz}}(\text{g/l})}{C_{x\ \text{composition}}(\text{g/l})} * 100\% \quad (1)$$

where $x$ denotes glucose (C6), xylose (C5) or glucose plus xylose (TS for total sugar), the $C_{xenz}$ is the concentration of $x$ measured after co-hydrolysis and the $C_{xcomposition}$ denotes the maximum possible concentration of hydrated $x$, calculated from compositional analysis and corrected for solid loading in the hydrolysis. Sugar release of the cultivars was evaluated by calculating the release of C6, C5 and TS in gram per g dm of raw biomass (g $g^{-1}$ dm). The standard deviation of the laboratory method (SDL) was based on the laboratory triplicates in the HTPH system and used to evaluate the well-plate technique (Eq. (2)):

$$\text{SDL} = \sqrt{\frac{\sum_{i=1}^{n}\sum_{j=1}^{m}\left(X_{ij} - \overline{X}_j\right)^2}{n * m - 1}} \quad (2)$$

where $i$ is the individual laboratory replicate out of $n$ replications ($n = 3$) and $j$ is the individual sample out of $m$ samples ($m = 79$).

Comparison of straw from wheat cultivars used Tukey simultaneous tests with proc GLM at a 95% confidence level [13]. A MANOVA statement was included in GLM models for all different pairs of chemical components, botanical fractions, sugar convertibility (%) and sugar release (g $g^{-1}$ dm) of the cultivars for estimation of correlations. The resulting sums of squares and cross products were used to calculate matrices of covariance and matrices of components of covariance for cultivar and error effects, respectively, based on expectations of mean squares. Coefficients of correlation between each pair of measurements were subsequently calculated from the matrices of components of covariance. As a result correlation coefficients were presented as either cultivar correlations coefficients or environmental correlation coefficients. Thus, cultivar correlation coefficients were based on the components of covariance of cultivars under the specific geographical conditions of the two growing sites in the particular harvest year. Environmental correlation coefficients were based on components of covariance of errors in the study, hence representing all unexplained variation.

A few coefficients of correlation exceeded the mathematical limit of ±1. They were checked by using the Restricted Maximum Likelihood method (REML) in proc varcomp [13] for estimation of the variance components but the numerical problem persisted and these correlation coefficients were subsequently adjusted to ±1.0. Also, heritabilities were calculated as the cultivar variance component relative to the sum of the cultivar and the error variance component. Heritability is the proportion of the phenotypic variance that is due to genetic causes [14].

## 3. Results and discussion

Average conversions and sugar release results from the HTPH system along with results of chemical composition, plant height, lodging, botanical distribution and grain yield are presented in Table 1. Before analyzing the 79 different samples the well-plate technique was validated for its ability to reproduce results with wheat material. The standard deviation of total sugar conversion resulting from analyzing our standard wheat material in all 96 wells was 3.0% of the average total sugar conversion. For comparison, the observed standard deviation of total sugar conversion was 4.1% on poplar wood when the report on the HTPH system was first published [2]. This demonstrated that the biomass milling, mixing, and plate loading approach was consistent. Reproducibility of total sugar release in the HTPH assay was further proved on the different cultivars as the SDL was 0.0129 g $g^{-1}$ dm in a range of total sugar averages from 0.36 to 0.43 g $g^{-1}$ dm. Accordingly a maximum uncertainty of the HTPH system of 3.4% was observed. Results generated with the HTPH system (Table 1) are in agreement with glucan and xylan conversions from wheat straw measured with larger-scale methods [15,16] and the HTPH system was a suitable method for cultivar studies in need of processing large sample sets at low uncertainties.

Please cite this article in press as: Lindedam J, et al., Cultivar variation and selection potential relevant to the production of cellulosic ethanol from wheat straw, Biomass and Bioenergy (2012), doi:10.1016/j.biombioe.2011.12.009

