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Genetic mapping and analysis of quantitative trait loci affecting fiber and lignin content in maize

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Abstract Plant cell walls of forage provide a major source of energy for ruminant animals. Digestion of cell walls is limited by the presence of lignin, therefore the improving the digestibility of forages by reducing lignin content is a major goal in forage crop breeding programs. A recombinant inbred line maize population was used to map quantitative trait loci (QTL) for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of leaf-sheath and stalk tissues. All traits were positively genetically correlated. The larger genetic correlations were between NDF and ADF in sheaths ($r = 0.84$), NDF and ADF ($r = 0.96$), ADF and ADL ($r = 0.83$), and NDF and ADL ($r = 0.76$) in stalks. Twelve QTL were detected for NDF and 11 QTL for ADF in leaf-sheaths. Eight QTL detected for both traits were defined by the same or linked marker loci. Eight QTL were associated with leaf-sheath ADL. Eleven QTL were detected for NDF and ADF, and 12 QTL for ADL in stalks. Nine of eleven QTL detected for both NDF and ADF in stalks coincided in their genomic position. A high proportion of QTL detected for these traits had the same parental effects and genomic locations, suggesting that it is only necessary to select on one fiber component (NDF or ADF) to improve digestibility. Favorable correlated responses of unselected fiber components are expected due to coincident genomic locations of QTL and the high genetic correlation between fiber components. Several QTL detected in this study coincided in their positions with putative cellulose synthase genes from maize.

Keywords Quantitative trait loci · Neutral detergent fiber · Acid detergent fiber · Acid detergent lignin · *Zea mays*

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Introduction

Plant cell walls are a major energy source for ruminants and thus, play a major role in forage utilization (Per Åman 1993). Cell walls are composed of cellulose fibrils embedded within a matrix of lignin and hemicellulose (Moore and Hatfield 1994); in addition, they contain inorganic solvents, phenolics, and proteins. Digestion of intact cell walls is limited by the presence of lignin and phenolic acids within the cell-wall matrix (Moore and Hatfield 1994). The fraction of the cell wall that is partially digested by ruminants is defined as fiber (Moore and Hatfield 1994), of which cellulose, hemicellulose and lignin are the major components. Cell-wall digestibility is negatively correlated with cell-wall lignin and fiber concentrations (Lundvall et al. 1994; Wolf et al. 1993).

Breeding for high digestibility in forage maize is an important goal because it would improve animal intake, growth rate, and milk production (Lundvall et al. 1994). There are several genetic approaches for enhancing the digestibility of forage maize: (1) using known mutants of the lignin pathway; (2) manipulating genes of the lignin, cellulose, and hemicellulose pathways via genetic engineering; and (3) breeding for lower fiber and lignin concentrations with conventional or with marker-assisted selection. Breeding for higher forage digestibility using the *Brown midrib* lignin mutant (*bm3)* has proven unsuccessful due to undesirable correlated effects of these genotypes on important agronomic traits (Coors et al. 1994).

Genetic engineering of forage digestibility requires a precise understanding of the biosynthetic pathways of

lignin, cellulose, and hemicellulose. Several genes of the lignin pathway have been cloned in a few plant species, including maize (Baucher et al. 1998; Whetten et al. 1998). Several attempts have been made to reduce the amount and change the composition of lignin in several plant species by down-regulating the expression of key enzymes in the lignin pathway via transgenic approaches. Conflicting results were observed among different experiments, suggesting that specific synthetic steps may vary among plant taxa and that our current understanding of this pathway is incomplete or incorrect (Baucher et al. 1998; Whetten et al. 1998). In addition, these experiments demonstrated that unexpected subunits are formed and incorporated in lignin, suggesting that plants have a high level of plasticity with respect to lignin production. Thus, increasing maize forage digestibility by genetically engineering key enzymes of the lignin biosynthetic pathway may not be a viable option until a better understanding of this pathway is achieved.

The enzymes and genes involved in cellulose and hemicellulose synthesis have not been as extensively characterized as those in lignin synthesis. Recently, nine putative cellulose synthase genes of maize have been cloned and partially analyzed, but their functions have not been completely defined (Holland et al. 2000). Hence, successful molecular manipulation of genes affecting these pathways for the purpose of improving maize forage digestibility seems unlikely until the function, cell-type specificity, and developmental expression of the genes are known.

In contrast, phenotypic selection for lower fiber and lignin contents in maize stover has been successful in increasing forage digestibility (Wolf et al. 1993). However, measurement of fiber and lignin is expensive and time-consuming, and methods to improve efficiency of selection for these traits are needed. Genetic mapping of factors associated with fiber and lignin variation could enhance breeding strategies and improve selection efficiency. Furthermore, genetic mapping will enhance the understanding of trait correlations, focus tests of candidate genes, and facilitate gene cloning. The objectives of the study reported here were to map and estimate the effects of quantitative trait loci (QTL) that affect cell-wall components (CWC) in the leaf-sheath and stalk of maize in a recombinant inbred line (RIL) population. A second objective was to study the tissue specificity of these QTL by comparing the results from the leaf-sheath and stalk. The specific CWC measured were the neutral detergent fiber (NDF), the acid detergent fiber (ADF), and the acid insoluble lignin (ADL) fractions (Goering and Van Soest 1970).

