Quantitative Trait Loci and Trait Correlations for Maize Stover Cell Wall Composition and Glucose Release for Cellulosic Ethanol

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ABSTRACT

In cellulosic ethanol production, the efficiency of converting maize (*Zea mays* L.) stover into fermentable sugars partly depends on the stover cell wall structure. Breeding for improved stover quality for cellulosic ethanol may benefit from the use of molecular markers. However, limited quantitative trait loci (QTL) studies have been published for maize stover cell wall components, and no QTL study has been published for glucose release (GLCRel) from stover by a cellulosic ethanol conversion process. Our objectives were to characterize the relationships among stover cell wall components and GLCRel, and to identify QTL with major effects, if any, influencing stover cell wall composition and GLCRel. Testcrosses of 223 intermated B73 × Mo17 recombinant inbreds and the parent lines were analyzed for cell wall composition and GLCRel after acid pretreatment and enzymatic hydrolysis. As expected, glucose (GLC), xylose (XYL), and Klason lignin (KL) composed the bulk (~72%) of the stover dry matter. Significant genetic variance and moderate heritability were observed for all traits. Genetic and phenotypic correlations among traits were generally in the favorable direction but also reflected the complexity of maize stover cell wall composition. We found 152 QTL, mostly with small effects, for GLCRel and cell wall components on both a dry matter and cell wall basis. Because no major QTL were found, methods that increase the frequency of favorable QTL alleles or that predict performance based on markers would be appropriate in marker-assisted breeding for maize stover quality for cellulosic ethanol.

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Abbreviations: ARA, arabinose; CW, cell wall; FA, ferulate esters; FDR, false discovery rate; GAL, galactose; GLC, glucose; GLCRel, glucose release; KL, Klason lignin; LOD, logarithm of the odds; LR, likelihood ratio; MAN, mannose; NDF, neutral detergent fiber; NIRS, near infrared reflectance spectroscopy; PCA, *p*-coumarate esters; QTL, quantitative trait loci; UA, uronic acids; XYL, xylose.

Maize (*Zea mays* L.) stover, the crop residue left after the grain is harvested, is an abundant and readily available substrate for cellulosic ethanol production (Dhugga, 2007; Vermerris et al., 2007; Lorenz et al., 2009). Like other potential substrates for cellulosic ethanol (e.g., bagasse, straw, and wood), maize stover consists largely of cell wall material, which in turn is composed mainly of cellulose, hemicelluloses, and lignin (Sticklen, 2007; Vermerris et al., 2007). Cellulose is a homopolymer of glucose molecules, whereas the predominant hemicellulose in maize and other grasses is arabinoxylan with varying degrees of glucuronic acid, hydroxycinnamate, and acetyl group substitution. Lignin is a complex phenylpropanoid polymer linked to arabinoxylan through ferulate cross-links (Jung and Deetz, 1993).

Converting cell wall polysaccharides into fermentable sugars for ethanol production involves two steps: (i) a harsh thermochemical pretreatment to modify lignin and disrupt cell wall structure, hydrolyze hemicellulose to free sugars, and render cellulose more

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accessible to hydrolytic enzymes; and (ii) enzymatic hydrolysis of cellulose to glucose (GLC) by cellulases (Wyman et al., 1992, 2005; Dien et al., 2006; Himmel et al., 2007). In addition to the type of pretreatment and enzymatic treatments used, the efficiency of converting biomass to fermentable sugars and then into ethanol is influenced by the characteristics and composition of the feedstock (McKendry, 2002; Sun and Cheng, 2002; Hamelinck et al., 2005; Dien et al., 2006). The ethanol yield from a biomass feedstock is a function of the available carbohydrates and limitations imposed on cell wall polysaccharide hydrolysis by biomass lignification (Dien et al., 2006; Dhugga, 2007). Lignin is known to limit both the enzymatic degradability of biomass (Yang and Wyman, 2004; Torney et al., 2007) and degradation of cell wall polysaccharides in the rumen (Jung and Deetz, 1993). In addition, cross-linking of lignin to arabinoxylan by ferulate bridges is believed to contribute to the reduced rumen degradability of maize cell walls (Jung, 1989, 2003; Grabber et al., 1998). However, whether such cross-linking inhibits bioethanol conversion processes is unknown.

Considering that current conversion technologies utilize GLC most efficiently for fermentation to ethanol (Hamelinck et al., 2005; Himmel et al., 2007), the ideal maize stover for cellulosic ethanol production would have a combination of three main traits: high concentration of cellulose in the dry matter, low concentration of lignin in the cell wall, and a large proportion of GLC released by enzymatic degradation (Lewis et al., 2010). In a previous study (Lewis et al., 2010), we found significant genetic variation for these three traits in the intermated B73 × Mo17 mapping population (Lee et al., 2002b). Furthermore, phenotypic analysis indicated no antagonistic relationships that would prevent breeding maize hybrids for grain yield, agronomic traits, and stover quality traits for cellulosic ethanol production. Quantitative trait loci (QTL) analysis provides further information useful in designing marker-assisted breeding strategies. For a trait influenced by a few QTL with large effects, a breeding strategy is to pyramid these QTL into elite germplasm to obtain improved cultivars. But for a trait influenced by many QTL with small effects, a useful breeding strategy is to increase the frequency of favorable QTL alleles by marker-assisted recurrent selection (Johnson, 2004; Eathington et al., 2007) or genome-wide selection (Meuwissen et al., 2001; Bernardo, 2008).

Limited studies have been published on the genetic relationships among the cell wall components of maize stover. In silage maize, many QTL with small to moderate effects have been reported for detergent fiber components in leaf sheath and stalk tissues (Cardinal et al., 2003; Krakowsky et al., 2005, 2006) and in whole plant samples (Méchin et al., 2001). However, the detergent fiber method has been shown to underestimate lignin and overestimate cellulose and hemicellulose (Theander and Westerlund, 1993; Dien et al., 2006). To our knowledge, no QTL results have been reported on stover cell wall composition or release of GLC for cellulosic ethanol production in maize. Our objectives were to (i) characterize the relationships among maize stover cell wall components and glucose release (GLCRel) at the phenotypic and quantitative trait level, and (ii) determine the chromosomal locations of QTL and identify QTL with major effects, if any, for maize stover cell wall composition and GLCRel.

MATERIALS AND METHODS

Plant Materials, Field Experiments, and Stover Quality Analysis

The plant materials, field evaluation procedures, and methods used for stover quality analysis have been reported in another study (Lewis et al., 2010) but are repeated here for convenience. A total of 223 intermated B73 × Mo17 recombinant inbreds (Lee et al., 2002b), along with the inbred parents B73 and Mo17, were testcrossed to a proprietary Monsanto inbred tester that combined well with both B73 and Mo17. The testcrosses were evaluated in 2007 at four Minnesota locations: two near Waseca, MN, and two near Lamberton, MN. The 225 testcrosses were divided into five sets and were evaluated in an augmented randomized complete block design (Federer, 1961), with five hybrid checks included in each set. The entries were planted in four 6.71 m rows spaced 0.76 m apart at a plant population density of 77,000 plants ha^{-1}.

Two rows of each plot were harvested to obtain grain yield and grain moisture data. Before harvest, seven plants were randomly chosen from the remaining two rows in each plot and cut approximately 10 cm above the soil surface. Ears, husks, and shanks were removed and discarded. The stover samples consisting of stalks, leaf blades, and leaf sheaths were then chopped with a yard waste chipper. Approximately 1-kg samples of each entry were obtained, dried at 60°C for 5 d, ground through a 6-mm screen in a cutting-type mill, and reground through a 1-mm screen in a cyclone-type mill.

Ground stover samples were scanned by near infrared reflectance spectroscopy (NIRS) with a Foss Model 6500 scanning monochrometer (Foss North America Inc., Eden Prairie, MN) with a range of 1100 to 2500 nm. A calibration subset consisting of 154 stover samples was selected: 70 calibration samples were selected using Intrasoft International (ISI, Port Matilda, PA) NIRS 3 ver. 4.0 software programs "Center" and "Select" to represent the spectral diversity of the testcrosses; 80 calibration samples were chosen by stratified random sampling within the 20 set-location combinations to ensure equal representation from the physical experimental design; and two samples for each of the inbred parent testcrosses were randomly selected because the parents had not been previously selected for inclusion. The hybrid checks were excluded from the calibration subset. The calibration samples were analyzed in duplicate for cell wall composition and GLCRel as described below.