**Table 1 – Average chemical compositions, conversions and release of sugar in the HTPH system, and plant characteristics of 20 wheat straw cultivars grown at two sites.**

| | Chemical composition | | | | Conversion to sugar | | | Release of sugar | | | Plant characteristics | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cell % | Hemi % | Lignin % | Ash % | C5 % | C6 % | TS % | C5 g g$^{-1}$ | C6 g g$^{-1}$ | TS g g$^{-1}$ | Leaf % | Ears % | Stem % | L:S ratio | Lodging | Height cm | Grain Mg ha$^{-1}$ |
| Abed site | 36.58 (2.00) | 25.61 (1.70) | 19.47 (0.73) | 6.64 (1.13) | 72.35 (4.83) | 56.96 (4.90) | 62.90 (4.65) | 0.17 (0.01) | 0.21 (0.01) | 0.39 (0.02) | 10.54 (2.94) | 6.86 (3.38) | 82.60 (4.47) | 0.13 (0.04) | 7.10 (1.22) | – | 9.55 (0.78) |
| Sejet site | 37.00 (1.54) | 25.40 (0.98) | 19.08 (0.81) | 6.28 (0.73) | 75.31 (3.15) | 56.96 (3.13) | 63.99 (3.01) | 0.18 (0.01) | 0.22 (0.01) | 0.40 (0.02) | 8.32 (1.99) | 4.63 (2.12) | 87.05 (3.17) | 0.10 (0.03) | 6.41 (4.00) | 84.44 (5.55) | 9.06 (0.69) |

Standard deviations are given in parenthesis ($n = 40$ for Abed and $n = 39$ for Sejet). Cell = Cellulose, Hemi = Hemicellulose, C5%, C6% and TS% = conversion of xylan, glucan or total sugar (xylan plus glucan) in percentage of the maximum available in raw material. C5, C6 and TS g g$^{-1}$ = release of xylose, glucose and total sugar (xylose plus glucose) in gram per gram dry matter biomass. L:S is the ratio of leaves-to-stem. The scale of lodging is between 0 and 10 for 0% of the plants in the parcel being lodged or 100% lodging. Height corresponds to plant height at harvest. Grain = grain yield.

### 3.1. *Effect of sites and blocks*

The two experimental sites had a highly significant effect on the convertibility and release of xylan ($P < 0.0001$) but not of glucan (Table 2), which seemed to be the result of a higher xylose conversion rate at Sejet site (75.31%) compared with Abed site (72.35%) (Table 1). Sites also had highly significant effects on the botanical distribution of plant material and the grain yield (Table 2), with cultivars at Abed producing more leaves and grain, while cultivars from Sejet produced more stem (Table 1). Less significant site effects were seen for the amount of lignin (Table 2). Blocks in the experiment showed a highly significant effect on the convertibility of xylan, and significant effects on the amount of hemicellulose and convertibility of glucan and total sugars (Table 2). These differences were caused mainly by differences between the two blocks at the Abed site, where one block had a low conversion of both xylan and glucan and a higher content of hemicellulose compared with the other block. Hence, local growth conditions affected chemical composition, sugar yields and botanical distribution, but these block effects were numerically very small, only significant because of their many replications in the experiment.

### 3.2. *Effects of cultivars on convertibility and sugar release*

Cultivar significantly affected the convertibility of xylan and total sugars plus the release of monomeric and total sugars (Table 2). Cultivar was also influential for the glucan conversion but at a less significant level. Table 3 lists the average values of the cultivars for selected traits.

Low sugar yielding cultivars were for example Dinosor, Glasgow, Robigus and Tuscan, while Ambition, Flair, and Inspiration were some of the best performing cultivars (Table 3). The ability of the cultivar to release sugar for ethanol production was not related to the grain yield, illustrated by the fact that for instance both Robigus and Inspiration were high grain yielding cultivars (Table 3). The maximum difference in total sugar release was found between cultivars Flair and Tuscan at Abed site (0.092 g g$^{-1}$ dm). The corresponding minimum and maximum ethanol yield from these cultivars can be estimated by assuming a 72% dry matter recovery after pretreatment of wheat straw [17] and equal fermentation efficiency (0.51 conversion factor) of the released sugar in both straw types. Under these circumstances, ethanol yields would range from 161 to 203 L ethanol t$^{-1}$ dm, potentially increasing the ethanol production by 26% by selecting the best performing cultivar. The results obtained from screening these cultivars in the small-scale HTPH system are consistent with the ethanol yields of approximately 153 L ethanol t$^{-1}$ dm wheat straw reported in pilot scale mass balance calculations of Kaparaju et al. [17].

Table 4 displays the cultivar and environmental correlation coefficients between all pairs of traits. The cultivar correlation coefficient between lignin and plant height could not be calculated due to a negative variance component estimate.

