

Genetic and Morphometric Analysis of Cob Architecture and Biomass-Related Traits in the Intermated B73×Mo17 Recombinant Inbred Lines of Maize

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Abstract Expected future cellulosic ethanol production increases the demand for biomass in the US Corn Belt. With low nutritious value, low nitrogen content, and compact biomass, maize cobs can provide a significant amount of cellulosic materials. The value of maize cobs depends on cob architecture, chemical composition, and their relation to grain yield as primary trait. Eight traits including cob volume, fractional diameters, length, weight, tissue density, and grain yield have been analyzed in this quantitative trait locus (QTL) mapping experiment to evaluate their inheritance and inter-relations. One hundred eighty-four recombinant inbred lines of the intermated B73×Mo17 (IBM) Syn 4 population were evaluated from an experiment carried out at three locations and analyzed using genotypic information of 1,339 public SNP markers. QTL detection was performed using (1) comparison-

wise thresholds with reselection of cofactors ($\alpha=0.001$) and (2) empirical logarithm of odds score thresholds ($P=0.05$). Several QTL with small genetic effects ($R^2=2.9\text{--}13.4\%$) were found, suggesting a complex quantitative inheritance of all traits. Increased cob tissue density was found to add value to the residual without a commensurate negative impact on grain yield and therefore enables for simultaneous selection for cob biomass and grain yield.

Keywords Cob biomass · Maize · Cob tissue density · QTL · IBM

Introduction

Cob biomass is a widely abundant residual in the US Corn Belt, and has received increased interest for generating renewable energy [1]. New cob harvest and conversion technologies are available, and cob biomass is considered for combustion or gasification in power plants and cellulosic ethanol production [2]. While grain usage for energy competes with food production on limited acreage, cobs can be harvested in addition to grain. Therefore, cobs do not require additional acreage, such as short rotation poplar or miscanthus. If stover remains in the field, harvest of cobs with nitrogen content below 1 % will hardly affect soil fertility or significantly change fertilizer management [3]. In order to further optimize economic feasibility of cob utilization, a denser cob biomass tissue will make harvest and transportation more efficient. Even though maize is among the best investigated crops, little is known about the genetic basis of cob biomass properties, defined by cob volume, density, and biomass quality.

Cob architecture can be simplified to a body with cylindrical or conical shape defined by cob length and cob diameter. Three distinguishable zones affect cob density [4, 5]. The inner zone (pith) consists of dead cells at

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maturity, resulting in spongy tissue of low density. The wooden zone surrounding this core consists of sclerenchyma cells with high density accounting for most of the cob biomass. The outer chaff of low weight and density directly connects to the grain and embodies the branched spikelets of the inflorescence. A complicated mazy channel system of interwoven bundles in the cob connects the vascular units with grain. These three zones have been characterized by Foley and Vander Hooven [4], who further divided chaff into a fine and coarse fraction. The reported average weight fractions were 1.9 % for pith, 60.3 % for the wooden ring, 33.7 % for coarse chaff, and 4.1 % for fine chaff. However, a large variation was found for distribution of those zones [5], as well as for cob architecture traits including length and diameter [2, 6, 7].

For the development of dual purpose maize that will be grown for high cob biomass combined with high grain yield, potential optimization of both traits must be pursued. Breeding for higher grain yield generally leads to higher cob yield, as both traits are positively correlated. However, genotypes with similar grain yield might differ significantly for cob biomass yield [2]. Cob biomass yield is a function of cob volume and density. Cob weight increases with volume and/or density. The relation between those three traits and, therefore, a possible tradeoff between them is mathematically well defined, but the underlying biology is unknown. Moreover, understanding of the relationship of cob biomass yield and as primary traits is fundamental for developing dual purpose maize.

Knowledge on location and effects of quantitative trait loci (QTL) related to cob biomass traits inform about the most appropriate breeding strategy. In case of QTL with major effects controlling these traits, marker-assisted selection or backcrossing are effective to introduce favorable regions into breeding materials. In case of multiple QTL with small effects, marker-assisted recurrent selection or genome-wide selection can be employed to increase the number of favorable alleles in a population [8, 9].

Several QTL have been reported for cob length (ear length), ear or cob diameter, and cob weight [6, 7, 10, 11] and reviewed by [2]. However, to our knowledge, no QTL have been reported for cob volume, cob density, or diameters of pith and wooden ring. Over the past 20 years, QTL for volume components such as ear and cob length and ear and cob diameter have been found across all ten chromosomes [6], [7], [11–14]. Yet, no QTL have been found for cob diameter on chromosomes 2, 3, 6, and 10. For cob weight, Upadyayula et al. [11] reported a total of nine QTL located on chromosomes 1–7.

QTL findings differ greatly between populations depending on the parents of a mapping population, population type and size, marker coverage, and environments used for phenotyping as well as phenotyping methods [15]. Furthermore, different mapping methods like single marker analysis, interval mapping, or composite interval mapping affect the outcomes of

QTL mapping studies [15, 16]. Some of the studies mentioned above were conducted before more advanced QTL mapping methods became available. For composite interval mapping, QTL results vary with choice and number of cofactors, as well as thresholds used to declare QTL significant [17].

