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Quantitative trait loci for cell wall components in recombinant inbred lines of maize (*Zea mays* L.) II: leaf sheath tissue

Abstract While maize silage is a significant feed component in animal production operations, little information is available on the genetic bases of fiber and lignin concentrations in maize, which are negatively correlated with digestibility. Fiber is composed largely of cellulose, hemicellulose and lignin, which are the primary components of plant cell walls. Variability for these traits in maize germplasm has been reported, but the sources of the variation and the relationships between these traits in different tissues are not well understood. In this study, 191 recombinant inbred lines of B73 (low-intermediate levels of cell wall components, CWCs) × De811 (high levels of CWCs) were analyzed for quantitative trait loci (QTL) associated with CWCs in the leaf sheath. Samples were harvested from plots at two locations in 1998 and one in 1999 and assayed for neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL). QTL were detected on all ten chromosomes, most in tissue specific clusters in concordance with the high genotypic correlations for CWCs within the same tissue. Adjustment of NDF for its subfraction, ADF, revealed that most of the genetic variation in NDF was probably due to variation in ADF. The low to moderate genotypic correlations for the same CWC across leaf sheath and stalk tissues indicate that some genes for CWCs may only be expressed in certain tissues. Many of the QTL herein were detected in other populations, and some are linked to candidate genes for cell wall carbohydrate biosynthesis.

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Introduction

Silage maize breeders have traditionally focused on improving the digestibility of the whole maize plant by performing phenotypic selection to increase the grain fraction or decrease the levels of compounds, particularly cell wall components (CWCs), which limit digestibility of the stover. Basing selection solely on increasing the ear-to-stover ratio may limit potential gains in dry matter (DM) yield and quality, so greater emphasis has been placed on whole-plant and stover digestibilities, and in particular on reducing DM concentrations of fiber and lignin (Hunter 1978; Hunt et al. 1992; Wolf et al. 1993a, b).

Fiber and lignin can be quantified as neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). NDF is composed of mostly cellulose, hemicellulose, and lignin, ADF of cellulose and lignin, and ADL primarily as lignin (van Soest 1994). Significant genetic variability for these traits has been reported for temperate and tropical maize germplasm (Roth et al. 1970; Bosch et al. 1994; Lundvall et al. 1994). While little information is available concerning the genetic basis of this variation, it is apparent that levels of CWCs are correlated within tissues but not necessarily between tissues (Lundvall et al. 1994; Beeghly et al. 1997). This would indicate tissue-specific expression of the genes involved in CWC biosynthesis, with further evidence provided by quantitative trait loci (QTL) studies in which tissues have been separated before analysis (Cardinal et al. 2003; Krakowsky et al. 2003).

In a companion paper, QTL for stalk CWCs were detected on nine chromosomes in recombinant inbred lines (RILs) of B73 × De811, mostly clustered in concordance with the high genotypic correlations between

NDF and ADF. Adjustment of NDF for ADF and ADF for ADL by covariance analysis revealed that most of the variability for stalk CWCs in the population was due to changes in ADF. The objectives of this study were to (1) assess genotypic and environmental components of variation for the concentration of CWCs in the leaf sheath of RILs of B73 × De811, (2) calculate the genotypic correlations between the concentrations of CWCs within the sheath and between the sheath and stalk, (3) map QTL for fiber and lignin concentrations and calculate relative efficiencies of marker assisted selection (MAS) and (4) compare QTL for CWCs mapped herein with those of F_3 lines of B73 × De811 and RILs of B73 × B52 for leaf sheath CWCs and with the QTL in the same population for stalk CWCs.

Materials and methods

Phenotypic data

The plant materials, field experiments, trait evaluations, analysis of phenotypic data, and detection of QTL have all been described previously in the companion paper (Krakowsky et al. 2005). In summary, 200 RILs were derived from a cross of inbreds B73 and De811 and used herein. Inbred B73 has low to intermediate levels of CWCs while inbred De811 has high levels of CWCs (Table 1). The experiments were planted in an alpha-lattice design in three environments: the Agronomy and Agricultural Engineering Research Center (AAERC) near Ames, IA and the Hinds farm near Ames, IA on May 5 and May 1, 1998, respectively (two replications each), and on May 20, 1999 at the AAERC (four replications). The entries in each experiment consisted of 200 RILs and five plots each of parental inbreds B73 and De811.

Entries were harvested approximately 1 week after 50% of the plots in an experiment had reached anthesis. Leaf sheaths were sampled from the three internodes above and one internode below the primary ear from the plants used for stalk tissue in the companion paper and dried for 1 week at 60°C. The samples were ground to pass through a 1 mm screen and scanned using NIR spectroscopy, and prediction equations were developed using data collected from a subset of samples analyzed using the van Soest detergent method. NDF, ADF, and ADL were measured in grams per kilogram DM.

