



Variation in *Miscanthus* chemical composition and implications for conversion by pyrolysis and thermo-chemical bio-refining for fuels and chemicals

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ABSTRACT

Different species and genotypes of *Miscanthus* were analysed to determine the influence of genotypic variation and harvest time on cell wall composition and the products which may be refined via pyrolysis. Wet chemical, thermo-gravimetric (TGA) and pyrolysis-gas chromatography–mass spectrometry (Py-GC–MS) methods were used to identify the main pyrolysis products and determine the extent to which genotypic differences in cell wall composition influence the range and yield of pyrolysis products. Significant genotypic variation in composition was identified between species and genotypes, and a clear relationship was observed between the biomass composition, yields of pyrolysis products, and the composition of the volatile fraction. Results indicated that genotypes other than the commercially cultivated *Miscanthus x giganteus* may have greater potential for use in bio-refining of fuels and chemicals and several genotypes were identified as excellent candidates for the generation of genetic mapping families and the breeding of new genotypes with improved conversion quality characteristics.

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1. Introduction

Due to the rise in demand for renewable energy, dedicated biomass energy crops such as *Miscanthus* are becoming more widely cultivated across Europe. At present the majority of *Miscanthus* planted commercially is *Miscanthus x giganteus*, the result of hybridisation between *Miscanthus sinensis* and *Miscanthus sacchariflorus*. *Miscanthus* has been shown to provide high biomass yields and to be a suitable feedstock for thermo-chemical conversion (Clifton-Brown et al., 2001; Lewandowski et al., 2003; Lewandowski and Kicherer, 1997). Major barriers to breeding *Miscanthus* varieties with improved energy conversion traits include a lack of knowledge regarding the degree of genotypic variation in cell composition and how this variation, positively or negatively, affects conversion efficiency and the range of products which may be achieved through bio-refining.

In this study, analytical pyrolysis experiments were carried out on several different genotypes of *Miscanthus* which were identified as significantly different in their cell wall composition, agronomic and combustion characteristics (Clifton-Brown et al., 2001; Hodgson et al., 2010a; Lewandowski et al., 2003).

In comparison with other lignocellulosic biomass crops, *Miscanthus* has also been shown to exhibit qualities favourable to thermo-

chemical conversion (Hodgson et al., 2010b; Lewandowski and Kicherer, 1997). The estimated energy ratio of *Miscanthus* grown for combustion under UK conditions (output vs. input) is estimated as being 37:1 at the farm gate. This makes the potential efficiency of *Miscanthus* derived energy production greater than for other major energy crops such as switchgrass, willow SRC, and reed canary grass (Powlson et al., 2005). In the UK it is current commercial practice for the harvest of *Miscanthus* grown for combustion to be delayed, until late winter/early spring, which reduces concentrations of moisture, ash, and alkali metals at the expense of dry matter yield (Lewandowski et al., 2003). For this reason, the plant material used in this study has been harvested at two time points corresponding to peak autumn yield and the delayed winter/spring harvest.

Thermo-gravimetric analysis (TGA) was used to examine feedstock properties and provide an indication of the distribution of main pyrolysis products (volatiles/char) which could be achieved under fast-pyrolysis conditions. This included the calculation of reaction kinetics to compare genotypic differences in apparent activation energy. Previous research identified that pyrolysis of *Miscanthus* produced lower yields of volatiles (~80%) and higher yields of char (~20%) than pine wood (86% and 14%, respectively) and also indicated that lignin concentration was the major contributor to the production of fixed carbon (de Jong et al., 2003).

Lignin concentration and the manner in which it binds the holocellulose within the cell wall matrix constitutes a major

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determinant of conversion efficiency in a number of different processes and greatly influences the range of products which may be derived through bio-refining (Gullu and Demirbas, 2001). High lignin feedstocks often confer a higher heating value which is preferable for combustion (Demirbas, 1997; Friedl et al., 2005). However for fast pyrolysis high lignin concentrations have been associated with the production of unstable and viscous bio-oils (Fahmi et al., 2008). High lignin concentrations have also been found to be inhibitory to biological conversion processes such as fermentation and anaerobic digestion due to an increased resistance to microbial and fungal degradation (Dixon et al., 2001; Esteghlalian et al., 1996).

At present, research into the extent and consequence of variation in cell wall composition within *Miscanthus* genotypes has not been fully explored. A combination of research on cell wall composition and its impact on thermal degradation would provide valuable information on how *Miscanthus* biomass may be matched to specific conversion processes and biorefinery applications. It may also provide information which can be used to identify target bioenergy traits which can be used in *Miscanthus* plant breeding programs to tailor specific *Miscanthus* varieties for different biological and thermochemical end uses.