Total sugar release showed a strong negative cultivar correlation with ash content ($r = -0.99^{***}$), and as expected total sugar release was positively correlated with straw content of cellulose ($r = 0.37^{*}$) and hemicellulose ($r = 0.57^{**}$),

Please cite this article in press as: Lindedam J, et al., Cultivar variation and selection potential relevant to the production of cellulosic ethanol from wheat straw, Biomass and Bioenergy (2012), doi:10.1016/j.biombioe.2011.12.009

**Table 2 – Mean square and level of significance for 17 variables related to site, block within site and cultivar effect and model error.**

Mean square

Chemical composition

| | DF | Cell % | Hemi % | Lignin % | Ash % |
|---|---|---|---|---|---|
| Site | 1 | 3.2 | 1.0 | 2.9* | 1.9 |
| Block (site) | 2 | 5.4 | 7.3** | 1.2 | 1.6 |
| Cultivar | 19 | 7.2*** | 4.2*** | 0.7 | 1.8*** |
| Error | 56 | 1.8 | 1.0 | 0.5 | 0.6 |

Conversion to sugar (%) and release of sugar (g $g^{-1}$ dm)

| | DF | C5 % | C6 % | TS % | C5 g $g^{-1}$ dm | C6 g $g^{-1}$ dm | TS g $g^{-1}$ dm |
|---|---|---|---|---|---|---|---|
| Site | 1 | 162*** | 0 | 20 | $8 \times 10^{-4}$*** | $1 \times 10^{-4}$ | $2 \times 10^{-3}$** |
| Block (site) | 2 | 64*** | 71** | 62* | $2 \times 10^{-6}$ | $4 \times 10^{-4}$* | $4 \times 10^{-4}$ |
| Cultivar | 19 | 39*** | 26* | 30*** | $2 \times 10^{-4}$*** | $3 \times 10^{-4}$*** | $1 \times 10^{-3}$*** |
| Error | 56 | 7 | 12 | 9 | $2 \times 10^{-5}$ | $9 \times 10^{-5}$ | $1 \times 10^{-4}$ |

Plant characteristics

| | DF | Leaves % | Ears % | Stem % | L:S ratio | Lodging % | Height[a] cm | Grain $Mg^{-1}$ ha |
|---|---|---|---|---|---|---|---|---|
| Site | 1 | 93*** | 100*** | 384*** | 0.02*** | 10 | – | 4.53*** |
| Block (site) | 2 | 7 | 0 | 11 | $1 \times 10^{-3}$ | 2 | 22 | 0.10 |
| Cultivar | 19 | 13*** | 13* | 31*** | $2 \times 10^{-3}$*** | 21*** | 44* | 1.39*** |
| Error | 56 | 4 | 7 | 10 | $7 \times 10^{-4}$ | 5 | 18 | 0.26 |

Analysis done over 79 observations. Significance values are marked with *** ($P = 0.001$), ** ($P = 0.01$) and * ($P = 0.05$). Cell = Cellulose, Hemi = Hemicellulose, C5%, C6% and TS% = conversion of xylan, glucan or total sugar (xylan plus glucan) in percentage of the maximum available in raw material. L:S is the ratio of leaves-to-stem.

a Height data only exist on Sejet, therefore this model only contain 39 observations and 1 DF (degrees of freedom) on block effect and error has 18 DF.

**Table 3 – Average chemical content, conversions, and release of sugar in the HTPH system of 20 wheat straw cultivars.**