For each trait and entry, least square means (lsmeans) were calculated with SAS Proc Mixed (SAS Institute 1999), considering complete and incomplete blocks as random effects and entries as fixed effects for each environment (Cardinal et al. 2003). Environments were also treated as random effects when calculating lsmeans across environments and these means were used for the QTL analysis. Genotype, genotype × environment, and error variances were estimated using a model that considered environments, complete and incomplete blocks, and entries and the entry × environment interaction as random effects (Cardinal et al. 2003). Broad-sense heritabilities on an entry-mean basis and their exact confidence intervals were calculated according to established procedures (Knapp et al. 1985; Fehr 1987). Genotypic correlations (rg) among traits and their standard errors were estimated using the method of moments (Mode and Robinson 1959). Sums of squares and cross products used to estimate the variance and covariance components were obtained with the MANOVA statement in Proc GLM of SAS, treating entries and environments as random effects (SAS Institute 1999). Due to the high genotypic correlations between NDF and ADF, least square means were also calculating for NDF using ADF as a covariate (Cochran and Cox 1957; Krakowsky et al. 2005) in the SAS Proc Glm model, creating a new trait labeled "NDF adjusted for ADF". The necessary sums-of-squares were calculated using the MANOVA statement in PROC GLM of the software package SAS, with entries and environments treated as random effects (SAS Institute 1999).

Detection of QTL

One hundred and eight genomic and cDNA probes detected 113 RFLP loci. In addition, segregation data for 33 loci defined by simple sequence repeats (SSRs) were collected according to a standard protocol (Senior et al. 1996). One hundred ninety-one RILs were used for linkage mapping. Linkage analysis was performed using MAPMAKER/EXP v. 3.0 (Lander et al. 1987).

Table 1 Means, variances, and heritabilities for CWCs

	B73	De811	RILs (g kg^{-1})								
			Mean	SE[a] of one RIL mean	Range	σ^2_g	95% CI	σ^2_{ge}	95% CI	h^2	95% CI
NDF	586 (9)[a]	626 (9)	612	12	556–664	386	316–482	29	18–55	0.94	0.92–0.95
NDF adjusted for ADF	611 (4)	604 (4)	611	6	589–637	95	77–121	16	11–27	0.88	0.85–0.91
ADF	306 (6)	350 (6)	330	8	296–363	178	145–222	13	8–26	0.93	0.91–0.95
ADL	26 (1)	28 (1)	27	2	19–33	2.7	2.0–3.8	1	0.5–2.6	0.67	0.57–0.75

[a] Standard error

QTL were detected using PlabQTL with cofactor selection performed as described in Utz and Melchinger (1996) and Austin et al. (2000). Outlier or influential observations were tested for based on statistics calculated by PlabQTL (Andrews-Pregibon statistic second factor, AP2 < 0.5; influential value of an observation, infl > 0.4; Studentized residual, stdRes > 3.5) but none were observed. The LOD threshold value of 2.5 was used to declare the presence of a QTL. This LOD threshold has been used in similar studies of QTL in maize (Cardinal et al. 2003; Krakowsky et al. 2003, 2005) and has the advantage of minimizing the risk of a Type II error (i.e., missing a QTL).

Fivefold cross validation (CV/G) was performed for the RILs as described in Papst et al. (2004) using PlabQTL. Briefly, the whole data set was randomly split into $k = 5$ subsets, four of which were combined to form the estimation set (ES) for QTL detection and estimation of genetic effects and the remaining of which formed the test set (TS) in which predictions derived from ES were tested for their validity by correlating predicted and observed data (TS.ES). Using a LOD threshold of 2.5, each CV/G run yielded different estimates for the number of QTL, their location, and genetic effects in the ES. Estimates of medians and percentiles and frequency of QTL detection in ES and TS were calculated over all replicated CV/G runs.

Digenic epistatic interactions between all pairs of loci were tested using *Epistacy*, which uses least-square statistics (Holland 1998). Interactions at $P < 0.00026$ were considered significant. Interaction terms were added to a model including all main effect QTL in PlabQTL, and those interactions that increased the AIC by at least 2.0 were deemed significant. Interactions between main effect QTL were also tested in PlabQTL by including additive × additive epistatic effects in the model. Effects that exceeded the F-to-Enter (and F-to-Drop) value obtained by using the Bonferroni bound at the significance level (P) of 0.05 (experiment-wise error rate) were included in the final model.

Joint mapping of QTL was used in determining whether multiple QTL that appear to be linked are distinct, linked QTL or single, pleiotropic QTL (Jiang and Zeng 1995). The multiple-trait analysis feature of QTL Cartographer was used to determine whether linked QTL for different traits that were detected in PLABQTL had a single peak (i.e., a single, pleiotropic QTL) or multiple peaks (i.e., multiple, linked, non-pleiotropic QTL) when mapped simultaneously (Wang et al. 2005). The traits were mapped individually and then simultaneously using the composite interval mapping (CIM) option, and a minimum one-LOD drop-off between QTL LOD-peaks was considered necessary to define two peaks as distinct.

The relative efficiency of MAS over conventional phenotypic selection was calculated as described in Lande and Thompson (1990) and Papst et al. (2004), with the efficiency of selection based solely on MAS calculated as RE $= \sqrt{p/h^2}$ and the efficiency of selection based on MAS and phenotypic data calculated as RE $= \sqrt{p/h^2 + (1-p)^2/(1-ph^2)}$, where h^2 is the heritability of the trait and p is the proportion of the additive genetic variance associated with the marker loci.