2. Methods

2.1. Plant material

The plant material used in this study came from a field experiment at Rothamsted Research (Herts, UK), which was established in 1997 under the European *Miscanthus* Improvement (EMI) project (Lewandowski et al., 2003) and comprised 15 *Miscanthus* genotypes (Greef et al., 1997; Matumura et al., 1985). At the field trial site, genotypes were planted using a randomised block design with three replicate plots per genotype giving a total of 45 sample plots. Sample material used for analysis was harvested from subsections of plots in November 2005 and February 2006 (delayed harvest). Five genotypes which best covered the range of chemical variation (Hodgson et al., 2010a) were selected for further analysis.

2.2. Sample preparation

Each plot was harvested separately and chipped using a rotary chaff-cutter. A 1 kg sub-sample of chipped material was taken from each plot, dried in an oven at 80 °C for 24 h and milled using a Fritsch 'Pulverisette 14' mill operating at 18,000 rpm with a 1 mm screen. Sample dry matter (DM) was determined gravimetrically after drying in an air circulated oven at 102 ± 2 °C for 16 h. Ash content was determined as loss on ignition after incineration in a muffle furnace at 550 °C for 5 h.

2.3. Determination of cell wall composition

Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) content was determined using the Van Soest (Vansoest and Wine, 1968) method and the cellulose and hemicellulose content calculated as: Cellulose = ADF – ADL; Hemicellulose = NDF – ADF. Holocellulose:Lignin (H:L) ratios were calculated as: (NDF – ADL)/ADL.

From the initial 15 genotype sample set, five genotypes which best covered the range of genotypic variation in cell wall composition were selected for further analysis (Table 1).

2.4. Thermo-gravimetric analysis (TGA)

Experiments were carried out using a Perkin–Elmer Pyris 1 thermogravimetric analyser. Samples were pyrolysed in Nitrogen

Table 1

Miscanthus species and genotypes selected for analysis including dry matter yields measured at the final harvest at Rothamsted Research (Harpenden, UK) in February 2006.

Code	Provenance	Details	Final harvest yield (t ha ⁻¹)
<i>M. x giganteus</i>			
EMI01	Larsen, Denmark	No. 16.05 in Greef et al. (1997)	9.84
<i>M. sacchariflorus</i>			
EMI05	Deuter, Germany	Matumura et al. (1985)	10.77
<i>M. sinensis</i>			
EMI08	Deuter, Germany	Hybrid of <i>M. sacchariflorus</i> and <i>M. sinensis</i>	9.15
EMI11	Brander, Denmark	Collected in Honshu, Japan in 1983, selected 1988	8.97
EMI15	Andersson, Sweden	Collected Hokkaido, Japan in 1990	7.44

Provenance and origin details adapted from Clifton-Brown et al. (2001).

at a flow rate of 20 mL min⁻¹ using the following temperature program: heat from 40 to 105 °C at 10 °C min⁻¹; hold at 105 °C for 10 min; heat from 105 to 905 °C at 10, 25, and 100 °C min⁻¹; hold at 905 °C for 15 min; cool from 905 to 105 °C at 25 °C min⁻¹.

A proximate analysis was performed on the TGA data to calculate the relative proportions (wt.%) of moisture, volatiles, and char (ash + fixed carbon) for each sample. The moisture content was calculated from the mass loss which occurred between 40 and 105 °C, the volatiles from the mass loss between 105 and 550 °C, and the char from 550 to 900 °C (Fig. 1).

Activation energies were calculated for each genotype over the 'active pyrolysis region' which was defined as the degradation which occurred between dehydration and the temperature at which maximum evolution of products occurred (T_{max}) as determined from the derivative curve (DTG) which is a function of mass-loss over time (Fig. 1). The kinetic analysis was performed according to the method suggested by Friedman (1965) using the following equation:

$$(-1/w_0)(dw/dt) = Ae^{-E/RT}f(w/w_0) \quad (1)$$

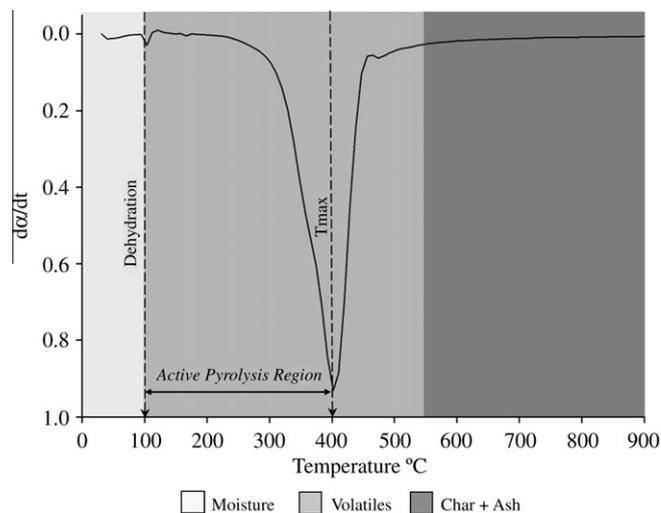


Fig. 1. Annotated example of a derivative (dx/dt) curve from TGA. The figure displays an example derivative curve produced from TGA analysis of a *Miscanthus* genotype at a single heating rate (100 °C min⁻¹). The figure includes the temperature ranges over which the proximate data was calculated and indicates the 'active pyrolysis region' from which the activation energies were calculated.

where: w = weight of organic material; w_0 = total weight of sample; t = time (h^{-1}); A_e = pre-exponential factor; E = activation energy; R = gas constant; T = absolute temperature.