Materials and methods

Population

A population of RILs was derived from the cross of inbred lines B73 and B52. B73 has lower NDF, ADF and lignin values than B52. Each RIL was derived from a single F_2 plant following the single seed descent method (Brim 1966) until the $F_{6:7}$ generation. Seed was increased for each line by selfing three plants in the $F_{6:7}$ generation and harvesting them in bulk to form the $F_{6:8}$ generation that was grown in the field experiments (Cardinal et al. 2001).

Phenotypic data

The description of plot sizes, seeding rates, experimental design and field management practices was as reported by Cardinal et al. (2001). Briefly, parental lines B52, B73, and 200 RILs were planted in single-row plots replicated twice at two locations near Ames, Iowa in 1997. Five entries each of B73 and B52 were included in each replication. The experiment was repeated at the same sites in 1998, but 14 RILs were discarded from the 1998 experiments because the DNA marker data revealed that they were contaminated.

Four internodes (one below and three above the primary grain-bearing ear) and their leaf-sheaths were harvested from the last three plants in each plot approximately 10 days to 2 weeks after 50% of the RILs reached anthesis. After harvest, samples were dried at 60 °C for approximately 7 days. Stalks and leaf-sheaths were kept separate for further processing and analysis.

Dried samples were ground with a Wiley mill and then reground with a UDY cyclone mill to less than a 1-mm particle size. Samples were scanned through a near-infrared reflectance spectrophotometer (NIRS) (model NIRS6500, FOSS NIRSystems, Eden Prairie, Minn.). Separate calibration sets were selected for each tissue type and year. Fifty to 52 samples were selected for each calibration set, representing 5% of the samples from each group. One gram of each sample was dried for at least 2 h at 100 °C to obtain dry matter percentages. Samples from each calibration set were analyzed, in triplicate, for NDF, ADF, and ash-free ADL. A 0.5-g sample was used for sequential detergent analysis to determine NDF, ADF, and ADL following the ANKOM filter bag method of fiber analysis (Anonymous 1998a, b, c). The ANKOM fiber analyzer (model no. ANKOM200, ANKOM, Fairport, N.Y.) was used for NDF and ADF determinations and the Daisy II incubator for ADL determinations (Anonymous 1998c).

Modifications were made to the NDF and ADF procedures: one hot water rinse was added for a total of four rinses of 5 min each, and the length of the final wash with acetone was increased to 5 min. Modifications were made to the ADL procedure: samples were rinsed with hot tap water in pippette washers five to six times to remove acid, and then they were rinsed for 5 min in acetone. Bags were dried at 100 °C overnight and weighed. NDF, ADF, and ADL determinations were corrected for dry matter content of each sample. Ash content was subtracted from the ADL determinations.

NIRS prediction equations were developed separately for NDF, ADF, and ADL for each tissue type and year using modified partial least squares (Shenk and Westerhaus 1991) with the Infrasoft International NIRS 3 ver. 3.0 software program (ISI, Port Matilda, Mass.). Criteria used to develop the equation were to include no more than eight terms in the regression equation, to use four to five cross validation groups, to have a coefficient of multiple determination between 0.83 and 0.99 and to have low standard errors of calibration and cross-validation. The NIRS prediction equations for leaf-sheath and stalk tissue samples explained most of the fiber variation observed in the calibration set for NDF, ADF, and ADL (data not shown). The R^2 ranged from 0.83 to 0.99. The worst prediction equation was for the ADL fraction of leaf-sheath tissue harvested in 1997. NDF, ADF, and ADL values were predicted for all samples using the prediction equations developed, and then the predicted values were used in the analysis of the data.

The trait "SHNDF" is defined as the predicted NDF content in g kg^{-1} in leaf-sheaths for each plot. Similarly, the traits "SHADF" and "SHADL" are defined as the predicted ADF and ADL content in g kg^{-1} in leaf-sheaths for each plot, respectively. The traits "STNDF", "STADF", and "STADL" are defined as the predicted NDF, ADF, and ADL contents in g kg^{-1} in stalks for each plot, respectively.

Phenotypic data analysis

The error variances for each trait at both locations were tested by homogeneity by Box's test (Milliken and Johnson 1992) to determine if a combined analysis was justified. A combined analysis was performed since error variances were not significantly different.

The data from each environment were analyzed with SAS Proc Mixed (SAS Institute 1997). Complete and incomplete blocks were considered to be random effects. Lines were fixed effects. The least squares means for each RIL from each environment were used for the overall analysis including environments and genotypes as factors. The combined analysis was performed using PROC GLM (SAS Institute 1990), with environments as a random effect. The least squares means (across all environments) for each RIL were used in QTL analysis.

