Available online at www.sciencedirect.com

ScienceDirect

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

Diverse cell wall composition and varied biomass digestibility in wheat straw for bioenergy feedstock

Zhiliang Wu [a,b,c], Huanhuan Hao [a,b,c], Zahoor [a,b,c], Yuanyuan Tu [a,b,c], Zheng Hu [a,b,c], Feng Wei [a,b,c], Yangyang Liu [a,b,c], Yuxia Zhou [c], Yanting Wang [a,b,c], Guosheng Xie [a,b,c], Chunbao Gao [d], Xiwen Cai [e], Liangcai Peng [a,b,c], Lingqiang Wang [a,b,c,*]

[a] National Key Laboratory of Crop Genetic Improvement, Huazhong Agriculture University, Wuhan 430070, China
[b] Biomass and Bioenergy Research Centre, Huazhong Agriculture University, Wuhan 430070, China
[c] College of Plant Science and Technology, Huazhong Agriculture University, Wuhan 430070, China
[d] Institute of Food Crops, Hubei Academy of Agricultural Sciences, 3 Nanhu Road, Wuhan 430064, China
[e] Department of Plant Science, North Dakota State University, Loftsgard Hall, P.O. Box 6050, Fargo, ND 58108, USA

ARTICLE INFO

Article history:
Received 14 January 2014
Received in revised form
30 May 2014
Accepted 22 August 2014
Available online 18 September 2014

Keywords:
Wheat straw
Cell wall
Sugar release
Germplasm
Chemical pretreatment
Enzymatic digestibility

ABSTRACT

Wheat straw has a vast potential as feedstock for biofuel production in China. However, little information is available regarding variation in cell wall composition and enzymatic digestibility of wheat straw. This study investigated cell wall compositions and biomass digestibility of the straw of 115 wheat accessions from central China using a 1% NaOH pretreatment and mixed enzymatic hydrolysis. Significant variation in cell wall composition and sugar release was observed, with a coefficient variation (CV) ranging from 4.7% to 21.2%. Cellulose, hemicelluloses, alkali detergent hemicelluloses (ADH), and acid insoluble lignin (AIL) positively correlated with each other, and they all negatively correlated with acid soluble lignin (ASL). Hexose yields had a negative correlation with ADH and AIL, and positive correlation with ASL. No apparent undesirable correlation was found between sugar release and grain yield, thus yield and biomass convertibility can be potentially improved simultaneously. Furthermore, the features of the cell wall constitutions were compared with other plants and their implication in determining the best possible conversion strategy was discussed. This initial study is essential to understand the cell wall composition of wheat straw and to explore the potential of wheat straw as feedstock for biofuel production.

1. Introduction

Biomass utilization is increasingly considered as a practical way of creating a sustainable energy supply while considering a long-term protection of the environment around the world. Biofuel production from lignocellulosic feedstock, oil plants and microalgae represents a promising source of renewable energy worldwide [1–4]. Lignocellulosic biomass can be acquired from dedicated biomass crops, or from agricultural and

* *Corresponding author*. Biomass and Bioenergy Research Centre, Huazhong Agriculture University, Wuhan 430070, China. Tel.: +86 27 87281765; fax: +86 27 87280016.
E-mail address: lqwang@mail.hzau.edu.cn (L. Wang).
http://dx.doi.org/10.1016/j.biombioe.2014.08.025
0961-9534/

forestry residuals. In China, approximately 700–900 million tons of agricultural residues are produced each year, half of which could be potential feedstock for biofuel production. Among these agricultural residues, nearly 75% of biomass resources were produced by rice, wheat and maize, three major food crops [5,6].

Because lignocellulosic biomass is made up of the complex structures of cellulose, hemicelluloses and lignin, such feedstock is highly resistant to bioconversion of its carbohydrates into ethanol, biochemical, or byproducts [7–9]. Pretreatment of lignocellulosic materials before saccharification and yeast fermentation is of great importance to break down the recalcitrance. In past years, much progress has been achieved in lignocellulose pretreatment technologies such as thermal pretreatment, acid pretreatment, lime pretreatment, oxidative pretreatment, and ammonia recycling percolation [10,11]. Despite their efficiency in unlocking cellulosic resources, most of the pretreatment technologies are energy-intensive, some of which even caused secondary environmental pollution [12,13]. Recent results have indicated that only a mild pretreatment is necessary in an industrial economically feasible system and some studies have demonstrated that dilute alkaline pretreatment is very effective for enzymatic saccharification of agricultural residues [14,15]. Much research on alkaline pretreatment focused on the optimal reaction conditions and reagent loading levels under higher temperatures or pressures for specific cellulosic biomass [16,17]. Few studies were conducted to compare the effects of the pretreatment methods on different cellulosic biomass. In essence, the lignocellulose conversion rate is determined by the wall polymer features and interaction styles such as cellulose crystallinity and lignin linking-styles [18–21]. The effect of the pretreatments largely depends on biomass composition and operating conditions [11]. The pretreatment methods should work for a wide range of lignocellulosic materials to provide a cellulosic stream that can be efficiently hydrolyzed with low concentrations of enzyme [22].

While optimizing processing techniques will initially pave the path to reduce biofuel production costs and increase ethanol yield of feedstock, further enhancement of the economics may be attained by improving feedstock quality [23]. To explore the potential of crop straw for biofuel production, it is essential to investigate the cell wall composition and capacity for producing fermentable sugars from straw of different cultivars and to estimate correlations between these traits. Recently, several studies on glucose release and stover or straw quality for cellulosic ethanol production have been published in maize and wheat [24–26]. It was found in corn stover samples that the cultivar variation of ethanol yield ranged between 45% and 73%, and it showed a strong negative correlation with lignin content [24]. Considerable differences in enzymatic digestibility were also found in wheat [25], the moderate heritability was observed for the sugar release from wheat straw that did not show an obvious adverse correlation to agronomic traits [26]. Therefore, screening wheat cultivars for less recalcitrance to enzymatic hydrolysis following pretreatment will facilitate selection of cultivars with improved biofuel feedstock of wheat straw.