This method was selected on the basis that it was developed to predict activation energies for the main degradation process of complex polymers without any prior knowledge of the form of the kinetic equation. It was identified that the use of multiple heating rates can aid in the development of equations to better estimate activation energy for the polymer under analysis. For this reason, three heating rates (10, 25, 100 $^\circ\text{C min}^{-1}$) were used to calculate mean activation energy for the main degradation process.

2.5. Pyrolysis-gas chromatography–mass spectrometry (Py-GC–MS)

Experiments were carried out using a CDS 2000 pyroprobe and a CDS AS-2500 autosampler (Chemical Data Systems, US). Sub-samples from the three biological replicates of each of the five selected genotypes were used in the analysis. A total of 0.5 ± 0.05 mg of sample was loaded into a quartz tube and heated to 500 $^\circ\text{C}$ at a heating rate of 300 $^\circ\text{C s}^{-1}$ and held at 500 $^\circ\text{C}$ for 10 s. The carrier gas used was Helium with a flow rate of 38 $\text{cm}^3 \text{s}^{-1}$. A Perkin–Elmer AutoSystem XL Gas Chromatograph fitted with a DB 1701 column (60 m \times 25 μm with 0.25 μm film thickness) was used to separate vapours produced with a split ratio of 1:25. The oven program held temperature at 45 $^\circ\text{C}$ for 4 min then heated to 240 $^\circ\text{C}$ at a rate of 4 $^\circ\text{C min}^{-1}$. The injector and detector temperatures were set at 280 $^\circ\text{C}$. Electron impact mass spectra were obtained by Perkin–Elmer MS GOLD (UK) at 70 eV.

Data processing was performed using National Institute of Science and Technology (NIST) Automated Mass spectral Deconvolution and Identification System (AMDIS, (v2.65) and compound identification was performed using Perkin–Elmer NIST Mass Spectral library in combination with referenced literature data (Fahmi et al., 2007; Faix et al., 1991a,b,1990a,b). Data reported for key marker compounds is presented in data tables as ‘amount’ and is the area of the component peak relative to the total ion count (TIC) for the entire chromatogram expressed as a percentage.

2.6. Statistical analysis

All statistical analyses applied to data were performed using Genstat statistical software package (9th edition, VSN International

Ltd.). One-way and two-way analyses of variance (ANOVA) were used to identify significance of differences between genotypes, both within and between harvests. Student Newman–Keuls (SNK) multiple comparison analysis ($P \leq 0.05$) was subsequently applied where significant differences were found to exist. Correlation of data sets was performed using the Pearson product moment correlation coefficient ($r = -1.0$ to 1.0).

3. Results and discussion

3.1. Variation in cell wall composition

Significant variation in cell wall composition was observed between *Miscanthus* genotypes at both harvest times (Table 2). There was a trend for lignin and cellulose concentration to increase and hemicellulose concentration to decrease, as a proportion of dry matter, between harvest dates. However only the difference in cellulose between harvest dates was significant ($P \leq 0.001$).

At both harvests significant variation in composition was observed between genotypes and clear species groupings were observed. For example, the *M. sinensis* genotypes were compositionally different from the *M. x giganteus* and *M. sacchariflorus* genotypes which were similar. Genotypic differences become more apparent when the data for the cell wall components was expressed in terms of holocellulose (cellulose + hemicellulose):lignin ratio (H:L). Whilst in some cases genotypic differences in concentrations of individual cell wall components did occur between harvest times, the overall ratio between structural carbohydrates and lignin remained largely consistent both between species and harvest times.

These differences between genotypes in cell wall composition are consistent with harvests from the same genotypes in 1999–2000 (Hodgson et al., 2010a) which indicates that these differences are consistent over growing seasons and at different ages of the crop. The *M. x giganteus* and *M. sacchariflorus* genotypes were consistently and significantly higher in lignin and cellulose and lower in hemicellulose than the *M. sinensis* genotypes (Table 2). One exception was the *M. sinensis* genotype EMI15 which was observed to contain cellulose in concentrations more similar to those observed for *M. x giganteus* and *M. sacchariflorus* and in the February harvest, hemicellulose concentrations more similar to *M. sacchariflorus*. However, despite being similar to *M. x giganteus* and

Table 2
Cell wall composition of *Miscanthus* species and genotypes in November and February harvests.