| | Cell % | Hemi % | TS % | TS g $g^{-1}$ dm | Grain Mg $ha^{-1}$ |
|---|---|---|---|---|---|
| Abika | 37.0 (0.3) | 26.3 (0.4) | 63.9 (3.5) | 0.40 (0.0) | 9.14 (0.4) |
| Ambition | 36.8 (1.2) | 25.6 (0.5) | 66.0 (2.6) | 0.41 (0.0) | 9.57 (0.7) |
| Audi | 36.9 (0.7) | 25.5 (0.7) | 64.0 (2.6) | 0.39 (0.0) | 9.49 (0.6) |
| Dinosor | 36.6 (1.6) | 24.7 (0.6) | 59.7 (3.1) | 0.36 (0.0) | 9.51 (0.8) |
| Flair | 37.4 (1.7) | 26.1 (0.2) | 67.4 (2.5) | 0.42 (0.0) | 8.32 (0.3) |
| Florett | 38.0 (2.4) | 26.8 (2.3) | 62.0 (5.8) | 0.39 (0.0) | 7.90 (0.8) |
| Glasgow | 37.9 (3.0) | 26.0 (2.4) | 57.4 (4.6) | 0.36 (0.0) | 9.48 (0.7) |
| Hattrick | 37.8 (0.7) | 26.0 (0.7) | 61.1 (3.5) | 0.38 (0.0) | 9.58 (0.4) |
| Inspiration | 39.7 (1.3) | 26.9 (1.6) | 62.1 (3.9) | 0.41 (0.0) | 9.83 (0.3) |
| Jenga | 38.7 (1.9) | 27.4 (1.7) | 61.2 (4.8) | 0.40 (0.0) | 9.53 (0.2) |
| Oakley | 37.6 (0.7) | 26.0 (0.8) | 60.2 (2.3) | 0.38 (0.0) | 10.4 (0.3) |
| Opus | 36.5 (0.9) | 25.1 (0.8) | 64.2 (2.5) | 0.39 (0.0) | 9.58 (0.5) |
| Penso | 35.3 (0.9) | 25.0 (0.9) | 66.0 (1.5) | 0.39 (0.0) | 8.92 (0.4) |
| Potenzial | 36.4 (1.2) | 25.5 (0.6) | 63.1 (1.6) | 0.39 (0.0) | 9.14 (0.8) |
| Robigus | 35.9 (0.2) | 24.0 (0.6) | 61.5 (1.3) | 0.36 (0.0) | 9.90 (0.5) |
| Samyl | 36.6 (0.5) | 25.3 (0.3) | 63.6 (0.9) | 0.39 (0.0) | 9.39 (0.8) |
| Skalmeje | 34.4 (1.3) | 23.5 (0.5) | 66.7 (3.0) | 0.38 (0.0) | 9.61 (0.4) |
| Smuggler | 35.1 (0.9) | 24.8 (0.3) | 67.8 (3.0) | 0.40 (0.0) | 9.41 (0.4) |
| Tommi | 35.1 (1.0) | 24.1 (0.5) | 66.4 (2.8) | 0.39 (0.0) | 8.34 (0.2) |
| Tuscan | 35.1 (1.0) | 24.5 (0.2) | 62.4 (4.0) | 0.37 (0.0) | 8.93 (0.2) |

Standard deviations are given in parenthesis ($n = 4$). Cell = Cellulose, Hemi = Hemicellulose, TS% = conversion of total sugar (xylan plus glucan) in percentage of the maximum available in raw material. TS g $g^{-1}$ dm = release of total sugar (xylose plus glucose) in gram per gram dry matter biomass. Grain = grain yield.

conversion of xylan and glucan ($r = 0.57$** and 0.66***, respectively) as well as separate release of xylose and glucose ($r = 0.99$***). Conversion rates of xylan and glucan and total conversion rate show strong negative cultivar correlation with ash content and content of cellulose in the cultivars, indicating less efficient conversion with higher cellulose components.

A prominent positive cultivar correlation of $r = 0.60$** is seen between total sugar release and plant height (Table 4), which may be the result of higher conversion of xylan ($r = 0.78$***) and glucan ($r = 0.86$***) from taller cultivars. Environmental correlations with total sugar release are dominated by the inherent positive correlations with conversion rates and release of separate sugars. In addition, environmental factors increasing percentage of ears ($r = 0.36$**) and stem ($r = -0.33$*) increase and decrease total sugar release, respectively. The only apparently undesirable cultivar correlation in this study is the negative cultivar correlation ($r = -0.60$**) between grain yield and conversion ratio of glucan (C6), indicating that sugars are less efficiently converted from higher grain yielding cultivars. The effect, however, is not strong enough to manifest itself in a significant cultivar correlation ($r = -0.32$ ns) between grain yield and total sugar release (Table 4). Obviously, the cultivar effects observed in this trail only apply to the particular year and the environmental conditions of this year and sites. However, we believe that results could be extended to other wheat growing sites in Denmark as climate variations within the country are reasonably small. Effectively accounting for differences caused by interactions between cultivar and year or cultivar and sites would require straw collection over multiple years and geographical locations.

Please cite this article in press as: Lindedam J, et al., Cultivar variation and selection potential relevant to the production of cellulosic ethanol from wheat straw, Biomass and Bioenergy (2012), doi:10.1016/j.biombioe.2011.12.009