China is the largest wheat producing country in the world. A total of 126.6 million tons wheat straw was harvested in 2009. The wheat straw accounted for 15.7% of the total yield of crop residues, which would be a huge source of crop residual biomass with a vast potential as feedstock for biofuel production in China [6]. Also there is a great genetic diversity in 11,694 wheat landraces and 11,441 cultivars in China [27,28]. Although the wall polymer features of wheat straw have been recently reported as predominant factors on biomass enzymatic digestibility [29], it remains obscured about the relationships among agronomic traits, cell wall composition and biomass saccharification, due to lack of large population of wheat biomass resource. In this study, hence, we performed an initial large-scale analysis of the traits including six agronomic traits, three major wall polymers, and sugar (hexoses and pentoses) release after alkali pretreatment and enzymatic hydrolysis in 115 wheat germplasm collections from central China, and then evaluated their correlations and breeding potential.

2. Materials and methods

2.1. Plant materials

A total of 115 wheat germplasm accessions from the collection at Hubei Agricultural Science Institute in Hubei Province, China, which represent a wheat gene pool adapted to central China and Yangzi River region, were planted in the experimental farm at Huazhong Agricultural University, Wuhan, China. Field management essentially followed normal local wheat cropping practices. The lines were harvested individually at maturity to prevent seed pollution. The mature stem tissues were collected and dried at 50 °C after inactivation at 105 °C for 10 min. The dried tissues were ground and sieved to less than 40-mesh and stored in a dry container until use.

2.2. Determination of biomass digestibility

2.2.1. Alkali (NaOH) pretreatment

Alkali (NaOH) pretreatment and enzymatic hydrolysis of the residues were performed as previously described by Huang et al. [30] with minor modification.

NaOH pretreatment: The well-mixed powder of biomass sample (0.3 g) was added with 6 mL 1% (w/v) NaOH, shaken at 150 rpm for 2 h at 50 °C, and centrifuged at 3000 *g* for 5 min. The pellet was washed three times with distilled water, and stored at −20 °C for enzymatic hydrolysis. All supernatants were collected for determination of total sugars released, and samples with 6 mL distilled water were shaken for 2 h at 50 °C as control. All samples were carried out in biological triplicate.

2.2.2. Enzymatic hydrolysis

The pretreated biomass samples were used for enzymatic hydrolysis as described by Xu et al. [21] with minor modification. The remaining residues from various pretreatments were washed 2 times with 10 mL distilled water, and once with 10 mL mixed-cellulases reaction buffer (0.2 M acetic acid–sodium acetate, pH 4.8). The washed residues were incubated with mixed-cellulases (containing β-glucanase $\geq 2.98 \times 10^4$ U and cellulase $\geq$ 298 U and xylanase $\geq 4.8 \times 10^4$ U from Imperial Jade Bio-technology Co., Ltd) with a final enzyme concentration of 1.6 g/L for the biomass samples harvested from field or 0.8 g/

L for the samples collected from hydroculture. During the enzymatic hydrolysis, the samples were shaken under 150 rpm at 50 °C for 48 h. After centrifugation at 3000 *g* for 10 min, the supernatants were collected for determining pentoses and hexoses released from enzymatic hydrolysis. The samples in 6 mL reaction buffer were shaken for 48 h at 50 °C as control. All samples were carried out in biological triplicate.

2.2.3. *Colorimetric assay of total hexoses and pentoses*

UV–vis spectrometer (V-1100D, Shanghai MAPADA Instruments Co., Ltd., Shanghai, China) was used for collecting absorbance. Hexoses were detected using the anthrone/H_2SO_4 method [31]. Briefly, 1.0 mL of aqueous sample (containing 20–200 μg of hexoses) was added to 0.2% anthrone (2.0 mL) in concentrated H_2SO_4, mixed well, and incubated in a boiling water bath for 5 min. After cooling the sample, its absorbance was tested at 620 nm wavelength. Pentoses were detected using the orcinol/HCl method [32]. Briefly, 1.0 mL of aqueous sample (containing 20–200 μg of pentoses) was added to 6% orcinol (134.0 μL) in ethanol, followed by 0.1% $FeCl_3 \cdot 6H_2O$ (2.0 mL) in concentrated HCl, mixed well, and incubated in a boiling water bath for 20 min. After cooling, the absorbance of the sample was detected at 660 nm. Anthrone was purchased from Sigma–Aldrich Co., LLC., and ferric chloride and orcinol were obtained from Sinopharm Chemical Reagent Co., Ltd. The standard curves for hexoses and pentoses were plotted using D-glucose and D-xylose as standards (purchased from Sinopharm Chemical Reagent Co., Ltd.), respectively.

2.3. *Plant cell wall fractionation and polysaccharide determination*

The plant cell wall fractionation method was used to extract cellulose and hemicelluloses, as described by Peng et al. [33] with minor modification. The soluble sugar, lipids, starch and pectin of the samples were successively removed by potassium phosphate buffer (pH 7.0), chloroform–methanol (1:1, v/v), DMSO–water (9:1, v/v) and 0.5% (w/v) ammonium oxalate. The remaining pellet was extracted with 4 M KOH with 1.0 mg/mL sodium borohydride for 1 h at 25 °C, and the combined supernatant with two parallel, one parallel was neutralized, dialyzed and lyophilized as KOH-extractable hemicelluloses monosaccharides; and one parallel was collected for determination of free pentoses as the KOH-extractable hemicelluloses. The remaining two parallel non-KOH-extractable residues, one parallel was sequentially extracted with TFA for monosaccharides; one parallel was further extracted with H_2SO_4 (67%, v/v) for 1 h at 25 °C and the supernatants were collected for determination of free hexoses and pentoses as total cellulose and non-KOH-extractable hemicelluloses. All experiments were carried out in biological triplicate.

