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Dry matter and relative sugar yield from enzymatic hydrolysis of maize whole plants and cobs

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With 1 figure and 6 tables

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Abstract

The objective of this work was to determine the potential of germplasm from the Germplasm Enhancement of Maize (GEM) programme for use as a biofuel feedstock, relative to commercial grain and silage hybrids. Eighteen maize genotypes including GEM varieties and commercial checks were evaluated in a 2-year field study for dry matter yield, moisture at harvest and sugar produced by hydrolysis of whole plants and cobs. There were no significant correlations between any of the traits measured, suggesting that it should be possible to improve yield with quality traits using a selection index. A *brown midrib* variety was in the top significance group for hydrolysis traits, underscoring the impact of this mutation on the digestibility of lignocellulosic biomass. Commercial varieties tended to have superior dry matter yield, while several GEM lines ranked highly for sugar produced by hydrolysis of whole plants. Selection indices that take both sugar produced by hydrolysis and dry matter yield into account produced rankings of the germplasm used in this study that were markedly different than rankings based on either trait alone.

Key words: lignocellulosic biomass — germplasm enhancement — biofuel — cobs

Models predict global oil production will peak before the middle of this century (for example, see Mohr and Evans 2008). It is therefore important to find alternative, renewable sources of liquid fuels. Sugar- and starch-based biomass are used for food and feed production, creating an undesirable competition. A potential solution to meeting global demands for carbon for liquid fuels is to develop systems based on lignocellulosic biomass such as crop residues or dedicated biomass crops. It may be possible to improve the economic viability of lignocellulosic ethanol production by breeding feedstock crops to improve their suitability for lignocellulosic ethanol production.

Corn stover is an abundant crop residue (Graham et al. 2007). However, corn stover removal can have negative effects on soil fertility, especially on erosion-prone soil (Lindstrom 1986, Hoskinson et al. 2007, Blanco-Canqui and Lal 2009), although stover removal impacts many soil quality parameters similar to the removal of grain in certain soil types or with certain management practices (Moebius-Clune et al. 2008). The cob is the organ to which the grain is attached. Cobs may be valuable as a feedstock for biofuel production (Varvel and Wilhelm 2008) because they are harvested with the grain and have a uniform shape and relatively high density that facilitates transport. Cobs comprise approximately 15% of the dry matter of corn stover biomass (Shinners and Binversie 2007). To improve corn stover and cobs for lignocellulosic ethanol production by breeding, it is important to identify sources of genetic variation for yield and quality of these plant fractions.

Germplasm with genetic diversity in traits related to lignocellulosic ethanol production would be valuable to maize breeders. The Germplasm Enhancement of Maize (GEM) Project is a cooperative effort between the USDA-ARS, private industry and public researchers to broaden and enhance the maize germplasm base (Pollak 2003, Blanco et al. 2009). The GEM programme seeks to develop corn-belt-adapted germplasm containing tropical (at least 25%), and temperate exotic (25% or 50%) germplasm by pedigree. The initial germplasm sources of GEM consisted of exotic sources from the Latin American Maize Project (LAMP), but the programme has expanded to include additional sources of germplasm from other regions (Trevisan and Blanco 2008). Novel sources of genetic diversity are critical for making progress in crop improvement, and tropical germplasm is an excellent source of alleles for numerous traits. Relatively, little germplasm is derived from tropical sources in the US Corn Belt because of lack of adaptation and the extensive resources required to develop and evaluate non-adapted germplasm (Nelson et al. 2006).

Silage corn is bred for dry matter yield and digestibility of plant material. These traits may make it superior for lignocellulosic ethanol production compared with varieties bred for grain production (Lorenz et al. 2009a). Several types of silage corn are commercially available in the United States. Brown Midrib (BMR) varieties contain a *brown midrib* (*bm*) mutation that results in reduced lignin content and altered lignin composition (Marita et al. 2003). Other hybrids have been bred specifically for maximum digestible forage tonnage, high nutritive value and milk production per unit area. These hybrids include products developed by the University of Wisconsin and private companies such as the Silage-Specific TMF products from Dow AgroSciences (http://www.dowagro.com).