Species	Genotype	Lignin	Cellulose	Hemicellulose	H:L	Ash
<i>November</i>						
<i>M. x giganteus</i>	EMI01	120.2 a [†]	503.4 a	248.3 a	6.3 a	26.7 b
<i>M. sacchariflorus</i>	EMI05	121.0 a	490.6 a	274.1 a	6.3 a	22.9 c
<i>M. sinensis</i> (hybrid)	EMI08	92.7 b	430.6 b	331.4 b	8.2 b	34.7 a
<i>M. sinensis</i>	EMI11	96.9 b	431.8 b	339.8 b	8.0 b	31.9 ab
<i>M. sinensis</i>	EMI15	92.3 b	475.9 a	330.0 b	8.8 b	24.4 c
Mean		104.6	466.5	304.7	7.5	28.1
SD		14.7	33.7	41.0	1.2	5.0
$P \leq$	(df 8)	***	***	***	***	***
<i>February</i>						
<i>M. x giganteus</i>	EMI01	125.8 a	521.3 a	257.6 a	6.2 a	27.4 a
<i>M. sacchariflorus</i>	EMI05	121.3 a	501.8 a	281.1 ab	6.5 a	21.6 b
<i>M. sinensis</i> (hybrid)	EMI08	97.0 b	453.6 b	329.9 c	8.1 b	27.1 a
<i>M. sinensis</i>	EMI11	103.2 b	455.2 b	338.3 c	7.7 b	30.4 c
<i>M. sinensis</i>	EMI15	93.4 b	522.0 a	305.6 ab	8.9 c	22.2 b
Mean		108.1	490.8	302.5	7.5	25.7
SD		14.6	34.2	33.6	1.1	3.7
$P \leq$	(df 8)	***	***	**	***	***

Data is reported in g kg^{-1} on a dry matter basis; H:L = holocellulose:lignin ratio; SD = standard deviation; df = degrees of freedom.

** $P \leq 0.05$.

*** $P \leq 0.001$.

[†] SNK multiple comparisons, where a, b, c etc. identify significant differences between genotypes/groups within harvests ($P \leq 0.05$).

Table 3
Thermogravimetric analysis and apparent activation energies of *Miscanthus* species and genotypes in the November and February harvests.

Species	Genotype	Moisture (% wt)	Volatiles (%wt ^{DMAF})	Char (%wt ^{DMAF})	Ash (%wt ^{DM})	E _a (kJ mol ⁻¹)
<i>November</i>						
<i>M. x giganteus</i>	EMI01	4.2	73.9 a [†]	19.3 a	2.7 b	76.3 a
<i>M. sacchariflorus</i>	EMI05	3.8	73.6 a	20.3 a	2.3 c	69.3 b
<i>M. sinensis</i> (hybrid)	EMI08	4.3	74.8 a	17.4 b	3.5 a	65.9 b
<i>M. sinensis</i>	EMI11	4.2	74.7 a	17.9 b	3.2 ab	64.6 b
<i>M. sinensis</i>	EMI15	4.2	78.2 b	15.1 c	2.4 c	66.3 b
Mean		4.2	75.1	18.0	2.8	68.5
SD		0.2	1.9	2.0	0.5	4.7
P ≤	(df 8)	n/s	***	***	***	**
<i>February</i>						
<i>M. x giganteus</i>	EMI01	4.9	72.6 a	19.8 a	2.7 a	76.7 a
<i>M. sacchariflorus</i>	EMI05	4.1	73.4 a	20.4 a	2.2 b	69.0 bc
<i>M. sinensis</i> (hybrid)	EMI08	4.5	75.6 b	17.2 b	2.7 a	67.7 bc
<i>M. sinensis</i>	EMI11	4.4	74.9 b	17.7 b	3.0 c	65.7 c
<i>M. sinensis</i>	EMI15	4.5	77.7 c	15.6 c	2.2 b	70.4 b
Mean		4.5	74.8	18.1	2.6	69.9
SD		0.3	2.0	2.0	0.4	4.2
P ≤	(df 8)	n/s	***	***	***	***

DM = dry matter basis; DMAF = dry matter ash free basis; SD = standard deviation; df = degrees of freedom. n/s = no significant difference.

** P ≤ 0.05.

*** P ≤ 0.001.

[†] SNK multiple comparisons, where a, b, c etc. identify significant differences between genotypes/groups within harvests (P ≤ 0.05).