Comparisons of QTL across populations, while complicated by sampling variation, differences in environments and methodology, and limited by the number of common genetic loci, provide an opportunity to further validate the association between a genomic region and a QTL (van Ooijen 1992; Jansen and Stam 1994; Zeng 1994; Visscher et al. 1996). The QTL herein were compared with those observed in F_3 lines derived from the same hybrid (109 of the RILs used herein were derived directly from F_3 lines used by Krakowsky et al. 2003) to ascertain some of the effects of population structure and environment on detection of QTL for CWCs, and with RILs of B73 × B52 that were grown in common environments (Cardinal et al. 2003). This is critical for evaluating differences in the genetics of CWCs between De811 and B52. Comparisons were made based on common loci between the genetic maps of the different populations, and QTL were considered to represent the same or closely linked loci if the most likely positions are within 30 cM.

Results

Phenotypic analysis

Significant differences between the inbred parents and among the RILs were observed for all CWCs (Table 1). Transgressive segregation was observed for all traits, indicating that at some loci the B73 allele is associated with higher levels of CWCs than the corresponding De811 allele. Entry mean heritabilities for NDF and ADF were very high, while that for ADL was much lower (Table 1).

Genotypic correlations for CWCs within the sheath or stalk were much higher than those for the same CWC between the sheath and stalk. The genotypic correlation for sheath NDF and ADF was 0.90 (S.E. = 0.32) while the values for stalk and sheath NDF and stalk and sheath ADF were 0.34 (S.E. = 0.14) and 0.40 (S.E. = 0.16), respectively. The genotypic variance of ADL was small and the estimates of the genotypic correlation between ADL and NDF or ADF were greater than one, therefore these correlation estimates were not considered reliable.

QTL analysis

One hundred ninety-one RILs were used for linkage mapping and QTL analysis. Eight RILs were excluded from all analyses due to detection of non-parental alleles at more than 5% of the loci, and one RIL was excluded

due to detection of heterozygotes at more than 10% of the loci. Non-parental alleles were observed at 0.5% of the total loci, and the eight lines removed from the study contained 83% of the non-parental alleles observed. These alleles may have resulted from any of a number of possibilities, including contamination of the RILs during inbreeding, mutations, use of parental lines that were still segregating at some alleles, or incomplete digestion of the DNA during RFLP analysis. No marker showed greater than 4% non-parental alleles.

QTL for NDF and ADF were detected on all ten chromosomes and the combined QTL effects were associated with greater than 50% of the phenotypic variation for each trait in the full data sets and approximately one-quarter of the phenotypic variation in the CV/G runs (Tables 2, 3; Fig. 1). For NDF one QTL (chromosome 9, *isu3*) was observed in 95% of the CV/G runs, with no other QTL observed in greater than 80% of the CV/G runs, while for ADF only two QTL (chromosomes 3, *umc26* and 8, *agrr21*) were observed in greater than 80% of the CV/G runs. Nine QTL for NDF are within 10 cM of QTL for ADF, in concordance with the high genotypic correlation, and in all of these cases a single peak was observed when the two traits were mapped simultaneously, indicating that there is likely a single pleiotropic QTL at each of these locations. The De811 allele was associated with an increase in NDF and ADF at eight (of 15 total) and nine (of 13 total) QTL, respectively. Twelve QTL associated with 44% of the phenotypic variation in the full data set and only 10% of the phenotypic data in the CV/G runs were observed for NDF adjusted for ADF, only six of which are within 10 cM of QTL for NDF (Table 4). Only one

QTL, on chromosome 10 (*umc44*), was detected in at least 80% of the CV/G runs. The De811 allele was associated with an increase in NDF adjusted for ADF at only four QTL (Fig. 1).

The QTL for sheath ADL were observed on 9 chromosomes and a simultaneous fit of the 13 QTL accounted for 51% of the phenotypic variation for the full data set and 18% for the cross validation runs (Table 5; Fig. 1). Only two QTL (chromosomes 3, *umc26* and 5, *bnlg609*) were observed in at least 80% of the cross-validation runs. The De811 allele was associated with an increase in sheath ADL at seven of the QTL and, as with the other CWCs, the transgressive segregation observed may be due to a high proportion of the QTL at which the allele from B73 is associated with an increase in ADL. Eight QTL for sheath ADL were within 20 cM of QTL for sheath ADF and NDF, and in only one instance, on chromosome 10 near *npi287*, does the allele from De811 confer a decrease in ADL and an increase in ADF and NDF. Single peaks were observed when the three traits were mapped simultaneously for seven of the eight instances, indicating that there is likely a single pleiotropic QTL at each of these locations. For the one exception, on chromosome 10 near *npi287*, it appears that two peaks are present, though the valley between the peaks does not meet the one-LOD minimum threshold.

Significant digenic epistatic effects were detected for all CWCs using *Epistacy*, but only one interaction for ADL and three interactions for NDF adjusted for ADF significantly improved the models containing all of the main effect QTL. An interaction for ADL between *umc33a* on chromosome 1 and *php20042* on chromo-

Table 2 Chromosomal locations, estimates of effects, and partial R^2 of QTL for NDF