Cell wall concentration and composition were measured using the Uppsala dietary fiber method (Theander et al., 1995). Starch-free alcohol insoluble residues were prepared and subjected to a two-stage sulfuric acid hydrolysis to hydrolyze the cell wall polysaccharides to their monosaccharide constituents. Neutral sugar components [GLC, xylose (XYL), arabinose (ARA), galactose (GAL), mannose (MAN), rhamnose, and fucose] of the cell wall polysaccharides were quantified by gas chromatography as alditol acetate derivatives after acid hydrolysis. Acidic sugars

(glucuronic, galacturonic, 4-*O*-methylglucuronic acids) were measured as total uronic acids (UA) by the colorimetric method of Ahmed and Labavitch (1977) with glucuronic acid as the reference standard. Klason lignin (KL) was estimated as the ash-free, acid-insoluble residue remaining after acid hydrolysis. Concentration of cell wall polysaccharides (corrected to an anhydro basis) was calculated as the sum of all neutral sugars and UA. All cell wall components were expressed on a 100°C dry matter basis.

Ferulate and *p*-coumarate esters were extracted from starch-free, alcohol insoluble residues with 2 mol NaOH at 39°C for 24 h and were quantified by high-pressure liquid chromatography (Jung and Shalita-Jones, 1990). Total ferulates (ester- and ether-linked) were extracted from similar starch-free, alcohol insoluble residues with 4 mol NaOH at 160°C for 3 h (Iiyama et al., 1990).

Measurement of sugars released after dilute acid/high temperature pretreatment and enzymatic saccharification was done using a modification of the method reported by Dien et al. (2006). Ground maize stover samples were pretreated at 121°C in an autoclave for 60 min with 1.5% (wt/wt) sulfuric acid. The pH of pretreated samples was adjusted to approximately 4.5 with KOH and citrate buffer. A mixture of cellulase (Celluclast) and β-glucosidase (Novozyme 188) (Novozyme A/S, Bagsvaerd, Denmark) was added to the samples to hydrolyze cellulose. The enzymes provided 50 filter paper units of cellulase and 200 IU β-glucosidase activity. Samples were then incubated in a 45°C water bath for 72 h and the reaction mixtures were freeze-dried. The nonhydrolized residue was subjected to the Uppsala dietary fiber method to measure residual polysaccharides. The proportion of cell wall sugars released was then calculated from the difference between the sugar components in the original cell wall material and the sugar components in the residue.

Prediction equations were developed from the analytical data and NIRS spectral data for the 154 calibration samples using the Intrasoft International NIRS 3 ver. 5.0 software program "Calibrate" with the modified partial least squares regression option and two passes to eliminate outliers (Shenk and Westerhaus, 1991a,b). Acceptable calibration statistics were obtained for KL, GLC, XYL, ARA, UA, GAL, MAN, *p*-coumarate esters (PCA), ferulate esters (FA), total sugars, and GLCRel (Table 1). Total ferulate (esters plus ethers) concentration resulted in a poor calibration R^2; therefore, this trait was excluded from further analysis. The calibration equations were used to predict the values for cell wall components and GLCRel for all entries in each location. From the predicted values, cell wall concentration was calculated as the sum of KL and total cell wall sugars. Cell wall components were expressed both on a g kg^{-1} dry matter basis and g kg^{-1} cell wall basis. Glucose release was expressed on a g kg^{-1} GLC basis.

Table 1. Near infrared reflectance spectroscopy (NIRS) calibration statistics for maize stover cell wall composition traits and glucose conversion.

Constituent†	*N*	Mean	Min.	Max.	R^2	SEC‡
KL	137	167	138	196	0.66	6
GLC	135	344	292	395	0.92	5
XYL	138	203	171	234	0.46	8
ARA	141	27.1	19.9	34.4	0.67	1.4
UA	138	26.7	22.0	31.4	0.50	1.1
GAL	138	11.4	8.0	14.8	0.88	0.4
MAN	139	10.0	7.8	12.2	0.63	0.4
PCA	148	18.5	12.8	24.2	0.95	0.4
FA	148	3.25	2.60	3.90	0.43	0.17
FA-total	148	8.15	5.38	10.92	0.04	0.95
Total sugars	137	622	548	696	0.81	11
GLCRel	138	518	398	638	0.83	16

†KL, Klason lignin; GLC, glucose; XYL, xylose; ARA, arabinose; UA, uronic acids; GAL, galactose; MAN, mannose; PCA, *p*-coumarate esters; FA, ferulate esters; FA-total, total ferulate esters and ferulate ethers; GLCRel, Glucose conversion. Cell wall components were expressed on a g kg^{-1} dry matter basis. GLCRel was expressed on a g kg^{-1} glucose basis.

‡Standard error of calibration.

Statistical Analysis and Quantitative Trait Loci Mapping

For each trait in each location, least squares means for each entry were obtained with SAS PROC GLM (SAS Institute, 2004) to adjust for set (or block) effects based on the performance of the checks in an augmented randomized complete block analysis (Scott and Milliken, 1993). Check and parental data were then removed and the data from all locations were combined. Testcross genetic variances (V_{TC}) and heritabilities on a testcross mean basis (h^2_{TC}; Bernardo, 2002) were calculated as appropriate for a one-factor mating design across environments. Data were analyzed using SAS PROC MIXED (SAS Institute, 2004) with location, testcross, and location by testcross interaction effects treated as random effects. Genetic correlations (r_A) between traits were calculated pairwise by multivariate restricted maximum likelihood estimation in SAS PROC MIXED according to Holland (2006). A test of significance for V_{TC} was obtained from the SAS PROC MIXED output. Approximate standard errors for h^2_{TC} and r_A were obtained with the delta method, on the basis of a Taylor's series expansion of up to second-order terms of the estimating functions (Lynch and Walsh, 1998; Holland et al., 2003; Holland, 2006). Approximate 95% confidence intervals for h^2_{TC} and r_A were then calculated from the estimated standard errors, and the h^2_{TC} and r_A estimates were declared significantly different from zero at $P = 0.05$ if the confidence interval did not include zero. Phenotypic correlations among traits were obtained as Pearson correlation coefficients of the means across locations.

Quantitative trait loci mapping was performed with Windows QTL Cartographer version 2.5 (Wang et al., 2007) using least squares means across locations for the phenotype data. Input files containing the linkage map and genotypes for 1339 markers for the intermated B73 × Mo17 population were retrieved from MaizeGDB (Lawrence et al., 2008). Composite interval mapping (Zeng, 1994) was used. Window size in the analysis was 10 cM and walk speed was 1 cM. Forward and backward stepwise regression with the default threshold probabilities ($P = 0.10$) was used to identify 10 control markers. The false discovery rate (FDR) criterion was used in determining thresholds for declaring the presence of QTL. Procedures controlling the FDR ensure that few false QTL are declared while increasing power for QTL detection for both single- and multiple-trait QTL mapping experiments (Benjamini and Yekutieli, 2005). The logarithm of the odds (LOD) threshold corresponding to a $q = 0.05$ FDR was determined separately for each trait. The *P*-value for each test was calculated from the corresponding likelihood ratio [LR, expressed as $-2ln(L_0/L_1)$ in Windows QTL Cartographer] assuming a χ^2_1 approximation. For a fixed testing position, the LR test statistic follows a χ^2_1 distribution (Zeng, 1994; Wu et al., 2007). Although the mixture of two parameters (QTL position

Table 2. Trait values for the parental lines, trait means and ranges for the testcrosses of intermated B73 x Mo17 recombinant inbreds, and testcross genetic variance (V_{TC}) and heritability on a testcross mean basis (h^2_{TC}) for maize stover cell wall composition and glucose release.

Trait†	B73	Mo17	(Intermated B73 x Mo17 inbreds) x Tester Mean	Range	LSD (0.05) ‡	V_{TC}§	h^2_{TC}§
	g kg^{-1} dry matter						
KL	168	169	167	160- 174	5	2	0.38
GLC	339	357	346	321- 363	14	25	0.51
XYL	202	209	204	194- 213	7	5	0.40
ARA	26.8	26.8	27.2	25.7- 29.1	1.2	0.14	0.45
UA	26.4	27.1	26.9	25.9- 27.9	0.8	0.05	0.37
GAL	12.0	10.3	11.3	10.1- 12.7	0.9	0.11	0.50
MAN	10.0	9.4	10.0	9.2- 10.7	0.6	0.02	0.29
PCA	17.4	20.4	18.7	16.3- 20.8	1.4	0.25	0.52
FA	3.11	3.23	3.19	3.00- 3.39	0.16	0.001	0.30
Cell wall	787	807	793	751- 820	24	59	0.44
	g kg^{-1} cell wall						
KL/CW	213	210	211	203- 219	5	6	0.65
GLC/CW	431	442	437	425- 445	5	9	0.71
XYL/CW	256	259	257	248- 265	5	5	0.64
ARA/CW	34.2	33.2	34.4	31.8- 37.0	2.1	0.24	0.31
UA/CW	33.5	33.6	34.0	32.4- 35.5	1.3	0.09	0.30
GAL/CW	15.2	12.7	14.3	12.3- 16.9	1.5	0.26	0.48
MAN/CW	12.7	11.6	12.6	11.7- 13.8	0.9	0.04	0.27
PCA/CW	22.1	25.2	23.6	21.0- 26.4	1.4	0.39	0.60
FA/CW	3.96	4.00	4.02	3.77- 4.44	0.28	0.006	0.37
	g kg^{-1} glucose						
GLCRel	520	511	515	481- 556	22	80	0.57

†KL, Klason lignin; GLC, glucose; XYL, xylose; ARA, arabinose; UA, uronic acids; GAL, galactose; MAN, mannose; PCA, *p*-coumarate esters; FA, ferulate esters; GLCRel, glucose conversion.

