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Comparison of lignocellulose composition in four major species of *Miscanthus*

Jingping Qin, Yong Yang, Jianxiong Jiang, Zili Yi*, Liang Xiao, Xin Ai and Zhiyong Chen

College of Bioscience and Biotechnology, Hunan Agriculture University, Changsha 410128, China.

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Miscanthus is a perennial grass rich in lignocellulose that has attracted interest as a non-food crop for renewable bioenergy with major environmental and economic benefits for China. The lignocellulose composition of whole stems of four major species of *Miscanthus* was assessed. The average values of total moisture content (TMC) (61.90%) and hemicelluloses (34.86%) were the highest while cellulose (32.71%) and acid detergent lignin (ADL) (8.90%) were the lowest in *Miscanthus floridulus*. On the contrary, the contents of cellulose (42.11%) and ADL (13.64%) were the highest and total ash (TA) (2.89%) was the lowest in *Miscanthus lutarioriparius*. The Shannon-Weaver diversity indices of components for the four species showed that hemicellulose content ($H' = 2.00 \pm 0.11$) was the most variable trait followed by cellulose ($H' = 1.84 \pm 0.07$), then ADL ($H' = 1.84 \pm 0.07$). The variational range of each component was relatively higher in *Miscanthus sacchariflorus*. In *M. lutarioriparius*, the diversity indices of each component were moderate. The diversity of cellulose was the highest and hemicellulose, ADL, TA and TMC were low in *Miscanthus sinensis*. By correlation analysis, neutral detergent fiber (NDF) significantly and positively correlated with ADF, cellulose and ADL at $P < 0.01$ as well as the relationship of cellulose and ADL in the four species. Hemicellulose showed significant ($P < 0.01$) but negative correlation with cellulose and ADL in *M. floridulus*, *M. lutarioriparius* and *M. sacchariflorus*. By principal component analysis (PCA), the components ADF and cellulose were the PC1 that were considered the foremost for the evaluation and selection of resource in the four species. The conclusions show that lignocellulose composition contents of *Miscanthus* culms were different. *M. floridulus* was more fit to ethanol fermentation. Though the components contents in *M. sinensis* and *M. sacchariflorus* were moderate, the range of choice was large. It provided a possible means to screen the appropriate materials according to different utilization. *M. lutarioriparius* had more superiorities relatively. So the four species of *Miscanthus* were appropriate for extension as excellent herbaceous energy plants, though, reasonable species choice should be employed according to the conversion approach and the growth characteristics, productivity levels and biomass quality characteristics of these tall grasses.

Key words: *Miscanthus*, bioenergy, lignocellulose compositions, detergent fiber, diversity analysis, PCA.

INTRODUCTION

Miscanthus Andersson (family Poaceae) is a perennial rhizomatous grass that is widely distributed in Eastern Asia. *Miscanthus* has been used as a traditional

ornamental plant (Hitchcock, 1901), but in recent years it has generated more attention as a potential bioenergy crop (Clifton-Brown et al., 2004). Indeed, the biomass yield from *Miscanthus* (Heaton et al., 2004) is much greater than switchgrass (Clifton-Brown et al., 2004; McLaughlin and Kszos, 2005), a grass native to the US (Hitchcock, 1951) that has also sparked interest as a potential biofuel source (McLaughlin and Kszos, 2005).

The use of *Miscanthus* as a biofuel source may have several environmental benefits. It meets many of the strict standards of a potential energy plant (Li et al., 2010),

*Corresponding author. E-mail: ziliyi@yahoo.com. Fax: +86-731-8461-8765.

Abbreviations: NDF, Neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; AIA, acid insoluble ash; TA, total ash; PCA, principal component analysis.

including its adaptability to marginal land that cannot sustain food crops, its efficient use of water (Clifton-Brown et al., 2008) and fertilizer (Davis et al., 2010), high photosynthetic rate (Farage et al., 2006; Naidu, 2003; Naidu and Long, 2004) and low emissions of CO₂ (Clifton-Brown et al., 2004) and other greenhouse gases (Davis et al., 2010).

Miscanthus is economically viable (Heaton et al., 2004) due to the rapid speed of rhizomatous creep and the high biomass yield relative to the low input requirements (Constanze et al., 2008; Heaton et al., 2004; Lewandowski and Clifton-Brown, 2000; Zub and Brancourt-Hulmel, 2010). *Miscanthus* contains lower water and sugar content when harvested, so conservation and transportation are more convenient than high-starch or sugar-producing crops like sweet sorghum (*Sorghum bicolor* L.). Furthermore, unlike food crops, harvesting *Miscanthus* for biofuel will not directly increase food prices (Muller, 2008; Vidal, 2007). Finally, growth of this tall grass has an added benefit of reducing soil erosion.

