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Significant Variation for Bio-oil Compounds After Pyrolysis/Gas Chromatography–Mass Spectrometry of Cobs and Stover Among Five Near-Isogenic Brown Midrib Hybrids in Maize

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Keywords
Py/GC-MS, Brown midrib, Bio-oil, Pyrolysis, Lignin

Disciplines
Agronomy and Crop Sciences | Genetics and Genomics | Plant Breeding and Genetics | Statistical Methodology

Comments
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Abstract

We analyzed five near-isogenic brown-midrib hybrids in maize via pyrolysis/gas chromatography-mass spectrometry (Py/GC-MS) in order to determine how differing lignin composition and structure impacts individual bio-oil compounds. Twenty six compounds were analyzed for differences among the five hybrids and between cob and stover materials. We found statistically significant differences for 9 compounds, when comparing the five hybrids, and 17 significant differences when comparing maize cobs with stover. Our data indicate that it may be possible to predict phenolic compounds within bio-oil based on cell wall lignin composition. The genetic variation observed in this study suggests that bio-oil quality can be improved by plant breeding.

Keywords

Py/GC-MS

Brown midrib

Bio-oil

Pyrolysis

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Introduction

In order to meet the renewable fuel directives established by the Energy Security and Independence Act, a variety of transportation fuel alternatives to petroleum are currently being investigated. Starch derived ethanol is currently the dominant alternative fuel in the United States [1]. However, it has several shortcomings for direct use as a transportation fuel. Ethanol is incompatible with much of the United States' current infrastructure, has a low heating value, and uses valuable food resources [2, 3]. Thermochemical processes that use cellulosic materials such as pyrolysis and gasification are alternatives to ethanol that have none of these disadvantages. Fast pyrolysis can be used to generate a wider variety of fuels and products (such as charcoal, fertilizers, gasoline, diesel, specialty chemicals, among others) than ethanol by fermentation. A variety of studies and laboratories have investigated the properties of bio-oil resulting from the pyrolysis of more than 100 types of biomass [4].

Previous studies have addressed agronomic practices/traits and their impact on bio-oil composition: soybean lines/cultivars [5], seasonal variation in kelp [6], maturity at harvest in switchgrass [7], and fertilizer treatments in *miscanthus* [8]. Relatively little research is available that addresses how much variation is available within one type of biomass. Of the three main plant cell wall constituents (cellulose, hemicelluloses, and lignin), cellulose has the most consistent structure as it is composed of β(1-4) linked D-glucose units. Hemicellulose is an inconsistent structure comprised of glucose, xylose, mannose, and β-(1→3,1→4)-glucans, that varies in amount, composition,

and structure between species and cell types [9]. Lignin is composed of p-hydroxyphenyl, guaiacyl, and syringyl units [10]. In addition, a variety of different linkages are involved in the final lignin structure [11]. As a result, we anticipate that variation in concentration of the chemical species analyzed, resulting from pyrolysis, will result from variation in lignin derived compounds. To address this question, we employed *brown midrib* (*bm*) mutants in maize. *Bm* mutants display a reddish brown color of the leaf midrib, are simple Mendelian recessive traits, are defective in some form of lignin biosynthesis, and show decreased lignin content and altered cell wall composition [12, 13].

Bm1 was first described by Jorgensen in 1931 and was later shown to exhibit altered cinnamyl alcohol dehydrogenase (CAD) activity [14, 15]. The *bm3* mutation results from structural changes in the caffeic acid *O*-methyltransferase (COMT) gene [16] and a number of deletion mutations have been identified [17]. The *bm2* and *bm4* mutations are less well characterized in terms of identifying single genes responsible for the phenotype. However, Guillaumie *et al*. [18] identified a number of maize cell wall genes that were up or down regulated within each of the individual *brown midrib* mutants. More recently, additional *bm* mutations (*bm5, bm6*) have been identified in maize, but were not included in this study [19].

The current study aims to investigate how different maize materials (cobs and stover) impact the product distribution of pyrolysis products. Compounds detected by pyrolysis/gas chromatography-mass spectrometry (Py/GC-MS) included carbohydrates, furans, carboxylic acids, aldehydes, and phenolic monomers. Py/GC-MS is a technique that allows a high sample throughput (20 or more per day) required in disciplines like plant breeding. In addition, near-isogenic hybrids with differing lignin composition/structure (*brown midrib* mutants 1, 2, 3 and 4) were analyzed to investigate if, and how, their pyrolysis products differed. Analyzing these hybrids could also provide insight into how genetic variation within a single biomass crop has the ability to impact the individual compounds within bio-oil. The objectives of this study were to (1) compare distribution of bio-oil compounds, as analyzed by Py-GC-MS, between maize stover and cobs, (2) address the question of whether there is significant variation for bio-oil compounds among different maize genotypes, represented by near-isogenic *bm* hybrids, and (3) discuss the implications for developing optimized plant materials for bio-oil production from lignocellulosic maize materials.