‡Approximate least significant difference at α = 0.05.

§V_{TC} and h^2_{TC} for all traits were significantly different from zero at α = 0.05.

and effect) under the null hypothesis in interval mapping suggests a χ^2_2 distribution for LR, the distribution of the maximum LR in a small interval is closer to a χ^2_1 distribution than with χ^2_2 (Wu et al., 2007). The LOD [= $LR/2ln(10)$] threshold for declaring significant QTL at q = 0.05 FDR was then identified using the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995) as described for the FDR1 procedure by Lee et al. (2002a) and as discussed by Benjamini and Yekutieli (2005). The 1-LOD and 2-LOD support intervals for the detected QTL were identified (Lander and Botstein, 1989).

RESULTS

Trait Means, Genetic Variances, and Heritabilities

Means, genetic variances, and heritability estimates for GLC, KL/cell wall (CW), and GLCRel have been reported in a previous study (Lewis et al., 2010) but are included here for convenience. The B73 and Mo17 parental inbred testcrosses had statistically significant differences for GLC, GAL, PCA, GLC/CW, GAL/CW, PCA/CW, and MAN/CW but not for the rest of the traits (Table 2). In contrast, significant differences were observed among the testcrosses of the intermated B73 × Mo17 recombinant inbreds for all traits. As expected, GLC, XYL, and KL were the dominant components of the cell wall in the recombinant inbred testcrosses. On a dry matter basis, GLC accounted for 346 g kg^{-1}, XYL accounted for 204 g kg^{-1}, and KL accounted for 167 g kg^{-1} of the stover dry matter on average. On a cell wall basis, GLC accounted for 437 g kg^{-1}, XYL accounted for 257 g kg^{-1}, and KL accounted for 211 g kg^{-1} of the total cell wall concentration on average. The remaining minor components on average accounted for only 95 g kg^{-1} of the cell wall. Ranges of trait values for the recombinant inbred testcrosses were outside the range of parental values for all traits.

Testcross genetic variance (V_{TC}) was significantly different from zero ($P < 0.05$) for all traits. Heritability on a testcross mean basis (h^2_{TC}) was significantly different from zero ($P < 0.05$) for all traits and ranged from 0.27 for MAN/CW to 0.71 for GLC/CW. For lignin, GLC, and XYL, h^2_{TC} was higher on a cell wall basis than on a dry matter basis. For the other cell wall components, h^2_{TC} was similar or higher on a dry matter basis. Heritability was moderate for cell wall concentration (h^2_{TC} = 0.44) and GLCRel (h^2_{TC} = 0.57).

Table 3. Phenotypic (above the diagonal) and genetic (below the diagonal) correlations among maize stover cell wall composition traits on a dry matter basis and glucose release.

	KL†	GLC	XYL	ARA	UA	GAL	MAN	PCA	FA	Cell wall	GLCRel
KL	–	0.53	0.52	−0.75	−0.30	−0.51	−0.11 NS‡	0.61	−0.16	0.54	−0.64
GLC	0.27 NS	–	0.80	−0.13 NS	0.27	−0.72	0.01 NS	0.44	−0.45	0.94	0.01 NS
XYL	0.40	0.74	–	−0.14	0.39	−0.72	−0.26	0.46	−0.17	0.77	−0.08 NS
ARA	−0.71	0.10 NS	−0.12 NS	–	0.73	0.40	0.15	−0.44	0.05 NS	−0.04 NS	0.48
UA	−0.32 NS	0.35	0.28 NS	0.78	–	−0.16	−0.23	0.04 NS	0.16	0.36	0.18
GAL	−0.39	−0.79	−0.86	0.25 NS	−0.29 NS	–	0.46	−0.60	−0.01 NS	−0.62	0.20
MAN	−0.13 NS	0.18 NS	−0.32 NS	0.08 NS	−0.36 NS	0.25 NS	–	−0.19	−0.63	−0.08 NS	0.31
PCA	0.57	0.32	0.36	−0.34	0.04 NS	−0.50	0.02 NS	–	0.15	0.37	−0.61
FA	0.15 NS	−0.53	−0.07 NS	−0.22 NS	−0.05 NS	0.12 NS	−0.83	0.20 NS	–	−0.40	−0.43
Cell wall	0.24 NS	0.92	0.63	0.31 NS	0.54	−0.63	0.09 NS	0.21 NS	−0.49	–	−0.02 NS
GLCRel	−0.74	0.21 NS	0.03 NS	0.42	0.18 NS	−0.12 NS	0.12 NS	−0.56	−0.54	0.17 NS	–

†KL, Klason lignin; GLC, glucose; XYL, xylose; ARA, arabinose; UA, uronic acids; GAL, galactose; MAN, mannose; PCA, *p*-coumarate esters; FA, ferulate esters; GLCRel, glucose release.

‡NS = Not significant; all other correlations were significantly different from zero at α = 0.05.

Phenotypic and Genetic Correlations

On a dry matter basis, KL had moderately positive phenotypic correlations ($r_P \sim 0.50$, Table 3) with GLC and XYL. Phenotypic correlation between GLC and XYL was strongly positive ($r_P = 0.80$). Phenotypic correlations between the major components (KL, GLC, XYL) with the minor components had different patterns: the three major components had moderate to strong positive correlations ($r_P \geq 0.40$) with PCA, whereas moderate to strong negative correlations ($r_P \leq -0.40$) were observed between the three major components and GAL, between KL and ARA, and between GLC and FA. Among the minor components, moderate to strong positive r_P were observed between ARA and UA, ARA and GAL, and MAN and GAL, whereas moderate to strong negative r_P were observed between ARA and PCA, GAL and PCA, and MAN and FA. Phenotypic correlations were strongly positive between cell wall and GLC, moderately positive for cell wall with both KL and XYL, and moderately negative between GAL and FA. Klason lignin, PCA, and FA all had moderately negative r_P with GLCRel.

Genetic correlation (r_A) on a dry matter basis (Table 3) was positive but nonsignificant between KL and GLC, moderately positive between KL and XYL, and strongly positive between GLC and XYL. Between the major components and minor components, moderately positive r_A was observed between KL and PCA, whereas moderate to strongly negative r_A were observed between KL and ARA, GLC and GAL, XYL and GAL, and GLC and FA. Among the minor components, strong positive r_A was observed between UA and ARA, and strong negative r_A was observed between GAL and PCA as well as between MAN and FA. Cell wall concentration had moderate to strongly positive r_A with GLC, XYL, and UA, and moderately negative r_A with GAL and FA. Glucose release had moderate to strong negative r_A with KL, PCA, and FA.

On a cell wall basis, r_P and r_A among the cell wall composition traits (Table 4) differed both in magnitude and direction for several trait combinations compared to the correlations on a dry matter basis. KL/CW had weak negative r_P with GLC/CW and weak positive r_P with XYL/CW. GLC/CW also had weak positive r_P with XYL/CW. Between the major components and minor components, GLC/CW had moderate to strong negative r_P with ARA/CW, UA/CW, GAL/CW, and FA/CW. Phenotypic correlations among the minor components were mostly moderately positive except for PCA/CW, which had weak negative r_P with other minor components. Only GLC/CW had a significant positive r_P with cell wall concentration; the rest of the components on a cell wall basis had nonsignificant to moderate or strong negative r_P with cell wall concentration. Klason lignin/CW and PCA/CW had moderate negative r_P with GLCRel, whereas correlation between FA/CW and GLCRel was weakly negative.

Genetic correlation on a cell wall basis (Table 4) was weakly negative between KL/CW and GLC/CW and was weak to moderately positive between XYL/CW and both KL/CW and GLC/CW. GLC/CW had moderate to strong negative r_A with most minor components, whereas KL/CW had moderate to strong positive r_A with PCA/CW and FA/CW. Moderate to strong positive r_A were found between most minor components. Among the cell wall components on a cell wall basis, only GLC/CW had a positive r_A with cell wall concentration. Klason lignin/CW, PCA/CW, and FA/CW had moderate to strong negative r_A with GLCRel.