While this tall grass has been traditionally used as either forage grass or as a raw material in papermaking, its high lignocellulosic biomass holds great potential for bioenergy production (Liu et al., 2008; Moukamnerd et al., 2010; Simon et al., 2009). It is now combusted and fermented in order to obtain thermal energy and biofuels (Clifton-Brown et al., 2008). Energy produced by lignocellulosic biomass is pure and renewable. Biofuel is a feasible approach (Moukamnerd et al., 2010) to reduce dependency on imported petroleum and to lower greenhouse gas emissions. Therefore, *Miscanthus* should be both environmentally and economically beneficial (Moon et al., 2010) by supplementing or even substituting for fossil fuels (Clifton-Brown et al., 2008).

The major components of lignocellulosic plants are cellulose (polymers of hexose sugars), hemicellulose (polymers of pentose sugars) and lignin (polyphenols) (Taherzadeh and Karimi, 2007). Both hexose and pentose can be fermented to produce biofuels, as is the case for corn ethanol (Himmel, 2009). The raw materials must be pretreated for conversion to monosaccharides before fermentation using either acidolysis, ammonium hydrolysis (Moukamnerd et al., 2010), or enzyme hydrolysis (Agbogbo and Wenger, 2006). These pretreatment steps are predicated by the relative polysaccharide composition, so it is necessary to analyze the content to determine the most suitable method for mass industrialization of *Miscanthus* biofuel (Thygesen et al., 2005). There are two popular methods to determine the lignocellulose composition of candidate cellulosic feed-stocks; detergent fiber analysis and dietary fiber analysis. The former detects the neutral detergent fiber (NDF) and acid detergent fiber (ADF) that reflect the content of cellulose and hemicelluloses. The latter method directly detects the content of structural carbohydrates (principally glucan and xylan). It had been demonstrated through near-infrared spectroscopy (NIRS) that the correlation between the two methods is high (Wolfrum et al., 2009).

In this study, we chose detergent fiber analysis to assess the lignocellulose composition of *Miscanthus*. The objectives of this study were to; (1) characterize the lignocellulose components of *Miscanthus*, (2) analyze the differences in lignocellulose composition between *Miscanthus* species and (3) provide a basis for the rational conservation of genetic diversity in *Miscanthus* as well as its utilization in breeding programs and industrial biofuel production.

MATERIALS AND METHODS

Sources of *Miscanthus* samples

We analyzed the lignocellulose composition of *Miscanthus floridulus* (n₁=118), *Miscanthus sinensis* (n₂=217), *Miscanthus lutarioriparius* (n₃=45), and *Miscanthus sacchariflorus* (n₄=130). All these material samples (510 in total) were collected from 25 provinces in China (Figure 1) from 2006 to 2008, and were planted in the *Miscanthus* germplasm repository of the Hunan Agricultural University, Changsha (N28°11'49", E 112°58'42"). The area has a subtropical monsoon climate, with an average annual temperature of 17.2°C, average annual precipitation of 1566.39 mm, total sunshine of 1661 h, and an average frost-free period of 274 days.

Miscanthus sample preparation methods

From each sample, we took about 100 to 200 g of whole stems (most still retained leaf sheath) from the nursery in January, 2010. During this period, the aerial parts of *Miscanthus* reached the senescent phase and mineral elements (such as nitrogen, phosphorus and potassium) could be returned to the soil (Heaton et al., 2008). Also, *Miscanthus* stems had lower moisture in the dry winter weather. These samples were placed in a convection oven at 45±3°C to dry to a constant weight (in about 40 to 48 h). Usually, the oven-dried samples had moisture content below 10%, suitable for detergent fiber analysis.

Samples were shredded by a kibbler, then passed through 0.38 mm screen, divided into nine portions, and weighed for lignocellulose composition analysis. Three portions (about 1.500 g each) were placed in ceramic crucibles for determination of dry matter (DM) and total ash (TA), three portions (about 0.500 g each) were placed in conical flasks for determination of neutral detergent fiber (NDF), and the other three portions (about 1.000 g each) were placed in conical flasks for the determination of acid detergent fiber (ADF), acid detergent lignin (ADL) and acid insoluble ash (AIA).

Lignocellulose composition analysis methods

To analyze the lignocellulose composition, we used detergent fiber analysis as described previously (Van Soest, 1963). The original method (Van Soest, 1963) made use of heating reflux for 1 h to determine NDF and ADF. We compared the heating reflux method with heating in an autoclave in order to reduce the cost and use the available experimental facilities. It turned out that there were no significant differences between these two methods. Our specific procedure is shown in Figure 2.