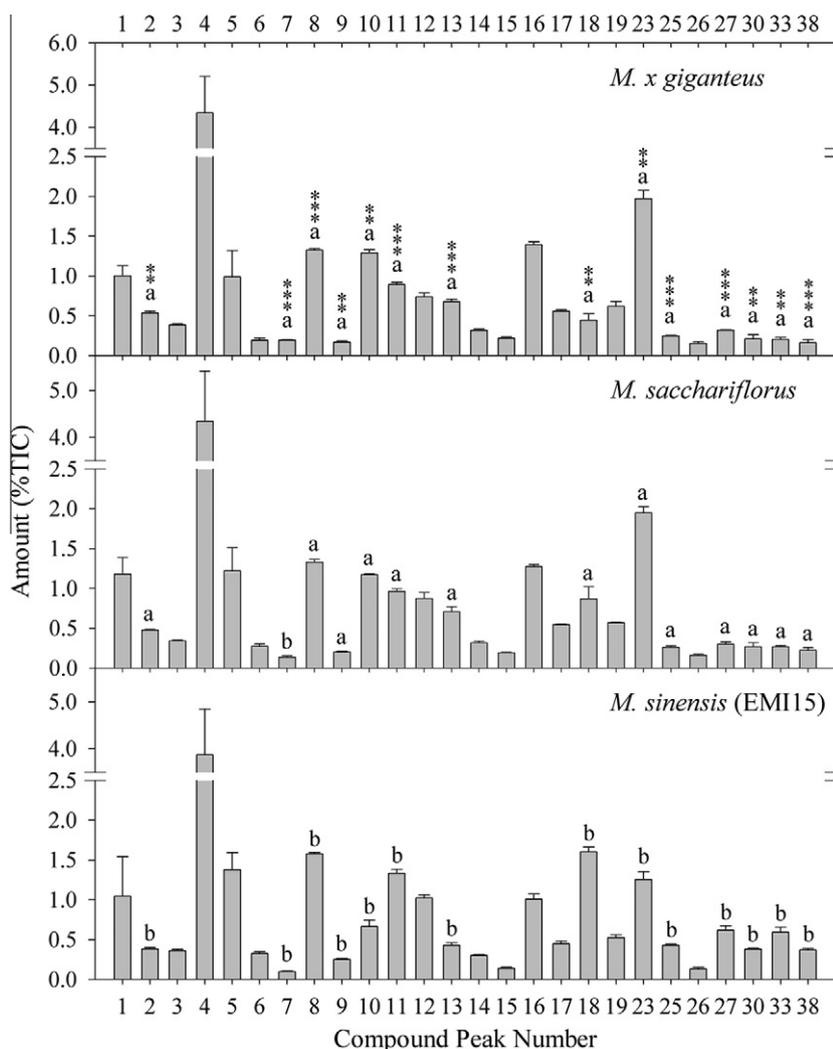


Fig. 2. Variation in amounts of the main identified holocellulose pyrolysis products between *Miscanthus* species and genotypes from the February harvest. Data for *M. x giganteus*, *M. sacchariflorus*, and *M. sinensis* (EMI11) are shown as the greatest variation was observed between these species/genotypes. Compounds are identified by number corresponding to Table 4 and are presented as amount (%TIC) including standard error, results of analysis of variance and SNK multiple comparisons. Levels of significance are included on the basis of all genotypes studied: ***P ≤ 0.001, **P ≤ 0.05.

M. sacchariflorus in the concentrations of structural carbohydrates, EMI15 contained significantly less lignin. EMI15 consistently exhibited the highest H:L ratio of all genotypes analysed and was significantly highest at the final harvest in February (Table 2).

Another key compositional characteristic for thermo-chemical conversion is the concentration of ash retained in the biomass at the time of harvest. As observed in a previous study (Lewandowski et al., 2003), concentrations of ash were significantly lower in the February harvested material than the November harvested material ($P = 0.003$). There was also significant genotypic variation in concentrations of ash in both harvests, though species differences were not as apparent as those observed for cell wall composition. At both harvest times the *M. sinensis* genotypes EMI08 and EMI11, and *M. x giganteus* were consistently high in ash (Table 2). In the February harvest, EMI11 was identified as containing significantly higher concentrations of ash than all other genotypes. In both harvests, no significant difference was identified between *M. sacchariflorus* and *M. sinensis* EMI15 which were significantly lower in ash concentration than the other genotypes.

3.2. Thermo-gravimetric analysis (TGA)

To determine the effects of the observed genotypic variation in cell wall composition on product distribution, which would result from conversion by fast-pyrolysis, sub-samples from both harvests were analysed using TGA (Table 3). Statistical analysis of the results identified *M. sinensis* EMI15 as having significantly greater volatile content and lower char content than all other genotypes in both harvests ($P \leq 0.001$). *M. x giganteus* and *M. sacchariflorus* were consistently lowest in volatile content and highest in char at both harvests, and significantly so in February ($P \leq 0.001$).

The genotypic differences in pyrolysis product distribution are clearly linked with the cell wall composition and are best explained by the H:L ratios which correlated positively with volatile yield ($r = 0.92$) and negatively with char ($r = 0.97$). Pyrolysis experiments performed on purified Kraft lignin at 500 °C have reported that 50–60% of the lignin was retained in the char (Sharma et al., 2004) and the observed yields of volatiles and char (Table 3) are in keeping with yields obtained in previous studies on *Miscanthus* (de Jong et al., 2003; Szabo et al., 1996). Ash concentration has also been shown to effect the pyrolysis product distribution under fast-pyrolysis conditions (Fahmi et al., 2008; Hodgson et al., 2010b), however no correlations between concentrations of ash and the yields of either volatiles or char was apparent from this data.