Quantitative Trait Loci Analysis

A total of 152 QTL were found (FDR ~ 0.05) for GLCRel and the stover cell wall composition traits. On a dry matter basis, four to nine QTL were found influencing the concentration of each cell wall component (Table 5). All QTL found for cell wall components on a dry matter basis had relatively low R^2 values ranging from 3% (XYL) to 10% (GLC). Nine QTL were found for cell wall concentration, with R^2 values ranging from 3 to 12%. Five to eight QTL were declared significant for each of the components expressed on a cell wall basis

Table 4. Phenotypic (above the diagonal) and genetic (below the diagonal) correlations among maize stover cell wall composition traits on a cell wall basis and glucose conversion.

	KL/CW†	GLC/CW	XYL/CW	ARA/CW	UA/CW	GAL/CW	MAN/CW	PCA/CW	FA/CW	Cell wall	GLCRel
KL/CW	–	−0.22	0.24	−0.31	−0.14	0.24	0.21	0.47	0.41	−0.49	−0.64
GLC/CW	−0.27	–	0.21	−0.52	−0.48	−0.71	−0.13	0.22	−0.56	0.56	0.08 NS‡
XYL/CW	0.45	0.22	–	−0.04 NS	0.25	−0.18	−0.18	0.28	0.26	−0.15	−0.09 NS
ARA/CW	−0.33	−0.58	−0.24 NS	–	0.87	0.71	0.43	−0.35	0.48	−0.60	0.40
UA/CW	0.06 NS	−0.63	0.12 NS	0.80	–	0.52	0.19	−0.07 NS	0.65	−0.61	0.18
GAL/CW	0.37	−0.79	−0.19 NS	0.64	0.50	–	0.71	−0.30	0.45	−0.78	0.17
MAN/CW	0.24 NS	−0.11 NS	−0.22 NS	0.21 NS	−0.05 NS	0.62	–	−0.14	0.02 NS	−0.54	0.27
PCA/CW	0.53	0.19 NS	0.35	−0.26 NS	0.08 NS	−0.11 NS	0.18 NS	–	0.25	−0.02 NS	−0.65
FA/CW	0.68	−0.57	0.46	0.29 NS	0.64	0.56	0.00 NS	0.37	–	−0.76	−0.30
Cell wall	−0.65	0.53	−0.33	−0.40	−0.60	−0.79	−0.54	−0.22 NS	−0.82	–	−0.02 NS
GLCRel	−0.72	0.21 NS	−0.16 NS	0.28 NS	−0.03 NS	−0.15 NS	0.00 NS	−0.58	−0.45	0.17 NS	–

†KL, Klason lignin; CW, cell wall; GLC, glucose; XYL, xylose; ARA, arabinose; UA, uronic acids; GAL, galactose; MAN, mannose; PCA, *p*-coumarate esters; FA, ferulate esters; GLCRel, glucose release.

‡NS = Not significant; all other correlations were significantly different from zero at $\alpha = 0.05$.

Table 5. Map locations and parameters for quantitative trait loci (QTL) influencing stover cell wall composition and glucose conversion in testcrosses of intermated B73 × Mo17 recombinant inbreds.

QTL	Chromosome	Bin	1-LOD† support interval	2-LOD support interval	Nearest marker	Distance‡	Position	LOD score	R^2 (%)§	*a*¶
			cM			cM				
KL# (2.7)††										
1	1	1.10	813–818	806–831	npi282b	0.6 (+)	816.8	4.4	6	–0.71
2	2	2.07	386–398	381–403	rz474c	1.0 (+)	395.4	3.3	5	–0.59
3	7	7.04	492–496	484–499	npi380	0.0	493.4	3.9	6	–0.61
4	8	8.01	68–93	62–97	umc1483	0.0	85.9	3.2	5	–0.54
GLC (2.3)										
1	1	1.03–1.04	288–294	286–296	mmp56	1.5 (+)	291.6	6.7	10	2.35
2	3	3.06	348–348	347–351	umc1027	0.0	348.3	4.2	5	–1.61
3	3	3.09	609–616	607–619	sho89	2.0 (–)	613.0	5.6	8	–2.06
4‡‡	6	6.05	338–348	334–349	rz444d	0.0	339.0	5.0	6	1.75
5	7	7.02	207–211	204–212	umc2092	2.0 (–)	208.5	6.6	10	–2.30
6	9	9.03	212–216	207–227	csu623	0.0	214.8	3.7	4	–1.53
7	10	10.01	64–73	57–76	umc2018	0.0	67.2	3.2	4	–1.40
XYL (2.3)										
1	1	1.02	163–176	150–181	bnlg1953	0.5 (+)	167.7	2.6	3	0.66
2‡‡	2	2.02	137–143	121–147	umc1261	0.0	142.4	4.9	6	–0.87
3	2	2.06	316–334	316–337	umc1079	1.0 (–)	327.6	3.6	5	–0.80
4	3	3.02	62–73	43–77	umc1458	0.2 (+)	67.7	3.2	4	–0.72
5	4	4.03	143–149	141–156	umc1926	0.0	145.6	3.6	4	–0.75
6‡‡	6	6.05	338–340	337–348	rz444d	0.0	339.0	5.3	7	0.93
7	7	7.02	155–172	146–172	psr371b	0.0	160.7	2.3	3	–0.60
8	8	8.05	339–351	337–357	bnl12.30a	0.0	342.8	4.5	6	1.01
9	8	8.07	428–450	424–451	bnlg1823	4.9 (+)	435.8	3.1	6	–0.88
ARA (3.0)										
1	1	1.02	152–163	146–167	umc1976	0.0	158.8	3.4	5	0.13
2	1	1.07	643–651	641–659	phi002	0.0	646.7	3.9	5	–0.13
3	7	7.03	294–301	290–314	mmp152	0.0	298.9	3.3	5	0.13
4	9	9.05	323–327	315–331	umc1231	0.0	325.9	3.5	5	–0.13
UA (2.5)										
1	2	2.01	15–27	11–30	isu144a	0.0	24.0	3.2	5	–0.08
2	2	2.07	360–367	347–367	bcd926b	0.8 (+)	365.6	3.9	6	0.09
3	3	3.01–3.02	48–60	43–77	asg30c	1.4 (+)	57.3	2.8	4	–0.08
4	5	5.06	429–445	429–449	bnlg609	0.0	441.2	2.9	4	–0.08
5	7	7.05	499–514	493–519	phi069	0.0	504.9	4.3	6	0.09
6	9	9.05	309–320	305–325	ufg63	0.0	315.1	2.6	3	–0.07
7	10	10.03	170–175	168–185	npi445a	0.0	172.8	3.8	5	–0.09
8	10	10.04	218–233	218–242	umc1330	4.0 (+)	226.6	2.7	5	0.09
GAL (2.5)										
1	1	1.05	422–430	415–433	umc1321	0.8 (+)	425.3	2.9	4	–0.10
2	2	2.02	120–134	115–142	bnlg2277	2.7 (+)	128.2	3.7	6	0.12
3	3	3.00	6–10	0–21	phi453121	0.0	7.3	3.2	4	0.10
4	3	3.06	355–362	353–375	rz538b	0.0	358.3	3.2	4	0.10
5	6	6.05	338–351	326–351	rz444d	1.0 (–)	340.0	3.2	4	–0.10
6	7	7.01–7.02	114–129	112–134	asg34a	1.2 (+)	121.2	2.5	3	–0.09
7	7	7.02	209–212	208–213	umc1393	1.5 (+)	210.5	6.2	9	0.16
8‡‡	9	9.06	474–481	469–486	isu049	0.0	479.0	4.2	5	–0.11
9	10	10.07	394–420	394–422	mmp181	2.8 (+)	400.8	2.5	4	0.09
MAN (2.3)										
1	1	1.03	258–268	256–269	mmp23	1.0 (–)	259.3	4.7	6	0.07
2	1	1.04	341–356	341–359	bnlg1811	2.0 (–)	350.6	3.4	5	0.06
3	1	1.05	419–432	400–446	umc1603	0.6 (+)	427.7	2.4	3	–0.05
4	1	1.07	626–632	622–633	bnlg1025	0.0	630.5	3.7	5	0.06
5	2	2.02	121–134	117–142	bnlg2277	0.0	130.9	4.5	6	0.06
6	4	4.05	283–289	278–294	bnl15.45	1.0 (–)	284.2	5.5	7	–0.07
7	7	7.03	279–293	274–298	npi394	0.0	284.9	2.5	3	–0.05
8	8	8.07	421–440	419–440	bnlg1823	7.9 (+)	432.8	2.9	6	0.06
9	9	9.03	198–210	195–217	psr160c	1.1 (+)	204.0	3.6	5	0.06

(cont'd)

Table 5. Continued.