Genetic variance components, heritabilities, and genetic and phenotypic correlations (r_g and r_p, respectively) were estimated from an analysis of variance performed with Proc GLM. Data collected from B52, B73, and the contaminated RILs were not included in those analyses. Standard errors of the genetic correlations were estimated according to Mode and Robinson (1959). Exact confidence intervals for heritabilities (H^2) on an entry-mean basis were calculated (Knapp et al. 1985). Approximate standard errors (SE) of plot-basis heritabilities were calculated according to Hallauer and Miranda-Fihlo (1981) and Mode and Robinson (1959).

Genotypic data

The procedures used for DNA isolation and for collection of restriction fragment length polymorphisms (RFLP) and simple sequence repeat (SSR) genotype data have been described previously by Cardinal et al. (2001). A total of 120 RFLP and 65 SSR loci were mapped in the population. One hundred and eighty-three RILs were used for linkage mapping and QTL analysis. Seventeen RILs were discarded from the original set of 200 because they were contaminated or had more than 10% of loci with non-parental alleles. Linkage analysis was performed with MAPMAKER/EXP ver. 3.0 (Lander et al. 1987). Loci were assigned to linkage groups (LG) with a minimum LOD score of 3.0 and a maximum Haldane distance of 40 centiMorgans (cM). Three-point linkage and multipoint analyses were performed for each linkage group (Cardinal et al. 2001).

QTL mapping

Composite interval mapping (CIM) (Zeng 1994) was conducted with PLABQTL ver. 1.1 (Utz and Melchinger 1996) and QTL CARTOGRAPHER ver. 1.13 (Basten et al. 1999). The programs were used to search for QTL that would be evaluated subsequently in multiple regression models. Procedures used for CIM analysis have been described in detail by Cardinal et al. (2001).

A LOD threshold of 2.50 was used to declare the presence of a QTL with PLABQTL. To declare the presence of a QTL with QTL CARTOGRAPHER, 500 permutations were performed for each trait to determine the genome-wise significance level at $\alpha = 0.05$ (Churchill and Doerge 1994). QTL detected by either program were then integrated in a single multiple regression model using PLABQTL. Model selection was performed using backward and forward stepwise regression using the Akaike's information criterion (AIC) to choose the best model (Jansen 1993). Two models were considered to be significantly different if their AIC values differed by more than 2.0. If two models were not significantly different in their AIC values, the model with the fewest parameters and highest R^2 was chosen.

Digenic epistasis between all pairs of loci for both stalk and sheath NDF, ADF, and lignin data were tested with two-locus analyses of variance using the SAS routine EPISTACY (Holland 1998). Interactions with $P < 0.00026$ were declared to be

Table 1 Mean and range of B73, B52, and $F_{6:8}$ lines, and heritabilities for cell-wall components of the stalk and leaf-sheath in the B73 × B52 RIL maize population

Generation	SHNDF		SHADF		SHADL		STNDF		STADF		STADL	
	Mean	Range[a]	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
	g kg^{-1} DM											
B73	555.5	550.5–559.8	271.5	267.7–273.6	19.7	19.4–20.2	482.1	480.0–483.9	273.2	271.2–274.9	32.2	31.5–32.9
B52	663.4	661.8–665.3	336.4	335.4–338.9	25.6	25.2–55.9	546.6	541.9–549.6	326.5	322.5–330.3	40.5	39.3–41.2
$F_{6:8}$[b]	606.5	539.3–658.8	305.3	249.0–335.8	22.8	15.6–29.0	510.6	425.4–604.9	293.1	229.7–363.8	34.2	20.7–48.0
5% LSD[c]	12.9		8.6		1.8		14.3		10.0		2.5	
5% LSD[d]	7.4		5.0		1.0		8.3		5.8		1.4	
	Entry-mean basis											
Heritability	0.94		0.92		0.87		0.96		0.96		0.93	
95% CI(H^2)[e]	0.95–0.93		0.94–0.90		0.90–0.84		0.97–0.94		0.97–0.95		0.95–0.92	
	Plot-basis											
Heritability	0.60		0.63		0.51		0.77		0.78		0.71	
SE(H^2)[f]	0.07		0.07		0.06		0.08		0.08		0.08	

[a] Range of the overall means, parents had five means each
[b] Overall average across years and locations of all $F_{6:8}$ lines, not including parents or contaminated lines
[c] LSD for comparison of a parental mean and a $F_{6:8}$ line mean
[d] LSD for comparison between parent means
[e] Exact confidence interval (Knapp et al. 1985)
[f] Approximate Standard Error (Hallauer and Miranda-Filho 1981)

significant. This threshold was chosen based on a conservative estimate of the minimum number of independent tests among 20 chromosome arms of maize (Holland et al. 1997). Significant interactions were sequentially added one at a time to a multiple regression model that included the loci closest to each QTL. Interactions significant at a $P < 0.05$ level in the multiple regression model were maintained in the final model. Models with up to three digenic epistatic interactions plus QTL main effects were developed. The final "best model" explained the greatest proportion of the phenotypic variance and had significant ($P < 0.05$) main effects of the initial QTL and interactions terms.

