Theor Appl Genet (2005) 111: 337–346
DOI 10.1007/s00122-005-2026-4

ORIGINAL PAPER

M. D. Krakowsky · M. Lee · J. G. Coors

Quantitative trait loci for cell-wall components in recombinant inbred lines of maize (*Zea mays* L.) I: stalk tissue

Received: 27 September 2004 / Accepted: 1 April 2005 / Published online: 19 May 2005

Abstract Maize silage is a significant energy source for animal production operations, and the efficiency of the conversion of forage into animal mass is an important consideration when selecting cultivars for use as feed. Fiber and lignin are negatively correlated with digestibility of feed, so the development of forage with reduced levels of these cell-wall components (CWCs) is desirable. While variability for fiber and lignin is present in maize germplasm, traditional selection has focused on the yield of the ear rather than the forage quality of the whole plant, and little information is available concerning the genetics of fiber and lignin. The objectives of this study were to map quantitative trait loci (QTLs) for fiber and lignin in the maize stalk and compare them with QTLs from other populations. Stalk samples were harvested from 191 recombinant inbred lines (RILs) of B73 (an inbred line with low-to-intermediate levels of CWCs) × De811 (an inbred line with high levels of CWCs) 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). The QTLs were detected on nine chromosomes, mostly clustered in concordance with the high genetic correlations between NDF and ADF. Adjustment of NDF for ADF and ADF for ADL revealed that most of the variability for CWCs in this population is in ADF. Many of the QTLs detected in this study have also been detected in other populations, and several are linked to candidate genes for cellulose or starch biosynthesis. The genetic information obtained in this study should be useful to breeding efforts aimed at improving the quality of maize silage.

Communicated by D.A. Hoisington

M. D. Krakowsky (✉) · M. Lee
Department of Agronomy, Iowa State University, Ames, IA 50011, USA
E-mail: mkrakowsky@tifton.usda.gov
Tel.: +1-229-3872341
Fax: +1-229-3872321

J. G. Coors
Department of Agronomy, University of Wisconsin, Madison, WI 53706, USA

Introduction

Maize silage is an important forage in animal production operations, specifically for beef and dairy cattle. Silage is an energy feed, the value of which is partially determined by the efficiency its conversion to animal product, which in turn is determined by the digestibility of the forage, animal intake, and the efficiency of feed utilization (Roth et al. 1970; Deinum and Struik 1986). Maize breeders have traditionally selected silage varieties on the basis of grain yield since grain is highly digestible, but more recent efforts have focused on improving the digestibility of the whole plant (Hunter 1978; Deinum and Struik 1986; Wolf et al. 1993). The change in breeding methods has lead to increased research on the factors limiting the digestibility of stover, in particular fiber and lignin (Lundvall et al. 1994; Lübberstedt et al. 1997; Méchin et al. 2001).

Fiber and lignin, which can be quantified as neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL), have been negatively correlated with digestibility ($-0.53 < r < -0.91$), and selection for reduced fiber and lignin should result in improved digestibility (Hunt et al. 1992; Wolf et al. 1993; van Soest 1994). Fiber and lignin are composed largely of plant cell-wall components (CWCs), with NDF consisting largely of cellulose, hemicellulose, and lignin and ADF consisting largely of cellulose and lignin. Genetic variability for these traits is present in temperate maize germplasm, and selection has successfully altered levels of CWCs (Buendgen et al. 1990; Lundvall et al. 1994; Beeghly et al. 1997).

A wide range of heritabilities has been reported for NDF, ADF, and ADL (0.24–0.96), indicating that differences in germplasm and evaluation methods can

greatly affect the analysis of CWCs (Ferret et al. 1991; Lübberstedt et al. 1997; Cardinal et al. 2003). Few studies of quantitative trait loci (QTLs) for CWCs have been reported, and while QTLs have been localized on 16 of the 20 chromosomes arms of maize, there is little consistency across studies (Lübberstedt et al. 1997; Cardinal et al. 2003; Méchin et al. 2001; Krakowsky et al. 2003). Several candidate genes for enzymes of the biosynthetic pathways involved in the synthesis of CWCs have been identified and mapped in the maize genome, such as cellulose synthases (*ZmCesA1–ZmCesA9*) and mutants in the lignin biosynthetic pathway (*bm1–bm4*), but these represent only a small fraction of the total number of genes involved. A greater understanding of the genetics of CWCs should enhance efforts to alter their concentration and composition and improve the value of maize as forage.

The objectives of this study were to assess genotypic and environmental components of variation for the concentration of CWCs in the stalk of recombinant inbred lines (RILs) of B73 × De811, calculate the genotypic correlations between the concentrations of CWCs, map QTLs for fiber and lignin concentration, and compare QTLs mapped herein with those of F_3 lines of B73 × De811 and with other populations.

Materials and methods

Plant materials

The RILs were derived from a cross between maize (*Zea mays* L.) inbreds B73 and De811. One F_1 plant was self-pollinated to produce F_2 individuals that were advanced by single-seed descent to the F_6 generation. Inbred B73 is widely used in temperate maize breeding programs and has low-to-intermediate levels of CWCs, while inbred De811 has high levels of CWCs (Table 1).