QTL	Chromosome	Bin	1-LOD† support interval	2-LOD support interval	Nearest marker	Distance‡	Position	LOD score	R^2 (%)§	a¶
			cM			cM				
Cell wall (2.3)										
1	1	1.03–1.04	287–294	285–296	mmp56	2.5 (+)	290.6	7.7	12	4.19
2	1	1.10	814–822	811–831	npi282b	0.0	817.4	3.2	4	–2.40
3	3	3.09	631–644	629–646	npi425a	2.0 (–)	640.3	6.0	9	–3.56
4	6	6.05	337–346	333–348	rz444d	0.0	339.0	4.7	5	2.79
5	7	7.03	347–363	347–370	isu150	6.0 (–)	352.7	2.5	4	–2.40
6	8	8.05	320–322	318–328	ufg74	0.0	320.9	4.8	6	3.23
7	9	9.04	250–250	246–251	umc1570	0.0	249.8	8.4	10	–5.67
8	9	9.04	268–273	263–281	umc1107	0.0	269.6	2.4	3	3.00
9	10	10.06	313–321	301–330	bnl10.13a	0.0	318.9	2.7	3	2.13
PCA (2.5)										
1	2	2.00–2.01	10–27	4–34	isu144a	4.0 (+)	20.0	3.0	7	0.19
2	2	2.02	123–135	120–139	bnlg2277	0.0	130.9	5.9	7	–0.19
3	2	2.05	300–303	292–316	umc131	0.0	302.5	2.7	3	0.14
4	4	4.06	349–359	343–363	rz273a	0.0	352.6	4.8	7	–0.18
5	5	5.03	192–199	189–205	csu340	0.0	195.6	3.4	4	–0.14
6	7	7.02	210–214	206–220	umc1393	0.0	212.0	4.5	5	–0.17
7	8	8.07	427–444	424–451	bnlg1823	4.9 (+)	435.8	2.9	6	–0.17
FA (2.4)										
1	1	1.03	257–264	247–267	bnlg1866	0.0	262.2	3.9	5	–0.02
2	1	1.04	326–337	323–345	Ufg77	1.8 (+)	333.2	3.2	4	–0.02
3	1	1.08	696–705	689–708	an1	0.0	699.7	5.8	7	–0.02
4	5	5.00	4–13	3–19	umc1423	0.5 (+)	8.0	3.4	4	–0.01
5	6	6.07	472–485	459–487	bnlg1136	0.0	475.9	2.7	3	0.01
6	8	8.04	282–289	278–299	gta101d	0.0	286.9	2.6	3	–0.01
7	9	9.04	277–283	275–287	bnlg1012	0.9 (+)	280.9	4.5	6	–0.02
8	10	10.04	241–248	240–254	umc1272	0.0	242.2	5.2	6	–0.02
GLCRel (2.3)										
1	1	1.04	301–311	299–312	asg75	0.0	308.7	8.2	10	3.81
2	1	1.06	489–500	485–507	mmp156	0.0	495.7	2.7	3	–2.12
3	1	1.10	820–836	820–839	mmp87	2.9 (+)	829.0	3.9	5	2.84
4	2	2.05–2.06	317–323	315–325	umc1028	1.0 (–)	319.5	6.7	9	–3.80
5	2	2.07	386–398	380–404	rz474c	0.0	396.4	3.5	4	2.51
6	2	2.08	439–444	437–454	umc137a	0.0	442.4	6.0	7	–3.32
7	5	5.01	54–83	53–96	lim407	0.0	63.3	2.5	3	2.01
8	7	7.00	0–11	0–84	csu582	0.7 (+)	2.0	2.3	3	–1.94
9	7	7.05	493–495	488–497	npi380	0.0	493.4	4.2	5	2.77
10	9	9.00	0–2	0–3	umc1957	0.0	0.0	5.4	6	3.01

†Logarithm of the odds.

‡Distance of the nearest marker from the QTL position; a negative sign in parentheses indicates that marker position is to the left of the QTL position, whereas a plus sign indicates that the marker position is to the right of the QTL position.

§Percentage of the phenotypic variance explained by the QTL, conditioned on the background markers (Basten et al., 2005).

¶Additive effect, half the difference between the two homozygous genotypes for the QTL.

#KL, Klason lignin; GLC, glucose; XYL, xylose; ARA, arabinose; UA, uronic acids; GAL, galactose; MAN, mannose; PCA, *p*-coumarate esters; FA, ferulate esters; GLCRel, glucose release.

††In parentheses is the LOD threshold at false discovery rate (FDR) ~ 0.05.

‡‡QTL mapped close to and in the same chromosome bin as QTL detected for pericarp cell wall by Hazen et al. (2003) in the intermated B73 x Mo17 recombinant inbred population.

(Table 6). Most of these QTL had small effects, with R^2 values less than 10%. However, one QTL each for KL/CW, GLC/CW, XYL/CW, and UA/CW had relatively large effects, with R^2 between 16 and 27%. Ten QTL were found for GLCRel, with R^2 values ranging from 3 to 10% (Table 6).

Even when the R^2 was low, the LOD score was high for the QTL with the highest R^2 within a trait (Tables 5 and 6). The QTL map position for most of the detected QTL was within 1 cM of the nearest marker and for many QTL the map position was located exactly on a marker position. The average lengths of support intervals for the QTL locations were 9.8 cM for a 1-LOD interval and 19.9 cM for a 2-LOD interval.

Although QTL were scattered across all 10 chromosomes, clustering of QTL was apparent (Tables 5 and 6). Five of the 10 QTL for GLCRel colocalized with a QTL for KL or KL/CW. For these colocalized QTL, the

Table 6. Map locations and parameters for quantitative trait loci (QTL) influencing stover cell wall composition on a cell wall basis in testcrosses of intermated B73 × Mo17 recombinant inbreds.

QTL	Chromosome	Bin	1-LOD† support interval	2-LOD support interval	Nearest marker	Distance‡	Position	LOD score	R^2 (%)§	*a*¶
			cM			cM				
KL/CW# (2.4)††										
1	1	1.02	152–162	149–164	umc1976	1.0 (+)	157.8	4.8	6	–0.79
2	1	1.04	301–306	297–309	bnlg2238	4.0 (–)	302.9	12.0	18	–1.33
3	1	1.11	897–912	892–912	umc1118	2.0 (–)	904.2	3.1	4	0.65
4	2	2.05	317–323	314–330	umc1028	0.0	318.5	3.5	4	0.71
5	2	2.07	354–367	347–379	bcd926b	2.8 (+)	363.6	2.6	4	–0.66
6	7	7.02	164–171	151–172	bnlg2203	0.5 (+)	168.2	3.9	5	0.70
7	9	9.01	82–97	65–97	umc1967	1.0 (–)	83.9	2.8	4	–0.58
8	9	9.05	311–318	303–321	ufg64	0.0	313.2	4.6	6	0.73
GLC/CW (2.4)										
1	1	1.04	333–338	322–345	ufg77	0.0	335.0	6.4	6	0.91
2	2	2.05–2.06	318–323	317–324	umc1028	2.0 (–)	320.5	18.2	22	–1.78
3	3	3.04	240–250	232–259	hac101a	0.0	245.9	2.4	2	–0.55
4	3	3.06	424–437	422–442	lim424	2.0 (+)	430.7	3.2	4	–0.69
5	3	3.09	607–616	595–621	sho89	1.0 (–)	612.0	4.8	5	–0.81
6	7	7.02	208–212	201–214	umc1393	2.5 (+)	209.5	4.9	6	–0.92
7	7	7.03	293–301	290–303	mmp152	0.0	298.9	3.4	3	–0.70
XYL/CW (2.3)										
1	1	1.04	305–312	302–321	umc1169	0.0	309.7	5.9	7	–0.77
2	2	2.05	317–322	316–323	umc1028	0.6 (+)	317.9	11.4	16	–1.55
3	3	3.02	49–79	43–79	umc1458	2.2 (+)	65.7	2.4	3	–0.52
4	3	3.05	276–284	269–289	rz296b	1.0 (–)	279.9	4.9	6	0.74
5	4	4.08	488–494	475–500	npi444	0.0	490.3	2.5	3	–0.51
6	8	8.05	336–342	336–358	umc1316	0.0	338.9	4.8	6	0.74
7	8	8.07	441–458	441–461	umc1268	1.0 (+)	451.0	4.4	6	–0.68
8	9	9.02	172–182	169–184	lim286	0.0	176.3	3.9	5	0.62
ARA/CW (2.5)										
1	1	1.07	643–650	641–659	umc1245	0.0	647.7	4.3	6	–0.21
2	2	2.07	388–398	384–403	rz474c	1.0 (+)	395.4	4.6	7	0.24
3	4	4.03	188–192	186–192	umc2039	0.0	189.4	5.1	7	0.33
4	4	4.04	204–214	204–217	npi386	1.0 (–)	204.7	2.7	5	–0.25
5	6	6.05	335–341	326–347	rz444d	0.0	339	3.3	4	–0.19
6	7	7.03	297–301	294–303	mmp152	0.0	298.9	5.2	7	0.26
7	7	7.05	493–495	479–497	npi380	0.0	493.4	4.0	5	0.21
8	9	9.05	323–326	315–328	umc1231	0.0	325.9	2.7	4	–0.18
UA/CW (2.6)										
1	1	1.05	404–406	400–407	umc1676	0.0	404.9	3.9	5	–0.16
2	1	1.08	697–705	689–709	an1	0.0	699.7	2.9	4	–0.11
3	3	3.04	253–270	250–270	umc2002	1.8 (+)	258.2	3.9	6	0.14
4	6	6.05	309–337	306–337	uaz121a	4.9 (+)	320.7	2.9	7	–0.15
5	7	7.04	457–472	454–478	ufg57	7.0 (–)	464.3	4.0	12	0.19
6	8	8.01	67–97	55–101	umc1483	0.0	85.9	2.6	4	0.11
7	9	9.05	323–327	318–331	umc1231	0.0	325.9	3.0	4	–0.12
8	9	9.06	470–482	463–483	mmp110	0.0	473.3	2.7	4	–0.11