Materials and Methods

Plant Materials

Five near-isogenic hybrids (NIH) were chosen from plant materials previously used to estimate theoretical ethanol yield (Kirkpatrick unpublished). Five W64 near-isogenic lines (W64 background genotype and four isogenic lines, each carrying one of the mutant brown midrib genes *bm1, bm2, bm3,* or *bm4*) were crossed with five A619 near-isogenic lines (A619 background genotype plus four lines with one each of *bm1, bm2, bm3,* or *bm4*) to create five W64 x A619 near isogenic hybrids (NIHs): W64 x A619, W64 x A619 *bm1* (*bm1*), W64 x A619 *bm2* (*bm2*), W64 x A619 *bm3* (*bm3*), and W64 x A619 *bm4* (*bm4*), with the four lines carrying *bm* mutations being homozygous for that trait. These NIHs were chosen to evaluate the impact of differing cell wall lignins on pyrolysis products. Brown midrib mutants are affected in lignin biosynthesis compared to wild type maize [20]. The main focus of this study is the impact of cell wall lignifications on the composition of bio-oil. The material was grown in Ankeny, IA in 2005 and Ames and Belmond, IA in 2006. A randomized complete block design with two replications was used in each of the three environments (i.e. combination of year and location) for a total of six biological samples. Plots included, on average, 66 plants. Ears were harvested by hand from each plot with ears (grain and cob) being harvested from all plants in each plot in 2005 and ears (grain and cob plus husk) from 20 plants per plot in 2006. Stover was harvested, at a height of approximately 6 cm, immediately after ear harvest with a commercial silage chopper modified for agronomic research, provided by Mycogen Seeds (Belmond, IA). Stover from 2005 included stalks, leaves, and husks, while stover from 2006 included stalks and leaves only as husks were harvested with the ears. Cob samples were ground by passing them through a wood chipper to reduce particle size and then ground in a Wiley Mill to pass through a 2mm screen. Cob and stover samples were ground (from 2mm) in a ball mill (Spex 200 Geno/Grinder) to reduce the size of the particles further (Figure 1) to achieve less variation between technical replications (Online Resource 1). Two genotypes (SGI912/W601S and Mycogen F697) were chosen from the Ames location in 2005 to compare the coarse (2mm) against the fine ground material.

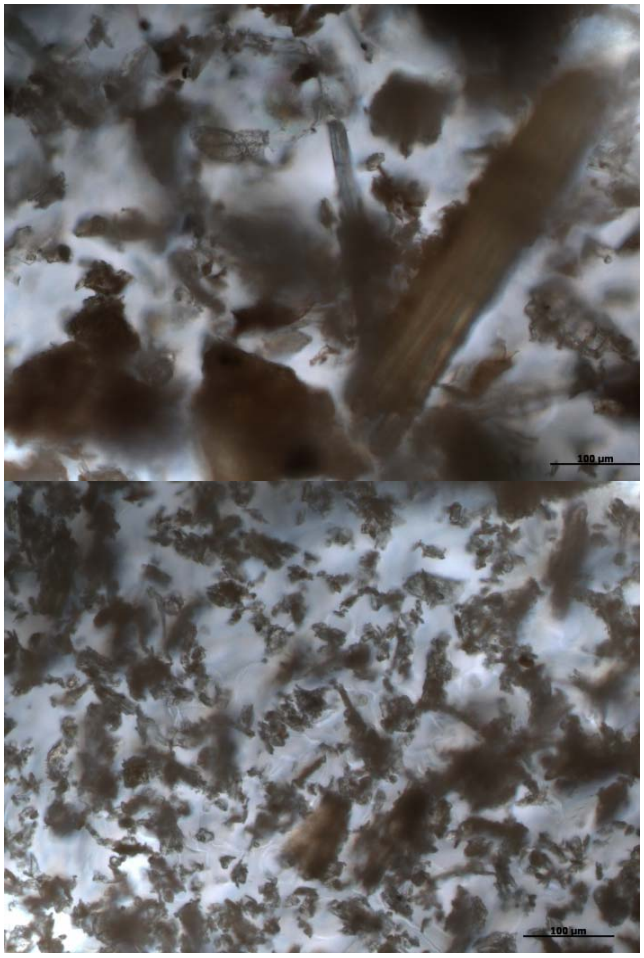

Fig. 1 Visual comparison between corn stover before (above) and after (below) being ground with a ball mill to reduce particle size. A 100µm scale bar is included in the lower right of each picture