Field experiments

Four trials were planted in three environments: one each at the Agronomy and Agricultural Engineering Research Center (AAERC) near Ames, Iowa, and the Hinds Farm near Ames on May 5th and May 1st, 1998, respectively, and two on May 20th, 1999, at the AAERC. Originally there were to be two different planting dates at the AAERC in 1999, but unfavorable weather compelled the planting of the two trials on the same date. Soil fertilization, weed control, and cultivation practices were consistent with optimum maize production for this region. The entries in each trial consisted of 200 RILs and five entries each of B73 and De811. The entries were evaluated in 3.8-m single-row plots arranged in a 14 × 15-alpha-lattice design with two replications per trial.

Trait evaluation

The harvest of stalk tissue samples has been described by Cardinal et al. (2003). Entries were harvested approximately 1 week after 50% of the plots in an experiment had reached anthesis, with anthesis defined at when 50% of the plants in the plot were shedding pollen. Stalks were sampled from the three internodes above and one internode below the primary ear and dried for 1 week at 60°C. In 1998, stalk tissue was only harvested from one replication at each location, while in 1999 it was harvested from both replications in each trial. We analyzed stalk tissue using the method of Beeghly et al. (1997). Briefly, the samples were scanned using NIR spectroscopy, and prediction equations were developed using data collected from a subset of samples analyzed using the van Soest detergent method (Robertson and van Soest 1980). The R^2 values of those equations for each year were 0.92–0.98 for ADF and NDF, and 0.72–0.84 for ADL. NDF, ADF, and ADL were measured in grams per kilogram dry matter (DM).

Analysis of phenotypic data

For each trait and entry, least-square means (lsmeans) were calculated, with trials and complete and incomplete blocks as random effects and entries as fixed effects (Cardinal et al. 2003). Adjustments of NDF for ADF and ADF for ADL were performed by including the correlated trait as a covariate in the model used for

Table 1 Means, variances (σ^2_g, genetic variance) and heritabilities (H^2) for cell-wall components in the stalk of the RILs of B73 × De811(*CI* confidence interval)

Cell-wall components[a]	Inbred line B73	Inbred line De811	RILs					
			Mean	Range	σ^2_g	95% CI	H^2	95% CI
NDF	563 (12)[b]	690 (12)	620 (18)	539–685	816	664–1029	0.92	0.90–0.94
NDF adjusted for ADF	617 (3)	621 (3)	626 (7)	603–650	62	49–82	0.81	0.73–0.85
ADF	316 (12)	421 (12)	357 (16)	302–408	453	368–571	0.92	0.89–0.94
ADF adjusted for ADL	324 (8)	411 (8)	359 (12)	318–408	262	210–336	0.89	0.85–0.91
ADL	40 (2)	53 (2)	46 (3)	36–55	10	7–13	0.74	0.62–0.78

[a]NDF, Neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin
[b] Mean (standard error of mean)

calculating the lsmeans (Cochran and Cox 1957; Krakowsky et al. 2004). Genotypic correlations (r_g) and their standard errors were calculated among traits by applying standard procedures (Mode and Robinson 1959). The necessary computations were performed using the MANOVA statement in PROC GLM of the SAS software package (SAS Institute 1999), with entries and trials treated as random effects. Entry means averaged across environments were used for the QTL analysis. Genotype, genotype × environment (G×E), and error variance were calculated treating environments, complete and incomplete blocks, and entries 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).

Detection of QTLs

The protocols we used for DNA isolation, Southern hybridization, and collection of segregation data at restriction fragment length polymorphism (RFLP) loci have been described by Veldboom et al. (1994). 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). The RILs with non-parental alleles at more than 5% of the loci or heterozygotes at more than 10% of the loci were excluded from the analyses. Segregation distortion was tested using the chi-square test.

Linkage analysis was performed using MAPMAKER/EXP ver. 3.0 (Lander et al. 1987). Loci were assigned to linkage groups using the program's default settings [minimum $\log_{10}$ of the likelihood odds ratio (LOD) score of 3.0; maximum distance between loci of 50 cM]. Multipoint analysis was performed using the ORDER command (informativeness criteria of 160 individuals, 2 cM between loci). The 146 loci comprised a genetic map of 1,551 cM, with an average distance of 11.2 cM between loci.

The QTLs were detected using PLABQTL with cofactor selection performed as described (Utz and Melchinger 1996; Austin et al. 2000). First the COV SELECT option was used to select cofactors using stepwise regression. Outlier or influential observations, 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), were eliminated from the data set. The LOD threshold value of 2.5 was used to declare the presence of a QTL. While previous reports suggest a LOD threshold value between 2 and 3 (Lander and Botstein 1989) or a permutation test to calculate the LOD threshold value for a specified type-I error rate (the probability of detecting a false QTL; Churchill and Doerge 1994), the LOD threshold value of 2.5 has been used in similar studies of QTLs in maize (Cardinal et al. 2003; Krakowsky et al. 2003) and (1) allows for comparisons with the B73 × De811 F_3 and B73 × B52 RIL populations (LOD > 2.5) and (2) minimizes the risk of a type-II error (i.e., missing a QTL). The critical value for the LOD score calculated in PLABQTL using the Bonferroni chi-square approximation (136 intervals, 2 *df*) was 3.5 for the experiment-wise error rate of $P < 0.05$, while the permutation test performed using PLABQTL calculated a LOD of greater than 4.5 at the 5% level for a type-I error. However, the goal of this study was to compare QTLs in the RILs of B73 × De811 with those observed in other studies, so the lower LOD value (2.5) was used and the risk of increasing the number of type-I errors accepted. For an LOD of 2.5, the experiment-wise error rate was 0.43 and the comparison-wise error 0.003. Then, the COV/+ SELECT and COV/– SELECT options were used to detect closely linked QTLs. All QTL were then integrated into a model using the SEQ/S option in PLABQTL. Model selection was performed using forward and backward stepwise selection. If the akaike information criterion (AIC) values of the two models differed by less than 2.0, the model with the fewest parameters was chosen (Jansen 1993; Cardinal et al. 2003).