Bin	Nearest marker locus	LOD	$\hat{a}$[a] (g kg^{-1})	Partial R^{2b} (%)	$\hat{a}$ (TS.ES)[c]		
					Median	(10, 90) Percentile	Frequency (%)
2.02	*isu147*	3.5	−3.4	5	−2.0	(−7.0, 2.4)	29
2.04–5	*umc8a*	3.7	5.6	13	3.4	(−1.1, 8.3)	69
2.08	*umc4*	4.7	−5.2	9	−2.5	(−7.5, 2.4)	32
3.04	*rz543a*	3.9	4.8	7	6.0	(1.4, 10.8)	77
3.05	*umc26*	3.2	3.6	4	2.1	(−3.5, 7.8)	27
4.00	*nc135*	4.2	−4.1	8	−0.2	(−4.8, 3.5)	18
4.02	*cdo520*	3.4	4.3	6	0.7	(−5.0, 5.1)	16
4.08	*bnl7.65*	2.7	−6.5	13	−5.0	(−9.6, 0.2)	63
4.09	*php10025*	3.5	−3.0	4	−4.5	(−8.6, −0.2)	74
5.06	*phi085*	2.8	−3.1	5	−1.0	(−5.8, 3.2)	25
7.04	*dupssr13*	3.1	2.8	4	−1.7	(−5.6, 3.0)	6
8.09	*agrr21*	5.7	−5.0	11	−3.5	(−7.8, 0.9)	64
9.06	*isu3*	13.5	9.3	29	8.6	(4.3, 13.1)	95
10.03	*npi105*	4.5	3.5	6	0.8	(−4.0, 5.7)	23
10.07	*umc1084*	9.1	7.4	18	6.7	(1.7, 11.6)	62
			Total adjusted R^{2d} = 58%			Total adjusted R^2 = 26% (10, 42)[e]	

[a] The allele from De811 is associated with an increase (+) or decrease (−) in the value of the trait. All effects were significant at $P < 0.01$
[b] Percentage of phenotypic variation explained by the QTL, maintaining all other QTL effects fixed
[c] Median, percentiles, and frequency of QTL detection were calculated based on 200 fivefold CV/G runs
[d] Percentage of phenotypic variation explained by a model including all QTL as main effects and adjusted for the number of parameters in the model
[e] Median (10, 90 percentile) based on 200 fivefold CV/G runs

Table 3 Chromosomal locations, estimates of effects, and partial R^2 of QTL for ADF

Bin	Nearest marker locus	LOD	$\hat{a}^a$ (g kg^{-1})	Partial R^2 (%)	$\hat{a}$ (TS.ES)[c]		
					Median	(10, 90) Percentile	Frequency (%)
1.01	phi097	3.0	2.8	7	1.6	(−0.8, 4.6)	47
3.04	rz543a	4.5	2.7	5	2.2	(−1.5, 5.8)	39
3.05	umc26	3.3	3.5	8	4.1	(0.8, 7.3)	81
4.02	cdo520	6.7	2.6	6	1.3	(−1.5, 4.4)	63
4.08	bnl7.65	3.8	−3.7	11	−3.6	(−6.8, −0.6)	58
5.06	bnlg609	4.4	−2.5	6	−2.1	(−4.8, 0.8)	44
6.07	phi123	2.7	2.7	7	1.2	(−1.3, 4.2)	31
8.05	bnl8.26	4.9	−3.2	10	−2.8	(−5.5, 0.1)	59
8.09	agrr21	6.7	−3.2	10	−2.9	(−5.7, −0.2)	88
9.02–3	npi567	2.5	2.6	5	0.7	(−1.3, 4.3)	14
9.06	isu3	6.5	3.7	12	4.3	(0.8, 7.3)	71
10.03	npi105	3.7	2.4	6	1.8	(−1.4, 4.9)	53
10.07	umc1084	10.4	4.6	15	4.2	(1.3, 7.4)	69
			Total adjusted R^{2d} = 54%		Total adjusted R^2 = 25% (10, 41)[e]		

[a]The allele from De811 is associated with an increase (+) or decrease (−) in the value of the trait. All effects were significant at $P < 0.01$
[b]Percentage of phenotypic variation explained by the QTL, maintaining all other QTL effects fixed
[c]Median, percentiles, and frequency of QTL detection were calculated based on 200 fivefold CV/G runs
[d]Percentage of phenotypic variation explained by a model including all QTL as main effects and adjusted for the number of parameters in the model
[e]Median (10, 90 percentile) based on 200 fivefold CV/G runs

some 3 had a partial R^2 of 8% and increased the phenotypic variance explained by the full data set by almost 3%. For this interaction, the lowest ADL value (25.6 g/kg) was observed when the B73 allele was present at both loci, while the highest value (27.7 g/kg) was observed when the De811 allele was present at *umc33a* and the B73 allele was present at *php20042*. Three interactions were observed for NDF adjusted for ADF, increasing the phenotypic variation explained by the full model by almost 10% (Table 6).

The relative efficiencies of MAS alone, based on the median adjusted R^2 from the CV/G models, ranged from RE = 0.38 for NDF adjusted for ADF to RE = 0.52 for NDF and ADF, while the relative efficiencies of MAS combined with phenotypic data ranged from RE = 1.00 for NDF and ADF to RE = 1.09 for ADL.