(cont'd)

direction of effects was favorable, i.e., the QTL allele from either B73 or Mo17 for high GLCRel was colocalized with the QTL allele for low KL or KL/CW. The QTL with the largest effect for GLCRel (R^2 = 10%) colocalized with the QTL with the largest effect for KL/CW (R^2 = 18%) and with a QTL with small effect for XYL/CW. These QTL were located between the 301 and 312 cM positions on chromosome 1. Quantitative trait loci with minor effects for GAL, MAN, PCA, GAL/CW, MAN/CW, PCA/CW, and XYL were found clustered between the 115 and 145 cM positions on chromosome 2. The QTL with the largest effect for GLC/CW (R^2 = 22%) clustered with the QTL with the largest effect for XYL/CW (R^2 = 16%), with the QTL with second largest effect for GLCRel (R^2 = 9%),

Table 6. Continued.

QTL	Chromosome	Bin	1-LOD† support interval	2-LOD support interval	Nearest marker	Distance‡	Position	LOD score	R^2 (%)§	a¶
			cM			cM				
GAL/CW (2.5)										
1	1	1.11	926–938	920–941	lim228	14.3 (+)	932.9	5.8	27	0.39
2	2	2.02	137–145	119–150	umc1261	0.0	142.4	3.0	4	0.15
3	3	3.04	244–247	242–249	hac101a	0.0	245.9	4.1	5	0.24
4	4	4.09	588–604	584–604	umc1573	2.0 (–)	596.1	3.5	5	0.17
5	6	6.05	334–348	326–351	rz444d	1.0 (–)	340	3.6	5	–0.17
MAN/CW (2.3)										
1	1	1.04	341–357	341–359	bnl9.11b	0.4 (+)	352.6	3.5	5	0.08
2	2	2.02	124–134	121–142	bnlg2277	0.0	130.9	5.8	8	0.11
3	3	3.01	0–21	0–22	bnl8.15	2.0 (–)	13.6	3.5	5	0.10
4	4	4.05	286–292	283–298	psr128	0.0	287.7	4.9	6	–0.12
5	6	6.01	64–69	56–70	umc1229	2.0 (–)	67.8	4.2	9	0.20
6	6	6.01	80–82	78–82	mmp160	0.0	80.8	6.5	9	–0.21
7	6	6.05	338–347	331–351	rz444d	1.0 (–)	340	3.5	5	–0.09
8	9	9.04	242–255	242–259	lim166	0.0	252.2	5.2	7	0.10
PCA/CW (2.5)										
1	1	1.03	216–218	208–221	umc230	0.0	216.6	2.5	3	–0.15
2	1	1.11	883–892	878–901	umc1129	0.2 (+)	889.1	3.2	4	0.18
3	2	2.02	121–140	114–142	bnlg2277	0.0	130.9	2.5	3	–0.14
4	2	2.04–2.05	293–303	292–309	umc131	1.1 (+)	301.4	3.5	5	0.19
5	4	4.06	343–360	337–363	rz273a	1.9 (+)	350.7	4.7	7	–0.22
6	5	5.03	190–196	189–199	bnl7.56	0.0	192.3	5.1	6	–0.22
7	6	6.02	124–135	121–138	psb108	1.0 (–)	131	3.7	5	–0.19
8	8	8.08	462–475	462–477	mmp64	2.1 (+)	470.1	4.1	6	–0.20
FA/CW (2.4)										
1	1	1.03	260–264	257–267	umc1598	0.0	262.8	4.4	5	–0.03
2	1	1.08	695–705	688–708	umc1928	0.6 (+)	697.3	6.4	8	–0.04
3	3	3.09	608–614	595–621	psb041	0.0	610.8	3.4	4	0.03
4	5	5.00	6–14	4–19	umc1423	0.0	8.5	4.0	5	–0.03
5	6	6.05	326–346	326–349	bnlg1732	0.4 (+)	336.6	2.5	3	–0.02
6	6	6.07	473–485	462–489	mmp105	0.8 (+)	477.9	3.0	4	0.03
7	8	8.04	282–290	279–298	gta101d	0.0	286.9	3.9	4	–0.03
8	10	10.04	241–247	240–253	umc1272	1.0 (–)	243.2	4.5	6	–0.03

†Logarithm of the odds.

‡Distance of the nearest marker from the QTL position; a negative sign in parentheses indicates that marker position is to the left of the QTL position, whereas a plus sign indicates that the marker position is to the right of the QTL position.

§Percentage of the phenotypic variance explained by the QTL, conditioned on the background markers (Basten et al., 2005).

¶Additive effect, half the difference between the two homozygous genotypes for the QTL.

#KL, Klason lignin; GLC, glucose; XYL, xylose; ARA, arabinose; UA, uronic acids; GAL, galactose; MAN, mannose; PCA, *p*-coumarate esters; FA, ferulate esters; FA-total, total ferulate esters and ferulate ethers; GLCRel, glucose release.

††In parentheses is the LOD threshold at false discovery rate (FDR) ~ 0.05.

and with a QTL with small effect for KL/CW. These QTL were located between the 317 and 323 cM positions on chromosome 2. Small-effect QTL for GLC, XYL, GAL, cell wall concentration, ARA/CW, GAL/CW, MAN/CW and FA/CW were clustered between the 325 and 350 cM positions on chromosome 6. Minor QTL for ARA, UA, ARA/CW, and UA/CW were also clustered between the 305 and 330 cM positions on chromosome 9. Other groups of two or more QTL for different traits were found colocalized in different chromosomal locations.

DISCUSSION

Stover Cell Wall Composition and Near Infrared Reflectance Spectroscopy Calibrations

In grasses, GLC (mainly from cellulose) and XYL (from xylans) residues, along with KL, are the three most abundant cell wall components, with ARA, UA, and other sugar residues present in smaller amounts (Åman, 1993). The composition of the stover of the B73 × Mo17 recombinant inbred testcrosses followed this trend expected for

a grass species. The mean concentrations of KL/CW, GL/CW, XYL/CW, ARA/CW, UA/CW, and FA/CW in the stover of recombinant inbred testcrosses were comparable to the mean concentrations reported by Jung and Casler (2006a) for mature internodes of three maize hybrids. The mean GLC, XYL, and KL content (dry matter basis) for the stover of recombinant inbred testcrosses in this study were also comparable to those reported for a diverse set of 49 maize hybrids (Lorenz et al., 2009).

During internode development, thick, lignin-rich secondary walls formed in sclerenchyma, rind-region parenchyma, and epidermal tissues in the internodes of three maize hybrids and accounted for increased cell wall concentration (Jung and Casler, 2006a). Secondary cell wall formation and lignification also occur in epidermis, parenchyma, parenchyma bundle sheath, and vascular tissues in the leaf blade, midrib, and sheath of mature C_4 grasses (Wilson, 1993) such as maize. Because the secondary cell wall of grasses is composed mostly of cellulose, xylans, and lignin (Åman, 1993), the bulk of the GLC, XYL, and KL in the maize stover must have come from these tissues with thick secondary cell walls.