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, while the remaining one subset formed the test set (TS) in which predictions derived from ES were tested for their validity by correlating predicted and observed data. By permuting the subsets, five different CV/G runs were possible for a fivefold CV/G. Subsets were formed randomly 200 times, yielding a total of 1,000 replicated CV/G runs. Using a LOD threshold of 2.5, each CV/G run yielded different estimates for the number of QTLs, 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 to be significant. This threshold was based on an estimate of the number of independent linkage groups in maize, with each chromosome arm representing one independent linkage group (Holland et al. 1997). Interaction terms were added to a model that included all main-effect QTLs in PLABQTL, and those interactions that increased the AIC by at least 2.0 were deemed significant.

Comparisons of QTLs across populations, while complicated by sampling variation and differences in environments and methodology and limited by the number of common genetic loci, can 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 QTLs herein can be 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 the detection of QTLs for CWCs, and with RILs of B73 × B52 that were grown in common environments (Cardinal et al. 2003) to evaluate 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 QTLs were considered to represent the same or closely linked loci if the most likely positions were within 20 cM.

Results

Phenotypic data

Significant differences between the inbred parents and among the RILs were observed for all CWCs (Table 1). Heritabilities for NDF, ADF, and ADF adjusted for ADL were very high, with those for NDF adjusted for ADF and ADL being lower (Table 1). The genetic correlation between NDF and ADF was 0.99 (standard error = 0.40); correlations could not be calculated with ADL, possibly due to the relatively large error variances associated with this trait (data not shown).

QTL analysis

One hundred and ninety-one RILs were used for linkage mapping and QTL analysis. Eight RILs were excluded from all analyses due to the detection of non-parental alleles at more than 5% of the loci, and one RIL was excluded due to the 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 more than 4% non-parental alleles.

The simultaneous fit of the 16 QTLs for NDF and the 18 QTLs for ADF explained 71 and 70% of the phenotypic variation, respectively, in the full data set, and 39% and 39% of the phenotypic variation, respectively, in the cross validation runs (Tables 2, 3). Only one QTL for NDF and two for ADF were observed in 90% or more of the cross validation runs. The allele from De811 was associated with increased NDF and ADF at 10 and 12 loci, respectively, and all QTLs for NDF were mapped within 25 cM of QTLs for ADF, which is in agreement with the high genetic correlation between the traits. The De811 allele was associated with an increase in ADL for six of the ten QTLs, and the simultaneous fit of all QTLs explained 43% of the variation in the full data set and 23% in the cross validation runs. Seven QTLs for ADL are linked to QTLs for NDF and ADF (Table 4). Nine QTLs were observed for NDF adjusted for ADF, three of which were not linked to QTLs for NDF, and 17 QTLs were observed for ADF adjusted for ADL, one of which was not linked to QTLs for ADF

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

Bin	Locus	LOD	$\hat{a}$[a] (g kg^{-1})	Partial R^2[b] (%)	$\hat{a}_{(TS.ES)}$[c]		
					Median	(10, 90) Percentile	Frequency (%)
1.02	*csu691*	9.8	11.4	31	10.0	(3.8, 16.0)	95
1.06	*umc58*	4.6	5.3	7	7.1	(0.2, 13.1)	44
1.07	*umc33a*	5.2	7.6	13	7.4	(0.3, 14.3)	51
1.11	*isu6*	2.6	5.3	6	5.1	(−1.7, 12.3)	36
2.02	*isu147*	4.9	−7.4	13	−4.5	(−13.5, 3.1)	40
2.04	*umc34*	11.7	9.6	23	8.9	(3.0, 15.8)	79
2.08	*umc4*	3.7	6.8	13	5.5	(−0.7, 12.1)	68
3.05	*bnlg420*	4.8	10.1	24	6.5	(0.2, 12.9)	63
4.06	*umc156*	2.8	−5.0	8	−2.9	(−9.1, 2.8)	24
4.10	*umc111*	2.8	−4.6	8	−2.6	(−8, 2.9)	37
5.02	*isu92*	6.1	7.3	15	5.0	(−0.9, 10.5)	50
5.06	*bnlg609*	5.0	−5.3	8	−2.8	(−9.3, 3.2)	21
5.07/8	*bnlg118*	6.3	−7.9	16	−8.5	(−15.5, −2.2)	68
6.07	*phi123*	3.2	4.8	9	5.0	(−0.8, 11.0)	66
7.04	*dupssr13*	8.0	6.8	13	6.9	(−0.2, 12.5)	68
7.05	*bnl8.44a*	4.3	−5.6	10	−4.2	(−12.1, 1.1)	69
		Total adjusted $R^2 = 71\%$[d]			Total adjusted $R = 39\%$ (22, 53)[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 QTLs 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 QTLs for ADF