**Table 4 – Environmental correlation coefficients (above the diagonal) and cultivar correlation coefficients (below the diagonal) among traits for 79 wheat straw samples.**

| Traits | Lignin % | Ash % | Cell % | Hemi % | C5 % | C6 % | TS % | C5 g g$^{-1}$ dm | C6 g g$^{-1}$ dm | TS g g$^{-1}$ dm | Leaves % | Ears % | Stem % | L:S ratio | Height cm | Lodging | Grain Mg ha$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lignin % | **10%** | −0.43*** | −0.09 | 0.03 | 0.19 | 0.16 | 0.19 | 0.31* | 0.12 | 0.20 | −0.27* | 0.23 | −0.01 | −0.25 | 0.40 | −0.00 | −0.02 |
| Ash % | 0.17 | **34%**** | −0.04 | −0.23 | −0.04 | −0.08 | −0.08 | −0.37** | −0.13 | −0.23 | 0.03 | −0.28* | 0.21 | 0.004 | 0.08 | 0.02 | 0.23 |
| Cell % | −1.00*** | −0.42* | **43%**** | 0.84*** | −0.69*** | −0.67*** | −0.73*** | 0.16 | −0.14 | −0.05 | 0.24 | −0.28* | 0.07 | 0.21 | 0.15 | −0.08 | −0.11 |
| Hemi % | −1.00*** | −0.61** | 0.93*** | **45%**** | −0.74*** | −0.53*** | −0.63*** | 0.29* | −0.10 | 0.03 | 0.27* | 0.12 | −0.28* | 0.30* | 0.12 | −0.05 | −0.04 |
| C5 % | 0.77*** | −0.50** | −0.58** | −0.36 | **53%**** | 0.70*** | 0.85*** | 0.41** | 0.43*** | 0.48*** | −0.36** | 0.13 | 0.13 | −0.35** | 0.06 | 0.10 | −0.03 |
| C6 % | 0.49* | −0.61** | −0.45* | −0.24 | 1.00*** | **24%*** | 0.97*** | 0.31* | 0.82*** | 0.74*** | −0.01 | 0.39** | −0.31* | 0.03 | −0.04 | 0.19 | 0.08 |
| TS % | 0.60** | −0.56** | −0.50** | −0.28 | 1.00*** | 1.00*** | **38%**** | 0.37** | 0.74*** | 0.70*** | −0.13 | 0.34** | −0.19 | −0.10 | 0.01 | 0.18 | 0.05 |
| C5 g g$^{-1}$ dm | −0.27 | −0.97*** | 0.24 | 0.51* | 0.62** | 0.75*** | 0.69*** | **71%**** | 0.52*** | 0.76*** | −0.15 | 0.38** | −0.21 | −0.10 | 0.18 | 0.10 | −0.11 |
| C6 g g$^{-1}$ dm | −0.56** | −1.00*** | 0.49* | 0.63** | 0.51* | 0.56** | 0.53** | 0.96*** | **37%**** | 0.95*** | 0.16 | 0.29* | −0.34** | 0.19 | 0.08 | 0.21 | 0.02 |
| TS g g$^{-1}$ dm | −0.42* | −0.99*** | 0.37* | 0.57** | 0.57** | 0.66*** | 0.62** | 0.99*** | 0.99*** | **57%**** | 0.06 | 0.36** | −0.33* | 0.11 | 0.14 | 0.19 | −0.03 |
| Leaves % | 0.35* | −0.04 | −0.01 | 0.09 | −0.28 | −0.06 | −0.16 | −0.21 | −0.05 | −0.13 | **33%**** | −0.10 | −0.57*** | 1.0*** | −0.20 | 0.14 | −0.05 |
| Ears % | −1.00*** | −0.08 | 0.43* | 0.14 | −0.06 | −0.08 | −0.08 | 0.04 | 0.30 | 0.17 | 0.48* | **19%*** | −0.76*** | 0.02 | −0.12 | 0.04 | 0.05 |
| Stem % | 0.33* | 0.07 | −0.22 | −0.13 | 0.20 | 0.08 | 0.14 | 0.11 | −0.13 | −0.01 | −0.88*** | −0.84*** | **31%**** | −0.67*** | 0.20 | −0.12 | −0.01 |
| L:S ratio | 0.27 | −0.06 | 0.00 | 0.08 | −0.25 | −0.04 | −0.14 | −0.19 | −0.02 | −0.11 | 1.0*** | 0.53** | −0.91*** | **34%**** | −0.22 | 0.15 | −0.01 |
| Height | N.D | −0.10 | −0.11 | −0.21 | 0.78*** | 0.86*** | 0.81*** | 0.38* | 0.72*** | 0.60** | 0.27 | 0.06 | −0.22 | 0.30 | **26%*** | 0.58*** | 0.54* |
| Lodging | −0.36* | −0.31 | 0.47* | 0.58** | −0.34 | −0.65*** | −0.48* | 0.15 | −0.20 | −0.02 | −0.04 | −0.20 | 0.13 | −0.08 | −0.40* | **46%**** | −0.10 |
| Grain | 0.03 | 0.31 | 0.24 | −0.03 | −0.36 | −0.60** | −0.49* | −0.28 | −0.35 | −0.32 | −0.18 | 0.10 | 0.05 | −0.19 | −0.11 | −0.17 | **53%**** |