Statistical analysis of the activation energies calculated for all genotypes and heating rates (Table 3) revealed that at both harvest times the activation energy required for decomposition of *M. x giganteus* was significantly higher than for all other genotypes ($P \leq 0.004$) and no differences were observed between the activation energies of *M. sacchariflorus* and the *M. sinensis* ($P \leq 0.05$). No relationship between activation energy and compositional differences could be determined. Whilst no correlation was observed between the activation energies and total concentrations of inorganics (ash) present, the composition of the ash may have differed between genotypes leading to differences in the catalytic effect caused under these pyrolysis conditions (Nowakowski and Jones, 2008; Nowakowski et al., 2008; Yorgun and Simsek, 2008).

3.3. Pyrolysis-gas chromatography–mass spectrometry (Py-GC–MS)

To examine whether genotypic differences in composition affected the pyrolysis products present in the volatile fraction, Py-GC–MS was performed on all genotypes from both harvest times. From the resulting spectra, 39 key marker compounds were selected which represented the most abundant pyrolysis degradation

products obtained from pyrolysis of the *Miscanthus* biomass (Figs. 2 and 3, Table 4).

Of the 39 key marker compounds identified, 26 were holocellulose derived and 13 were lignin derived. The holocellulose fraction (cellulose + hemicellulose) comprised the major proportion of the biomass. Therefore holocellulose derivatives would be expected to yield the greater proportion of pyrolysis products as reflected by the relationship between H:L ratio and total volatile yield in TGA analyses. However, it is known that at pyrolysis temperatures of 500 °C complete pyrolysis of lignin does not occur (Alves et al., 2006; Caballero et al., 1996; Del Rio et al., 2001). The key lignin markers identified in this research represented the main pyrolysis products observed to result from pyrolysis of lignin at temperatures between 400 and 600 °C (Alen et al., 1996) and the overall compound product distribution is in keeping with results obtained from analysis of bio-oil produced from the fast-pyrolysis of *M. sinensis* at 500 °C (Heo et al., 2010).

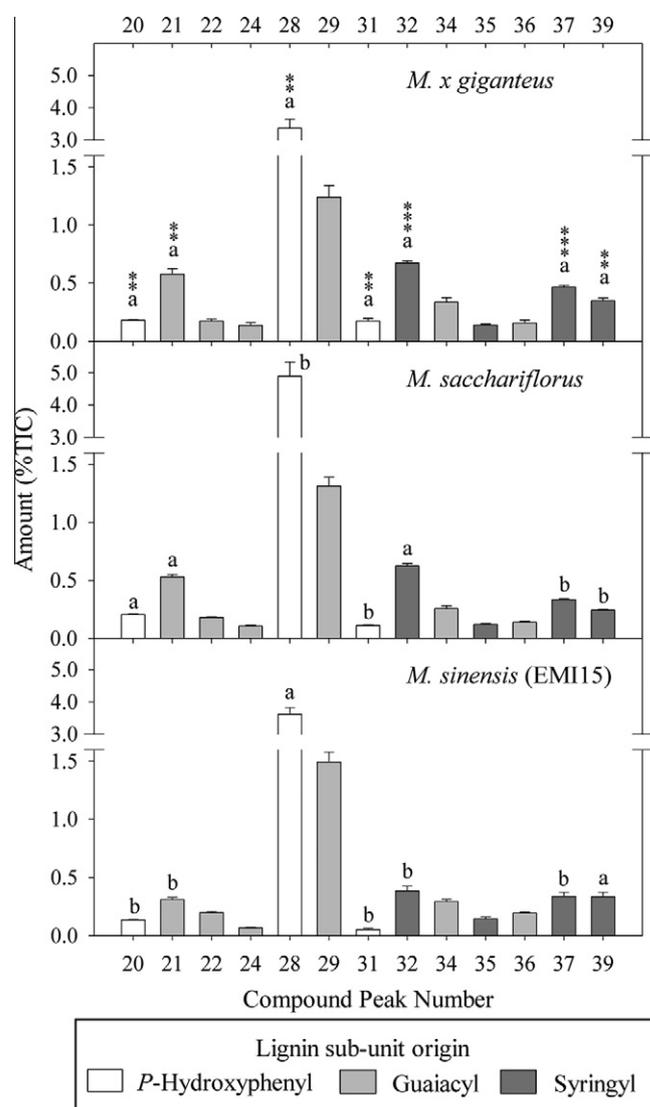


Fig. 3. Variation in amounts of the main identified lignin pyrolysis products between *Miscanthus* species and genotypes from the February harvest data for *M. x giganteus*, *M. sacchariflorus*, and *M. sinensis* (EMI11) are shown as the greatest variation was observed between these species/genotypes. Compounds are identified by number corresponding to Table 4 and are presented as amount (%TIC) including standard error, sub-unit origin, results of analysis of variance and SNK multiple comparisons. Levels of significance are included on the basis of all genotypes studied: *** $P \leq 0.001$, ** $P \leq 0.05$.

Table 4
Key marker compounds identified by Py-GC–MS analysis of *Miscanthus* species and genotypes.