Bin	Locus	LOD	â[a] (g kg^{-1})	Partial R^{2b} (%)	â$^{c}_{(TS.ES)}$		
					Median	(10, 90) Percentile	Frequency (%)
1.02	*csu691*	5.2	6.3	21	5.4	(1.1, 9.6)	88
1.07	*umc33a*	3.8	5.9	16	6.5	(−1.2, 8.9)	50
1.11	*isu6*	5.9	6.0	14	5.1	(−0.5, 10.1)	66
2.02	*isu147*	3.8	−5.9	13	−4.7	(−9.9, 1.1)	54
2.04	*umc34*	5.5	6.9	20	6.0	(0.8, 10.9)	82
2.08	*umc4*	4.8	4.2	10	4.0	(−0.8, 9.0)	72
3.05	*bnlg420*	9.8	8.6	30	6.6	(1.5, 11.4)	97
4.06	*umc156*	4.0	−4.0	8	−2.5	(−6.8, 2.3)	20
4.10	*umc111*	3.1	−3.6	9	−1.9	(−6.0, 2.2)	40
5.02	*isu92*	3.1	3.4	5	3.0	(−1.6, 7.5)	36
5.03	*bnl5.02*	4.3	4.2	8	5.0	(−0.1, 9.9)	67
5.06	*bnlg609*	4.2	−4.7	11	−3.5	(−8.6, 1.4)	54
5.07/8	*bnlg118*	5.4	−5.7	14	−5.9	(−10.7, −1.2)	80
6.05	*bnl5.47*	3.9	2.7	5	0.7	(−3.9, 5.5)	23
6.07	*phi123*	4.3	3.7	8	3.8	(−0.2, 8.6)	69
7.04	*dupssr13*	10.4	6.7	21	5.6	(0.7, 9.9)	90
7.05	*bnl8.44a*	3.4	−4.2	9	−3.6	(−8.8, 1.3)	74
10.03	*npi105*	4.1	4.5	11	4.1	(−0.8, 8.1)	68
		Total adjusted $R^2 = 70\%$[d]			Total adjusted $R^2 = 39\%$ (22, 54)[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 QTLs, 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 QTLs 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 4 Chromosomal locations, estimates of effects, and partial R^2 of QTLs for ADL

Bin	Locus	LOD	â[a] (g kg^{-1})	Partial R^{2b} (%)	â$^{c}_{(TS.ES)}$		
					Median	(10, 90) Percentile	Frequency (%)
1.07	*umc33a*	4.7	1.3	17	1.1	(0.4, 1.8)	84
2.08	*phi127*	7.4	1.1	14	0.9	(0.2, 1.6)	95
3.02	*php20042*	3	0.8	6	0.8	(−0.1, 1.6)	56
5.03	*bnl5.02*	5.4	1.2	12	0.8	(0.0, 1.6)	56
5.04	*bnl7.71*	3.4	−0.9	7	−0.4	(−1.5, 0.7)	28
5.07	*phi128*	3.5	−1.3	16	−1.1	(−1.9, −0.2)	41
6.05	*bnl5.47*	11.3	1.3	17	1.6	(0.7, 2.4)	94
6.07	*phi123*	3.1	0.7	6	0.4	(−0.4, 1.0)	18
7.06	*umc168*	3.5	−0.7	6	−0.4	(−1.2, 0.3)	54
9.03	*bnlg127*	2.6	−0.7	6	−0.4	(−1.1, 0.4)	35
		Total adjusted $R^2 = 50\%$[d]			Total adjusted $R^2 = 23\%$ (8, 38)[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 QTLs, 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 QTLs 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

(Tables 5, 6). Significant digenic epistatic effects were detected for all CWCs, but no interactions remained significant when incorporated into the models.

Several QTLs were mapped in close proximity of each other (within 30 cM; e.g., NDF on chromosome 1), and it was not possible to distinguish whether these QTLs represent multiple linked loci or a single loci with a large effect that could not be conclusively mapped to one location. Therefore, they are presented as two separate QTLs in the tables and on the map.

The heritabilities for NDF and ADF herein (Table 1) were higher than those observed in the F_3 lines (0.74 and 0.76, respectively), while the value for ADL herein was slightly lower than that reported in an earlier study

Table 5 Chromosomal locations, estimates of effects, and partial R^2 of QTLs for NDF adjusted for ADF

Bin	Locus	LOD	â[a] (g kg^{-1})	Partial R^{2b} (%)	â[c]$_{(TS.ES)}$		
					Median	(10, 90) Percentile	Frequency (%)
1.02	*csu691*	14.1	4.5	25	4.2	(2.2, 6.1)	89
1.08	*umc83*	2.6	2.9	12	2.2	(0.3, 4.0)	52
2.04/5	*umc8a*	4.9	2.7	12	2.0	(0.1, 3.7)	56
5.05	*bnl5.71*	3.4	−1.8	9	−0.6	(−2.3, 1.3)	36
6.02/3	*npi565*	3.5	−2.2	6	−2.4	(−4.2, −0.3)	37
6.05	*bnl5.47*	4.0	−2.2	7	−1.4	(−3.7, 0.5)	26
7.04	*bnl7.61*	4.0	−2.3	8	−1.4	(−4.0, 1.2)	8
7.04	*umc80*	5.1	2.7	6	1.1	(−0.6, 3.4)	57
9.03	*bnlg127*	3.0	−1.7	4	−0.8	(−2.5, 1.5)	30
		Total adjusted $R^2 = 47\%$[d]			Total adjusted $R^2 = 19\%$ (7, 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 QTLs, 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 QTLs 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 QTLs for ADF adjusted for ADL