QTL for the same trait in different tissues (e.g., NDF in the leaf sheath and NDF in the stalk) were mapped simultaneously with the multiple-trait mapping procedure. For the six genomic regions in which QTL for NDF in the sheath and stalk appeared to be linked (with 20 cM), distinct peaks were observed for each tissue for QTL on chromosomes 2 (*umc34-umc8*) and 5 (*bnlg609*), a single peak was observed on chromosome 7 (*dupssr7*), and for the other three regions (chromosome 2, *isu147* and *phi127-umc4* and chromosome 3, *rz543a-umc26*), QTL for NDF in either the sheath or the stalk that were detected in PLABQTL were not significant in the multiple trait analysis using CIM in QTL Cartographer. In the case of ADF, linkages appear in five genomic regions, with single peaks observed for QTL on chromosomes 3 (*rz543a-umc26*) and 4 (*umc156-bnl7.65*), and distinct peaks for each tissue on chromosome 5 (*bnlg609*). On chromosome 6 (*phi123*) the sheath QTL was not detected, and on chromosome 10 (*npi105*) no

QTL were detected. Linkages between QTL for the different tissues only appeared in three genomic regions for ADL, with distinct peaks observed for each tissue on chromosomes 3 (*php20042-rz543a*) and 5 (*bnlg609-phi128*) and a single peak observed for chromosome 7 (*umc168*).

The QTL herein can be compared with those observed in F$_3$ lines derived from the same hybrid (B73 × De811) to ascertain some of the effects of population structure and environment on detection of QTL for CWCs. One hundred and nine of the RILs were derived directly from F$_3$ lines used in the previous study, reducing the effect of sampling on comparisons between the studies. Ten QTL each were observed for sheath NDF and ADF in the F$_3$ lines of B73 × De811 (sheath ADL was not measured in the F$_3$), five each of which are within 10 cM of QTL herein, respectively (Fig. 1). Of the seven QTL each for NDF and ADF that had the highest frequencies of detection in the cross validation runs, four each were observed in the F$_3$ lines, with two others located in genomic regions that were not covered by marker loci in that population.

The RILs of B73 × B52 provide an opportunity for comparisons of QTL for CWCs across studies that have a common parent (B73) and that were grown in common environments (Cardinal et al. 2003). Heritabilities were similar across studies for NDF and ADF and significantly lower herein for ADL, probably due to the relatively small genotypic variance for ADL observed herein. Eleven QTL each for sheath NDF and ADF were observed in the RILs of B73 × B52, five and four of which are within 30 cM of QTL for the respective CWCs herein, while 2 of the 12 QTL for sheath ADL in the RILs of B73 × B52 are within 30 cM of QTL for the respective CWCs herein. Of the seven QTL each for
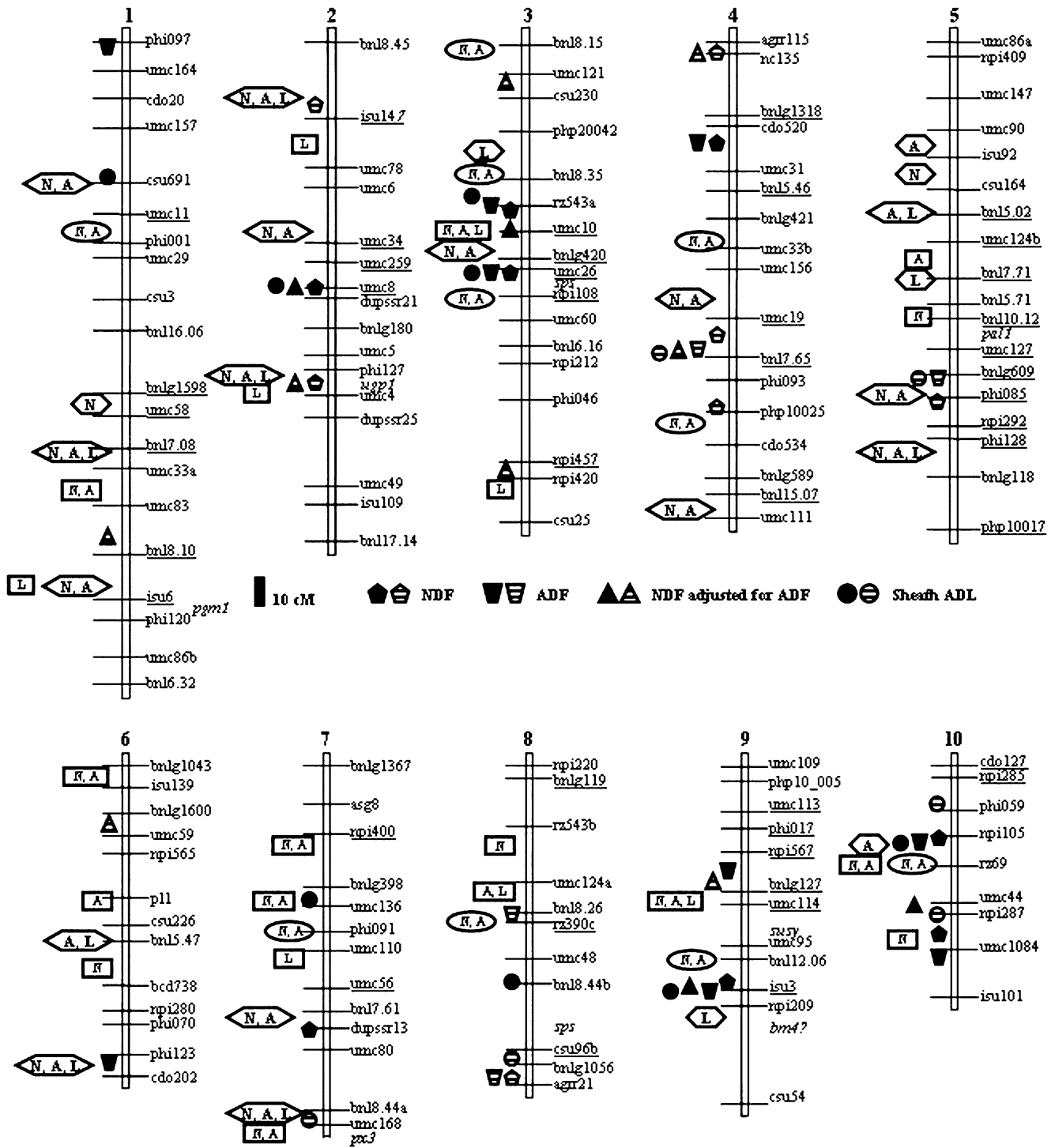
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Fig. 1 Linkage map of B73 × De811 RIL population. *Underlined* loci exhibited segregation distortion (*P* < 0.05). *Solid shapes* (e.g. *filled square*) denote QTL for which the allele from De811 is associated with an increase in the trait, while *striped shapes* (e.g. *filled square*) denote QTL for which the allele from B73 is associated with an increase in the trait. Candidate genes are listed in *bold italic pgm*, phosphoglucomutase; *ugp*, UDP-glucose pyrophosphorylase; *sps*, sucrose phosphate synthase; *pal*, phenylalanine ammonia lyase; *px3*, peroxidase; *susy*, sucrose synthase; *bm4, brown midrib4, open circle* represent QTL for sheath CWCs from the F3 lines of B73 × De811, *open square* represent QTL for the sheath CWCs from the RILs of B73 × B52, *open square* represent QTL for stalk CWCs from the RILs of B73 × DE811; *N* NDF, *A* ADF, *L* ADL