In this study we developed NIRS calibrations to measure the different stover-quality traits, and the calibration R^2 values were lower for some traits (XYL, UA, and FA) than for others. We could have increased the R^2 values simply by increasing the variation in the concentration of cell wall components. This could have been accomplished by sampling different genetic backgrounds or harvesting at different stages of plant maturity. However, having NIRS calibrations from more diverse material would not have necessarily improved the predictions in our experiment, which comprised a single population sampled after grain harvest. Our NIRS calibrations were therefore specifically developed for our experiment and are not a global calibration for use in other experiments. The ability to detect statistically significant effects as well as QTL in our study indicated that the genetic variation among testcrosses of recombinant inbreds was greater than the random variation in the imperfect NIRS predictions. As such we believe that the NIRS calibrations were sufficiently robust for the purposes of our study.

Relationships among Stover Cell Wall Components

The phenotypic (r_P) and genetic (r_A) correlations among the cell wall components reflected the complexity of plant cell wall development (Jung and Buxton, 1994). Most of the observed r_P and r_A were as expected. The positive r_P among GLC, XYL, KL, and cell wall concentration on a dry matter basis imply that high cell wall concentration was associated with increased concentrations of GLC, XYL, and KL, as expected. The r_P among GLC, XYL, and KL on a dry matter basis were comparable to those reported by Lorenz et al. (2009) for a set of maize hybrids. The positive r_P among GLC, XYL, KL, and cell wall concentration on a dry matter basis may have been due to the accumulation of secondary cell walls and to the proportion of tissues in the stover containing large amounts of secondary cell walls. The positive r_A among GLC, XYL, and cell wall concentration imply that these traits had a common genetic basis to some extent. KL had a positive but nonsignificant r_A with both GLC and cell wall concentration, suggesting that the genetic control of KL is independent to that of GLC and cell wall concentration. The nonsignificant r_A between KL and cell wall concentration was a surprising result given that KL was the third largest component of the cell wall in maize stover. The r_A of KL and XYL was positive, indicating some possible common genetic control for these two cell wall components. Lignin was the last major cell wall component to begin rapidly accumulating in a developing maize internode, with GLC being the first major component to accumulate and XYL being intermediate (Jung, 2003). This accumulation pattern may explain why XYL was more strongly correlated with KL than was GLC in the current study.

The r_P values suggested that increased cell wall concentration was associated with increased GLC/CW and also with reduced KL/CW and XYL/CW concentrations in the cell wall. The strong positive r_P between cell wall concentration and GLC on a dry matter basis may therefore be attributed to concentration of GLC in the cell wall, in addition to secondary cell wall accumulation. On the other hand, the positive association between cell wall concentration and XYL and KL on a dry matter basis may be attributed to secondary cell wall accumulation, but not with the concentration of these components in the cell wall. The r_A between cell wall concentration and the three major components on a cell wall basis indicated that these were influenced to some extent by linked or pleiotropic genes, but in opposite direction of effects, such that an increase in cell wall concentration was associated with increase in GLC/CW and reduction in both XYL/CW and KL/CW. The negative but weak r_A between KL/CW and GLC/CW may have simply been due to these traits being expressed as a proportion of the cell wall: when one component increases in concentration one or more other components must decrease because the sum must equal 100%.

Notable among the correlations between the major components and minor polysaccharide residues were the moderate to strong negative r_P and r_A between KL and ARA, for GAL with the three major components on a dry matter basis, and for GLC/CW with ARA/CW, UA/CW, and GAL/CW. These inverse relationships for concentration of major and minor cell wall components are indicative of dilution of primary cell wall material (rich in ARA, GAL, MAN, and UA) by the accumulation of secondary wall material (rich in GLC, XYL, and KL) (Carpita, 1996; Jung, 2003). Among the minor polysaccharide residues,

the strong positive r_P and r_A between UA and ARA and between UA/CW and ARA/CW may be expected if the majority of the UA and ARA residues occurred together in polysaccharides such as glucuronoarabinoxylans. Similarly, the moderate r_P and r_A of GAL/CW with ARA/CW, UA/CW, and MAN/CW can be due to the co-occurrence of these residues in other cell wall polysaccharides such as galactoglucomannans, polygalacturonic acid, and arabinogalactans (Åman, 1993; Carpita, 1996).

Lignin and hemicellulose are cross-linked in the cell wall by ferulate molecules with ester bonds to arabinoxylan and various covalent linkages to lignin (Grabber et al., 1998; Jung and Casler, 2006a). The moderately positive r_P and r_A between PCA and KL both on a dry matter and cell wall basis may be expected if most PCA is esterified to lignin (Jung and Buxton, 1994; Jung and Casler, 2006a). On the other hand, r_P and r_A of FA with XYL and ARA, the primary polysaccharide residues of hemicellulose, were nonsignificant on a dry matter basis but were moderately positive on a cell wall basis, probably because FA is a component of arabinoxylan, the major hemicellulose in grass cell walls (Carpita, 1996).

Stover Glucose Conversion

As expected, GLCRel had moderate negative r_P and r_A with KL and PCA both on a dry matter basis and cell wall basis. Removal of lignin was shown to significantly enhance the enzymatic hydrolysis of cellulose in maize stover (Yang and Wyman, 2004), and Dien et al. (2006) likewise reported a negative relationship between GLCRel and KL. Forage digestibility in ruminants is known to be influenced by KL concentration and cross-linking of KL to other cell wall polymers (Jung and Deetz, 1993), with 60 to 80% of the variability in rumen cell wall degradability typically explained by lignin concentration. It had been proposed that lignin structure, as measured by the syringyl-to-guaiacyl monolignol ratio, influences the degree to which lignin inhibits rumen cell wall degradability (Jung and Deetz, 1993). However, subsequent studies strongly suggested that even large shifts in syringyl-to-guaiacyl monolignol ratio did not alter the impact of lignin concentration on rumen cell wall degradability (Grabber et al., 1997; Jung et al., 1999). Based on these reports it seems unlikely that lignin structure as measured by monolignol composition would impact bioethanol conversion processes.

The moderate negative r_P and r_A for FA with GLCRel was unexpected because FA only measured esterified ferulates, not ferulate cross-links. This result was also contrary to the higher concentration of FA in smooth bromegrass (*Bromus inermis* Leyss.) plants with high rumen neutral detergent fiber (NDF) digestibility than in plants with low digestibility (Jung and Casler, 1990). In a study of maize internode maturity, ferulate ester concentration was not correlated with rumen cell wall polysaccharide degradability of young, elongating internodes but was positively correlated in mature, postelongation internodes (Jung et al., 1998). Ferulate cross-links have also been shown to limit enzymatic and rumen degradability of cell walls (Grabber et al., 1998; Casler and Jung, 1999).

The poor NIRS calibration for total ferulates prevented us from evaluating ferulate cross-links in the current study. While FA slow the rate of cell wall polysaccharide degradation by rumen microbes, it is the cross-links that actually limit the extent of degradation (Grabber et al., 1998; Jung and Casler, 2006b). Data for 92 maize stover calibration samples analyzed by wet chemistry indicated that ferulate ethers (cross-links) were not correlated with GLCRel (data not shown). We speculate that this lack of an effect was not unexpected because of the impact of the pretreatment process utilized in our GLCRel conversion test. The high temperature/dilute acid pretreatment is designed to hydrolyze hemicellulose, thereby increasing accessibility of the subsequently added cellulases to the cellulose. Because all ferulate molecules are esterified to arabinoxylan, after the pretreatment those ferulates with cross-links to lignin would still be attached to lignin but would no longer be attached to hemicellulose because the xylan had been converted to free XYL. This destruction of the cross-link should eliminate any inhibitory impact of the ferulate cross-links on subsequent cellulase activity on cellulose.

Removal of xylans was shown by Yang and Wyman (2004) to result in greater enzymatic hydrolysis of maize stover cellulose. Phenotypic and genetic correlations between GLCRel and the polysaccharide residues were mostly nonsignificant, suggesting that cell wall polymers other than lignin might not have a large impact on release of GLC (which is primarily from cellulose), or that the pretreatment process used was effective in removing most of the inhibitory effects of the other polymers on cellulose degradability. Given that the noncellulosic polysaccharides were hydrolyzed by acid, rather than enzymatically, the latter hypothesis is more likely. Unlike rumen digestion of maize cell wall polysaccharides where xylan degradability was lower than cellulose degradation (Jung and Casler, 2006b), the opposite response was observed in the current study (data not shown) and by Dien et al. (2006) for a variety of biomass crops. These results indicate that the pretreatment (dilute acid and high temperature) used in the conversion test was relatively effective at removing noncellulosic polysaccharides from the cell wall matrix, but leaving lignin residues that still interfered with cellulose hydrolysis.

Quantitative Trait Loci for Stover Cell Wall Composition and Glucose Conversion

Many QTL, mostly with small effects, were detected across all 10 chromosomes for the concentration of stover cell wall components, total cell wall concentration, and GLCRel. Only a few QTL (one each for KL/CW, GLC/

CW, XYL/CW, and GAL/CW) had R^2 values greater than 15%. The R^2 values in QTL mapping experiments are typically inflated (Beavis, 1994; Schön et al., 2004), and the lack of QTL with inordinately large effects hinders a candidate gene approach for identifying genes for the concentration of cell wall components.