2.4. *Total lignin assay*

Total lignin content was determined by two-step acid hydrolysis method according to Laboratory Analytical Procedure of the National Renewable Energy Laboratory [34]. The lignin includes acid-insoluble and acid soluble lignin. The acid insoluble lignin was calculated gravimetrically after correction for ash, and the acid soluble lignin was measured by UV spectroscopy.

Acid insoluble lignin determination: 0.5 g sample was recorded as W1. The sample was extracted with benzene/ethanol (2:1, v/v) in a Soxhlet for 4 h, and then air-dried. The sample was hydrolyzed with 10 mL 72% H_2SO_4 (v/v) in shaker at 30 °C for 1.5 h. After hydrolysis, the acid was diluted to a concentration of 2.88%, and then placed in the autoclave for 1 h at 121 °C (15 psi). The autoclaved hydrolysis solution was vacuum-filtered through the previously weighed filtering crucible. The filtrate was captured in a filtering flask for acid soluble lignin. The lignin was washed free of acid with hot distilled water and the crucible and acid-insoluble residue was dried in an oven at 80 °C until constant weight was achieved. Then, the samples were removed from the oven and cool in a dry-container. The weight of the crucible and dry residue was recorded to the nearest 0.1 mg (W2). At last the dried residue was ashed in the muffle furnace at 200 °C for 30 min and 575 °C for 4 h. The crucibles and ash were weighed to the nearest 0.1 mg and recorded the weight (W3). Acid insoluble lignin (AIL) on original sample was calculated as the following: AIL (%) = (W2 − W3) × 100/W1%. Each sample was tested in biological triplicate.

Acid soluble lignin determination: The acid soluble lignin was solubilized during the hydrolysis process, and was measured by UV spectroscopy. The hydrolysis liquor obtained previously was transferred into 250 mL volumetric flask and brought up to 250 mL with 2.88% sulfuric acid. The absorbance of the sample was read at 205 nm on an UV–Vis spectroscopy (Beckman Coulter Inc., Du800), and 2.88% sulfuric acid was used as blank. The method of calculation about the amount of acid soluble lignin was as follows: ASL (%) = ($A \times D \times V$/1000 × $K \times$ W1) × 100%. *A* (absorption value), *D* (dilution ratio of the sample), *K* (absorptivity constant) = 110 L/g/cm. Total lignin (%) = ASL% + AIL%. All experiments were carried out in triplicate.

2.5. *Statistical calculation of correlation coefficients*

Superior Performance Software Systems software package (SPSS 17.0, Inc., Chicago, IL) was used for statistical analysis. Correlative analysis was performed using Spearman's rank correlation analysis at the two-sided 0.05 level of significance ($^*P < 0.05$, $^{**}P < 0.01$). The variation and regression analysis are developed using Origin 8.0 software (Microcal Software, Northampton, MA) for the best fit curve from the experimental data. This analysis used the average values calculated from all original determinations for a given trait pair.

3. Results

3.1. *Phenotypic correlation of six agronomic traits in the wheat germplasm collections*

Wheat is one of the staple crops in China. Wheat biomass production is closely related to multiple agronomic traits. In the present study, we evaluated the wheat germplasm collections for six agronomic traits, including plant height, fertile spikes per plant, spike length, kernels per spike, grain yield per plant, and 1000 grain weight, in addition to biofuel-related traits. The observed values of the six agronomic traits, along with the results of sugar release, chemical composition, and

sugar convertibility are presented in Tables 1 and 2. The means of the six agronomic traits, plant height, fertile spikes per plant, spike length, kernels per spike, grain yield per plant, 1000 grain weight, were 86.33, 5.43, 9.72, 46.64, 6.54 and 32.53 in the wheat germplasm collections, respectively. Significant phenotypic variation was detected in the agronomic traits among the accessions with a coefficient of variation (CV) ranging from 16.7% to 47.4%. Yield showed a very strong positive correlation with fertile spike per plant ($r = 0.645^{**}$), followed by kernels per spike ($r = 0.622^{**}$), while the weakest correlation was found with plant height ($r = 0.169$) (Table S1). Because plant height and fertile spike number per plant contribute largely to the yield of wheat biomass, the results of correlation analysis suggest that grain and other biomass yield can be potentially improved simultaneously.

3.2. *Variation in sugar release of the wheat germplasm collections*

Sugar release of the wheat accessions was evaluated by calculating the amount of released hexoses (C6) and pentoses (C5) as percentages of the raw biomass (%). The average value of pentoses removed after a 1% NaOH pretreatment (C5/NaOH) was 5.59%, much higher than the hexoses released (C6/NaOH, 1.98%). This indicated that alkaline pretreatment partially solubilized the hemicellulose fraction, leaving the material enriched in cellulose. The hexoses released in the pretreatment process were significantly different among the wheat cultivars in this study, though the amounts were relatively small (Table 2).

Hexoses released (C6/1.6 g/L) from pretreatment and 1.6 g/L enzyme hydrolysis was 12.98% on average, lower than the pentoses released (C5/1.6 g/L, 18.02%). The higher enzyme loading resulted in an obvious increase in sugar release for both hexoses (average 22.54%, increment 9.55%) and pentoses (average 21.37%, increment 3.35%). The increment in hexose release was 74.0% on average (ranging from 38.0% to 118.2%), more significant than in pentoses (19.0% on average). A high conversion rate (sugar convertibility) of cellulose to hexoses was achieved in a 3.2 g/L dose, with sugar convertibility ranging from 55.1% to 94.0%, while in 1.6 g/L dose the maximum conversion from cellulose to hexoses was only 54.6%. These results indicated that the cellulose can be almost completely digested into sugar in the wheat straw at the higher dose of enzymatic hydrolysis. The hexose release varied in a range of 9.7–16.9% (1.6 g/L) and 18.2–27.4% (3.2 g/L) at different enzyme doses.