Py/GC-MS

Each sample was pyrolyzed at 500 °C using a double shot pyrolyzer (Multi-Functional Pyrolysis System PY-2020iD, Frontier Laboratories Ltd., Japan). For each plant sample, 500 µg were loaded into a deactivated stainless steel cup. The cups were loaded into an autosampler, which allowed the cups to quickly fall freely into the preheated furnace to ensure rapid heating. Samples were heated at the pyrolysis temperature of 500 °C for 30 seconds, after which helium was used to directly carry the pyrolysis vapors into the gas chromatograph (GC) (450-GC, Bruker Corporation, United States) via the micropyrolyzer ‘s deactivated needle, which was inserted into the GC injector (set at 300°C). The compounds within the pyrolysis vapors were separated using an alloy (UltraAlloy^{+}-1701, Frontier Labs Ltd., Japan) capillary column (60m, 0.25mm internal diameter) coated with 14% cyanopropyl polysiloxane (0.25µm). A split (vent) ratio of 1/100 was used. The GC temperature program held for 3 minutes at

45°C and then increased at a rate of 4 °C/min to a final temperature of 270 °C (held for 45 seconds) for a 60 minute total run time. The separated compounds were identified using a mass spectrometer (MS) (320-MS, Agilent Technologies, United States). The MS was operated in the electron ionization mode in the extended dynamic range (EDR) at a mass to charge ratio (m/z) of 40 to 650. Samples were processed with MS Workstation (Agilent Technologies, United States). Peak areas were obtained from the total ion current (TIC) chromatogram. Individual compounds in the spectra were identified using a National Institute of Standards and Technology (NIST) library search and were subsequently confirmed by injecting the pure compounds into the GC-MS setup. Retention times and spectra from the pure compounds were compared to those obtained in our samples in order to confirm compound identity.

Statistical Analysis for the comparison of bio-oil profiles from cob and stover samples among different *bm* genotypes

Raw area values for each compound were divided by the total area in the TIC chromatogram. In addition to individual peak analysis, compounds were grouped into one of six groups for analysis: carbohydrate, furan, acid, phenol, guaiacol, or syringol (Table 1). Compounds were grouped based on previous studies with some carbohydrate derived compounds being further classified as acids or furans [21]. For each of these groups, individual peak values (area as a percentage of the total chromatogram) were summed to achieve a group value. A linear mixed-effects model was fit separately to the data from each peak using SAS (SAS Institute 2003) PROC MIXED. Each linear mixed-effects model included fixed effects for genotypes, materials, and interactions between genotypes and materials. Random effects were included for environments, field replications within environments, plots within field replications, material samples within plots, and technical replicate measurements within material samples.

A power analysis was conducted (for each peak) in order to determine the number of technical replications necessary to achieve acceptable power for detecting a difference between two genotype means. Variance components associated with each of the random factors included in the linear mixed-effects model were set equal to their estimates, and the power of a 0.05-level pairwise mean contrast was estimated for a given number of technical replicates and a given difference in genotype means. As a result of these analyses, two technical replications were performed as little power was gained with additional replications (Online Resource 2).

Results

A typical chromatogram resulting from the Py-GC-MS is shown in Figure 2. The 26 compounds identified and analyzed are listed in Table 1 along with each compound's CAS number and retention time. Table 2 gives the area % value of each compound and compound group for each genotype and material. Table 2 also includes the genotypic and material standard errors. Table 1 indicates whether results remain significant after applying a Bonferroni correction.