Heritability is given diagonally for each trait. Significance on heritability is the significance of cultivar in the ANOVA F-test on each trait, also presented in Table 2. Coefficients marked with * is significant at $P = 0.05$, ** at $P = 0.01$ and *** at $P = 0.001$. N.D. = correlation coefficient could not be determined.

### 3.3. *Chemical compositions in correlation with sugar yield*

Cultivar selection for increased conversion rate of glucan, xylan, and total sugar would lead to a reduction of ash and cellulose content, but an overall increase in sugar release based on dry matter (Table 4). Similarly negative influences of cellulose content on conversion were reported by Habib et al. [18] on in vitro digestibility of 15 wheat straw cultivars and on theoretical ethanol yield of maize stover [4]. A possible explanation for this relationship could be that higher cellulose content is strongly associated with higher hemicellulose content (Table 4). Numerous authors have shown that hemicellulose has the ability to restrict enzymatic access to cellulose [19,20], and since we are not adding extra xylanases in the experiment, hemicellulose could indirectly cause the low sugar convertibility at high cellulose levels.

In a study of fundamental factors affecting biomass enzymatic reactivity, Chang and Holtzapple [21] found enzymatic digestibility of wheat straw inversely correlated with lignin content. Likewise, we found a negative cultivar correlation between lignin and sugar release (Table 4). It should be noted that finding significant correlations between lignin and HTPH data, even though lignin content did not significantly differ between cultivars in the present study (Table 2), can only be explained by assuming that the wet chemistry method for measuring lignin was insufficiently precise in recording cultivar differences, whereas lignin differences might still have existed and affected the HTPH sugar results for the cultivars. Surprisingly, results showed a positive cultivar correlation between sugar conversion rates and lignin content (Table 4). We have speculated that this positive correlation is an artifact caused by the use of the same enzyme loading to all samples. Since high lignin contents are naturally associated with lower cellulose and hemicellulose contents, these samples could have a relative higher conversion due to higher enzyme-to-substrate ratio. The lignin content in the material used by Chang and Holtzapple [21] ranged from 1% to 26%, whereas lignin content in our wheat cultivars ranged from 17% to 23%. Most correlations with lignin in this study are therefore believed to be hampered with numerical problems, perhaps because the cultivar variation for lignin content was non-significant (Table 2).

Summarizing the results in Table 4, we found that high amounts of carbohydrates influenced the sugar release positively provided that the accessibility to cellulose is maintained by proper pretreatment or enzyme mixtures. Furthermore, high contents of ash influenced sugar release in a negative manner.

### 3.4. *Does distribution of botanical parts affect the sugar yield?*

Previous reports on ruminal digestibility of wheat straw have confirmed that botanical fractions differ in digestibility in the order leaves > chaff, nodes > internodes [22,23]. The literature results on the contribution of the differences in degradability of separate leaf and stem versus the differences in their proportion within the plants are contradictory but well presented in Ramanzin et al. [24]. As an example, Anderson et al.

Please cite this article in press as: Lindedam J, et al., Cultivar variation and selection potential relevant to the production of cellulosic ethanol from wheat straw, Biomass and Bioenergy (2012), doi:10.1016/j.biombioe.2011.12.009

[25] found that three of five grasses pretreated with sulfuric acid did not yield significantly different ethanol levels from leaf and stem, while leaf gave significantly more ethanol than stem from the remaining two species. The authors contemplated that the species-specific leaf and stem sugar yield was more important in predicting the overall sugar yield than the leaf-to-stem ratio *per se*. Our data could support this since cultivar correlations between leaf-to-stem ratio and all sugar yields were insignificant (Table 4). However, in the environmental correlations there was consistently at least one botanical trait (leaves, ears, stem or L:S ratio) that significantly affected the sugar conversion or sugar release (Table 4). Such environmental effects may well be the reason for diverging reports in the literature on the performance of botanical fractions in enzymatic hydrolysis.