Rapid near-infrared spectroscopy methods have been developed to evaluate corn stover composition (Templeton et al. 2009); however, it has been shown that composition explains only part of the variation in suitability for ethanol yield (Lorenz et al. 2009b) with much of the variation being explained by differences in stover digestibility. Thus, measures that mimic ruminant digestion (Lorenz et al. 2009b) or large-scale biofuel production (Weimer et al. 2005, Isci et al. 2008)

are good predictors of suitability for biofuel production. A genetically engineered microbial system for rapid evaluation of sugars produced in the course of enzymatic hydrolysis of biomass was developed (Haney et al. 2008), and this system can be coupled with a small-scale enzymatic hydrolysis to predict relative sugar yields from hydrolysis of stover.

The objective of this work was to determine the potential of germplasm from the GEM programme for use as a biofuel feedstock, relative to commercial grain and silage hybrids. We measured dry matter yield of each genotype as well as the amount of sugar produced by enzymatic hydrolysis of this whole-plant material. In addition to examining whole-plant material, we examined relative sugar yield from hydrolysis of cobs as well.

Methods

Experimental design: Eighteen genotypes were selected for evaluation (Table 1) including 16 hybrids, one breeding cross and one open pollinated population. Most of the hybrids have one elite corn-belt-adapted inbred parent and one parent from the GEM programme. The exotic tropical and temperate entries selected for the study were chosen based on previous observations of whole-plant biomass, grain yield and agronomic traits. Two entries were selected with large cobs (Choclero race and the pipe corn hybrid). These traits may be advantageous for biofuel production. In addition, a commercial and a public grain hybrid were included as well as several commercial or public hybrids intended for silage production. The 18 entries consisted of tropical-derived exotics (nine entries) and temperate-derived exotics (four entries) from the GEM programme or University of Wisconsin silage hybrids bred from GEM germplasm. The remaining entries in the study included commercial and public check hybrids bred specifically for silage or grain.

Each entry was planted in one plot (two rows) in a randomized complete block design, with two blocks (replications) each containing all entries in the experiment at each location. The experiment was carried out at two locations near Davenport, Iowa, in each of 2 years (2008 and 2009). Thus, eight observations were made on each entry (two reps × two locations × 2 years). The locations were approximately eight kilometres apart. Data were collected on four traits on an individual plot basis: Dry matter yield, moisture at harvest, whole-plant quality and cob quality.

Production of plant material for analysis: Experimental plots consisted of two rows that were 5.3 m long. Plant density was 81 700 plants per hectare, and the field was well fertilized at 200 kg of nitrogen per hectare, with medium to high levels of phosphorous and potassium applied. The experiment was harvested close to the time for normal grain harvest. A John Deere 5400 silage chopper equipped with a Hege scale was used for whole-plant harvest. This device is designed to obtain representative samples of whole plants and we have validated that it does this. It is important to note that our whole-plant samples contain grain, so caution should be used when comparing these results to studies examining whole plants without grain. Prior to harvest, five ears were randomly selected and removed from each plot and sent to the USDA-ARS laboratory in Ames, IA, for cob analysis. Whole-plant samples were collected in the field from each plot, dried to near 0% moisture and samples (0.5 kg each) were sent to the Ames laboratory for analysis.

Measurement of relative sugar levels produced by enzymatic hydrolysis: The coarsely chopped whole-plant and cob samples were finely ground prior to analysis using a Thomas Wiley Laboratory Mill Model 4, equipped with a rotary grinder and stationary blades with a 1-mm screen. Cob quality and whole-plant quality are defined by the sugar level produced from a sample using a hydrolysis process commonly used in lignocellulosic ethanol production. Generally, sugar levels limit ethanol production in the ligocellulose conversion processes, so this measure is a reasonable proxy for suitability for biofuel production. Five-milligram samples were evaluated by measuring the sugars produced during hydrolysis by commercial grade enzymes (GC220 and Multifect Xylanse; Genecor International, Palo Alto, CA, USA) following treatment with 1150 µl of 0.5% (v/v) per cent sulphuric acid at 100°C for 1 h using the simultaneous saccharification and catabolism method described by Haney et al. (2008). In this method, enzymatic hydrolysis was carried out in a carbon-limited bacterial growth medium inoculated with a strain of *Escherichia coli* that produces the fluorescent reporter protein GFP. The fluorescence of the culture is proportional to the rate of bacterial growth, which is in turn proportional to the amount of sugars produced by hydrolysis. Acid treatment is required to break linkages between lignin and sugars in the cell wall, and a sufficiently strong treatment can facilitate the conversion of nearly of the cell wall polysaccharides to sugars. Complete conversion would make all genotypes appear similar with regard to digestibility, although genotypic differences in the total amount of polysaccharide would still be apparent. We decided to define our measure of quality as a composite of both the amount of cell wall polysaccharide and the digestibility of this polysaccharide. Thus, we chose a mild acid treatment to facilitate partial hydrolysis of the samples to allow differences in both digestibility and polysaccharide content among samples to be evaluated. Relative cob and stover sugar