NDF and ADF that had the highest frequencies of detection in the cross validation runs, only one each was observed in the RILs of B73 × B52. The relatively small fraction of the QTL in common between two populations may reflect the genetic heterogeneity between De811 and B52.

Table 4 Chromosomal locations, estimates of effects, and partial R^2 of QTL for NDF adjusted for ADF

Bin	Nearest marker locus	LOD	$\hat{a}$[a] (g kg^{-1})	Partial R^{2b} (%)	$\hat{a}$ (TS.ES)[c]		
					Median	(10, 90) Percentile	Frequency (%)
1.09	Bnl8.10	6.2	−2.5	6	−0.8	(−3.4, 1.8)	30
2.04–5	umc8a	4.0	2.4	7	0.5	(−1.9, 3.1)	19
2.08	umc4	4.4	−3.3	11	−1.5	(−4.0, 1.0)	70
3.01	umc121	3.0	−2.3	8	−1.1	(−3.2, 0.9)	37
3.04	umc10	3.8	1.7	4	0.9	(−1.5, 3.5)	39
3.09	npi457	2.7	−1.9	5	0.2	(−2.0, 2.5)	11
4.00	Nc135	3.6	−2.1	7	−1.2	(−3.3, 0.9)	62
4.08	bnl7.65	4.0	−4.0	16	−2.0	(−4.3, 0.4)	29
6.01	bnlg1600	3.4	−3.1	11	−1.8	(−4.0, 0.5)	45
9.03	bnlg127	5.3	−3.3	11	−1.9	(−4.5, 0.9)	46
9.06	isu3	7.7	4.2	17	3.2	(0.9, 5.5)	58
10.06	umc44	4.2	2.5	9	2.5	(0.2, 4.7)	85
				Total adjusted R^{2d} = 44%		Total adjusted R^2 = 10% (0, 22)[e]	

[a]The allele from De811 is associated with an increase (+) or decrease (−) in the value of the trait. All effects were significant at $P < 0.01$
[b]Percentage of phenotypic variation explained by the QTL, maintaining all other QTL effects fixed
[c]Median, percentiles, and frequency of QTL detection were calculated based on 200 fivefold CV/G runs
[d]Percentage of phenotypic variation explained by a model including all QTL as main effects and adjusted for the number of parameters in the model
[e]Median (10, 90 percentile) based on 200 fivefold CV/G runs

Discussion

Cross validation runs were performed to calculate the upward bias in the values of the additive effects and total adjusted R^2 for the full data sets. Simulation studies have shown an increase in the overestimation of QTL effects as the actual size of the effects and the size of the population sampled decrease (Beavis 1994; Georges et al. 1995; Utz et al. 2000). While the median QTL effects calculated from CV/G were for the most part similar to the values calculated from the full data set, the

10 and 90 percentile values indicate potentially large variances associated with the effects. The median total adjusted R^2 values from the CV/G are much lower than those for the full data set, indicating a strong upward bias in the calculation of the phenotypic variance explained by the models. The low values reduced the relative efficiencies of MAS alone to 50% or lower, indicating that the cost of collecting phenotypic data would have to far exceed the cost of collecting molecular data for MAS alone to be an efficient selection tool. Combining phenotypic and molecular data was only

Table 5 Chromosomal location, estimates of effects, and partial R^2 of QTL for ADL