Table 1 – Agronomic traits of wheat germplasm ($n = 115$).

Traits	Mean	Maximum	Minimum	SD	CV (%)
Plant height (cm)	86.33	145.1	63.2	14.87	17.2
Fertile spikes per plant	5.43	14.3	1.3	2.33	42.9
Spike length (cm)	9.72	21.0	6.2	1.62	16.7
Kernels per spike	46.64	81.7	16.8	11.19	24.0
Grain yield per plant (g)	6.54	16.7	0.6	3.10	47.4
1000 grain weight (g)	32.53	46.9	12.6	6.54	20.1

SD: standard deviation; CV (%): coefficient of variation (SD*100/mean).

With the lower loading doses, the variation in sugar release (both hexoses and pentoses) was higher than that with the higher dose as indicated by CV values, perhaps reflecting the variation in the cell wall composition. In general, a moderate variation in sugars released from wheat straw was observed. The variation of sugar released in different germplasms could be potentially used in the development of wheat cultivars with higher sugar release from straw.

3.3. *Diversity of the cell wall composition in the wheat germplasm collections*

To investigate the relationship between cell wall composition and sugar release, 58 wheat accessions were randomly selected for cell wall composition analysis. The results were presented in Table 2. The average contents of cellulose and hemicelluloses in the wheat straw of the accessions were about 31.77% and 31.27%, respectively. The hemicelluloses fraction totaled 31.27% of the dry biomass, with alkali detergent hemicelluloses (ADH) being the major component (24.27%) and the remaining 6.99% being alkali insoluble hemicelluloses (AIH). The major component of lignin was acid insoluble lignin (AIL, ranged from 16.3 to 22.6%), accounting for 19.59% out of the 21.73% total lignin on average, and the remaining was acid soluble lignin (ASL) (2.14%). The total lignin content in the wheat accessions ranged from 19.3 to 24.5%, similar to the range of 17–23% reported by Lindedam et al. [26]. These accessions exhibited a moderate variation in cell wall composition with the CV values ranging from 4.7% to 13.6%, comparable to the variation in sugar release. Compared to the agronomic traits, the variation in cell wall composition and sugar release from the wheat straw were relatively small, indicating the necessity of genetic improvement for further increasing sugar content of the wheat straw.

3.4. *Correlation among the cell wall composition, sugar yield, and agronomic traits*

We found that cellulose, hemicelluloses, alkali detergent hemicelluloses (ADH), and acid insoluble lignin (AIL) positively correlated with each other, and they all negatively correlated with acid soluble lignin (ASL) (Table 3). Similar results were obtained in maize stover that a higher cellulose content was closely associated with a higher hemicelluloses content [35]. These results suggest that cellulose, hemicelluloses (mainly ADH), and AIL might simultaneously be synthesized in secondary cell wall fraction in wheat straw. The hexoses released in pretreatment (1% NaOH) exhibited significantly negative correlation with the total lignin, AIL, and ADH (Table 4), respectively, indicating that hexose release in the pretreatment process was suppressed by these factors. A slightly negative correlation between hexoses released in the pretreatment process and cellulose content was also observed. A possible explanation for this relationship could be due to a strong positive correlation of cellulose content with the contents of hemicelluloses, and AIL (Table 3).

The hexoses yielded after enzyme hydrolysis for two enzyme loadings were negatively correlated with the alkali detergent hemicelluloses (ADH) ($r1.6 = -0.304^{*}$, $r3.2 = -0.150$), acid insoluble lignin (AIL) ($r1.6 = -0.279^{*}$, $r3.2 = -0.190$), while

Table 2 – Sugar release and cell wall composition of wheat straw.

Traits	Mean	Maximum	Minimum	SD	CV (%)
Sugar release (% dry matter)					
C6 (NaOH[a])	1.98	4.1	1.3	0.42	21.2
C5 (NaOH)	5.59	7.7	4.0	0.74	13.2
C6 (1.6 g/L[b])	12.98	16.9	9.7	1.66	12.8
C5 (1.6 g/L)	18.02	21.0	15.3	1.27	7.0
C6 (3.2 g/L)	22.54	27.4	18.2	2.13	9.5
C5 (3.2 g/L)	21.37	23.6	17.4	1.40	6.6
Increment	9.55	13.7	5.4	1.67	17.5
Increment%	75.10	118.2	38.0	17.54	23.4
Cell wall composition (% dry matter)					
Cellulose	31.77	36.4	27.5	2.04	6.4
Hemicelluloses	31.27	35.1	28.4	1.48	4.7
ADH	24.27	27.8	22.1	1.39	5.7
AIH	6.99	9.2	5.9	0.91	13.0
Lignin	21.73	24.5	19.3	1.06	4.9
ASL	2.14	2.9	1.5	0.29	13.6
AIL	19.59	22.6	16.3	1.19	6.1
Sugar convertibility (% cellulose)					
C6 convertibility (1.6 g/L)	42.08	54.6	29.6	7.21	17.1
C6 convertibility (3.2 g/L)	72.89	94.0	55.1	9.63	13.2

SD: standard deviation; CV (%): coefficient of variation (SD*100/mean); ADH: alkali detergent hemicelluloses; AIH: alkali insoluble hemicelluloses; ASL: acid soluble lignin; AIL: acid insoluble lignin.
[a] Pretreated by 1% NaOH.
[b] Concentration of enzymatic hydrolysis.

Table 3 – Correlation between the cell wall composition ($n = 58$).