Table 1: Description, origin and race of germplasm evaluated in this study

Germplasm description	Country/race	Description	Figure 1 code
CHOCLERO	Chile/Choclero	Open pollinated population	H
BMR	US/Corn Belt Dent	*bm3* exp hybrid	E
(Mo15W/Mo16W) × (MoPipe(E1))	US/Corn Belt Dent	Pipe corn hybrid	A
B73 × Mo17	US/Corn Belt Dent	Public check grain hybrid	D
GEMS-0113 × GEMN-0097	(Mexico/Tuxpeno) × (US/S. Corn Belt)	GEM hybrid	F
W608S × LH244	Chile/Camelia	U of Wisconsin silage hybrid	G
GEMN-0039 × LH200	Brazil/Cateto	GEM hybrid	N
W606S × LH244	St. Croix/St. Croix race	U of Wisconsin silage hybrid	O
GEMN-0132 × T8	Brazil/(tropical hybrid)	GEM hybrid	C
GEMN-0133 × HC33	Brazil/(tropical hybrid)	GEM hybrid	B
MDI022:N99d	Peru/Cuban yellow	GEM breeding cross	L
GEMN-0033 × LH198	Brazil/Cateto	GEM hybrid	M
GEMN-0145 × LH200	Peru/Cuban yellow	GEM hybrid	K
LH200 × LH262	US/Corn Belt Dent	Check grain hybrid	J
GEMS-0115 × GEMN-0097	(Mexico/tropical hybrid) × (US/S. Corn Belt)	GEM hybrid	I
TMF 2	US/Corn Belt Dent	Silage check hybrid	Q
W605S × LH244	Argentina/Cristalino Colorado	U of Wisconsin silage hybrid	R
TMF 1	US/Corn Belt Dent	Silage check hybrid	P

BMR, Brown Midrib; GEM, Germplasm Enhancement of Maize.

Table 2: Degrees of freedom, sums of squares and significance levels for yield, moisture at harvest, whole-plant quality and cob quality from ANOVA of 18 genotypes in two locations for 2 years

	df	Yield	Moisture at harvest	Whole-plant quality[1]	Cob quality
Genotype	17	1395**	2868**	324 528*	449 926**
Year	1	149**	12^{ns}	51 718*	12 712^{ns}
Location [year]	2	488**	6841**	132 693**	28 426^{ns}
Genotype × Year	17	1346**	2062**	471 823**	336 926**
Location [year] × Genotype	34	370*	1269**	217 741^{ns}	187 332^{ns}
Error[2]	72	434	573	690 313	335 424
Total	143	4182	12 626	1 888 815	1 350 746
Model *R* squared		0.90	0.96	0.63	0.75
Repeatability[3]		0.33	0.23	0.17	0.33
Proportion G × E[4]		0.41	0.26	0.37	0.39

*, ** and ^{ns} indicate significance at $\alpha = 0.05$, $\alpha = 0.01$ or not significant.
[1]Our measure of quality was mass of sugars per mass of tissue following enzymatic hydrolysis.
[2]The error term contains the effect of blocks (replications at a location) in the model.
[3]Repeatability was calculated as the fraction of the total variance explained by the genotypic variance.
[4]Proportion of G × E was calculated as the fraction of the total variance explained by the Genotype × Year and Location [year] × Genotype effects.

yields were calculated on a per mass basis are presented in standardized form (experiment-wide mean = 0 and standard deviation = 1).

Data analysis: Response variables for each plot were subjected to ANOVA by fitting a linear model using a standard least squares modelling personality. The parameters in the model are listed in Table 2. The block (i.e. rep) effect was considered as error in the linear model. The inference space of this experiment is limited to the genotypes and environmental conditions observed. We therefore considered these parameters as fixed effects in the ANOVA. Least squares means for each genotype were used to estimate correlations between traits and to rank samples using pairwise Student's *T*-tests (Tables 3 through 5).