Data processing

All descriptive statistics were calculated using Excel 2007. We analyzed the variance (ANOVA) and correlations among different

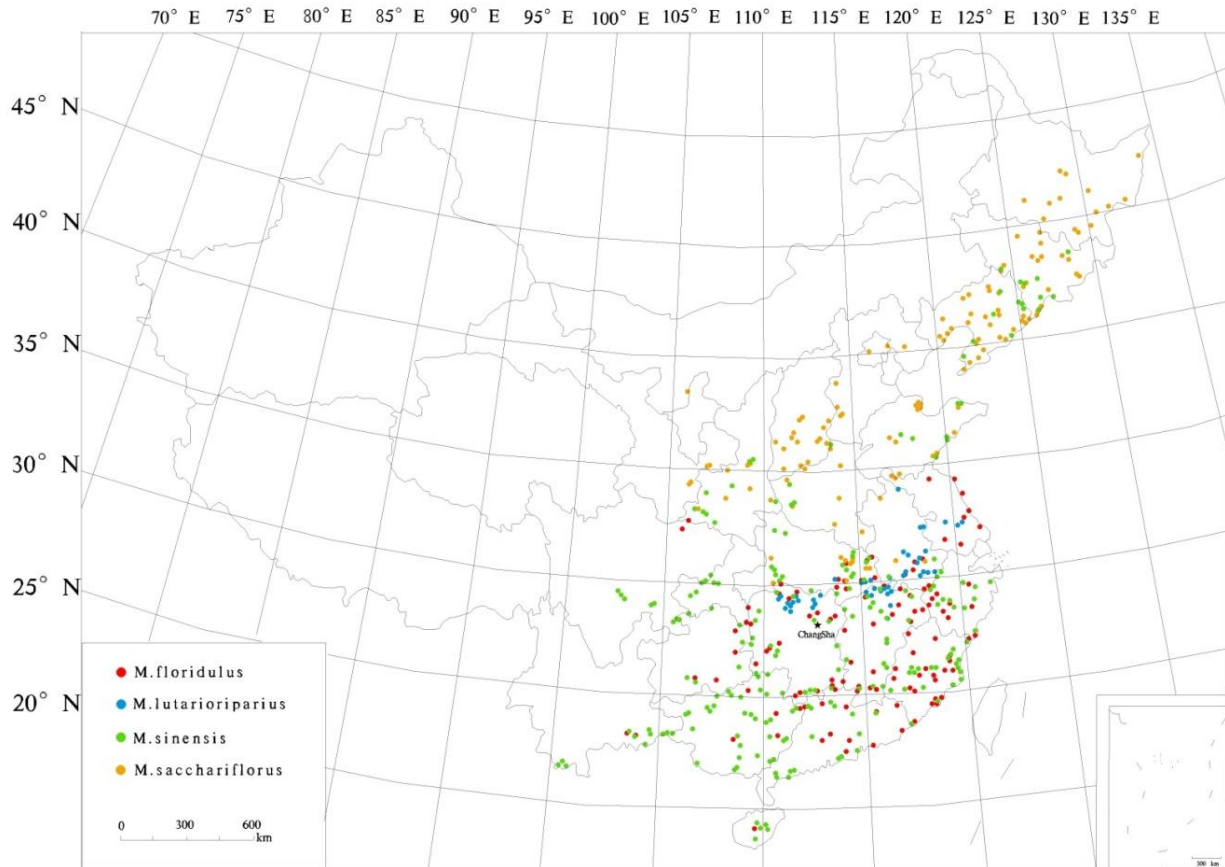


Figure 1. Locations and sources of *Miscanthus* samples used in this study

compositions and species of *Miscanthus* using SPSS 13.0. The parameters were subjected to correlations using Pearson correlation coefficient (r) for pairwise comparisons. Accordingly, the seven quantitative traits were also used to generate indices among population diversity using the Shannon–Weaver method.

RESULTS

Statistical analysis

Lignocellulose composition was determined in triplicate from the dry matter of whole stems (Table 1). Hemicellulose (HC) content was estimated by the difference between NDF and ADF. Cellulose (C) content was estimated by the difference between ADF and the acid detergent residue (ADL+ AIA).

The mean for TMC values among the four species was highest in *M. floridulus* (61.90%), followed by *M. sinensis* with 59.58%. *M. lutarioriparius* and *M. sacchariflorus* had 50.47 and 48.99%, respectively. There were no significant difference ($P > 0.05$) in TMC between *M. floridulus* and *M. sinensis*, or between *M. lutarioriparius* and *M. sacchariflorus*. The rank order of mean hemicellulose values was similar to TMC that *M. floridulus* had the

highest mean of 34.86%. The rank order of mean NDF, ADF, ADL and cellulose were all the same regular pattern, that is, *M. lutarioriparius* > *M. sacchariflorus* > *M. sinensis* > *M. floridulus*; furthermore there were significant differences in NDF, ADF and cellulose components ($P < 0.01$) between the four species except for ADL. There was no significant difference ($P > 0.05$) in ADL content between *M. floridulus* and *M. sinensis*. The lowest TA content was *M. lutarioriparius* (2.89%) and the highest was *M. sacchariflorus* (5.69%). The difference was significant ($P < 0.01$), but the mean for TA content between *M. floridulus* and *M. sinensis* did not differ significantly.