The 1-LOD and 2-LOD support intervals spanned on average about 10 and 20 cM. However, 4 cM in the linkage map of the intermated B73 × Mo17 population corresponds roughly to 1 cM in a linkage map for an F_2–derived population that did not undergo any intermating (Lee et al., 2002b; Balint-Kurti et al., 2007). Therefore, the 1-LOD and 2-LOD support intervals in this study were actually on the order of 2.5 and 5 cM on a usual maize linkage map for a nonintermated F_2-derived mapping population. This precise mapping of QTL can be attributed to intermating the F_2 population before the inbreds were developed and the use of a high marker density. The intermated B73 × Mo17 recombinant inbred population was developed by intermating the F_2 progeny of the B73 × Mo17 cross for four generations before recombinant inbreds were derived by selfing, resulting in an expanded and high resolution linkage map (Lee et al., 2002b). With 1339 markers, the average distance between adjacent markers was only 4.7 cM, given that the size of the linkage map was about 6240 cM.

Several studies have been conducted to map QTL for cell wall components measured by the detergent fiber method (Van Soest, 1982) in maize leaf sheath and stalk samples (Cardinal et al., 2003; Krakowsky et al., 2005, 2006) and whole plant samples (Méchin et al., 2001). Cardinal et al. (2003) found a large number of QTL with small to large effects (R^2 = 4 to 25%) influencing the concentration of NDF (mostly cellulose, hemicellulose, and lignin), acid detergent fiber (ADF; mostly cellulose and lignin), and acid detergent lignin (ADL; mostly lignin) in stalks and leaf sheaths of B73 × B52 recombinant inbreds. In the B73 × De811 cross, QTL with small to large effects (R^2 = 4 to 31%) were found for NDF, ADF, and ADL concentrations in maize stalk and leaf sheath (Krakowsky et al., 2005, 2006). Méchin et al. (2001) found few QTL for NDF and ADL in whole plant samples in the F2 (flint) × Io (dent) cross. The results from these studies, however, cannot be directly compared with the results from this study because the detergent fiber method substantially overestimates cellulose and hemicellulose and severely underestimates lignin (Theander and Westerlund, 1993; Dien et al., 2006). Nevertheless, the results from these previous studies suggest that quantitative variation in cell wall composition in maize stalk and leaf sheath is due to segregation of QTL with mostly small effects.

In the intermated B73 × Mo17 population, Hazen et al. (2003) mapped QTL for cell wall composition in the grain pericarp. Interestingly, based on the positions of flanking markers in the intermated B73 x Mo17 linkage maps in MaizeGDB (Lawrence et al., 2008), two QTL for XYL on chromosomes 2 and 6, one QTL for GLC on chromosome 6, and one QTL for GAL on chromosome 9 (the four QTL that had the highest R^2 in their study) mapped close and in the same chromosome bins to the positions of QTL detected in our study for the same components in the stover cell wall (Table 5). The composition of the pericarp cell walls reported by Hazen et al. (2003) differed greatly from the composition of the maize stover in this study, and it can be speculated that the QTL common to both studies represent genes that influence the concentration of XYL, GLC, and GAL across tissue types in maize.

Glucose release in this study was measured by a two-step process of pretreatment and enzymatic saccharification to release glucose residues from cell wall polysaccharides. Obviously, maize does not have genes for release of GLC from the cell wall via such an industrial process. Therefore, any QTL detected for GLCRel was actually QTL influencing cell wall structure, which affected the efficiency of the process used to extract GLC from the cell wall polysaccharides. Five of the 10 QTL for GLCRel colocalized with QTL for KL and/or KL/CW, in agreement with the known inhibitory effect of lignin on enzymatic degradability of the cell wall (Dien et al., 2006; Jung and Casler, 2006b). For all of these colocated QTL, the effects were in opposite but favorable directions, i.e., a QTL with negative effect on lignin was colocated with a QTL with positive effect on GLCRel. The opposite direction of effects for the colocalized QTL for GLCRel and either or both KL and KL/CW is consistent with the negative genetic correlation of GLCRel with both KL and KL/CW. Only one QTL for GLCRel may be linked to QTL for PCA and PCA/CW, and no FA QTL colocalized with any QTL for GLCRel. These results were surprising because PCA and FA on a dry matter and cell wall basis had significant negative phenotypic and genetic correlations with GLCRel.

Genetic correlations between traits are due to pleiotropic or linked genes (Falconer and Mackay, 1996). The colocated QTL having relatively large R^2 and in the same direction of effects for GLC/CW and XYL/CW may explain the small but positive genetic correlation (r_A = 0.22) between these traits. Colocated QTL for other traits such as ARA/CW and UA/CW, MAN and GAL, and other combinations may also partly explain the r_A observed for these traits. However, few colocated QTL were found for most trait combinations. For instance, GLC and cell wall concentration had very high r_A, but only three pairs of QTL with small effects colocated. Although many QTL with small effects were found influencing the traits in this study, additional QTL with smaller effects may not have been detected.

Several QTL for XYL and minor cell wall residues were found between the 120 and 145 cM positions in chromosome 2, and several QTL for GLC, XYL, cell wall concentration, and minor components were found between the 325 and 350 cM positions in chromosome 6. These QTL

might represent linked or pleiotropic genes that simultaneously influence the concentrations of several cell wall components, and may also warrant further characterization.

Implications in Breeding for Stover Quality for Cellulosic Ethanol

The results presented in this study were for maize stover that included stem, leaf sheath, and leaf blade tissues. Cobs, husks, and shanks were excluded because of the additional large labor requirement for removing and shelling the cobs when the stover samples were collected. Tissue types and proportions vary among different plant organs (Wilson, 1993), and the cell wall composition of the stem, leaf sheaths, and leaf blades may vary substantially (Åman, 1993). Differences in cell wall composition among organs were therefore confounded in the results. On the other hand, considering the composition of each organ separately in improving maize stover quality would be difficult.

The significant genetic variance and moderate heritability for all of the traits in the recombinant inbred testcrosses indicated that the amounts of cell wall components, cell wall concentration, and GLCRel can be improved by selection in this population. As mentioned before, the ideal maize stover for cellulosic ethanol would have high GLC content, low lignin content in the cell wall, and high GLCRel. The genetic correlations among the cell wall components indicate that selection for higher GLC may lead to concomitant increase in XYL and cell wall concentration. This result is important because in the future, technologies may be developed that will allow efficient utilization of the pentose sugars XYL and ARA to produce additional ethanol from biomass (Sun and Cheng, 2002; Saha, 2003; Hamelinck et al., 2005). As expected, KL and PCA had significant negative genetic correlations with GLCRel, both on a dry matter and cell wall basis. GLCRel may therefore be increased by selecting for low KL/CW and/or PCA concentrations. The phenotyping costs associated with PCA and GLCRel analysis are substantially greater than measuring KL/CW. If resources are limited, selection for low KL/CW may be preferable to direct selection for high GLCRel. Also, PCA is generally considered an indicator of extent of cell wall lignification rather than as a direct effecter of enzymatic hydrolysis of cellulose (Jung and Deetz, 1993; Jung and Casler, 2006b).

Molecular markers have been shown to be useful in breeding for quantitative traits in crops (Johnson, 2004; Eathington et al., 2007; Bernardo, 2008), particularly when phenotyping is expensive, as is the case with breeding for improved stover quality. Because very few QTL with relatively large effects were found in this study, pyramiding the QTL with largest effects into elite lines may not be the most efficient method for marker-assisted breeding for enhanced stover quality in this population. Instead, use of molecular markers in breeding for stover quality may be more efficient through methods that increase the frequency of favorable marker alleles linked to QTL and that rely on prediction of genotypic value from marker data, such as marker-assisted recurrent selection (Johnson, 2004; Eathington et al., 2007) or genomewide selection (Meuwissen et al., 2001; Bernardo and Yu, 2007).

CONCLUSIONS

As expected, GLC, XYL, and KL were the main components of the maize stover cell wall. Genetic variances existed and heritabilities for most traits were moderate. Phenotypic and genetic correlations among the stover cell wall components were generally in favorable directions and reflected the complexity of maize stover cell wall composition. Glucose release was negatively associated with KL, as expected. Many QTL with mostly small effects were found for stover cell wall components, cell wall concentration, and GLCRel. Because there were no major QTL, the appropriate methods in marker-assisted breeding for maize stover quality for cellulosic ethanol would be methods that increase the frequency of favorable QTL alleles or predict performance based on markers.

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