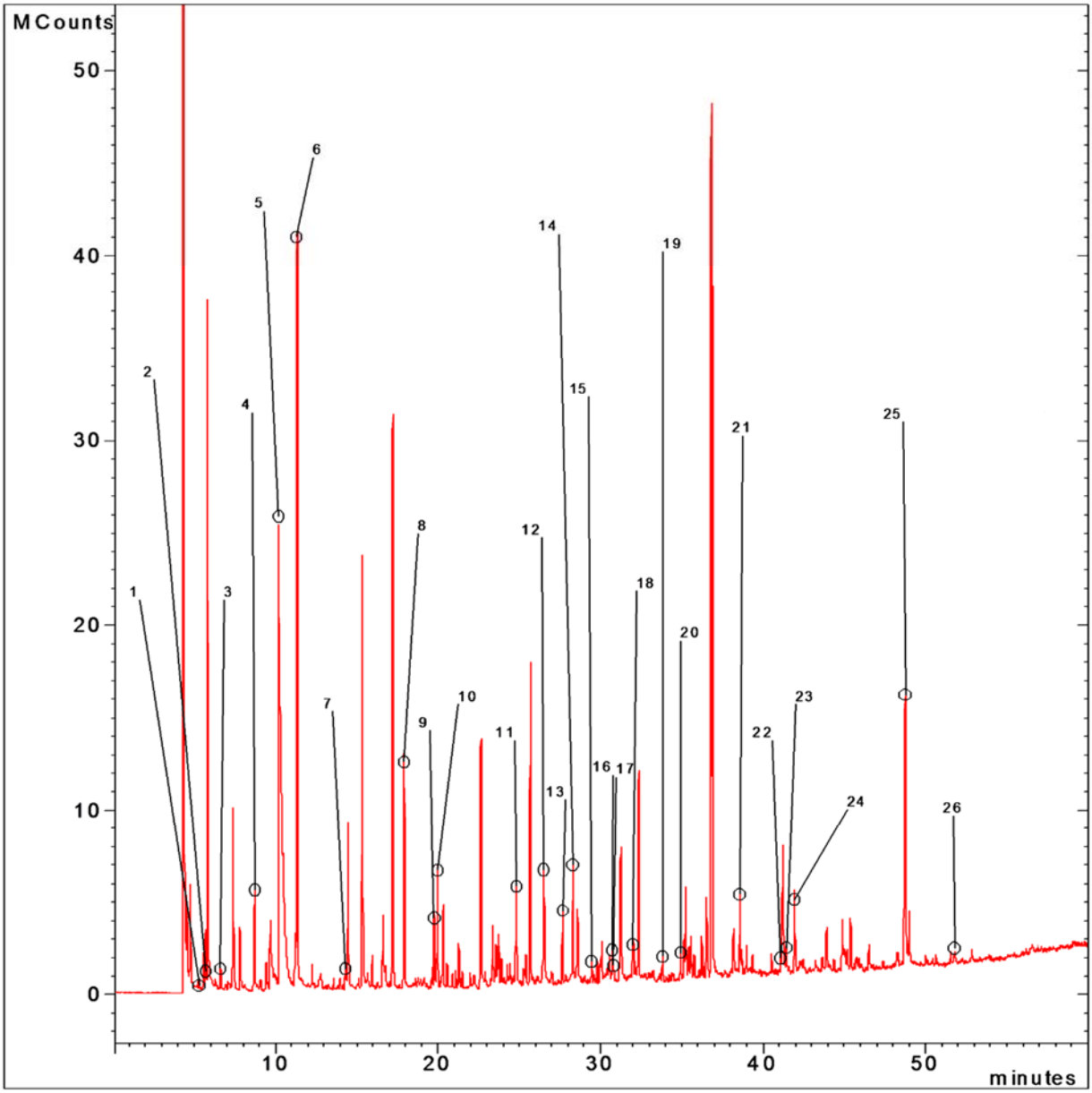

Fig. 2 A chromatogram resulting from the Py-GC-MS analysis. The peak extending off the chromatogram is carbon dioxide, which measures around 190 MCounts. Compounds are numbered according to retention time with the peak apex circled. (1) Furan, (2) Acetone, (3) 2-Methylfuran, (4) Hydroxyacetaldehyde, (5) Acetic acid, (6) Hydroxyacetone, (7) Propanoic acid, (8) 2-Furancarboxaldehyde, (9) 2-Furanmethanol, (10) Acetoxyacetone, (11) 2(5H)-Furanone, (12) 3-Methyl-1,2-Cyclopentanedione, (13) Phenol, (14) 2-Methoxyphenol, (15) 2-Methylphenol, (16) 4-Methylphenol, (17) 3-Methylphenol, (18) 4-Methyl-2-methoxyphenol, (19) 3-Ethylphenol, (20) 4-Ethyl-2-methoxyphenol, (21) 2,6-Dimethoxyphenol, (22) Iso-eugenol, (23) 4-Methyl-2,6-dimethoxyphenol, (24) Vanillin, (25) Levoglucosan, (26) Acetosyringone

Table 1. The 26 compounds identified and analyzed in our maize samples via Py-GC-MS. The results for each of the identified 26 pyrolysis compounds are shown. Tests were performed for fixed effects differences among genotypes, between plants materials (cob vs. stover) and for interaction between genotype and plant material