Diversity analysis

The Shannon-Weaver diversity index (H') (Shannon, 1949) was calculated to evaluate the diversity for the seven quantitative traits in the different species (Table 2). Hemicellulose content was the most variable trait across the populations with a mean diversity index (H') of 2.00. The same trait varied highly in *M. sacchariflorus* ($H' = 2.11$) followed by *M. lutarioriparius* ($H' = 2.05$). The populations from *M. sinensis* and *M. floridulus* had H'

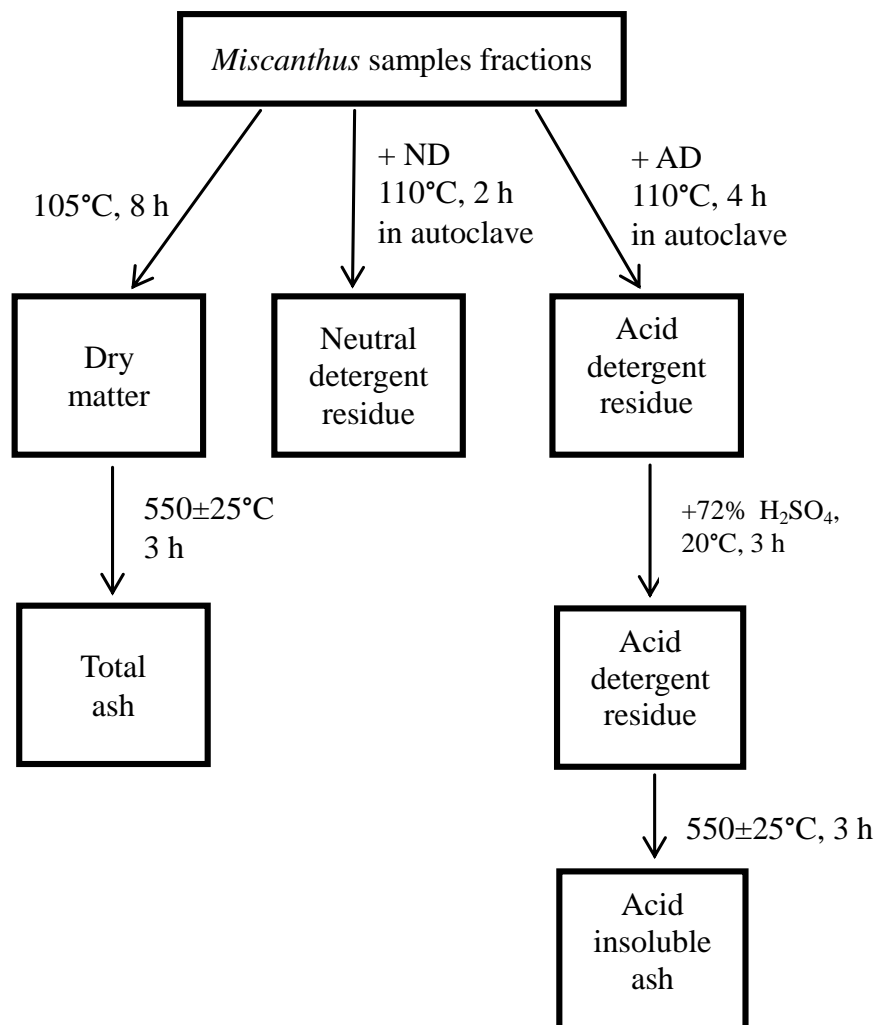


Figure 2. Lignocellulose composition analysis procedure used in this study.

values of 1.99 and 1.86, respectively. The lowest mean diversity index score was 1.43 for TA content. The H' values for this trait ranged from 1.07 in *M. sinensis* populations to 1.91 in *M. sacchariflorus* populations. *M. lutarioriparius* and *M. floridulus* populations had H' values of 1.11 and 1.63, respectively. A mean H' value of 1.80 was recorded for ADL content. *M. sacchariflorus* varied the most ($H' = 1.88$) followed by *M. floridulus* (1.85), *M. sinensis* (1.79) and *M. lutarioriparius* (1.67), respectively. Cellulose content had a mean H' value of 1.84 with *M. sacchariflorus* and *M. lutarioriparius* populations recording a similar H' value of 1.84. TMC which was also an important trait with *M. sacchariflorus* ($H' = 1.94$) recorded the biggest population diversity index. The most varied population based on all the quantitative traits measured was *M. sacchariflorus* ($H' = 1.87$). The least varied population based on the same criteria was *M. sinensis* ($H' = 1.67$). The rank order of mean H' values among the four species was *M. sacchariflorus* > *M. floridulus* > *M. lutarioriparius* > *M. sinensis*.