Bin	Locus	LOD	â[a] (g kg^{-1})	Partial R^{2b} (%)	â[c]$_{(TS.ES)}$		
					Median	(10, 90) Percentile	Frequency (%)
1.02	*csu691*	7.9	5.6	26	5.6	(2.1, 9.4)	96
1.07	*bnl7.08*	4.1	5.7	19	5.6	(2.0, 9.4)	54
1.11	*isu6*	2.7	3.5	7	3.1	(−0.6, 6.6)	32
2.02	*isu147*	4.1	−4.4	11	−3.4	(−7.0, 0.2)	36
2.04	*umc34*	9.0	6.9	28	6.0	(2.5, 9.8)	91
2.08	*umc4*	2.6	2.2	4	1.1	(−2.5, 4.5)	24
3.05	*bnlg420*	7.0	4.9	14	4.1	(−0.7, 8.7)	68
3.06	*umc60*	3.3	3.1	8	3.7	(−0.2, 7.6)	69
4.07	*umc19*	3.7	−2.7	6	−2.6	(−6.6, 1.3)	31
5.02	*isu92*	3.3	4.3	16	3.2	(−0.8, 6.7)	38
5.06	*bnlg609*	4.5	−2.8	7	−1.1	(−4.9, 1.9)	65
5.07/8	*bnlg118*	5.2	−4.2	15	−4.9	(−8.6, −1.1)	91
6.07	*phi123*	2.7	2.4	6	2.1	(−1.3, 5.5)	56
7.02	*bnlg398*	3.4	2.6	6	2.7	(−0.9, 5.9)	65
7.04	*dupssr13*	5.5	4.5	14	3.7	(0.1, 7.4)	83
7.05	*bnl8.44a*	2.7	−3.5	9	−2.3	(−5.8, 1.3)	56
10.03	*npi105*	2.7	3.9	13	2.8	(−0.8, 6.1)	59
		Total adjusted $R^2 = 71\%$[d]			Total adjusted $R^2 = 43\%$ (26, 56)[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 QTLs, 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 QTLs 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

(0.72; Beeghley et al. 1997). Eleven and twelve main-effect QTLs for NDF and ADF, respectively, were observed in the F_3 lines (Fig. 1; Krakowsky et al. 2003), of which eight and nine were linked to the QTLs for NDF and ADF, respectively. For ADL, nine QTL were observed in the F_3 lines, three of which were linked to QTLs.

Genotypic variance for CWCs in the RILs of B73 × B52 (Cardinal et al. 2003) was similar to the variance we observed in the present investigation for ADF, but it was higher for NDF and ADL. Heritabilities were significantly lower in the present investigation for NDF, ADF, and ADL, but the actual differences were relatively small for NDF and ADF, with heritabilities in both studies above 0.90. A large number of QTLs are located in the same genomic regions in both populations (Fig. 1), and for the majority of the QTLs in common, the allele associated with in-