Compound Name	CAS Number	Retention Time (Min)	Genotype	Material	Interaction	Group
Acetic acid	64-19-7	10.206		c*		Acid
Propanoic acid	79-09-4	14.267		c		Acid
Acids (Group)	NA	NA		c*		
Acetone	67-64-1	5.688				Carb
Hydroxyacetaldehyde	141-46-8	8.756		s		Carb
Hydroxyacetone	116-09-6	11.282				Carb
Acetoxyacetone	592-20-1	19.961		c*		Carb
3-Methyl-1,2-cyclopentanedione	765-70-8	26.457		c		Carb
Levoglucosan	498-07-7	48.710				Carb
Carbohydrates (Group)	NA	NA				
Furan	110-00-9	5.300		s*		Furan
2-Methylfuran	534-22-5	6.692				Furan
2-Furancarboxaldehyde	98-01-1	17.915		c*		Furan
2-Furanmethanol	98-00-0	19.727		c*	x*	Furan
2(5H)-Furanone	497-23-4	24.793	x	c		Furan

Furans (Group)	NA	NA		c*	x*	
2-Methoxyphenol	90-05-1	28.272	x	c*		Guaiacol
4-Methyl-2-methoxyphenol	93-51-6	31.996	x*	s*		Guaiacol
4-Ethyl-2-methoxyphenol	2785-89-9	34.914		c*		Guaiacol
Iso-eugenol	97-54-1	41.045	x*	s*	x	Guaiacol
Vanillin	121-33-5	41.917	x*	c*	x	Guaiacol
Guaiacols (Group)	NA	NA	x*	c*		
Phenol	108-95-2	27.638		c*		Phenol
2-Methylphenol	95-48-7	29.421				Phenol
4-Methylphenol	106-44-5	30.717	x*	s*	x	Phenol
3-Methylphenol	108-39-4	30.809				Phenol
3-Ethylphenol	620-17-7	33.867				Phenol
Phenols (Group)	NA	NA	x*	c*		
2,6-Dimethoxyphenol	91-10-1	38.565	x*		x*	Syringol
4-Methyl-2,6-dimethoxyphenol	6638-05-7	41.409	x*		x	Syringol
Acetosyringone	2478-38-8	51.791	x*	s*		Syringol
Syringols (Group)	NA	NA	x*		x*	
Rest of chromatogram	NA	NA		s*		
Total (Compounds only)			9(7)	17(13)	6(2)	

x indicates a significant ($p < 0.05$) result, and x* indicates a significant result after applying a Bonferroni correction ($p < 0.0019$ for compounds and $p < 0.008$ for compound groups). For the material comparison, c (or s) indicates that cob (or stover) had the significantly higher mean for that compound with the * indicating a p-value that is still significant after applying a Bonferroni correction ($p < 0.0019$ for compounds and $p < 0.008$ for compound groups)

Comparison of Cob and Stover

Out of the six compound groups, cobs had significantly higher ($p < 0.05$) area percentages for four of the groups: furans (2.45% to 2.20%), acids (10.21% to 8.49%), phenols (1.71% to 1.28%), and guaiacols (2.02% to 1.63%)

(Table 1, Table 2). The remaining two compound groups, carbohydrates and syringols, were not significantly different between cob and stover materials.

Out of the 26 individual compounds tested, 11 compounds showed significantly ($p < 0.05$) higher cob area percentages (Table 1, Table 2): acetic acid (9.97% to 8.28%); propanoic acid (0.23% to 0.20%); 2-furancarboxaldehyde (1.21% to 1.15%); 2-furanmethanol (0.43% to 0.27%); acetoxyacetone (0.77% to 0.66%); 2(5H)-furanone (0.61% to 0.59%); 3-methyl-1,2-cyclopentanedione (0.95% to 0.90%); phenol (0.59% to 0.46%); 2-methoxyphenol (0.84% to 0.63%); 4-ethyl-2-methoxyphenol (0.17% to 0.090%); and vanillin (0.59% to 0.30%).

Six compounds showed significantly ($p < 0.05$) increased stover area percentages (Table 1, Table 2): furan (0.065% to 0.055%); hydroxyacetaldehyde (0.90% to 0.84%); phenol, 4-methyl (0.17% to 0.15%); phenol, 2-methoxy-4-methyl (0.44% to 0.33%); iso-eugenol (0.16% to 0.087%); and acetosyringone (0.088% to 0.048%).

Genotype Comparisons

When testing for genotypic differences among the five hybrids for the 6 compound groups, significant ($p < 0.05$) differences were found for the phenol (area % range of 0.34, standard error of 0.038), guaiacol (area % range of 0.36, standard error of 0.038), and syringol (area % range of 0.54, standard error of 0.052) compound groups (Table 1, Table 2).