Principal component analysis

Principal component analysis (PCA) is used to identify the variables contributing to phenotypic diversity (Upadhyaya et al., 2002; 2006). Correlation coefficients (r) between the lignocellulose traits of *Miscanthus* are presented in Table 3.

In *M. floridulus* populations, TMC significantly and negatively correlated with NDF ($r = -0.425$), ADF ($r = -0.460$), cellulose ($r = -0.405$) and ADL ($r = -0.447$) at $P < 0.01$ level of significance, but positively with TA ($r = 0.398$, $P < 0.01$). Obviously, there was significant positive correlation ($P < 0.01$) between NDF and ADF ($r = 0.829$), cellulose ($r = 0.792$), ADL ($r = 0.724$) and hemicellulose ($r = 0.253$), but negatively with TA ($r = -0.377$, $P < 0.01$). TA had significant correlation with the rest of components except hemicellulose. Hemicellulose showed significant ($P < 0.01$) but negative correlation with cellulose ($r = -0.306$) and ADL ($r = -0.241$). Cellulose was significantly and positively correlated to ADL ($r = 0.667$, $P < 0.01$).

Table 1. Lignocellulose composition of *Miscanthus* stem.

Species	Component	% TMC	% DM basis					
			NDF	ADF	HC	C	ADL	TA
<i>M. floridulus</i> n ₁ = 118	Mean	61.90 ^{Aa}	77.82 ^{Dd}	42.96 ^{Dd}	34.86 ^{Aa}	32.71 ^{Dd}	8.90 ^{Cd}	3.75 ^{Bb}
	Std.D	7.64	4.92	5.04	2.91	3.55	1.98	1.03
	Minimum	36.96	63.29	32.07	27.45	25.69	4.61	1.72
	Maximum	79.88	91.25	54.93	42.80	43.59	15.35	8.17
	Range	42.92	27.96	22.86	15.35	17.90	10.74	6.45
<i>M. sinensis</i> n ₂ = 217	Mean	59.58 ^{Aa}	81.15 ^{Cc}	46.33 ^{Cc}	34.82 ^{Aa}	35.06 ^{Cc}	9.51 ^{Cc}	4.02 ^{Bb}
	Std.D	8.49	5.75	5.51	3.26	4.33	1.82	1.24
	Minimum	31.67	69.67	32.48	25.12	26.38	4.90	2.12
	Maximum	79.50	94.92	58.78	43.38	47.00	15.48	12.69
	Range	47.83	25.25	26.30	18.26	20.62	10.58	10.57
<i>M. lutarioriparius</i> n ₃ = 45	Mean	50.47 ^{Bb}	89.87 ^{Aa}	57.53 ^{Aa}	32.34 ^{Bb}	42.11 ^{Aa}	13.64 ^{Aa}	2.89 ^{Cc}
	Std.D	7.89	5.08	7.80	4.43	6.19	2.33	0.89
	Minimum	26.20	78.52	43.95	18.17	32.50	9.59	1.94
	Maximum	65.20	97.51	74.11	40.87	53.94	18.33	6.12
	Range	39.00	18.99	30.16	22.70	21.44	8.74	4.18
<i>M. sacchariflorus</i> n ₄ = 130	Mean	48.99 ^{Bb}	85.69 ^{Bb}	52.71 ^{Bb}	32.98 ^{Bb}	38.50 ^{Bb}	11.22 ^{Bb}	5.69 ^{Aa}
	Std.D	11.35	4.68	5.05	4.06	4.24	2.24	1.73
	Minimum	17.67	69.50	43.12	21.67	30.47	7.03	2.90
	Maximum	68.42	95.32	68.14	42.79	51.57	18.64	11.23
	Range	50.75	25.82	25.02	21.12	21.10	11.61	8.33

Any means in the same row having a common letter are not significantly different at the 5% and 1% level of significance. NDF, Neutral detergent fiber; ADF, acid detergent fiber; HC, hemicellulose; C, cellulose; ADL, acid detergent lignin; TA, total ash.

Table 2. Shannon-Weaver diversity index (*H'*) of lignocellulose composition for different species of *Miscanthus*.