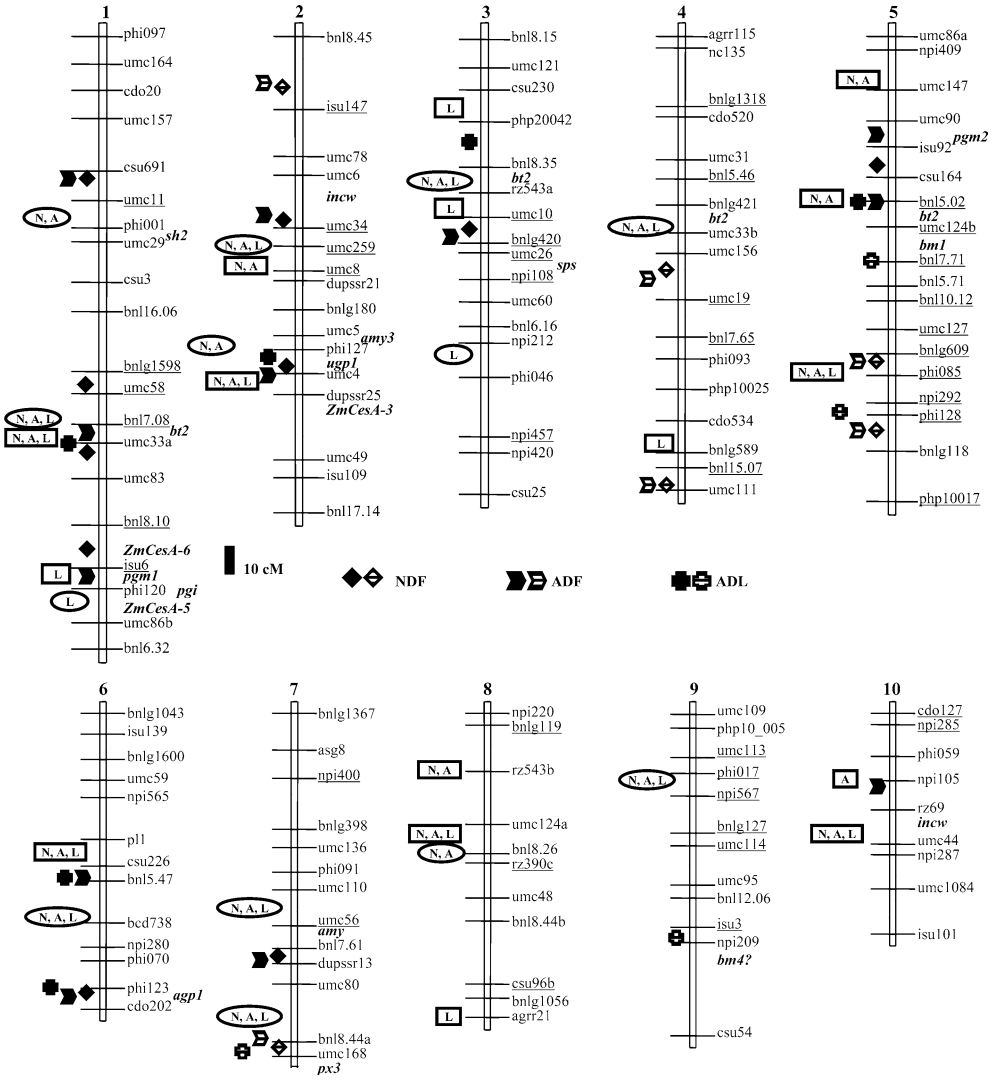

Fig. 1 Linkage map of the B73 × De811 RIL population. *Underlined* loci exhibited segregation distortion ($P < 0.05$). *Solid squares* denote QTLs for which the allele from De811 was associated with an increase in the trait, while *striped squares* denote QTLs for which the allele from B73 was associated with an increase in the trait. Candidate genes are listed in *bold italics*: *sh2*, *bt2*, *agp1* ADP-glucose pyrophosphorylase subunits, *ZmCesA-3*, *-5*, *-6* cellulose synthases, *pgm* phosphoglucomutase, *pgi* glucose-6-P isomerase, *bm* brown midrib mutants, *incw* cell-wall-bound invertase, *amy* beta-amylase, *ugp* UDP-glucose pyrophosphorylase, *sps* sucrose phosphate synthase, *px3* peroxidase. *Ovals* represent QTLs from the F_3 lines of B73 × De811, *rectangles* represent QTLs from the RILs of B73 × B52. *N* NDF, *A* ADF, *L* lignin

creased levels of CWCs was derived from the non-B73 parent.

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, and while the median QTL effects calculated from CV/G were mostly 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. 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). The large number of QTLs with small effects observed herein may have contributed to the upward bias, and a larger population size may have reduced the effects of multicollinearity in the data.

The clustering of QTLs appears to be non-random, and data from other studies provide further evidence for this hypothesis (Cardinal et al. 2003; Krakowsky et al. 2003). Some possible explanations for non-random clustering are that the QTLs are regulatory genes controlling cell-wall synthesis; that cell-wall synthesis is limited by one factor, and an increase in that factor leads to an increase in all components; that detection of a QTL for a CWC is based on QTLs for its sub-fraction(s) (e.g., ADF is a sub-fraction of NDF; see Cardinal et al. 2003 and Krakowsky et al. 2003). A test of this last possibility was provided by adjusting NDF for ADF and ADF for ADL. Significantly lower genetic variation and fewer QTLs were observed for NDF adjusted for ADF than for NDF, indicating that most of the variability in NDF may be due to the ADF fraction. The results for ADF adjusted for ADL were quite different, with a much smaller decrease in genetic variation and

number of QTLs as compared with ADF. This was not unexpected as the genetic variation for ADL is low in this population.

Many QTLs in this study may have sequence homology due to large-scale chromosomal duplications present in the maize genome, a pattern also observed in the F_3 lines of B73 × De811 (Krakowsky et al. 2003). 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 QTLs appear to be present in duplicate copies [e.g., chromosomes 1 (*isu6*) and 5 (*isu92*)], while others appear to be present in multiple copies in the genome [e.g., chromosomes 1 (*umc58*), 3 (*bnlg420*), 7 (*bnl8.44a*), and 10 (*npi105*)]. The detection of QTLs in homologous sequences in different regions of the genome provides further evidence of an association between those sequences and the expression of CWCs.

The QTLs 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 QTLs were observed. The QTLs for ADF that we identified in the present investigation on chromosomes 1 (*umc33a* and *isu6*), 2 (*umc34*), 3 (*bnlg420*), 4 (*umc156*), 5 (*bnlg609*), and 7 (*bnl8.44a*) are linked to QTLs 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). One QTL was detected for NDF in the top-cross, and it is linked to a QTL identified herein for NDF on chromosome 1 (*isu6*). No QTLs were detected for NDF in the RILs per se, and the QTLs for ADL in the top-cross and RILs per se were not linked to those observed here.

Some of the QTLs and candidate genes for enzymes postulated to have direct [cellulose synthase (CS), UDP-glucose pyrophosphorylase (UGP) and sucrose synthase (SuSy)] and indirect [glucose-6-P isomerase (PGI), phosphoglucomutase (PGM), sucrose phosphate synthase (SPS)] roles in cellulose biosynthesis have been identified in the maize genome (Preiss 1982; Causse et al. 1995a, 1996; Delmer and Amor 1995; Prioul et al. 1999; Holland et al. 2000). 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 CS. Enzymes such as UGP, PGI, PGM, and SPS facilitate in the recycling of the fructose back into sucrose (Delmer and Haigler 2002). The QTLs for NDF and ADF on chromosomes 1 (*csu691* and *umc33a*) and 5 (*isu92* and *bnl5.02*) are linked (within 20 cM) to QTLs for SuSy, while those on chromosomes 1 (*umc33a*), 2 (*umc4*), and 5 (*bnlg118*) are linked to QTLs for SPS. Candidate genes *ugp, pgm*, and *ZmCesA* (CS in maize), among others, are also linked to QTLs for NDF and ADF (Fig. 1). The detection of QTLs 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.