Bin	Nearest marker locus	LOD	$\hat{a}$[a] (g kg^{-1})	Partial R^{2b} %	$\hat{a}$ (TS.ES)[c]		
					Median	(10, 90) Percentile	Frequency (%)
1.02	csu691	4.1	0.3	4	0.1	(−0.3, 0.6)	28
2.04–5	umc8a	3.2	0.4	6	0.1	(−0.3, 0.5)	30
3.04	rz543a	6.2	0.5	6	0.3	(−0.3, 0.8)	31
3.05	umc26	3.1	0.5	6	0.7	(0.2, 1.2)	81
4.08	bnl7.65	5.0	−0.5	9	−0.4	(−0.9, 0.0)	60
5.06	bnlg609	3.5	−0.5	11	−0.5	(−0.8, −0.1)	80
7.02	umc136	4.3	0.5	10	0.5	(0.0, 0.8)	70
7.06	umc168	4.3	−0.4	7	−0.3	(−0.7, 0.2)	52
8.06	bnl8.44b	2.8	0.3	4	0.1	(−0.4, 0.6)	15
8.07	csu96b	3.4	−0.7	16	−0.4	(−0.8, 0.0)	33
9.06	isu3	3.5	0.4	8	0.3	(−0.1, 0.7)	49
10.02	phi059	3.7	−0.5	6	−0.2	(−0.8, 0.4)	25
10.03	npi105	4.9	0.6	8	0.2	(−0.4, 0.8)	40
10.06	umc44	6.2	−0.5	9	−0.3	(−0.7, 0.1)	50
				Total adjusted R^{2d} = 51%		Total adjusted R^2 = 18% (4, 33)[e]	

[a]The allele from De811 is associated with an increase (+) or decrease (−) in the value of the trait. All effects were significant at $P < 0.01$
[b]Percentage of phenotypic variation explained by the QTL, maintaining all other QTL effects fixed
[c]Median, percentiles, and frequency of QTL detection were calculated based on 200 fivefold CV/G runs
[d]Percentage of phenotypic variation explained by a model including all QTL as main effects and adjusted for the number of parameters in the model
[e]Median (10, 90 percentile) based on 200 fivefold CV/G runs

Table 6 Chromosomal locations, estimates of effects, and partial R^2 of QTL for NDF adjusted for ADF including epistatic effects

Bin	Nearest marker locus	$\hat{a}$[a] (g kg^{-1})	Partial R^{2}[b] (%)	Allelic classes[c] (g kg^{-1})			
				AA	AB	BA	BB
1.09	*bnl8.10*	−1.8	4				
2.04–5	*umc8a*	1.7	4				
2.08	*umc4*	−3.2	12				
3.01	*umc121*	−2.6	11				
3.04	*umc10*	1.6	4				
3.09	*npi457*	−1.3	3				
4.00	*nc135*	−2.3	9				
4.08	*bnl7.65*	−3.5	16				
6.01	*bnlg1600*	−2.9	13				
9.03	*bnlg127*	−2.9	10				
9.06	*isu3*	3.6	16				
10.06	*umc44*	2.7	11				
1.05–6	*bnl16.06*	−0.1	0				
5.03	*bnl5.02*	−0.1	0				
5.07	*npi292*	−0.7	1				
	*umc4*umc121*	−2.6	8	613	612	613	606
	*bnl7.65*bnl5.02*	−2.1	5	611	613	617	606
	*bnl16.06*npi292*	−2.4	7	608	613	615	604

Total adjusted R^{2}[d] = 53%

[a] The allele from De811 is associated with an increase (+) or decrease (−) in the value of the trait. All effects were significant at $P < 0.01$
[b] Percentage of phenotypic variation explained by the QTL, maintaining all other QTL effects fixed
[c] A represents allele derived from B73, B represents allele derived from De811
[d] Percentage of phenotypic variation explained by a model including all QTL as main effects and adjusted for the number of parameters in the model

slightly more efficient than collecting phenotypic data alone and would likely not be cost-effective. The large number of QTL with small effects observed herein may have contributed to the upward bias, and a larger population size may have reduced the effects of multi-collinearity in the data.

The high genotypic correlation between NDF and ADF prompted us to calculate the trait labeled "NDF adjusted for ADF", and significant differences in genotypic variation and QTL were observed between NDF and NDF adjusted for ADF. Physiologically the difference between NDF and ADF is specific cell wall compounds such as hemicellulose that are bound by lignocellulosic bonds. Genetic variation in NDF adjusted for ADF may be associated with genes for enzymes that break these bonds as well as genes associated with the concentrations of those cell wall compounds. The difference between the parental lines for NDF adjusted for ADF was very small, and the genetic variability was only about one-quarter that of NDF. Only 7 of the 12 QTL for NDF adjusted for ADF are closely linked to QTL for NDF; the other five are not within 40 cM of QTL for NDF. Increased levels of NDF adjusted for ADF were, for the most part, associated with the allele derived from B73, which corresponds with the slightly higher concentrations of NDF adjusted for ADF in B73. The clustering of QTL for NDF and ADF may have resulted from the fact that ADF is a subfraction of NDF, with evidence for this hypothesis provided by the simultaneous mapping of QTL for leaf sheath NDF, ADF and ADL, which indicated that almost all instances of linked QTL between the different traits were due to pleiotropic QTL affecting multiple traits rather than distinct QTL for each trait.