Species	TMC	NDF	ADF	HC	C	ADL	TA	Mean±SD
<i>M. floridulus</i>	1.75	1.80	1.76	1.86	1.75	1.85	1.63	1.77±0.08
<i>M. sinensis</i>	1.15	1.97	1.78	1.99	1.91	1.79	1.07	1.67±0.39
<i>M. lutarioriparius</i>	1.67	1.74	1.79	2.05	1.84	1.67	1.11	1.70±0.29
<i>M. sacchariflorus</i>	1.94	1.77	1.64	2.11	1.84	1.88	1.91	1.87±0.15
Mean±SD	1.63±0.34	1.82±0.10	1.74±0.07	2.00±0.11	1.84±0.07	1.80±0.09	1.43±0.41	

NDF, Neutral detergent fiber; ADF, acid detergent fiber; HC, hemicellulose; C, cellulose; ADL, acid detergent lignin; TA, total ash.

In *M. sinensis* populations, TMC significantly and negatively correlated with NDF ($r = -0.443$), ADF ($r = -0.482$), cellulose ($r = -0.353$) and ADL ($r = -0.368$) at $P < 0.01$ level of significance. There was significant positive correlation ($P < 0.01$) between NDF and ADF ($r = 0.833$), cellulose ($r = 0.833$), ADL ($r = 0.342$) and hemicelluloses ($r = 0.355$). TA had no correlation with the rest of components except ADL ($r = -0.256$, $P < 0.01$). Hemicellulose showed no correlation with cellulose but negatively with ADL ($r = -0.313$, $P < 0.01$). Cellulose was significantly and positively correlated to ADL ($r = 0.246$, $P < 0.01$).

In *M. lutarioriparius* populations, TMC had no correlation with the rest of components. NDF significantly and positively correlated with ADF ($r = 0.846$), cellulose ($r = 0.830$) and ADL ($r = 0.656$) at $P < 0.01$ level of significance, but negatively with hemicelluloses ($r = -0.343$, $P < 0.05$). There was significant negative correlation ($P < 0.05$) between TA and ADL ($r = -0.306$). Hemicellulose showed significant ($P < 0.01$) but negative correlation with cellulose ($r = -0.756$) and ADL ($r = -0.691$). Cellulose was significantly and positively correlated to ADL ($r = 0.677$, $P < 0.01$).

In *M. sacchariflorus* populations, TMC had no

Table 3. Correlation coefficient (r) between lignocellulos traits of *Miscanthus*.

Component	Species	TMC	NDF	ADF	HC	C	ADL	TA
TMC	<i>M. floridulus</i>	1						
	<i>M. sinensis</i>	1						
	<i>M. lutarioriparius</i>	1						
	<i>M. sacchariflorus</i>	1						
NDF	<i>M. floridulus</i>	-0.425**	1					
	<i>M. sinensis</i>	-0.443**	1					
	<i>M. lutarioriparius</i>	0.153	1					
	<i>M. sacchariflorus</i>	0.087	1					
ADF	<i>M. floridulus</i>	-0.460**	0.829**	1				
	<i>M. sinensis</i>	-0.482**	0.833**	1				
	<i>M. lutarioriparius</i>	0.077	0.846**	1				
	<i>M. sacchariflorus</i>	0.171	0.654**	1				
HC	<i>M. floridulus</i>	0.079	0.253**	-0.331**	1			
	<i>M. sinensis</i>	0.034	0.355**	-0.221**	1			
	<i>M. lutarioriparius</i>	0.040	-0.343*	-0.791**	1			
	<i>M. sacchariflorus</i>	-0.113	0.339**	-0.490**	1			
C	<i>M. floridulus</i>	-0.405**	0.792**	0.949**	-0.306**	1		
	<i>M. sinensis</i>	-0.353**	0.833**	0.928**	-0.100	1		
	<i>M. lutarioriparius</i>	0.089	0.830**	0.970**	-0.756**	1		
	<i>M. sacchariflorus</i>	0.231**	0.703**	0.912**	-0.323**	1		
ADL	<i>M. floridulus</i>	-0.447**	0.724**	0.845**	-0.241**	0.667**	1	
	<i>M. sinensis</i>	-0.368**	0.342**	0.542**	-0.313**	0.246**	1	
	<i>M. lutarioriparius</i>	-0.013	0.656**	0.820**	-0.691**	0.677**	1	
	<i>M. sacchariflorus</i>	0.027	0.320**	0.677**	-0.473**	0.346**	1	
TA	<i>M. floridulus</i>	0.398**	-0.377**	-0.335**	-0.056	-0.282**	-0.481**	1
	<i>M. sinensis</i>	0.019	0.008	0.066	-0.098	0.067	-0.256**	1
	<i>M. lutarioriparius</i>	0.191	-0.228	-0.218	0.122	-0.262	-0.306*	1
	<i>M. sacchariflorus</i>	0.029	-0.383**	-0.289**	-0.082	-0.292**	-0.324**	1