On an individual compound basis, 9 of the 26 compounds were significantly ($p < 0.05$) different among all five genotypes (Table 1): 2(5H)-furanone (area % range of 0.056, standard error of 0.013); 2-methoxyphenol (area % range of 0.12, standard error of 0.023); 4-methylphenol (area % range of 0.034, standard error of 0.0054); 4-methyl-2-methoxyphenol (area % range of 0.079, standard error of 0.014); 2,6-dimethoxyphenol (area % range of 0.41, standard error of 0.044); iso-eugenol (area % range of 0.061, standard error of 0.0049); 4-methyl-2,6-dimethoxyphenol (area % range of 0.083, standard error of 0.011); vanillin (area % range of 0.26, standard error of 0.012); and acetosyringone (area % range of 0.053, standard error of 0.0045).

Pairwise Genotype Comparisons

The Tukey-Kramer method was used to compare all possible pairwise genotype comparisons among the 26 individual compounds and among the 6 compound groups (Table 1). There were no significant ($p < 0.05$) genotype comparisons for the carbohydrate, acid, and furan compound groups. For the phenol compound group, *bm3* had a significantly ($p < 0.01$) lower value than the other four genotypes. For the guaiacol compound group: *bm1* had a significantly ($p < 0.05$) higher area % value than all other genotypes, and W64 x A619 had a significantly ($p < 0.05$) higher area % value than *bm2* and *bm3*. For the syringol compound group: W64 x A619, *bm2*, and *bm4* all had a significantly ($p < 0.01$) higher values than *bm1* and *bm3*, with *bm1* having a significantly ($p < 0.01$) higher value than *bm3*.

When comparing the W64 x A619 genotype against the average of the four *bm* genotypes, four of the 26 compounds were significantly ($p < 0.05$) different: 4-methyl-2,6-dimethoxyphenol, acetosyringone, 2,6-dimethoxyphenol, and iso-eugenol. In addition, W64 x A619 had a significantly ($p < 0.05$) higher area % for the syringol and guaiacol compound groups when comparing it against the average of the four *bm* genotypes (Table 2).

On an individual compound basis, there were eight compounds that contained significant pairwise genotype comparisons (Table 2). For 2(5H)-Furanone, *bm1* had a signicantly ($p < 0.05$) higher area % than *bm3*. W64 x A619, *bm2*, and *bm4* all had similar area % values for 4-methyl-2,6-dimethoxyphenol that were significantly higher than that of *bm1* and *bm3*. When comparing genotypes for acetosyringone, the four other genotypes had significantly ($p < 0.05$) higher area percentages than *bm3*, with the *bm4* genotype having a higher area % than both the *bm1* and *bm2* genotypes. For 2,6-dimethoxyphenol; W64 x A619, *bm2*, and *bm4* all had significantly ($p < 0.05$) higher area % values than *bm1* and *bm3*. *Bm1* had a significantly higher area % than *bm2* and *bm4* for 2-methoxyphenol. For iso-eugenol, W64 x A619 and *bm3* had similar area % values that were significantly ($p < 0.05$) higher than the other three genotypes. In addition, *bm2* and *bm4* had similar values that were significantly ($p < 0.05$) higher than *bm1*. For 4-methyl-2-methoxyphenol, *bm1* had a significantly ($p < 0.05$) higher area % value than *bm2* and *bm3*. W64 x A619, *bm2*, and *bm4* all had significantly ($p < 0.05$) higher area % values than *bm3* for 4-methylphenol. For vanillin, *bm3* had a significantly ($p < 0.05$) lower area % value than the rest of the genotypes, with *bm1* having a significantly ($p < 0.05$) higher area % value than all other genotypes.

Genotype by Plant Material Interaction Effects

At the compound group level, two compound groups displayed significant ($p < 0.05$) interaction effects (Table 1): syringols and furans.

Of the 26 individual compounds, 6 showed statistically significant ($p < 0.05$) interaction effects (Table 1): 2-furanmethanol; 4-methylphenol; 2,6-dimethoxyphenol; iso-eugenol; 4-methyl-2,6-dimethoxyphenol; and vanillin.

Table 2. Area % values for each compound/compound group by genotype and material and standard error for genotype (GSE) and material (MSE) mean estimates. Also included are notations indicating significant pairwise genotypic comparisons