Some QTLs identified in the present investigation may also be associated with enzymes that have pleiotropic effects on the synthesis of CWCs, such as ADP-glucose pyrophosphorylase (AGP), beta-amylase (AMY), and cell-wall-bound invertase (INCW). While cell-wall and starch synthesis may not compete for carbon entering the stalk tissue cells due to temporal differences (Delmer and Haigler 2002), the quantities of starch in the stalk could affect the concentrations of CWCs observed in the stalk, with higher starch quantities diluting the CWC concentrations overall. The locations of QTLs and candidate genes for these enzymes have been localized on nine chromosomes in the maize genome (Causse et al. 1995b, 1996; Prioul et al. 1999). The AGP is a regulating enzyme in starch synthesis and consists of two subunits, coded by *bt2* and *sh2* in the grain and *agp1* and *agp2* in the embryo, while AMY is directly involved in the synthesis of starch (Buchanan et al. 2000). INCW appears to play an important role in phloem loading of sucrose (Shanker et al. 1995). The QTLs for NDF and ADF on chromosomes 1 (*isu6*), 2 (*umc34*), 3 (*bnlg420*), 5 (isu92, bnl5.02, and *phi128*), and 10 (*npi105*) are linked to QTLs for AGP, while the QTLs on chromosomes 2 (*umc34*) and 5 (*isu92* and *bnl5.02*) are linked to QTL for INCWs. Candidate genes for AGP, AMY, and INCW are also linked to QTLs detected herein (Fig. 1). The effect of starch levels on CWC concentrations is an important consideration in selecting germplasm for forage quality, since a dilution of CWC concentrations through increased starch levels in the stalk may result in decreased grain yield.

Only two main-effect QTLs for ADL are linked to candidate genes. A QTL on chromosome 5 (bnl7.71) is linked to *bm1*, one of the brown midrib mutants, and the QTL on chromosome 7 (bnl8.44a) is linked to peroxidase (PX), 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 QTLs for ADL as compared to the many candidate genes linked to QTLs for NDF and ADF is probably attributable to the low variability for ADL in this population.

An understanding of the genetic basis of CWCs should be a powerful tool for improving the quality of maize forage. In the present study, QTLs for CWCs were observed on nine chromosomes of the maize genome, many of which are linked to QTLs observed in F_3

lines of the same population and other populations as well. Due to the large number of QTLs observed and the relatively small effects of most of the QTLs, marker assisted selection (MAS) for a few QTLs with large effects along with phenotypic selection for lower CWCs would likely be more effective than MAS alone. 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. Some QTLs were linked to candidate genes for enzymes directly involved in the synthesis of CWCs, such as cellulose synthase and sucrose synthase, or to enzymes implicated in other pathways of carbon metabolism, such as starch synthesis. Most of the research on starch synthesis has focused on the developing kernels on the ear, but analysis of the stalk may provide some clues about the relationship between starch and cellulose synthesis, and how important a role each plays in determining the quality of forage maize.

Acknowledgments The research reported here was carried out in partial fulfillment of the Ph.D. degree by M.D. Krakowsky. This journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa, Project No. 3134, was supported by Hatch Act and State of Iowa funds and The R.F. Baker Center for plant breeding.

References

Austin DF, Lee M, Veldboom LR, Hallauer AR (2000) Genetic mapping in maize with hybrid progeny across testers and generations: grain yield and grain moisture. Crop Sci 40:30–39

Beavis WD (1994) The power and deceit of QTL experiments: lessons from comparative QTL studies. In: ASTA (ed) 49th Annu Corn Sorghum Industry Res Conf. ASTA, Washington, D.C., pp 250–266

Beeghly HH, Coors JG, Lee M (1997) Plant fiber composition and resistance to European corn borer in four maize populations. Maydica 42:297–303

Buchanan BB, Gruissem W, Jones RL (2000) Biochemistry and molecular biology of plants. ASPP, Rockville, Md.

Buendgen MR, Coors JG, Grombacher AW, Russell WA (1990) European corn borer resistance and cell wall composition of three maize populations. Crop Sci 30:505–510

Cardinal A, Lee M, Moore KJ (2003) Genetic mapping and analysis of quantitative trait loci (QTL) affecting fiber and lignin content in maize. Theor Appl Genet 106:866–874

Causse M, Rocher J, Henry AM, Charcosset A, Prioul J, de Vienne D (1995a) Genetic dissection of the relationship between carbon metabolism and early growth in maize, with emphasis on key-enzyme loci. Mol Breed 1:259–272

Causse M, Rocher J, Pelleschi S, Barriére Y, de Vienne D, Prioul J (1995b) Sucrose phosphate synthase: an enzyme with heterotic activity correlated with maize growth. Crop Sci 35:995–1001

Causse M, Santoni S, Damerval C, Maurice A, Charcosset A, Deatrick J, de Vienne D (1996) A composite map of expressed sequences in maize. Genome 39:418–432

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Cochran WG, Cox GM (1957) Experimental designs, 2nd edn. Wiley, New York

Deinum B, Struik PC (1986) Improving the nutritive value of forage maize. In: Dolstra O, Miedema P (eds) Breed Silage Maize. Proc 13th Cong Maize Sorghum Sect EUCARPIA. Pudoc, Wageningen, pp 77–90

Delmer DP, Amor Y (1995) Cellulose biosynthesis. Plant Cell 7:987–1000

Delmer DP, Haigler CH (2002) The regulation of metabolic flux to cellulose, a major sink for carbon in plants. Metab Eng 4:22–28

Fehr WR (ed) (1987) Principles of cultivar development. McGraw-Hill, New York

Ferret A, Casañas F, Verdú AM, Bosch L, Nuez F (1991) Breeding for yield and nutritive value in forage maize: an easy criterion for stover quality, and genetic analysis of Lancaster variety. Euphytica 53:61–66