Results

Four traits were evaluated: dry matter yield, moisture content at harvest, whole-plant quality and cob quality. For the purposes of this manuscript, we define quality as the relative mass of sugar produced by enzymatic hydrolysis of the sample. No significant correlations ($\alpha = 0.05$) were observed among the mean values of any of the traits (Fig. 1). For all traits, genotype main effects were significant (Table 2). These significant differences among genotypes suggested that it would be valuable to examine the differences in the genotypes in greater detail. The environmental main effects (Year and Location [year]) were significant for dry matter yield and whole-plant quality. The Genotype × Year effect was significant for all traits, and the Genotype × Location [year] effect was not significant for the quality traits. These terms represent genotype by environment interactions, and their significance indicates the likelihood of changes in trait value because of differences in environment. The significance of these terms indicates the ranking of this germplasm for yield and quality is likely to vary from year to year, which is not unusual in evaluating germplasm for yield and quality traits.

Together, the genotype by environment interaction effects explain between 26% and 41% of the total variance in the experiment. For all traits, the genotype by environment

Table 3: Significance groupings of genotypes ranked from highest to lowest based on relative sugar yield from hydrolysis of whole plants

Genotype	Significance grouping[1]					Standardized LSM[2]
MDI022:N99d	A					2.05
GEMS-0113 × GEMN-0097	A	B				1.79
BMR	A	B	C			1.31
(Mo15W/Mo16W) × (MoPipe(E1))	A	B	C	D		0.59
GEMN-0039 × LH200	A	B	C	D		0.49
W605S × LH244	A	B	C	D		0.40
GEMS-0115 × GEMN-0097	A	B	C	D		0.26
CHOCLERO		B	C	D	E	−0.08
TMF 1		B	C	D	E	−0.11
GEMN-0033 × LH198		B	C	D	E	−0.13
TMF 2		B	C	D	E	−0.19
GEMN-0145 × LH200			C	D	E	−0.50
GEMN-0132 × T8			C	D	E	−0.57
W608S × LH244				D	E	−0.70
W606S × LH244				D	E	−0.76
B73 × Mo17				D	E	−0.78
LH200 × LH262				D	E	−1.24
GEMN-0133 × HC33					E	−1.80

BMR, Brown Midrib.
[1]Significance groupings determined by pairwise *T*-tests at $\alpha = 0.05$. Entries not connected by the same letter are significantly different.
[2]Least squares mean (LSM) values were adjusted by subtracting the group mean and dividing by the group standard deviation to create a distribution with mean 0 and standard deviation of 1.

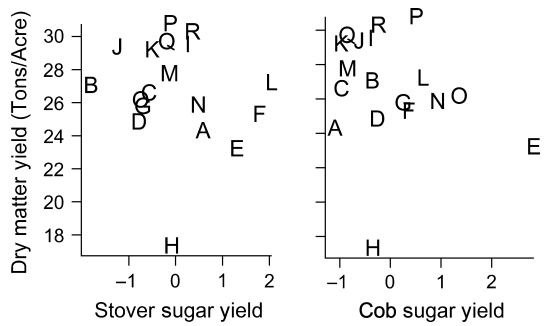

Fig. 1: Scatter plot of genotype mean dry matter yield vs. stover relative sugar yield and cob relative sugar yield. Letters correspond to genotypes as described in Table 1

interaction effects explain more of the variance than the genotype effect alone. This presents a challenge to breeders and producers and emphasizes the importance of multiple year evaluation at several locations.

Given the significant genotype effect, we examined the ranking of the genotypes in more detail. While low in dry matter yield, the BMR hybrid was in the top significance grouping for relative sugar yield from hydrolysis of both the cob and whole-plant fractions (Tables 3 and 4), although several other entries were in the top group when considering whole-plant hydrolysis. The superiority of the BMR variety in relative sugar yield from hydrolysis of the cob fraction may be because of the *bm3* mutation. If this were the case, this mutation may function in cobs as well as in other portions of the plant. The TMF hybrids were superior in yield (Table 5), but in the middle of the range for whole-plant quality. The two

Table 4: Significance groupings of genotypes ranked highest to lowest based on relative sugar yield from hydrolysis of cob

Genotype	Significance grouping[1]						Standardized LSM[2]
BMR	A						2.83
W606S × LH244		B					1.36
GEMN-0039 × LH200		B	C				0.94
MDI022:N99d		B	C	D			0.65
TMF 1		B	C	D	E		0.51
GEMS-0113 × GEMN-0097		B	C	D	E		0.37
W608S × LH244		B	C	D	E	F	0.25
W605S × LH244			C	D	E	F	G	−0.23
B73 × Mo17				D	E	F	G	−0.26
CHOCLERO				D	E	F	G	−0.34
GEMN-0133 × HC33				D	E	F	G	−0.35
GEMS-0115 × GEMN-0097				D	E	F	G	−0.38
LH200 × LH262					E	F	G	−0.61
GEMN-0033 × LH198						F	G	−0.85
TMF 2						F	G	−0.85
GEMN-0132 × T8							G	−0.97
GEMN-0145 × LH200							G	−0.97
(Mo15W/Mo16W) × (MoPipe(E1))							G	−1.09