Peak	RT	Compound name	Origin	Mw	m/z
1	4.98	Propanal-2-one	C	72	43/42/72/44
2	6.4	2,3-Butanedione	C	86	43/86/42/44
3	6.79	3-Pentanone	C	86	57/42/86/39
4	8.77	Acetic acid	C	60	45/43/60/42
5	10.22	1-Hydroxypropan-2-one	C	74	43/74/42/45
6	13.72	2-Propenoic acid methyl ester	C	86	55/58/57/86
7	14.69	1-Hydroxy-2-butanone	C	88	57/56/88/42
8	14.91	3-Hydroxypropanal (isomer of 5)	C	74	43/42/73/74
9	16.27	Tetrahydro-2-furanmethanol	C	84	54/84/55/42
10	16.92	Butanedial	C	86	58/57/43/44
11	17.12	2-Hydroxy-3-oxobutanal	C	102	43/42/102/44
12	17.78	Furan-2-carbaldehyde (furfural)	C	96	96/95/39/38
13	20.00	2-Furamethanol	C	98	98/41/39/42
14	20.51	1-(2-Acetyloxy)-2-propanone	C	116	43/86/42/116
15	20.80	Tetrahydro-4-methyl-3-furanone	C	100	43/72/57/42
16	23.40	Dihydro-methyl-furanone	C	98	98/55/42/41
17	26.22	(5H)-Furan-2-one	C	84	55/84/54/39
18	27.29	4-Hydroxy-5,6-dihydro-(2H)-pyran-2-one	C	114	58/114/57/42
19	28.34	2-Hydroxyl-3-methyl-2-cyclopenten-1-one	C	112	112/55/69/41
20	29.98	Phenol	H	94	94/66/65/39
21	30.79	2-Methoxyphenol (guaiacol)	G	124	109/81/124/53
22	35.54	2-Methoxy-4-methylphenol	G	138	123/138/95/73
23	35.78	1,6-Anhydro-3,4-dideoxy-D3-b-D-pyranosen-2-one (levoglucosenone)	C	132	44/57/43/41
24	39.33	4-Ethyl-2-methoxyphenol	G	152	137/43/41/152
25	39.65	3-Methyl-2,4-furandione	C	114	56/42/114/84
26	40.79	1,4:3,6-Dianhydro- α -D-glucopyranose	C	144	69/57/41/70
27	41.18	1,5-Anhydro-arabinofuranose	C	132	57/43/73/44
28	41.79	4-Ethenylphenol (4-vinyl-phenol)	H	120	120/91/65/39
29	41.88	4-Ethenyl-2-methoxyphenol (4-vinyl-guaiacol)	G	150	150/135/77/107
30	43.47	5-Hydroxymethyl-2-furaldehyde	C	126	41/97/39/126
31	43.83	1,2-Benzenediol (catechol)	H	110	110/64/63/81
32	44.12	2,6-Dimethoxy-phenol (syringol)	S	154	154/139/96/93
33	47.08	1,5-Anhydro- β -D-xylofuranose	C	132	57/73/43/44
34	47.33	2-Methoxy-4-(1-propenyl)-phenol	G	164	164/77/103/91
35	47.83	2,6-Dimethoxy-4-(2-propenyl)-phenol	S	168	168/153/125/53
36	48.17	4-Hydroxy-3-methoxybenzaldehyde (vanillin)	G	162	151/152/81/109
37	52.99	4-Ethenyl-2,6-dimethoxyphenol (4-vinyl-syringol)	S	180	180/165/137/77
38	56.83	1,6-Anhydro- β -D-glucopyranose (levoglucosan)	C	162	60/57/73/43
39	57.67	2,6-Dimethoxy-4-(2-propenyl)-phenol	S	194	194/91/77/119

Origin: C = holocellulose; H = *p*-hydroxyphenyl; G = Guaiacyl; S = Syringyl.

The most abundant key marker compounds produced from pyrolysis of *Miscanthus* biomass were acetic acid (4), 4-ethenylphenol (28) and 1,6-anhydro-3,4-dideoxy-D3-b-D-pyranosen-2-one (levoglucosenone) (23). Harvest time had little overall effect on the relative amounts of pyrolysis products produced from the *Miscanthus* biomass. However mean amounts of 2-propenoic acid methyl ester (6), 3-hydroxypropanal (8), (2H)-furan-3-one (9), 2-hydroxy-3-oxobutanal (11), and 1,5-anhydro- β -D-xylofuranose (33) were all significantly higher from pyrolysis of the February harvested material ($P \leq 0.03$).

Analyses of variance performed on the relative amounts of key marker compounds identified that the *Miscanthus* genotypes differed significantly in amounts of 16 of the 39 key marker compounds from the November harvested material and 24 in February. Overall, statistical analysis of the Py-GC–MS data highlighted the same species and genotype groupings as observed from cell wall and TGA analyses. For example similarities were observed between *M. x giganteus* and *M. sacchariflorus*, and these were different to *M. sinensis*, and EMI15 in particular (Figs. 2 and 3).