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed). NDF, Neutral detergent fiber; ADF, acid detergent fiber; HC, hemicellulose; C, cellulose; ADL, acid detergent lignin; TA, total ash.

correlation with the other component traits except cellulose ($r = 0.231$, $P < 0.01$). NDF significantly and positively correlated with ADF ($r = 0.654$), cellulose ($r = 0.703$), hemicelluloses ($r = 0.339$) and ADL ($r = 0.320$) but significantly negative with TA ($r = -0.383$) at $P < 0.01$ level of significance. Obviously, there was significantly negative correlation ($P < 0.01$) between TA and NDF ($r = -0.383$), ADF ($r = -0.289$), C ($r = -0.292$) and ADL ($r = -0.324$). Hemicellulose showed significant ($P < 0.01$) but negative correlation with cellulose ($r = -0.323$) and ADL ($r = -0.473$). Cellulose was significantly and positively correlated to ADL ($r = 0.346$, $P < 0.01$).

In the subsequent PCA, we determined the percentage of variation explained by the first two or three principal components (PCs) and the vector loadings for each trait (Table 4).

In *M. floridulus*, the first two PCs accounted for approximately 74.385% of the total variation with PC1 and PC2 explaining 56.454 and 17.931% of the total variation, respectively. The PC1, which was the most important component, explained the variability in NDF, ADF, C and ADL, while PC2 explained the variability in HC only.

In *M. sinensis*, the first three PCs accounted for approximately 82.541% of the total variation with PC1, PC2 and

Table 4. Vector loadings and percentage of variation explained by the first two principal components for *Miscanthus*.

Species	Component	Principal component		
		1	2	3
<i>M. floridulus</i>	NDF	0.865	0.354	
	ADF	0.962	-0.195	
	HC	-0.205	0.934	
	C	0.897	-0.203	
	ADL	0.885	-0.05	
	TA	-0.527	-0.392	
	TMC	-0.608	-0.152	
	% of Variance	56.454	17.931	
	% Cumulative	56.454	74.385	
<i>M. sinensis</i>	NDF	0.886	0.434	-0.092
	ADF	0.974	-0.067	0.145
	HC	-0.085	0.878	-0.406
	C	0.892	0.155	0.204
	ADL	0.58	-0.571	-0.324
	TA	-0.009	0.199	0.898
	TMC	-0.618	0.081	0.129
	% Variance	46.492	19.417	16.633
	% Cumulative	46.492	65.908	82.541
<i>M. lutarioriparius</i>	NDF	0.832	0.159	
	ADF	0.991	0.079	
	HC	-0.791	0.044	
	C	0.954	0.075	
	ADL	0.864	-0.1	
	TA	-0.328	0.649	
	TMC	0.06	0.85	
	% of Variance	58.093	17.046	
	% Cumulative	58.093	75.139	
<i>M. sacchariflorus</i>	NDF	0.716	0.637	0.11
	ADF	0.975	-0.125	0.021
	HC	-0.388	0.89	0.101
	C	0.895	0.065	0.221
	ADL	0.707	-0.294	-0.376
	TA	-0.463	-0.418	0.388
	TMC	0.223	-0.144	0.837
	% of Variance	45.401	21.442	15.202
	% Cumulative	45.401	66.843	82.045

NDF, Neutral detergent fiber; ADF, acid detergent fiber; HC, hemicellulose; C, cellulose; ADL, acid detergent lignin; TA, total ash.

PC3 explaining 46.492, 19.417 and 16.633% of the total variation, respectively. The PC1 explained the variability in ADF, NDF and C, while PC2 explained the variability in HC and PC3 explained in AT.

In *M. lutarioriparius*, the first two PCs accounted for approximately 75.139% of the total variation with PC1 and PC2 explaining 58.093 and 17.046% of the total variation,

respectively. The PC1 explained the variability in NDF, ADF, C and ADL, while PC2 explained the variability in TMC. In *M. sacchariflorus*, the first three PCs accounted for approximately 82.045% of the total variation with PC1, PC2 and PC3 explaining 45.401, 21.442 and 15.202% of the total variation, respectively. The PC1 explained the variability in ADF, C, while PC2

explained the variability in HC and PC3 explained in TMC.

DISCUSSION

The components of lignocellulosic biomass varied including cellulose, hemicelluloses, lignin and so on. These materials could be utilized through a variety of ways such as solidification, direct combustion, co- combustion with coal, ethanol fermentation, pyrolysis and gasification. To make certain the characteristics of different components is benefits to exploit lignocellulosic biomass. As raw materials for ethanol fermentation, the selection criterias are higher cellulose hemicelluloses, and lower lignin (Nigan, 2001). As materials for combustion, the content of lignin has a direct relationship with calorific value. Both the content of moisture and ash are prejudicial to calorific value and technological process (Lewandowski et al., 2003).