Both sugar conversion rates and sugar releases in this study had a clear positive correlation with plant height (Table 4). Proportionally, taller plants should have more stem than shorter plants and this would theoretically result in lower digestibility [8]. However, we found no clear correlations between plant height and any of the botanical components or L:S ratio in this study (Table 4) and the effect of plant height on sugar yield was therefore deemed a result of differences in structure. Since taller plants had unchanged content of carbohydrates but still released more sugar based on dry matter, we must conclude that taller plants had better sugar conversions due to something not measured, such as looser cell wall structures because of rapid growth. Consequently, taller plants had a preferred structure for pretreatment or enzymatic hydrolysis, which impact conversion to sugar positively compared to shorter plants.

### 3.5. *Selection of cultivars with improved sugar release*

Enzymatic digestibility of wheat straw has previously been studied for 106 winter wheat cultivars grown at the same two sites the year before our harvest, and the heritability in narrow sense was estimated to approximately 29% [5]. For most traits the present study showed higher heritability, including very promising estimates of 53%, 71%, 37% and 57% for grain yield, sugar release of xylose, glucose, and the sum of the two, respectively. This would translate into a good gain of selection toward improved sugar release, and the estimated coefficient of cultivar correlations provide strong evidence that such breeding could be accomplished with few adverse effects due to the observed small undesirable cultivar correlations between traits (Table 4). Selection for high total sugar release would be genetically disposed for producing progeny with more carbohydrates and less ash. Such selection would not influence the grain yield but would be promoted by increased plant height.

We concluded that by selecting the best performing cultivars from within this relatively narrow range of commercially grown cultivars, there was a potential total sugar gain from the progeny of 15% (26% sugar difference * 57% heritability). Since the economic value of products from the straw is still low compared to grain value, grain yield and associated traits will still be the primary goal for breeding of new wheat cultivars. However, if a method to efficiently assess the quality of straw for sugar release could be provided, then the selection of cultivars with high release of sugars may be included in practical breeding and could impart additional value to the straw.

## 4. Conclusions

This study demonstrated the presence of considerable varietal differences in sugar yield from 20 wheat straw cultivars. As such, we found a difference in total sugar release based on dry matter of up to 26%. Cellulose content was found to be negatively correlated to the sugar conversion efficiency, but enhanced the overall sugar release. Generally, high amounts of carbohydrates influenced the sugar release positively, provided that the accessibility to cellulose was maintained, while high amounts of ash influenced sugar release negatively. Our study supports the theory that cultivar-specific relationships of leaf and stem sugar yield is more important in predicting the overall sugar yield than the leaf-to-stem ratio *per se*. Taller plants had a preferred structure for pretreatment and enzymatic hydrolysis, which impact conversion to sugar positively compared to shorter plants. Cultivar selection for an improved biofuel feedstock within wheat straw is possible, with a total sugar release heritability of 57%. No relationship between grain yield and sugar release was found, which suggests that it should be possible to select wheat cultivars that produce high quality straw without scarifying grain yield.

## Acknowledgments

The current project was funded through the OPUS project (case file 2117-05–0064) funded by the Danish Strategic Research Council. The extensive work of measuring the chemical composition of all wheat materials was done by the lab technicians at Dept. of Forest & Landscape, University of Copenhagen. Support by the Office of Biological and Environmental Research in the DOE Office of Science for the BioEnergy Science Center (BESC) was essential to the development of the HTPH system applied in this research. The Ford Motor Company is also acknowledged for funding the Ford Motor Company Chair in Environmental Engineering at the University of California, Riverside that makes possible many research projects such as this one possible.

## REFERENCES

[1] Wyman CE. What is (and is not) vital to advancing cellulosic ethanol. Trend Biotechnol 2007;25(4):153–7.
[2] Studer MH, DeMartini JD, Brethauer S, McKenzie HL, Wyman CE. Engineering of a high-throughput screening system to identify cellulosic biomass, pretreatments, and enzyme formulations that enhance sugar release. Biotechnol Bioeng 2010;105(2):231–8.
[3] Decker SR, Brunecky R, Tucker MP, Himmel ME, Selig MJ. High-throughput screening techniques for biomass conversion. Bioenergy Res 2009;2(4):179–92.