At the November harvest *M. sacchariflorus* was identified as significantly higher in tetrahydro-4-methyl-3-furanone (15), levoglucosenone (23), 1,4:3,6-dianhydro-glucopyranose (26), phenol (20), and 2-methoxyphenol (guaiacol) (21) contents ($P \leq 0.04$) and *M. x giganteus* significantly lower in tetrahydro-2-furanmethanol (9), 1,6-anhydro- β -D-glucopyranose (levoglucosan) (38), and 2-methoxy-4-methylphenol (22) contents ($P \leq 0.03$). All *M. sinensis* geno-

types were significantly higher in 1,5-anhydro-arabinofuranose (27) content ($P \leq 0.001$), and EMI15 also significantly higher in 3-hydroxypropanal (8), 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one (18), and 1,5-anhydro- β -D-xylofuranose (33) contents ($P \leq 0.05$) but lower in butanedial (10) content ($P \leq 0.01$).

At the February harvest *M. x giganteus* and *M. sacchariflorus* exhibited a greater degree of similarity and were identified as being significantly higher in 2,3-butanedione (2), butanedial (10), 2-furanmethanol (13), levoglucosenone (23), 2-methoxyphenol (guaiacol) (21), and 2,6-dimethoxy-phenol (syringol) (32) contents than the *M. sinensis* genotypes. All *M. sinensis* genotypes were significantly higher in compounds 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one (18), 3-methyl-2,4-furandione (25), and 1,5-anhydro-arabinofuranose (27) ($P \leq 0.004$). EMI11 was significantly higher in compound dihydro-methyl-furanone (16) ($P \leq 0.04$), and EMI11 and EMI15 significantly higher than other genotypes in compounds: 3-hydroxypropanal (8) and 1,6-anhydro- β -D-glucopyranose (levoglucosan) (38) ($P \leq 0.002$). Of all the *M. sinensis* genotypes, EMI15 was the most distinct and was higher than other genotypes in tetrahydro-2-furanmethanol (9), 2-hydroxy-3-oxobutanal (11), 5-hydroxymethyl-2-furaldehyde (30), and 1,5-anhydro- β -D-xylofuranose (33) contents ($P \leq 0.03$).

Notably genotype influenced the type and amount of compounds produced during pyrolysis. For example it was evident that the pyrolysis products derived from *M. sinensis* genotypes were distinct from pyrolysis products derived from *M. x giganteus* and

M. sacchariflorus particularly in relation to holocellulose compounds (Fig. 2). A smaller degree of variation was observed between genotypes in the amounts of lignin derived compounds but this was not surprising as a substantial proportion of the lignin is un-pyrolysed at 500 °C and is not volatilised (Fig. 3).

3.4. Implications for use of *Miscanthus* as a feedstock for bio-refining

The similarities and differences between the commercially cultivated *M. x giganteus* and *M. sacchariflorus*, and those between *M. sacchariflorus* and the *M. sinensis* EMI15 genotype are encouraging from a plant breeding perspective and indicate a potential for breeding to produce new genotypes with compositions suited to different bioenergy conversion routes. For example the low H:L ratio, low-ash *M. sacchariflorus* genotype exhibited quality characteristics favourable to combustion whilst the high H:L ratio of the *M. sinensis* EMI15 genotype exhibited characteristics more suitable for fast-pyrolysis to bio-oil and/or biological conversion by fermentation to alcohols. *M. sinensis* EMI15 exhibited characteristics of low lignin and char, and was high in holocellulose and volatiles. It was higher in valuable product chemicals currently in use as food additives, adhesives, and other platform chemicals suitable for use in green energy applications such as levoglucosan and 5-hydroxymethyl-2-furaldehyde which can be further converted to higher value chemicals such as levulinic and formic acid (Girisuta et al., 2006).

The data suggest that *M. sinensis* EMI15 and *M. sacchariflorus* (EMI05), or similar genotypes, would make excellent parents for a mapping population to dissect cell wall composition. Ideally, in a breeding program the cell wall characteristics of EMI15 would be combined with the dry matter yields of *M. sacchariflorus*. Research into traits such as senescence is also required as it is likely to be a major factor in determining the accumulation and retention of inorganic species (ash), and the concentrations of structural and non-structural carbohydrates present in biomass at the time of harvest. Studies such as this indicate that energy crop selection can have considerable influence on dry matter yield, and enable the matching of biomass to different conversion processes or the range and abundance of products/chemicals which may be refined.

4. Conclusions

Significant variation in cell wall composition was identified between *Miscanthus* genotypes and harvest times. The variation was found to have a significant effect on the yields of char and volatiles produced under pyrolysis conditions. Analysis of the volatile fraction by Py-GC–MS revealed significant genotypic differences in the range of products which may be derived via pyrolysis. Moreover it was indicated that genotypes other than the current commercially cultivated *M. x giganteus* have great potential for use in energy conversion processes and as feedstocks for the bio-refining of chemicals. This study identified several genotypes which would make excellent candidates for parents of genetic mapping families and for use in the breeding of new *Miscanthus* varieties which would be matched to specific conversion pathways and end uses.

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