According to the test results, the Shannon–Weaver diversity indices of hemicelluloses, cellulose and lignin contents all varied highly in the four species of *Miscanthus*. So it provided the reasons for choice. *M. floridulus* was more fit to ethanol fermentation because it had lower biomass content of lignin and higher water than those of the others and would be a disadvantage for transport, storage and combustion. Though the components' content in *M. sinensis* and *M. sacchariflorus* were moderate, the range of choice was large. Judging by different utilization, the raw materials with higher cellulose and hemicelluloses could be good for ethanol fermentation, while with higher lignin and lower water and ash could be prepared for combustion, solidification or some other approaches. *M. lutarioriparius* had more superiorities relatively. It would be perfect for cellulosic ethanol conversion due to higher biomass content of cellulose. It would be good for combustion because of the highest lignin, lowest moisture and ash contents.

Based on the higher lignin content, *M. lutarioriparius* had some other virtues. Lignin exists in the cellulose mesh structure of the plant cell wall. It not only enhances the rigidity of the cell wall but also strengthen the stress resistance (Jones et al., 2001; Pedersen et al., 2005). The role of lignin content in determining the physical properties of plant stems had been confirmed in rice and spring wheat (Tripathi et al., 2003). The increase of lignifications of plant could enhance the draught resistance because of the fewer loss of water (Cruz et al., 1992) and expression of relative enzymatic activity (Riccardi et al., 1998). Stems of *M. lutarioriparius* had 4 to 7 m straight leaves. Leaves turned to yellow and dry naturally in winter. Rapid speed of rhizomatous creep and less water helped to strength the draught, disease and insect resistance so that the high biomass yield could be ensured. Therefore, *M. lutarioriparius* was one kind of good potential biomass source.

Contrast to switchgrass (*Panicum virgatum* L.) and giantreed (*Arundo donax* L.), the four species of

Miscanthus showed superiority in the way of higher cellulose, hemicelluloses and lignin (Fan et al., 2010). In brief, the four species of *Miscanthus* were appropriate for extension as excellent herbaceous energy plants, but reasonable species choice should be employed according to the conversion approach and the growth characteristics, productivity levels, and biomass quality characteristics of these tall grasses.

Conclusion

Miscanthus samples (n = 510, four major species) from 25 provinces in China were analyzed by detergent fiber analysis to determine the lignocellulose composition. The determined components were reported as the fractional percentage of whole stem dry matter.

(1) Lignocellulose composition contents of *Miscanthus* culms were difference by the analysis of variance. The average values of TMC were the highest, while cellulose and ADL were the lowest in *M. floridulus*. On the contrary, the contents of cellulose, ADL were the highest and TA was the lowest in *M. lutarioriparius*. Furthermore, TMC was fewer in *M. lutarioriparius*. On the whole, there was little difference in each component content within *M. sinensis* and *M. floridulus*. Each component content of *M. sacchariflorus* was similar to *M. lutarioriparius*.

(2) The Shannon–Weaver diversity indices of components for the four species showed that hemicellulose content was the most variable trait followed by cellulose, then ADL. The variation of TA was very stable within species. The diversity of cellulose was the highest and hemicellulose, ADL, TA and TMC were low in *M. sinensis*. By comparison, the diversity of hemicelluloses, ADL, TA and TMC were the highest in *M. sacchariflorus*. There were little variations about hemicellulose and cellulose but more variation about TMC and TA in *M. floridulus*. In *M. lutarioriparius*, the diversity indices of each component were moderate. On the basis of these characteristics, we should evaluate the natural resources reasonable and would screen the most appropriate materials according to different utilization.

(3) By correlation analysis, NDF significantly and positively correlated with ADF, cellulose and ADL at $P < 0.01$ as well as the relationship of cellulose and ADL in the four species. Hemicellulose showed significant ($P < 0.01$) but negative correlation with cellulose and ADL in *M. floridulus*, *M. lutarioriparius* and *M. sacchariflorus*. The relationship of TMC and cellulose was negative in *M. floridulus* but was positive in *M. sacchariflorus*. There was no correlation between TMC and the others in *M. lutarioriparius*. In addition, TA was negatively correlated to ADL. These also provided a basis for the choice of biomass.

(4) By PCA, the components ADF and cellulose were the PC1 that were considered the foremost for the evaluation and selection of resource in the four species. Moreover,

ADL, hemicellulose, TMC and TA were also the important factors successively.

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