	ASL	AIL	Lignin	AIH	ADH	Hemicelluloses	Cellulose
ASL	1	−0.474**	−0.273*	−0.103	−0.476**	−0.479**	−0.381**
AIL		1	0.963**	−0.032	0.308*	0.203	0.298*
Lignin			1	−0.077	0.190	0.072	0.199
AIH				1	−0.206	0.360**	0.190
ADH					1	0.790**	0.478**
Hemicelluloses						1	0.597**
Cellulose							1

* and ** indicated the significant correlation coefficient values at $P < 0.05$ and 0.01, respectively.

positively correlated with acid soluble lignin (ASL) ($r1.6 = 0.327^*$, $r3.2 = 0.220$) (Table 4, Fig. 1). We noticed that the cellulose content had a strong positive correlation with the contents of hemicellulose, and AIL in this study (Table 3). This may suggest that cellulose degradation can be affected by these factors with which cellulose is closely associated. Although hexose yield in the treatment with 3.2 g/L enzyme is also positively correlated to the contents of hemicelluloses

Table 4 – Correlation between sugar release and the cell wall composition ($n = 58$).

	ASL	AIL	Lignin	AIH	ADH	Hemicellulose	Cellulose
C6 (NaOH[a])	0.220	−0.391**	−0.362**	0.010	−0.270*	−0.140	−0.220
C5 (NaOH)	−0.060	−0.080	−0.110	−0.220	0.130	0.030	0.230
C6 (1.6 g/L[b])	0.327*	−0.279*	−0.240	0.010	−0.304*	−0.200	−0.040
C5 (1.6 g/L)	−0.080	−0.080	−0.090	−0.200	−0.070	−0.110	−0.020
C6 (3.2 g/L)	0.220	−0.190	−0.170	−0.220	−0.150	−0.210	−0.100
C5 (3.2 g/L)	−0.050	0.010	0.030	−0.240	0.250	0.150	0.100
C6 convertibility (1.6 g/L)	0.455**	−0.395**	−0.321*	−0.070	−0.434**	−0.382**	−0.363**
C6 convertibility (3.2 g/L)	0.370**	−0.319*	−0.261*	−0.295*	−0.354**	−0.468**	−0.540**

* and ** indicated the significant correlation coefficient values at $P < 0.05$ and 0.01, respectively.
[a] Pretreated by 1% NaOH.
[b] Concentration of enzymatic hydrolysis.

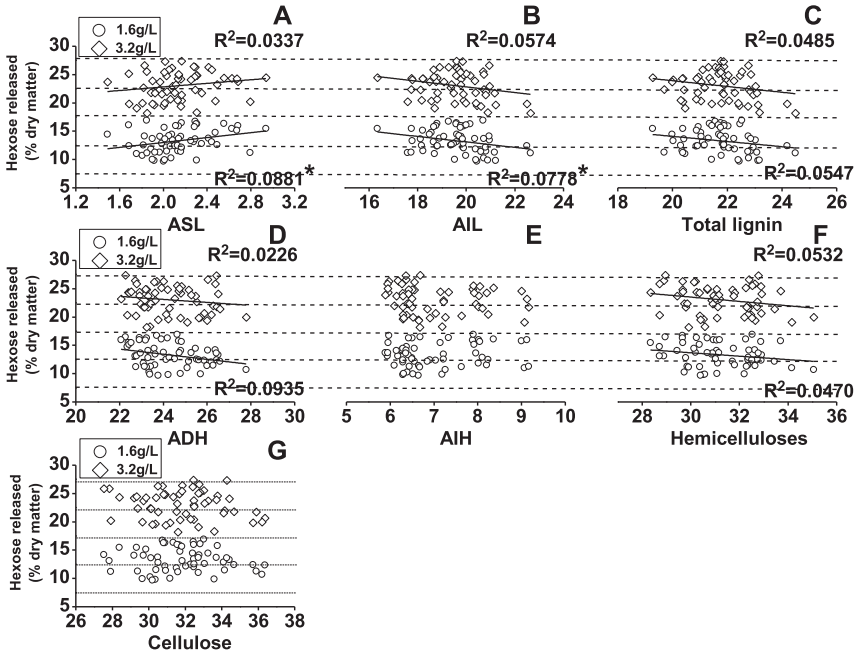

Fig. 1 – Correlation between cell wall compositions with hexose yield (% dry matter) released from enzymatic hydrolysis after NaOH pretreatment in wheat ($n = 58$). (A) ASL; (B) AIL; (C) total lignin; (D) ADH; (E) AIH; (F) hemicelluloses; (G) cellulose. * and ** indicated the significant correlation coefficient values at $P < 0.05$ and 0.01, respectively.

and lignin, the correlation coefficient is not as significant as that under the treatment with 1.6 g/L enzyme, indicating that the enhanced loading of the mixed enzymes that contain xylanase helps further remove xylans and lignin and thus impair their recalcitrance. No negative effect was found on sugar yield with the cellulose content of wheat straw in this study. We expect that removal of hemicelluloses and lignin in the cell wall of wheat straw either by chemical reagent, xylanase, or by genetic modification will be very valuable in reaching high sugar convertibility at high cellulose levels.

Almost all of the cell wall component traits, including ADH and AIL, which affect sugar yield, have no significant correlation with agronomic traits. Similarly, except for a weak negative correlation between hexoses yielded at a 1.6 g/L enzyme loading and the 1000 grain weight ($r = -0.185$*), no apparent undesirable correlation was found between sugar release and agronomic traits (Table S2). A previous study also found that the ability of the cultivar to release sugar for ethanol production was not related to the grain yield [26]. So, it implies that grain yield and biomass convertibility can be potentially improved simultaneously and commercial cultivars exhibiting improved fermentation production can be developed without sacrificing grain yield.