Compound	W64 x A619	*bm1*	*bm2*	*bm3*	*bm4*	GSE	Cob	Stover	MSE
Acetic acid	9.106	9.446	8.890	9.221	8.974	0.149	9.974	8.281	0.095
Propanoic acid	0.213	0.215	0.215	0.233	0.220	0.012	0.234	0.204	0.007
Acids (Group)	9.319	9.661	9.104	9.454	9.195	0.153	10.208	8.485	0.098
Acetone	0.454	0.467	0.474	0.494	0.446	0.017	0.481	0.453	0.011
Hydroxyacetaldehyde	0.878	0.878	0.883	0.821	0.877	0.022	0.837	0.897	0.015
Hydroxyacetone	5.657	5.641	5.758	5.686	5.574	0.173	5.763	5.564	0.110
Acetoxyacetone	0.690	0.721	0.717	0.737	0.702	0.016	0.769	0.658	0.010
3-Methyl-1,2-cyclopentanedione	0.916	0.912	0.934	0.940	0.909	0.024	0.947	0.897	0.015
Levoglucosan	1.510	1.620	1.539	1.353	1.648	0.216	1.446	1.622	0.182
Carbohydrates (Group)	10.104	10.240	10.304	10.030	10.156	0.200	10.243	10.091	0.140
Furan	0.057	0.062	0.059	0.061	0.061	0.003	0.055	0.065	0.002
2-Methylfuran	0.127	0.140	0.138	0.140	0.133	0.005	0.137	0.134	0.004
2-Furancarboxaldehyde	1.177	1.194	1.159	1.200	1.171	0.023	1.214	1.146	0.018
2-Furanmethanol	0.337	0.348	0.356	0.357	0.352	0.014	0.434	0.265	0.012

2(5H)-Furanone	0.597	0.629[3]	0.614	0.574	0.602	0.013	0.614	0.592	0.010
Furans (Group)	2.295	2.372	2.325	2.331	2.319	0.036	2.454	2.203	0.029
2-Methoxyphenol	0.750	0.804[2,4]	0.679	0.768	0.680	0.023	0.841	0.631	0.015
4-Methyl-2-methoxyphenol	0.402	0.434[2,3]	0.355	0.357	0.385	0.014	0.330	0.443	0.009
4-Ethyl-2-methoxyphenol	0.132	0.134	0.121	0.121	0.133	0.005	0.167	0.090	0.003
Iso-eugenol	0.150[1,2,4]	0.091	0.112[1]	0.152[1,2,4]	0.120[1]	0.005	0.087	0.163	0.003
Vanillin	0.453[3]	0.598[0,2,3,4]	0.428[3]	0.334	0.432[3]	0.012	0.594	0.304	0.010
Guaiacols (Group)	1.887[2,3]	2.060[0,2,3,4]	1.696	1.731	1.749	0.038	2.019	1.631	0.028
Phenol	0.553	0.513	0.543	0.481	0.524	0.020	0.587	0.459	0.013
2-Methylphenol	0.245	0.251	0.245	0.221	0.238	0.008	0.234	0.245	0.005
4-Methylphenol	0.165[3]	0.155	0.170[3]	0.137	0.166[3]	0.005	0.151	0.166	0.004
3-Methylphenol	0.144	0.150	0.142	0.140	0.136	0.004	0.144	0.140	0.002
3-Ethylphenol	0.232	0.212	0.225	0.201	0.230	0.014	0.230	0.210	0.009
Phenols (Group)	1.547[3]	1.627[3]	1.508[3]	1.292	1.487[3]	0.038	1.707	1.278	0.025
2,6-Dimethoxyphenol	0.667[1,3]	0.378	0.581[1,3]	0.260	0.569[1,3]	0.044	0.476	0.506	0.037
4-Methyl-2,6-dimethoxyphenol	0.146[1, 3]	0.093	0.131[1, 3]	0.063	0.134[1, 3]	0.011	0.12	0.11	0.009
Acetosyringone	0.083[3]	0.068[3]	0.067[3]	0.035	0.087[1,2,3]	0.004	0.048	0.088	0.003
Syringols (Group)	0.896[1,3]	0.538[3]	0.779[1,3]	0.358	0.791[1,3]	0.052	0.643	0.701	0.046
Rest of chromatogram	73.589	73.281	73.894	74.3721	73.920	0.307	72.388	75.233	0.194

[0,1,2,3,4] Numeric notations indicate a significant (Tukey-Kramer adjusted $p < 0.05$) genotypic comparison between the genotype in the column and the genotype notated, with 0 indicating the W64 x A619 genotype and the subsequent numbers matching the genotype with that *bm* mutation

Discussion

The amount of primary cell wall constituents cellulose, hemicellulose, and lignin within a biomass feedstock are known to play a significant role in the quality of resulting bio-oil [22]. In the thermal decomposition of biomass, cellulose is degraded into sugars and water, and the majority of its mass contributes to bio-oil production, whereas

hemicellulose produces a larger amount of char and acids than cellulose [23], as well as contributing a large amount of gases [24]. Lignin decomposes mostly into phenolic compounds [25] and higher molecular weight oligomers [26, 27] and confers a higher heating value to the bio-oil [28]. A higher lignin concentration results in a lower quality bio-oil in regards to stability, but a higher bio-oil yield [27]. In addition, lignin content and metals content seem to compete within a feedstock, with a lower lignin content meaning a higher metals content. The resulting bio-oil would then have a higher water content and lower heating value [27]. Fahmi *et al.* [27] suggest that feedstock limits for metals, ash, and lignin need to be developed in order to create a bio-oil that is stable, but still has a commercially viable yield and heating value.