Results

Fiber analysis

The parental lines, B73 and B52, and the mean of the RILs differed significantly for all traits (Table 1). Transgressive segregants beyond both parental lines were observed for SHADL, STNDF, STADF, and STADL and below the B73 value for SHNDF and SHADF.

The entry-mean heritabilities were very high (0.87–0.96) for all traits (Table 1). The plot-basis heritabilities for fiber and lignin were intermediate (0.51–0.63) in the leaf-sheath tissue and were high (0.71–0.78) in the stalk tissue.

All CWCs were positively correlated (Table 2). The larger genetic correlations were between STNDF and STADF ($r = 0.96$), SHNDF and SHADF ($r = 0.84$), STADF and STADL ($r = 0.83$) and STNDF and STADL ($r = 0.76$). The smaller genetic correlations were between SHNDF and STADL ($r = 0.39$) and SHADL and STNDF ($r = 0.32$).

QTL analysis

Eight to twelve QTL were detected for each CWC (Tables 3–6) and these explained 45% to 66% of the phenotypic variation. The majority of QTL for each CWC had positive additive effects, indicating that B52 alleles increased the content of the component.

Many QTL for different CWC were clustered. Map locations of eight QTL were coincident for both SHNDF and SHADF (Table 3, Fig. 1). Similarly, positions of nine QTL overlapped for both STNDF and STADF (Table 5, Fig. 1). Three QTL for SHADF and SHADL mapped to similar positions, whereas seven QTL coincided for both STADF and STADL. Finally, three QTL for SHNDF and SHADL overlapped, and six QTL for this pair of components in stalks overlapped. At each of these coincident QTL positions for pairs of CWC, the same parental allele contributed to an increase in both components. A region on chromosome 9 near UMC95-UMC81 is an exception to these observations. QTL were detected for SHNDF, SHADF, and SHADL in this region, but the allele that increased the NDF and ADF content, decreased the ADL content in sheaths (Tables 3, 4).

Two to four digenic epistatic interactions were significant at $P < 0.00026$ for each of the fiber and lignin measurements in leaf-sheaths and stalks. Most interaction terms were not significant in the full multiple regression model, or their inclusion made some of the main QTL effects non-significant. Interaction terms only remained significant in full QTL models of two traits (SHADL and STADL). ISU6 interacted with phi087 such that genotypes homozygous for the B73 allele at ISU6 and homozygous for the B52 allele (or vice versa) had the largest amount of lignin in their leaf sheaths, 23.4 g kg^{-1} drymatter (DM) versus 22.1 g kg^{-1} DM (Table 4). At UMC128 and UMC65, lines that were homozygous at both loci for the B52 allele had the largest lignin amount in their leaf-sheaths, 24.3 g kg^{-1} DM versus 22.5 g kg^{-1} DM (Table 4). Individuals homozygous for the B73 allele at UMC16 and homozygous for the B52 allele at bnlg669 had the largest lignin content in their leaf-sheaths, 24.2 g kg^{-1} DM versus 22.5 g kg^{-1} DM (Table 4). The full QTL model including the interactions accounted for 57% of the phenotypic variation in SHADL. The model estimated by PROC GLM including only the main effects of QTL explained 42% of the phenotypic variation in SHADL because the QTL were fit at the marker locus position.

ISU101 significantly interacted with phi027 for STADL (Table 6). Lines homozygous for either parental allele at both loci had the lowest STADL, 32.6 g kg^{-1} DM versus 35.2 g kg^{-1} DM. The interaction explained 3% of the phenotypic variation.

Table 2 Genotypic correlations between entry-means for SHNDF, SHADF, SHADL, STNDF, STADF, and STADL in the B73 × B52 maize RIL population. Standard errors are in parenthesis

	SHNDF	SHADF	SHADL	STNDF	STADF	STADL
SHNDF		0.837 (0.024)	0.419 (0.067)	0.510 (0.058)	0.473 (0.060)	0.393 (0.067)
SHADF			0.624 (0.050)	0.529 (0.057)	0.536 (0.056)	0.455 (0.063)
SHADL				0.320 (0.072)	0.437 (0.065)	0.610 (0.053)
STNDF					0.964 (0.006)	0.762 (0.034)
STADF						0.827 (0.025)

Table 3 Genetic positions, additive effects, and partial R^2 for each QTL for SHNDF and SHADF in the B73 × B52 maize RIL population based on a multiple regression model