4. Discussion

4.1. *Variation and potential of the wheat germplasms collections for bioenergy*

This study has identified considerable variation among 115 winter wheat accessions with respect to agronomically important traits, sugar yield, and cell wall composition. For example, the high variability in hexose release between germplasms ranging from 9.7% to 16.9% (1.6 g/L) and 18.2% to 27.4% (3.2 g/L), with coefficients of variation between cultivars of 9.5% and 12.8% at two different enzyme doses. As indicated by coefficients of variation, the variation observed for the current set of winter wheat germplasms seems to be in the highest end of the range reported from other studies [24,36,37]. The reasons for lower diversity found in previous studies might be due to the selection of the specific wheat genotypes (such as cultivars with high yield and lodging resistance) and relative small size of the populations. Furthermore, the variation in straw degradability we identified in this study is comparable to that observed in a previous wheat study [26]. Also, similar results have been reported in other cereals [38–40]. These results optimistically indicate a potential for the selection of energy crop varieties in China. However, wheat straw exhibited less variation in both sugar yield and cell wall composition when compared with the agronomic traits in this study. It implies that to further increase sugar yield, genetic engineering and/or induced mutation of the selected energy crops from the best performing natural germplasm resources are necessary. The six best performing cultivars have been selected, with the average sugar release being 3.5% higher than the average and higher grain yield (Table S3). Currently, we have selected twelve materials including six best performing cultivars to re-test and optimize their performance under six different nitrogen fertilizer levels. By characterizing the wheat cultivars that are less recalcitrant to enzymatic hydrolysis following pretreatment, we may be able to identify genes and biochemical characteristics that enhance sugar yield. Ultimately, this research is expected to set a foundation for the engineering and development of superior feedstock.

4.2. Sugar release profiles of wheat straw under different enzyme dosages

In this study, saccharification was performed with two different enzyme dosages (1.6 g/L and 3.2 g/L). This allowed us to obtain a better understanding of the sugar release dynamic profile and its relationship with cell wall composition. To avert large and rapid sugar releases which could potentially mask any variance among wheat germplasms, the relatively low enzyme dose (1.6 g/L) combined with mild pretreatment (1% NaOH) was designed to better augment, and thus evaluate the diversity of the sugar releases, and to identify key cell wall components associated with the degradability. We have demonstrated that with this lower enzyme loading, a higher diversity of sugar release can be observed, at same time reflecting its strong association with cell wall composition. We can speculate that sugar release with this loading dose of enzyme is sensitive to the changes in both cell wall composition and pretreatment methods, and thus it is beneficial to determine the effect of enzyme model on cellulosic biomass conversion in future studies. For comparison, the 3.2 g/L loading was designed to maximize sugar release, and thus allow us to explore the potential of sugar release in wheat germplasm. Furthermore, the comparison of these two different loading conditions allows us to understand the function of the enzyme, yield difference and, release dynamics in two major sugar components, i.e. hexoses and pentoses. We are currently optimizing the pretreatment and enzyme loading model to reach the maximum sugar yield.

4.3. Features of cell wall composition in wheat straws

Quite limited information is currently available for the lignocellulosic biomass of wheat straw. Our studies have characterized the cell wall compositions of a large number of mature straws from wheat germplasms and showed substantial difference in contents of main cell wall constituents. The cellulose content varied between 27.5% and 36.4% while the hemicelluloses content varied in a range of 28.4–35.1% and lignin in a range of 19.3–24.5% of dry matter. The wheat germplasm collections showed similar levels of cellulose and hemicelluloses, with a mean of about 31.5% in this study. The cellulose content is lower than and the hemicellulose content is similar to some previous studies [41–43]. The total lignin content in the wheat germplasm collections was similar to those in some previous studies [26,41,42], but much higher than those in another study [43]. Such discrepancy in reported lignin contents could be partly due to the differences in the analytical procedures currently used for quantifying lignin in cell walls.

In addition, we have analyzed the main cell wall constituents of the straws from *Miscanthus* [21,44,45] and rice [29,46] with same pretreatment and analytical procedures as we used for wheat straws. It is interesting to note that straws of paddy rice are less in the contents of three main cell wall constituents, but are rich in pectins and silicon, compared with wheat straws. Wheat straws have lower or similar levels of cellulose, but higher or similar levels of lignin and hemicelluloses compared with *Miscanthus* [21,44,45,47], corn stover, and switchgrass [3]. The lignin content in wheat straws can reach to as high as that in the trees like *Eucalyptus saligna* and Monterey pine, but trees were featured in much higher levels of cellulose and much lower levels of hemicelluloses [3]. In many previous studies, the effectiveness of enzymatic saccharification on pretreated material is principally evaluated by the conversion rate of cellulose to glucose monomers. In this study, we used alkaline-based pretreatment processes and mixed enzymes containing xylanase. This also included the release of monomeric pentose (xylose and arabinose) sugars from preserved hemicelluloses. Therefore, quantifying individual sugar components in enzyme-treated hydrolyzates permits evaluation of their fermentation potential and assists in determining the best possible conversion strategy. The features of the wheat straw with relatively high contents of lignin and hemicelluloses attract attentions on their release and integrated biorefinery concept to improve the overall efficiency of biomass utilization which gained tremendous interest in recent years in production of both bio-fuels and profitable products and in environment protection [48,49].

We found a negative correlation between contents of lignin and hemicelluloses and sugar release. This result is consistent with some previous studies, showing that enzymatic digestibility of wheat straws is inversely correlated with lignin content [24,26], and ethanol yield negatively correlated with the hemicellulose and lignin content of the straw, amount of the lignin phenolics syringic acid, and coniferyl alcohol [43]. This result is also consistent with the fact that hemicelluloses have the ability to restrict enzymatic access to cellulose [39,50]. However, this result differs from a recent finding in *Miscanthus* that hemicelluloses level has a strong positive effect on lignocellulose enzymatic digestion after NaOH or H_2SO_4 pretreatment [21]. It is also different from another recent report that sugar release was positively correlated with straw content of cellulose and hemicelluloses in wheat [26]. Thus, further studies are needed to resolve the different findings in those studies. When partitioning the total lignin into acid soluble lignin and acid insoluble lignin, we found that their correlations with sugar release were reversed. It was shown that the levels of acid insoluble lignin and alkali detergent hemicelluloses were better correlated with sugar yield and convertibility than the total lignin and hemicelluloses, respectively. This suggests that acid insoluble lignin and alkali detergent hemicellulose content may be a useful indicator of sugar yield potential from wheat straws.