Bm Genotypes

The results of the genotypic and contrast comparisons would suggest that there is significant variation available that could be exploited in a breeding program in order to increase the levels of lignin derived compounds favorable to bio-oil quality and decrease those with a negative impact. The majority of these comparisons resulting in significant differences are in phenolic compounds (includes all of the compounds classified as phenol, guaiacol, or syringol), which have been shown to be products of lignin pyrolysis [29]. While Ralph and Hatfield [29] identified a number of compounds that derive from lignin, hemicelluloses, and cellulose, our data (along with others) suggests that it may be possible to develop prediction equations for high value or quality impacting compounds in bio-oil from the plant cell wall lignins. It is reasonable then to suggest that our results could extend to cellulose and hemicelluloses derived compounds and should be further investigated. Fahmi *et al*. [30] and others have used Py/GC-MS to predict lignin values for biomass, but prediction of bio-oil compounds, or prediction of an industry established quality index, from cell wall components would be useful for bio-oil applications and plant breeding programs designed to maximize those applications.

Previous information (in a near-isogenic background) on the effects of brown midrib genes upon the content of *p*-hydroxyphenyl, guaicyl, and syringyl lignin monomers is available [31]. We found 17 statistically significant ($p < 0.05$) comparisons among pairwise genotype tests for phenol, syringol, and guaiacol compound groups. For the syringol compound group, our data matches up extremely well with that of Barrière *et al*. [31] and all 7 of our significant comparisons are supported by this previous data. In both studies, the background genotype, *bm2*, and *bm4* genotypes had similar syringol levels that were all higher than that of the *bm1* and *bm3* genotypes, with the *bm3*

genotype consistently measuring the lowest. This is consistent with what is known of the *bm3* mutant, as it is a result of altered COMT acitivity, which is involved in syringyl unit synthesis [16, 31]. In the phenol compound group, all of our significant comparisons result from the *bm3* genotype having a lower area % than the other genotypes. This result is also supported by the hydroxyphenyl content found in the F292 hybrids by Barrière *et al*. [31]. The comparison of data for guaiacols between the two studies is muddled, as we found the *bm1* genotype to have a much higher value than the previously mentioned study. This could be due to the specific compounds we measured (5 guaiacols) versus the thioacidolysis method employed by Barrière *et al*. [31] or the materials measured (we measured cobs and stover while they measured only stems).

The *bm* mutants have long been studied for their impact on lignin composition, but recently they have been examined for ethanol conversion [32]. The use of mutants, such as maize cob architecture traits and maize starch digestibility [33, 34], has been proposed and is likely to contribute to bioenergy conversion. Lignin modification has received particular attention, due to lignin's impedance on polysaccharide degradation [35].

Further studies including more genetically diverse materials (10 maize hybrids) are underway to further explore the genetic variation available in maize in regards to the compound profile after pyrolysis. In addition we will explore to what degree individual compounds in the bio-oil resulting from pyrolysis can be predicted from the plant cell wall components cellulose, hemicelluloses, and lignin.

Cob vs. Stover

A large number of the compounds we measured, 17 out of 26, had significantly higher or lower area % values for maize cobs when comparing to maize stover. In our study, one clear difference between stover and cobs is a lower area % value for acids in stover (8.49% vs. 10.24% with a standard error of 0.098%), which results, largely, from a lower acetic acid value. This is likely caused by a difference between content of hemicelluloses between cobs and stover, with Saha [36] reporting 35% for cobs against 25% for stover. Cellulose and lignin content also differ between cobs and stover [36], which is supported by finding many compounds with differing area % values. The relatively low pH of bio-oil, often 2-3, means that bio-oil is often corrosive to construction and some sealing materials [37]. This can cause storage and direct use (burning whole bio-oil in turbines or diesel engines) issues; however, acetic acid is also one of the chemicals in bio-oil that can be extracted and sold [26].

We also found a higher area % value for phenols and guaiacols for cobs, as compared to stover. Depending on desired bio-oil characteristics, one material type may be desired over the other. Although we analyzed stalks and leaves together, it is also possible that maize stalks and leaves may differ substantially as stalks contain more lignin and fewer metals than leaves [38]. Maize could then contribute stalks, leaves, husks, cobs, or some combination thereof, to the production of a quality bio-oil.

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