Chromosome	Locus[a]	Position[b]	Additive effect[c]	Standard error	Partial R^{2d}	Position	Additive effect	Standard error	Partial R^2
		SHNDF				SHADF			
		cM	g kg^{-1} DM		%	cM	g kg^{-1} DM		%
1	bnlg615	118	10.09	1.34	24.9	124	4.45	0.76	16.6
3	dupssr5	63	8.39	1.19	22.6	62	4.70	0.75	18.7
5	NPI104					64	3.49	0.71	12.3
5	BNL10.12	94	3.23	1.13	4.6				
6	phi126	0	–3.30	1.15	4.6	0	–2.42	0.71	6.4
6	UMC21					70	2.52	0.76	6.0
6	BNL9.08a	113	–4.17	1.30	5.7				
7	ISU84b	54	–6.90	1.43	12.0	54	–3.25	0.87	7.5
7	phi034	69	7.77	1.49	13.8	70	4.66	0.92	13.1
7	UMC35	169	4.03	1.17	6.5	170	2.74	0.73	7.6
8	UMC103	34	4.20	1.21	6.6				
8	UMC31					61	1.90	0.70	4.1
9	UMC95	58	8.33	1.25	20.7	66	2.64	0.80	6.0
10	BNL7.49c					76	4.59	0.72	19.0
10	UMC64	82	6.92	1.20	16.3				
10	BNL7.49a	148	4.34	1.55	4.4				
	Total R^2		AIC				Total R^2		AIC
	65.8 ± 4.4%		–148.49				58.9 ± 4.7%		–118.60

[a] Closest marker locus to QTL position
[b] Position, Maximum peak in centiMorgans, relative to the first locus on each chromosome
[c] Additive effect is the regression coefficient of the QTL at the specific position from the multiple regression analysis. Positive additive effects indicate that the B52 allele increases the value of the trait
[d] Partial R^2, Coefficient of determination between the respective QTL and the phenotypic observations, maintaining all other QTL effects fixed

Table 4 Genetic positions, additive effects, and partial R^2 for the eight QTL for SHADL based on a multiple regression model (Additive model) in the B73 × B52 maize RIL population. Position, partial R^2, and probability value of the *F* statistic in the epistasic model

Chromosome	Locus[a]	Position[b]	Additive effect[c]	Standard error	Partial R^{2d}	Position[e]	Partial R^{2f}	Pr > *F*
	Additive model					Epistasic model		
		cM	g kg^{-1} DM		%	cM	%	
1	ISU6	188	0.46	0.15	5.4	184	5.4	0.0002
2	BNL7.49b	30	0.81	0.19	9.7	17	3.5	0.0022
2	UMC4	113	0.55	0.14	7.7	113	2.4	0.0113
3	dupssr5	70	0.93	0.15	17.7	65	12.2	0.0001
3b	dupssr33	18	0.54	0.17	5.7	24	2.1	0.0161
7	dupssr9	86	0.58	0.14	9.0	87	1.9	0.0206
8	phi115	62	0.54	0.13	8.6	62	5.2	0.0002
9	UMC81	52	–0.85	0.14	18.0	52	9.5	0.0001
5	phi087					115	2.4	0.0110
	ISU6*phi087						2.1	0.0164
1	UMC128					130	0.2	0.4199
6	UMC65					56	0.2	0.4539
	UMC128*UMC65						2.0	0.0194
	UMC16					131	1.0	0.0918
8	bnlg669					52	0.2	0.4765
	UMC16*bnlg669						2.9	0.0048
Total R^2 = 44.5 ± 5.6%			AIC = –75.59			Total R^2 = 56.9% (Model without interactions R^2 = 42.0%)		

[a] Closest marker locus to QTL position
[b] Position, Maximum peak in centiMorgans relative to the first locus on each chromosome
[c] Additive effect is the regression coefficient of the QTL at the specific position from the multiple regression. Positive additive effects indicate that the B52 allele increases the value of the trait
[d] Coefficient of determination between the respective QTL and the phenotypic observations, maintaining all other QTL effects fixed
[e] Marker position in centiMorgans, relative to the first locus on each chromosome

Table 5 Genetic positions, additive effects, and partial R^2 for each QTL for STNDF and STADF in B73 × B52 maize RIL population based on a multiple regression model

Chromosome	Locus[a]	Position[b]	Additive effect[c]	Standard error	Partial R^{2d}	Position	Additive effect	Standard error	Partial R^2
		STNDF				STADF			
		cM	g kg^{-1} DM		%	cM	g kg^{-1} DM		%
1	bnlg615	122	9.71	2.03	11.8	122	9.41	1.27	24.4
1	ISU119	138	7.32	2.11	6.6				
2	ISU7	66	–7.07	1.63	9.9	66	–4.13	1.27	5.8
2	UMC4	112	9.73	1.77	15.1	114	6.24	1.36	10.9
3	NPI212	122	–5.75	1.71	6.2				
5	UMC147	0	4.46	1.61	4.3	0	3.08	1.24	3.5
5	ISU106d	58	5.73	1.68	6.4	58	4.82	1.31	7.4
5	phi087	116	–5.88	1.69	6.6	116	–6.03	1.33	10.7
6	PL1	68	8.51	1.70	12.8				
6	UMC21	70					5.86	1.32	10.4
8	BNL9.11	26	8.21	1.65	12.6	30	4.02	1.50	4.0
8	phi081	64					4.55	1.33	6.4
10	bnlg210	72					4.82	1.43	6.2
10	NPI232	108	–6.63	1.75	7.7	106	–7.65	1.45	14.1
Total R^2 = 61.2 ± 4.5%			AIC = –129.14			Total R^2 = 54.1 ± 5.0%		AIC = –98.65	