5. Conclusion

China is the largest producer of wheat and also one of the most diverse centers of wheat germplasms in the world. It is essential to understand the biological variation of wheat feedstocks in order to optimize cellulosic ethanol yield, and to find valuable genetic materials for energy crop breeding. This study examined the variance in the capability of sugar release and the cell wall composition of wheat straw collection in central China, which allowed us to understand the factors affecting sugar release and to evaluate the potential of energy crop breeding. Also, it could be very useful for further studies of wheat feedstock. For example, using the data of cell wall traits coupled with molecular marker genotyping data of the

germplasm population, the genetic loci underlying the cell wall composition and sugar release can be mapped by association mapping. Also, these germplasm can be used to further elucidate the fine structure of the wheat cell wall and its effect on sugar release.

Acknowledgements

This work was supported in part by grants from the National Natural Science Foundation of China (30900890 and 31171524), the Programme of Introducing Talents of Discipline to Universities (B08032), the China Postdoctoral Science Foundation (20070420917), the Fundamental Research Funds for the Central Universities China (2011PY047), the Natural Science Foundation of Hubei Province (2009CDB324), and China Scholarship Council (File No. 201208420115).

Appendix A. Supplementary material

Supplementary material related to this article can be found at http://dx.doi.org/10.1016/j.biombioe.2014.08.025.

REFERENCES

[1] Dhugga KS. Maize biomass yield and composition for biofuels. Crop Sci 2007;47:2211–27.
[2] Searchinger T, Heimlich R, Houghton RA, Dong FX, Elobeid A, Fabiosa J, et al. Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land-use change. Science 2008;319:1238–40.
[3] Sannigrahi P, Ragauskas AJ, Tuskan GA. *Poplar* as a feedstock for biofuels: a review of compositional characteristics. Biofuels Bioprod Biorefining 2010;4:209–26.
[4] Singh A, Pant D, Olsen SI, Nigam PS. Key issues to consider in microalgae based biodiesel production. Energy Educ Sci Technol Part A Energy Sci Res 2012;29:563–76.
[5] Chen LG, Xing L, Han LJ. Renewable energy from agro-residues in China: solid biofuels and biomass briquetting technology. Renew Sustain Energy Rev 2009;13:2689–95.
[6] Jiang D, Zhuang DF, Fu JY, Huang YH, Wen KG. Bioenergy potential from crop residues in China: availability and distribution. Renew Sustain Energy Rev 2012;16:1377–82.
[7] Somerville C, Youngs H, Taylor C, Davis SC, Long SP. Feedstocks for lignocellulosic biofuels. Science 2010;329:790–2.
[8] Abramson M, Shoseyov O, Shani Z. Plant cell wall reconstruction toward improved lignocellulosic production and processability. Plant Sci 2010;178:61–72.
[9] ElMekawy A, Diels L, Wever HD, Pant D. Valorization of cereal based biorefinery by-products: reality and expectations. Environ Sci Technol 2013;47:9014–27.
[10] Jørgensen H, Kristensen JB, Claus FC. Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. Biofuels Bioprod Biorefining 2007;1:119–34.
[11] Hendriks A, Zeeman G. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol 2009;100:10–8.
[12] Boudet AM, Kajita S, Grima-pettenati J, Goffner D. Lignins and lignocellulosics: a better control of synthesis for new and improved uses. Trends Plant Sci 2003;8:576–81.
[13] Xie GS, Peng LC. Genetic engineering of energy crops: a strategy for biofuel production in China. J Integr Plant Biol 2011;53:143–50.
[14] McIntosh S, Vancov T. Optimisation of dilute alkaline pretreatment for enzymatic saccharification of wheat straw. Biomass Bioenergy 2011;35:3094–103.
[15] Chen BY, Chen SW, Wang HT. Use of different alkaline pretreatments and enzyme models to improve low-cost cellulosic biomass conversion. Biomass Bioenergy 2012;39:182–91.
[16] Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY. Comparative sugar recovery data from laboratory scale application of lead pretreatment technologies to corn stover. Bioresour Technol 2005;96:2026–32.
[17] Kumar R, Wyman CE. Effect of xylanase supplementation of cellulase on digestion of corn stover solids prepared by leading pretreatment technologies. Bioresour Technol 2009;100:4203–13.
[18] Lewandowski I, Clifton-Brown JC, Scurlock J, Huisman W. *Miscanthus*: European experience with a novel energy crop. Biomass Bioenergy 1983;19:209–28.
[19] Murnen HK, Balan V, Chundawat S, Bals B, Sousa LC, Dale BE. Optimization of ammonia fiber expansion (AFEX) pretreatment and enzymatic hydrolysis of *Miscanthus* x *giganteus* to fermentable sugars. Biotechnol Prog 2007;23:846–50.
[20] Sørensen A, Teller PJ, Hilstrøm T, Ahring BK. Hydrolysis of *Miscanthus* for bioethanol production using dilute acid presoaking combined with wet explosion pre-treatment and enzymatic treatment. Bioresour Technol 2008;99:6602–7.
[21] Xu N, Zhang W, Ren SF, Liu F, Zhao CQ, Liao HF, et al. Hemicelluloses negatively affect lignocellulose crystallinity for high biomass digestibility under NaOH and H_2SO_4 pretreatments in *Miscanthus*. Biotechnol Biofuels 2012;5:58.
[22] Chandra RP, Bura R, Mabee WE, Berlin A, Pan X, Saddler JN. Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics. Advence Biochem Eng Biotechnol 2007;108:67–93.
[23] Wyman CE. What is (and is not) vital to advancing cellulosic ethanol. Trends Biotechnol 2007;25:153–7.
[24] Isci A, Murphy PT, Anex RP, Moore KJ. A rapid simultaneous saccharification and fermentation (SSF) technique to determine ethanol yields. Bioenergy Res 2008;1:163–9.
[25] 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;35:1114–20.
[26] Lindedam J, Andersen SB, DeMartini J, Bruun S, Jørgensen H, Felby C, et al. Cultivar variation and selection potential relevant to the production of cellulosic ethanol from wheat straw. Biomass Bioenergy 2012;37:221–8.
[27] Dong YS, Cao YS, Zhang XY, Liu SC, Wang LF, You GX, et al. Development of candidate core collections in Chinese common wheat germplasm. J Plant Genet Resour 2003;4:1–8 [in Chinese].
[28] Wang J, Sun JZ, Liu DC, Yang WL, Wang DW, Tong YP, et al. Analysis of *Pina* and *Pinb* alleles in the micro-core collections of Chinese wheat germplasm by Ecotilling and identification of a novel *Pinb* allele. J Cereal Sci 2008;48:836–42.
[29] Wu ZL, Zhang ML, Wang LQ, Tu YY, Zhang J, Xie GS, et al. Biomass digestibility is predominantly affected by three factors of wall polymer features distinctive in wheat accessions and rice mutants. Biotechnol Biofuels 2013;6:183.
[30] Huang JF, Xia T, Li A, Yu B, Li Q, Tu YY, et al. A rapid and consistent near infrared spectroscopic assay for biomass enzymatic digestibility upon various physical and chemical pretreatments in *Miscanthus*. Bioresour Technol 2012;121:274–81.