Low genotypic correlations between the same CWC in the sheath and the stalk have been observed previously and may be indicative of tissue specific expression of CWCs (Lundvall et al. 1994; Cardinal et al. 2003; Krakowsky et al. 2003). Simultaneous mapping of QTL for the same trait in different tissues indicated that even when QTL are localized to the same genomic region, in some cases these linked QTL represent distinct genes associated with tissue-specific expression. This information could impact the methods used for selection of reduced fiber concentrations in forage maize varieties. While phenotypic selection based on whole-plant fiber concentrations should be effective without the need to break the plant down into its component parts, selection based on DNA markers may not be very effective if the whole plant fiber concentrations are used for mapping QTL, as tissue-specific effects may be masked in whole plant samples.

As in the companion study (Krakowsky et al. 2005), many QTL in this study may have sequence homology due to large-scale chromosomal duplications present in the maize genome. These duplications may be the result of an ancient tetraploid event or internal duplication and have been characterized with molecular markers (Helentjaris et al. 1988; Gaut 2001). Some regions containing QTL appear to be present in duplicate copies (e.g., *umc4* on chromosome 2 and *agrr21* on chromosome 8, or *rz543a* on chromosome 3 and *bnl8.44a* and *b* on chromosomes 7 and 8, respectively). Detection of QTL in homologous sequences in different regions of the

genome provides further evidence of an association between those sequences and the expression of CWCs.

QTL for forage maize quality have also been observed in two studies using European germplasm. In the first study, F_3 lines derived from two elite flint inbreds were top-crossed to two testers and evaluated for six forage traits in whole plant samples, including ADF and metabolizable energy content (MEC; Lübberstedt et al. 1997). Due to the high phenotypic and genotypic correlations between ADF and MEC (−0.99 and −0.93, respectively) QTL analysis was only performed for MEC, and 12 QTL were observed. The QTL for ADF herein on chromosomes 3 (rz543a), 4 (bnl7.65), 5 (bnlg609), 7 (bnl8.44a), 8 (agrr21) and 9 (isu3) are within 30 cM of QTL detected for MEC. In the second study, RILs were evaluated per se and in top crosses for nine forage quality traits on a whole-plant basis, including NDF and ADL (Méchin et al. 2001). Few QTL for NDF and ADL were observed in that study (one on chromosome 1 and two on chromosome 4) and none were within 30 cM of QTL observed herein.

QTL and candidate genes for enzymes postulated to have roles in biosynthesis of cell wall carbohydrates (UDP-glucose pyrophosphorylase (UGP), sucrose synthase (SuSy), sucrose phosphate synthase (SPS) and phosphoglucomutase, (PGM)) have been identified in the maize genome (Preiss 1982; Causse et al. 1995; Delmer and Amor 1995; Causse et al. 1996; Prioul et al. 1999). A proposed model for the pathway of carbon from sucrose to cellulose in plants involves the conversion of sucrose and UDP into UDP-glucose and fructose by SuSy, followed by linkage of the glucose to the growing cellulose molecule by cellulose synthase. Enzymes such as UGP, SPS and PGM facilitate in the recycling of the fructose back into sucrose (Delmer and Haigler 2002). The QTL for ADF on chromosome 9 (npi567) is within 30 cM of QTL for SuSy, while the QTL for NDF and ADF on chromosomes 2 (umc8) and 4 (bnl7.65) are within 30 cM of QTL for SPS. Candidate genes ugp, sps and susy, among others, are also linked to QTL for NDF and ADF (Fig. 1). The detection of QTL in genomic regions associated with genes for enzymes involved in cellulose biosynthesis suggests that these genes may be associated with the variability for CWCs in this population.

Two main effect QTL for ADL are linked to candidate genes. The QTL on chromosome 5 (bnlg609) is linked to phenylalanine ammonia lyase, an enzyme involved in the lignin biosynthesis pathway, while the QTL on chromosome 7 (umc168) is linked to peroxidase, an enzyme that may be involved in the lignification process (Whetten and Sederoff 1998). The QTL on chromosome 9 (npi209) was in the same region as bm4, but due to a large marker interval in that region it is not possible to determine how close the QTL maps to the candidate gene. That so few candidate genes were linked to QTL for ADL is probably attributable to the low variability for ADL in this population.

Due to the large number of QTL observed and the relatively low efficiency of MAS, phenotypic data would be more useful for selection than DNA marker data unless collecting the molecular data is significantly more economical. The presence of epistatic interactions in NDF adjusted for ADF and ADL indicate that in cases were MAS was more economical, selection for reduced CWCs would need to be based on pairs of loci (e.g., umc4 and umc121) selected in tandem, since selection for one locus without selection for the other would reduce potential gains. While there are limited possibilities for using the QTL observed herein for conducting MAS, QTL that have been observed in more than one population that are not linked to known candidate genes could provide clues as to the general locations of new candidate genes. The smaller number of CWC QTL detected in sheath versus stalk tissue was also observed in the F3 lines of B73 × De811, suggesting a greater effect of environment on CWCs in the sheath than the stalk. Indications that a large fraction of the variability for CWCs in this population is related to ADF, which is largely composed of cellulose and has low digestibility, may signify that reducing overall levels of CWCs (selecting for reduced NDF or ADF) could increase digestibility in this population and possibly in others as well, where adjustment of NDF for ADF has not been performed.

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