[a] Closest marker locus to QTL position
[b] Position, Maximum peak in centiMorgans relative to the first locus on each chromosome
[c] Additive effect is the regression coefficient of the QTL at the specific position from the multiple regression analysis. Positive additive effects indicate that the B52 allele increases the value of the trait
[d] Partial R^2, Coefficient of determination between the respective QTL and the phenotypic observations, maintaining all other QTL effects fixed

Table 6 Genetic positions, additive effects, and partial R^2 for the 12 QTL for STADL in B73 × B52 maize RIL population based on a multiple regression model (Additive model). Position, Partial R^2, and probability value of the *F* statistic for the epistatic model

Chromosome	Locus[a]	Position[b]	Additive effect[c]	Standard error	Partial R^{2d}	Position[e]	Partial R^{2f}	Pr > *F*
		Additive model				Epistasic model		
		cM	g kg^{-1} DM		%	cM	%	
1	bnlg615	124	1.57	0.27	16.3	122	7.7	0.0001
1	ISU106a	179	0.72	0.25	4.5	176	1.8	0.0165
2	UMC4	110	0.91	0.27	6.3	113	4.2	0.0003
3	UMC121a	24	–1.32	0.37	7.1	14	4.5	0.0002
3	dupssr5	62	1.71	0.29	17.0	65	10.4	0.0001
4	bnlg589	182	–0.68	0.27	3.5	180	4.4	0.0002
5	BNL7.43	62	1.18	0.25	11.4	63	4.7	0.0001
5	phi087	116	–0.65	0.26	3.5			
6	UMC21	70	1.23	0.27	11.0	72	6.6	0.0001
8	phi081	64	0.65	0.25	3.8	65	2.2	0.0083
8	phi080	156	0.67	0.25	4.1	157	1.4	0.0306
10	NPI232	110	–1.42	0.28	12.9	107	5.5	0.0001
2	ISU101					0	1.6	0.0223
9	phi027					40	0.3	0.3039
	ISU101*phi027						2.9	0.0025
Total R^2 = 53.3 ± 5.0%			AIC = –91.45			Total R^2 = 59.5% (Model without epistasis R^2 = 51.0%)		

[a] Closest marker locus to QTL position
[b] Position, Maximum peak in centiMorgans relative to the first locus on each chromosome
[c] Additive effect is the regression coefficient of the QTL at the specific position from the multiple regression analysis. Positive additive effects indicate that the B52 allele increases the value of the trait
[d] Coefficient of determination between the respective QTL and the phenotypic observations, maintaining all other QTL effects fixed
[e] Marker position in centiMorgans, relative to the first locus on each chromosome
[f] Partial R^2 = SS marker/SS Total. SS Total = Type-III Sum of Squares from the full model under consideration