BMR, Brown Midrib.
[1]Significance groupings determined by pairwise *T*-tests at $\alpha = 0.05$. Entries not connected by the same letter are significantly different.
[2]Least squares mean values were adjusted by subtracting the group mean and dividing by the group standard deviation to create a distribution with mean 0 and standard deviation of 1.

Table 5: Significance groupings of genotypes ranked highest to lowest based on dry matter yield

Genotype	Significance grouping[1]								LSM value (tons/acre)
TMF 1	A								30.8
W605S × LH244	A								30.2
TMF 2	A	B							29.7
GEMS-0115 × GEMN-0097	A	B	C						29.5
LH200 × LH262	A	B	C	D					29.3
GEMN-0145 × LH200	A	B	C	D					29.1
GEMN-0033 × LH198		B	C	D	E				27.7
MDI022:N99d			C	D	E	F			27.2
GEMN-0133 × HC33				D	E	F			27.0
GEMN-0132 × T8					E	F	G		26.6
W606S × LH244					E	F	G		26.1
GEMN-0039 × LH200					E	F	G		25.8
W608S × LH244					E	F	G		25.8
GEMS-0113 × GEMN-0097						F	G	H	25.3
B73 × Mo17						F	G	H	24.8
(Mo15W/Mo16W) × MoPipe(E1)							G	H	24.3
BMR								H	23.2
CHOCLERO									I 17.3

BMR, Brown Midrib.
[1]Significance groupings determined by pairwise *T*-tests at $\alpha = 0.05$. Entries not connected by the same letter are significantly different.

Table 6: Comparison of ranking (1 is highest) of germplasm in this study based on dry matter yield and whole-plant quality

Genotype	Rank by				
	Dry matter yield	Whole-plant quality[1]	1× yield, 1× quality	2× yield, 1× quality	4× yield, 1× quality
TMF 1	1	9	4	2	1
W605S × LH244	2	6	2	1	2
TMF 2	3	11	6	5	4
GEMS-0115 × GEMN-0097	4	7	5	4	3
LH200 × LH262	5	17	12	9	7
GEMN-0145 × LH200	6	12	7	6	6
GEMN-0033 × LH198	7	10	10	8	8
MDI022:N99d	8	1	1	3	5
GEMN-0133 × HC33	9	18	17	16	12
GEMN-0132 × T8	10	13	13	11	11
W606S × LH244	11	15	14	14	13
GEMN-0039 × LH200	12	5	9	10	10
W608S × LH244	13	14	15	15	14
GEMS-0113 × GEMN-0097	14	2	3	7	9
B73 × Mo17	15	16	16	17	17
(Mo15W/Mo16W) × (MoPipe(E1))	16	4	11	13	15
BMR	17	3	8	12	16
CHOCLERO	18	8	18	18	18

BMR, Brown Midrib.
[1]Our measure of whole-plant quality was the mass of sugars produced by enzymatic hydrolysis of the sample per mass of tissue.

grain hybrid check samples varied in dry matter yield, but ranked low for whole-plant quality.

Genotype rankings differed depending on whether dry matter yield or whole-plant quality was used as the ranking factor. If dry matter yield and whole-plant quality are considered together using an index, the rankings of the genotypes are different from those based on dry matter yield or whole-plant quality alone. Weighted rankings can be used if yield and quality are not considered equally important; however, even if yield is considered to be four times as important as quality, there is minor variation in the rankings (Table 6). This illustrates that information about quality may be valuable when making selection decisions, even if quality is not the main target of selection.

Discussion

Development of improved feedstocks for lignocellulosic ethanol production will increase the economic viability of the process. This can be done with plant breeding but requires information about the genetic variation for traits of interest. To this end, we examined dry matter yield and amount of sugar produced by enzymatic hydrolysis of a diverse set of germplasm that included commercial varieties bred for grain or silage production as well as varieties derived from tropical germplasm available through the GEM programme.