[4] Isci A, Murphy PT, Anex RP, Moore KJ. A rapid simultaneous saccharification and fermentation (SSF) technique to determine ethanol yields. Bioenergy Res 2008;1(2):163–9.
[5] Jensen JW, Magid J, Hansen-Møller J, Andersen SB, Bruun S. Genetic variation in degradability of wheat straw and potential for improvement through plant breeding. Biomass Bioenergy 2011;35(3):1114–20.
[6] Lewis MF, Lorenzana RE, Jung HJG, Bernardo R. Potential for simultaneous improvement of corn grain yield and stover quality for cellulosic ethanol. Crop Sci 2010;50(2):516–23.
[7] Lorenzana RE, Lewis MF, Jung HJG, Bernardo R. Quantitative trait loci and trait correlations for maize stover cell wall composition and glucose release for cellulosic ethanol. Crop Sci 2010;50(2):541–55.
[8] Capper BS. Genetic-variation in the feeding value of cereal straw. Anim Feed Sci Technol 1988;21(2–4):127–40.
[9] Tolera A, Tsegaye B, Berg T. Effects of variety, cropping year, location and fertilizer application on nutritive value of durum wheat straw. J Anim Physiol Anim Nutr 2008;92(2):121–30.
[10] Duguid KB, Montross MD, Radtke CW, Crofcheck CL, Shearer SA, Hoskinson RL. Screening for sugar and ethanol processing characteristics from anatomical fractions of wheat stover. Biomass Bioenergy 2007;31(8):585–92.
[11] Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, et al. Determination of structural carbohydrates and lignin in biomass. National renewable energy laboratory 1617 Cole Boulevard. Golden, Colorado: National Renewable Energy Laboratory; 2008. Apr, 15 pp., Report No. TP 510 42618.
[12] Overend RP, Chornet E. Fractionation of lignocellulosics by steam-aqueous pretreatments. R Soc Lond Philos Trans Ser A Math Phys Eng Sci 1987;321(1561):523–36.
[13] SAS Institute. SAS/STAT user's guide, release 6.03 edition. Cary, NC: SAS Institute Inc; 1988. pp.1028.
[14] Sleper DA, Poehlman JM. Quantitative inheritance in plant breeding. Breeding field crops. 5th ed. Ames: Wiley-Blackwell; 2006. Blackwell publishing professional 2121 State AvenueIowa 50014pp.53–67.
[15] Jørgensen H, Vibe-Pedersen J, Larsen J, Felby C. Liquefaction of lignocellulose at high-solids concentrations. Biotechnol Bioeng 2007;96(5):862–70.
[16] Thomsen MH, Thygesen A, Thomsen AB. Hydrothermal treatment of wheat straw at pilot plant scale using a three-step reactor system aiming at high hemicellulose recovery, high cellulose digestibility and low lignin hydrolysis. Bioresour Technol 2008;99(10):4221–8.
[17] Kaparaju P, Serrano M, Thomsen AB, Kongjan P, Angelidaki I. Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. Bioresour Technol 2009;100(9):2562–8.
[18] Habib G, Shah SBA, Inayat K. Genetic-variation in morphological-characteristics, chemical-composition and in-vitro digestibility of straw from different wheat cultivars. Anim Feed Sci Technol 1995;55(3–4):263–74.
[19] Berlin A, Maximenko V, Gilkes N, Saddler J. Optimization of enzyme complexes for lignocellulose hydrolysis. Biotechnol Bioeng 2007;97(2):287–96.
[20] Selig MJ, Knoshaug EP, Adney WS, Himmel ME, Decker SR. Synergistic enhancement of cellobiohydrolase performance on pretreated corn stover by addition of xylanase and esterase activities. Bioresour Technol 2008;99(11):4997–5005.
[21] Chang VS, Holtzapple MT. Fundamental factors affecting biomass enzymatic reactivity. Appl Biochem Biotechnol 2000;84-6:5–37.
[22] Shand WJ, Ørskov ER, Morrice LAF. Rumen degradation of straw .5. Botanical fractions and degradability of different varieties of oat and wheat straws. Anim Prod 1988;47:387–92.
[23] Ramanzin M, Ørskov ER, Tuah AK. Rumen degradation of straw .2. Botanical fractions of straw from 2 barley cultivars. Anim Prod 1986;43:271–8.
[24] Ramanzin M, Bailoni L, Beni G. Varietal differences in rumen degradation of barley, wheat and hard wheat straws. Anim Prod 1991;53:143–50.
[25] Anderson WF, Dien BS, Brandon SK, Peterson JD. Assessment of bermudagrass and bunch grasses as feedstock for conversion to ethanol. Appl Biochem Biotechnol 2008;145(1–3):13–21.

Please cite this article in press as: Lindedam J, et al., Cultivar variation and selection potential relevant to the production of cellulosic ethanol from wheat straw, Biomass and Bioenergy (2012), doi:10.1016/j.biombioe.2011.12.009