[31] Fry SC. The growing plant cell wall: chemical and metabolic analysis. London: Longman; 1988. p. 95–7.
[32] Dische Z. Color reactions of carbohydrates. In: Whistler RL, Wolfrom ML, editors. Methods in carbohydrate chemistry. New York: Academic Press; 1962. p. 477–512.
[33] Peng LC, Hocart CH, Redmond JW, Williamson RE. Fractionation of carbohydrates in *Arabidopsis* root cell walls shows that three radial swelling loci are specifically involved in cellulose production. Planta 2000;211:406–14.
[34] Sluiter A, Hames B, Ruiz R, Scralata C, Sluiter J, Templeton D, et al. Determination of structural carbohydrates and lignin in biomass. NREL/TP-510-42618. Golden, Colorado: National Renewable Energy Laboratory (NREL); 2008.
[35] Habib G, Shah S, Inayat K. Genetic variation in morphological characteristics, chemical composition and in vitro digestibility of straw from different wheat cultivars. Anim Feed Sci Technol 1995;55:263–74.
[36] Knapp JS, Parton JH, Walton NI. Enzymic saccharification of wheat straw – differences in the degradability of straw derived from different cultivars of winter wheat. J Sci Food Agric 1983;4:433–9.
[37] White LM, Hartman GP, Bergman JW. In vitro digestibility, crude protein, and phosphorus content of straw of winter wheat, spring wheat, barley, and oat cultivars in Eastern Montana. Agron J 1981;73:117–21.
[38] Chang VS, Holtzapple MT. Fundamental factors affecting biomass enzymatic reactivity. Appl Biochem Biotechnol 2000;84–86:5–37.
[39] Berlin A, Maximenko V, Gilkes N, Saddler J. Optimization of enzyme complexes for lignocellulose hydrolysis. Biotechnol Bioeng 2007;97:287–96.
[40] 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;105:231–8.
[41] Sidiras D, Koukios E. Simulation of acid-catalysed organosolv fractionation of wheat straw. Bioresour Technol 2004;94:91–8.
[42] Sun XF, Sun R, Tomkinson J, Baird M. Degradation of wheat straw lignin and hemicellulosic polymers by a totally chlorine-free method. Polym Degrad Stab 2004;83:47–57.
[43] Ali SS, Khan M, Mullins E, Doohan F. The effect of wheat genotype on ethanol production from straw and the implications for multifunctional crop breeding. Biomass Bioenergy 2012;42:1–9.
[44] Li FC, Ren SF, Zhang W, Xu ZD, Xie GS, Chen Y, et al. Arabinose substitution degree in xylan positively affects lignocellulose enzymatic digestibility after various NaOH/H_2SO_4 pretreatments in *Miscanthus*. Bioresour Technol 2013;130:629–37.
[45] Zhang W, Yi ZL, Huang JF, Li FC, Hao B, Li M, et al. Three lignocellulose features that distinctively affect biomass enzymatic digestibility under NaOH and H_2SO_4 pretreatments in *Miscanthus*. Bioresour Technol 2013;130:30–7.
[46] Feng YQ, Zou WH, Li FC, Zhang J, Zhang H, Xie GS, et al. Study on biological Characterization of Rice Brittle Culm mutants and their biomass degradation efficiency. J Agric Sci Technol 2013;15:77–83 [in Chinese].
[47] Lygin AV, Upton J, Dohleman FG, Juvik J, Zabotina OA, Widholm JM, et al. Composition of cell wall phenolics and polysaccharides of the potential bioenergy crop-*Miscanthus*. GCB Bioenergy 2011;3:333–45.
[48] Pant D, Adholeya A. Concentration of fungal ligninolytic enzymes by ultrafiltration and their use in distillery effluent decolorization. World J Microbiol Biotechnol 2009;25:1793–800.
[49] ElMekawy A, Diels L, Bertin L, Wever HD, Pant D. Potential biovalorization techniques for olive mill biorefinery wastewater. Biofuels Bioprod Biorefining 2014;8:283–93.
[50] 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;99:4997–5005.