Gaut BS (2001) Patterns of chromosomal duplication in maize and their implications for comparative maps of the grasses. Genome Res 11:55–66

Georges MD, Nielsen D, Mackinnon M, Mishra A, Okimoto R, Pasquino AT, Sargeant LS, Sorensen A, Steele MR, Zhoa Z, Womack JE, Hoeschele I (1995) Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. Genetics 139:907–920

Helentjaris T, Weber D, Wright S (1988) Identification of genomic locations of duplicate nucleotide sequences in maize by analysis of restriction fragment length polymorphisms. Genetics 118:353–363

Holland JB (1998) EPISTACY: a SAS program for detecting two-locus epistatic interactions using genetic marker information. J Hered 89:374–375

Holland JB, Moser HS, O'Donoughue LS, Lee M (1997) QTLs and epistasis associated with vernalization responses in oat. Crop Sci 38:1306–1316

Holland N, Holland D, Helentjaris T, Dhugga KS, Xoconostle-Cazares B, Delmer DP (2000) A comparative analysis of the plant cellulose synthase (*CesA*) gene family. Plant Physiol 123:1313–1324

Hunt CW, Kezar W, Vinande R (1992) Yield, chemical composition, and ruminal fermentability of corn whole plant, ear, and stover as affected by hybrid. J Prod Agric 5:286–290

Hunter RB (1978) Selection and evaluation procedures for whole-plant corn silage. Can J Plant Sci 58:661–678

Jansen RC (1993) Interval mapping of multiple quantitative trait loci. Genetics 135:205–211

Jansen RC, Stam P (1994) High resolution of quantitative traits with multiple loci via interval mapping. Genetics 136:1447–1455

Knapp SJ, Stroup WW, Ross WM (1985) Exact confidence intervals for heritability on a progeny mean basis. Crop Sci 25: 192–194

Krakowsky MD, Beeghly HH, Coors JG, Lee M (2003) Characterization of quantitative trait loci affecting fiber and lignin in maize (*Zea mays* L). Maydica 48:283–292

Krakowsky MD, Lee M, Woodman-Clikeman WL, Long MJ, Sharpova N (2004) QTL mapping of resistance to stalk tunneling by the European corn borer in RILs of maize population B73 × De811. Crop Sci 44:274–282

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln ES, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lübberstedt T, Melchinger AE, Klein D, Degenhardt H, Paul C (1997) QTL mapping in testcrosses of European flint lines of maize: II. Comparison of different testers for forage quality traits. Crop Sci 37:1913–1922

Lundvall JP, Buxton DR, George JR (1994) Forage quality variation among maize inbreds: in vitro digestibility and cell-wall components. Crop Sci 34:1672–1678

Méchin V, Argillier O, Hébert Y, Guingo E, Moreau L, Charcosset A, Barrière Y (2001) Genetic analysis and QTL mapping of cell wall digestibility and lignification in silage maize. Crop Sci 41:690–697

Mode CJ, Robinson HF (1959) Pleiotropism and the genetic variance and covariance. Biometrics 15:518–537
Ooijen JW van (1992) Accuracy of mapping quantitative trait loci in autogamous species. Theor Appl Genet 84:803–811
Papst C, Bohn M, Utz HF, Melchinger AE, Klein D, Eder J (2004) QTL mapping for European corn borer resistance (*Ostrinia nubilalis* Hb.), agronomic and forage quality traits of testcross progenies in early-maturing European maize (*Zea mays* L.) germplasm. Theor Appl Genet 108:1545–1554
Preiss J (1982) Regulation of the biosynthesis and degradation of starch. Annu Rev Plant Physiol 33:431–454
Prioul JL, Pelleschi S, Séne M, Thévenot C, Causse M, deVienne D, Leonardi A (1999) From QTLs for enzyme activity to candidate genes in maize. J Exp Bot 50:1281–1288
Robertson JB, van Soest PJ (1980) Detergent system of analysis and its application to human foods. In: James WPT, Theander O (eds) The analysis of dietary fiber in food. Marcel Dekker, New York, pp 123–158
Roth LS, Marten GC, Compton WA, Stuthman DD (1970) Genetic variation of quality traits in maize (*Zea mays* L.) forage. Crop Sci 10:365–367
SAS Institute (1999) SAS OnlineDoc, version 8. SAS Institute, Cary, N.C.
Senior ML, Murphy JP, Goodman MM, Stuber CW (1996) Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci 38:1088–1098
Shanker A, Salazar RW, Taliercio EW, Chourey PS (1995) Cloning and characterization of full-length cDNA encoding cell-wall invertase from maize. Plant Physiol 108:873–4
van Soest PJ (1994) Nutritional ecology of the ruminant, 2nd edn. Cornell University Press, Ithaca
Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTL. JQTL 2:1
Veldboom LR, Lee M, Woodman W (1994) Molecular marker-facilitated studies in an elite maize population: I linkage analysis and determination of QTL for morphological traits. Theor Appl Genet 88:7–16
Visscher PM, Thompson R, Haley CS (1996) Confidence intervals in QTL mapping by bootstrapping. Genetics 143:1013–1020
Whetten R, Sederoff R (1998) Lignin biosynthesis. Plant Cell 7:1001–1013
Wolf DP, Coors JG, Albrecht KA, Undersander DJ, Carter PR (1993) Forage quality of maize genotypes selected for extreme fiber concentrations. Crop Sci 33:1353–1359
Zeng Z (1994) Precision mapping of quantitative trait loci. Genetics 136:1457–1468