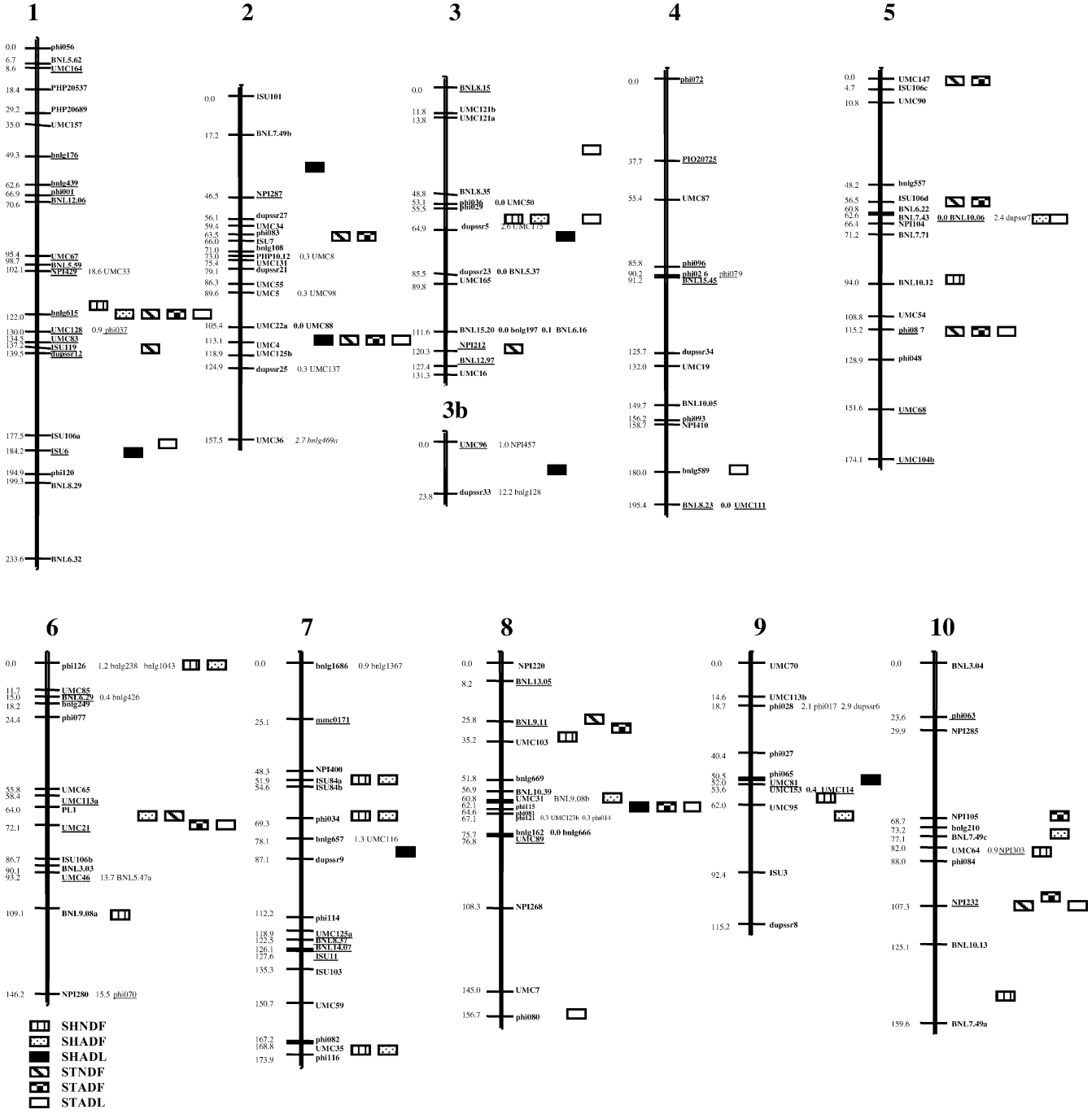

Fig. 1 Genetic map of B73 × B52 RILs maize population. Chromosomes are identified by *numerals* (*1–10*) at the *top* of each linkage group. RFLP loci are in *uppercase letters*, SSR loci are in *lowercase letters* and *underlined* loci indicate segregation distortion ($P < 0.001$). Loci placed at LOD < 2.0 are in *italics*. Values to the *left* of each linkage group indicates a locus position in centiMorgans. *Boxes* to the *right* of each linkage group indicate the position of a QTL

Discussion

Heritabilities for CWC herein and in previous studies are high, suggesting that phenotypic selection for decreased CWC content should be effective (Table 1) (Beeghly et al. 1997). Heritabilities for CWC were high even when estimated on a plot-basis, suggesting that extensive replication over environments would not be necessary to effectively decrease NDF, ADF, and ADL via selection. Genetic correlation of SHNDF and SHADF and of STNDF and STADF were extremely high (0.84 and 0.96, respectively). Consequently, selection in this population for one trait is expected to result in a correlated response in the other fiber trait. Thus, for breeding

purposes, analysis of only one of the two fiber components should be necessary, and the elimination of half of the lab analyses would improve the cost-efficiency of selection programs.

The QTL mapping of CWCs presented here is congruent with the high genetic correlations. Several QTL coincided in their genomic positions and additive effects for SHNDF and SHADF, STNDF and STADF, STADF and STADL, STNDF and STADL, SHADL and STADL (Tables 2–6, Fig. 1). These results are further evidence that suggests that selection to decrease the value of one trait will decrease the value of the correlated traits.

Tissue-specific QTL clusters were also observed in this study (Fig. 1). For example, QTL near phi087 on chromosome 5 and near NPI232 on chromosome 10 affected lignin, NDF, and ADF but only in the stalk tissue. Conversely, QTL on chromosome 7 near phi034-dupssr9 and on chromosome 9 near UMC81-UMC95 affected all three CWC, but only in leaf-sheath tissues.

The majority of QTL for a CWC within leaf-sheaths and stalks coincided in their genomic locations with QTL for other CWC. There are several possible explanations for this result. First, ADL is a fraction of ADF, and ADL and ADF are fractions of NDF. Therefore, a QTL detected in a particular fiber fraction could be detected because there is a QTL in one or both of its subfractions. For example, the QTL near phi126 has almost the same additive effect for both SHNDF and SHADF. Therefore, the QTL effect on NDF is most probably a result of the difference in ADF content between genotypes homozygous for different parental alleles in this region. The QTL with similar effects on both SHNDF and SHADF on chromosome 7 near UMC35 is another example (Table 3). Similarly, one QTL for both SHADF and SHADL on chromosome 8 near UMC31-phi115 may be due primarily to differences in ADL. Finally, the QTL near bnlg615 (C1), near UMC147 (C5), near ISU106d (C5), near phi087 (C5), and near NPI232 (C10) affected STNDF and STADF similarly, suggesting that their primary effect is on STADF.