Germplasm Enhancement of Maize germplasm was selected for this study in part because of its contributions to silage breeding programmes. Extensive research has been conducted on silage yield and nutritional quality of GEM germplasm (Nass and Coors 2003, Pollak 2003). Inbred W605S was released by the University of Wisconsin and was derived from the GEM breeding cross, AR17056:N1019. This line has high silage yield, with improved in vitro digestibility (IVD), and neutral detergent fibre digestibility (NDFD).

Evaluation of the quality of plant material for production of biofuels from lignocellulosic biomass is hampered by the lack of a standard industrial process. Until a standard method is agreed upon, it is difficult to evaluate the appropriateness of methods that have been proposed for the evaluation of lignocellulosic biomass. We chose to use a microbial assay to measure the release of sugars from biomass using commercially available hydrolytic enzymes. This method has the advantages of high throughput (144 samples per day) and repeatability while using enzymes similar to those that may be used for industrial purposes. Our purpose was to make comparisons within the germplasm included in this study, and this method is well suited to this purpose.

Several other studies have examined the quality of maize for lignocellulosic biofuel production; however, experimental variables such as environmental variation and experimental design reduce the value of comparison among experiments. Such comparisons should be interpreted with caution. The rankings of BMR, TMF, and commercial grain check samples in our study are consistent with the results obtained by Lorenz et al. 2009a and Sheaffer et al. 2006. Although the traits were measured differently, in general BMR hybrids had low yield and high quality and the reverse was true of the TMF hybrids. Similarly, in a comparison of BMR, TMF and normal hybrids, Ballard et al. (2001) determined that the BMR hybrid led to higher milk production in lactating cows, while the TMF hybrid had a greater dry matter yield. The germplasm derived from tropical sources varied widely for both dry matter yield and whole-plant quality, and some of this germplasm was competitive with commercial germplasm for dry matter yield or whole-plant quality, underscoring the potential value of this germplasm.

We elected to harvest whole plants rather than corn stover because infrastructure is in place for this practice in corn silage production systems, and whole-plant ethanol production has been demonstrated to be a feasible ethanol production strategy (Shao et al. 2010). The main difference between corn stover and whole plants is the presence of the grain. Our assay for whole-plant quality should be effective in hydrolysing grain polysaccharide, which is >90% starch. The high proportion of polysaccharide in grain is predicted to increase the amount of sugars produced in our assay. Thus, our measure of sugars produced by hydrolysis of whole plants is likely influenced by

the harvest index (the proportion of the plant that is grain) of the genotypes examined. A review of literature on harvest index as it relates to biofuel production (Lorenz et al. 2010) concluded that harvest index in maize is generally constant in corn-belt-adapted germplasm; however, this may not be the case for unadapted germplasm. While the germplasm in this study is of tropical origin, it has been adapted to the corn belt by recurrent selection.

The observation that none of the correlations between traits were statistically significant suggests it should be possible to improve these traits by selection without impacting the other traits in this study. For example, it should be possible for cobs and whole plants to be developed for independent uses and for yield to be improved simultaneously with cob and whole-plant quality. By contrast, Lorenz et al. (2009a) found many correlations between traits related to yield and composition. The difference between these results may be because our study examined sugar yields per unit of biomass from hydrolysis, which is likely to be impacted by composition and availability (Lorenz et al. 2009b), while the previous study examined a measure of composition only, TEP (theoretical ethanol potential). In addition, our study was smaller than the study by Lorenz et al. (2009a), which would limit the ability to declare significance for a correlation. Another study (Lewis et al. 2009) reported significant phenotypic correlations between glucose release and grain yield.

Our observation that the rankings for quality measures differed from those for dry matter yield is consistent with the findings by Lewis et al. (2009), who examined selection indices involving grain yield and whole-plant quality based on data from a set of recombinant inbred lines. This observation has important economic implications for end-users. As markets develop, it will be important to determine the value of quality vs. quantity. If both high yield and quality are desired, it will be important to develop markets that provide incentives for improving both traits. To this end, it will be important to develop accepted measures of quality that can be applied in the marketing chain.

The large Location [year] and Location [year] × genotype effects are surprising considering the two locations are only 8 km apart. We attribute these effects to differences in planting date, rainfall, and local soil properties including drainage and slope.

In conclusion, we succeeded in differentiating germplasm based on relative sugar yields from hydrolysis of whole plants and cobs as well as yield. Which varieties are superior depends on the relative importance assigned yield and quality. GEM germplasm was competitive with commercial germplasm in all ranking strategies. In breeding maize for lignocellulosic biofuel production, the large genotype × environment interactions make it important to carry out evaluations in many environments.

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