Alternatively, a small modification of lignin composition can have a large influence on lignin and cell-wall behaviors in traditional methods of lignin determination (Whetten et al. 1998). Small changes in lignin composition can lead to important changes in forage digestibility (Moore and Hatfield 1994), chemical pulping yields (Whetten et al. 1998) and lignin determination by thiocidolysis (Whetten et al. 1998). A change in lignin composition or content could cause changes in fiber digestibilities without necessarily altering fiber content because fiber digestibility is influenced by the crosslinking between lignin polymers and cell-wall polysaccharides mediated by *p*-coumaric acid, ferulic acids and phenolic dimers (Moore and Hatfield 1994). It is not clear if increases and modifications of lignin will change NDF and ADF solubilities estimated by the ANKOM chemical method used in this study. If so, then we would expect that a QTL with small but significant effects on lignin content would coincide with QTL having larger effects on NDF and ADF. This was observed for QTL on chromosomes 3 (near dupssr5) and 7 (near phi034-dupssr9) that were associated with SHNDF, SHADF, and SHADL. QTL for STNDF, STADF, and STADL on chromosome 2 near UMC4, on chromosome 5 near phi087, and on chromosome 10 near NPI232 also act as expected if this were true.

Finally, genes for hemicellulose, cellulose, and lignin synthesis may be linked in the maize genome. Many genes of the lignin biosynthesis pathways are known and have been cloned and sequenced in maize but their chromosomal locations are currently not published. The brown midrib mutants (*bm*) of maize are an exception, and they do not map to same genomic regions [Baucher et al. 1998; MaizeDB (http://nucleus.agron.missouri.edu/ssr.html)]. Nine putative cellulose synthase genes have been cloned in maize (Holland et al. 2000). Unfortunately, there is very little information on the genes involved in hemicellulose synthesis.

One putative cellulose synthase gene (ZmCesA-1) mapped to the region on chromosome 8.02 where QTL for SHNDF, STNDF, and STADF were detected in this study (Holland et al. 2000, Fig. 1). This gene was expressed in internal ground tissue of stalk internodes, suggesting that the QTL detected in our study could be associated with this gene. The gene ZmCesA-2 was found in a leaf-expressed sequence tag library and mapped to a genomic region on chromosome 6.05 near QTL for SHADF, STNDF, STADF, and STADL QTL detected in this study (Holland et al. 2000, Fig. 1). These observations suggest that at least the QTL for SHADF could represent allelic differences of the ZmCesA-2 gene. The QTL detected for SHADL, STNDF, STADF, and STADL on chromosome 2 near UMC22a-UMC4 coincided in their genomic position with the reported map location of ZmCesA-3 (Holland et al. 2000, Fig. 1). This gene was expressed in internal ground tissue of stalk internode and leaf tissue. Finally, three cellulose synthase genes (ZmCesA-4, ZmCesA-8, and ZmCesA-9) mapped to chromosome 7 (7.01 and 7.02) near QTL for SHNDF and SHADF detected in this study (Holland et al. 2000, Fig. 1). These genes were expressed in internal ground tissue of stalk internodes and in shank/stalk tissue, indicating that the QTL detected in this region may not be related to these genes or that the QTL are expressed in the leaf-sheath but not in the leaf tissue. Overall, these observations suggest that the QTL detected for ADF in this study could represent allelic differences in some of the cellulose genes reported by Holland et al. (2000). Direct mapping of these genes in this RIL population would be required to determine if they localize to QTL positions detected for stalk and leaf-sheath NDF and ADF.

Two QTL detected in this study coincided with known mutants of lignin synthesis. The *bm2* mutant of maize maps to bin 1.11, close to a QTL for SHADL near ISU6 and a QTL for STADL near ISU106a. Locus *bm1* maps to bin 5.04, close to the QTL for STADL near BNL7.43. The *bm1* mutants have reduced lignin content and reduced cinnamyl alcohol dehydrogenase (CAD) activity (Bauch-

er et al. 1998) but do not have improved digestibility (Barriére et al. 1994).

Heritability and genetic correlation estimates for CWC from this study are comparable to those reported in other maize populations (Beeghly et al. 1997). Similarly, several QTL for metabolizable energy concentration (identical to ADF) of whole plant material mapped by Lübberstedt et al. (1997) in European flint germplasm coincided with QTL for SHADF and STADF in this study. QTL for STADF on chromosome 2 near ISU7, chromosome 5 near phi087, and chromosome 6 near UMC21 coincided with those reported for metabolizable energy concentration. QTL for SHADF on chromosome 3 near dupssr5, chromosome 6 near UMC21, chromosome 7 near UMC35, and chromosome 9 near UMC95 coincided with those reported by Lübberstedt et al. (1997).

The biological cause of clustering of QTL affecting CWC is unimportant if the objective is to improve forage digestibility and intake via phenotypic or MAS. Plant breeders can exploit this clustering by selecting the trait and analysis method that is least expensive and fastest for evaluating many breeding lines, and most highly correlated to in vivo digestibility. These results suggest that if fibers are considered important in predicting maize forage digestibility, then evaluation of only one component, either NDF or ADF, is necessary to select for improving digestibility. On the other hand, understanding the regulation of cellulose, hemicellulose, and lignin synthesis will require a better understanding of the causes behind the QTL clustering reported in this study.

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