The parents B73 and Mo17 of the intermated B73×Mo17 (IBM) Syn4 population [18] used in this study play a key role in US breeding programs and maize research [19, 20]. IBMSyn4 is expected to segregate for cob architectural traits, since ears of B73 are shorter with wider diameter than cobs of Mo17 [21]. The IBMSyn4 population has been intermated five generations resulting in a 3.9-fold larger mapping distance due to an average 2.7-fold higher recombination fraction compared to F₂ derived populations [18].

Our objectives were to (1) evaluate the relationships among cob architecture traits, and their relationship to grain yield at the phenotypic and genetic level, (2) identify QTL and their effects for cob length, weight, diameter (including for the pith and wooden ring fractions), volume, density, as well as grain and cob yield, and (3) discuss our findings with regard to developing dual purpose maize varieties.

Materials and Methods

Plant Materials, Field Experiments, and Phenotyping

Field trials for this study were performed in Wisconsin in 2007 (two locations and two replications) and for two traits (cob length and cob diameter) also in Iowa in 2006 (one location and two replications). Materials and methods for the Wisconsin locations have been reported by Lorenz and colleagues [22], who performed genetic analysis on maturity, yield, and composition traits. Herein, cob trait data were collected from these plants and analyzed in the context of the additional component traits underlying the quality of cob residuals. Briefly, 206 recombinant inbred lines (RILs) of the IBMSyn4 per se population [18] and their parents Mo17 and B73 were grown in two locations in Wisconsin [Arlington (ARL) and Madison (MAD)] in 2007 [22] and phenotyped for cob traits. Identity of lines was confirmed as described by Lorenz et al. [22]. Trials were planted May 7 and May 21 on Plano silt loam soil and harvested 135 and 156 days after planting at ARL and MAD, respectively. Randomized complete block design was applied at each location with two replications using single-row plots (6.08×0.76 m) with 79,040 plants/ha [22]. B73 and Mo17 were planted in two plots per block, resulting in eight single rows of each; parental phenotype data were used for comparisons to the IBM RILs, but not for QTL analyses.

Ten plants were hand-harvested from each plot. All ears were dried and shelled. After shelling, cobs were dried in a forced-air dryer at 55 °C for 1 week. Total cob and grain

weight of those ten plants was used to determine cob and grain weight per plant and cob and grain yield (CY and GY) from multiplication with stand counts. Cob and grain weight per cob (CWC and GWC) relate to the weight of all ten plants, including ears and cobs without seed set. Grain weights were adjusted to 15.5 % moisture content.

Three representative cobs per plot were sampled to measure average cob length, single cob weight, total cob diameter, volume, and tissue density (Fig. 1). Diameters were measured within cobs for central pith, the thickness of the wooden part excluding pith and chaff and for the total diameter at the middle of the cob section (Fig. 1). Cob volume estimates were calculated from cob length and diameter using the formula for cylindrical volume:

$$\text{Cob volume} = \left(\frac{\text{Diameter}}{2}\right)^2 \times \text{Length} \times \pi \quad (1)$$

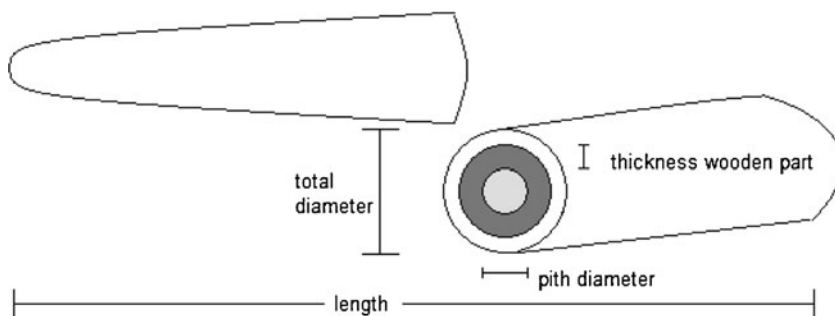
Cob tissue density (DEN1) for the entire cob was calculated as the ratio between single cob weight and volume. Cob tissue density (DEN2) was calculated from a cylindrical mid-cob section (about 1–4 cm long) using the weight of the piece and its volume calculated based on formula (1), but using the piece’s length instead of cob length. Values from three cobs were averaged for each plot after calculating values for all traits for single cobs.

The Iowa experiment was grown in 2006 at the Agronomy Agricultural Engineering Research Center in Ames, IA, USA (AME; [21]; total cob diameter and cob length have been determined. Parents B73 and Mo17 were planted in AME eight and seven plots, respectively. In this experiment, an alpha-lattice design with two reps was used and 12 plants were grown per plot (single row, 0.76×3.8 m). Data were obtained for the same 184 RILs used in the Wisconsin experiment.

Statistical Analyses

For cob diameter and cob length, analyses have been carried out for the two Wisconsin locations (diameter and length) and for three environments including the Ames experiment as third environment (TD3 and CL3).

Fig. 1 Scheme for phenotyping cob traits. Shown are the measure points for pith and total diameter, thickness of the wooden part, and cob length



Cob data analyses were carried out using SAS PROC MIXED (SAS Institute Inc., Cary, NC, USA) fitting locations and lines, interactions of locations and line (G×E), and blocks as random effects. Heritability was calculated on an entry mean basis derived from variance components using SAS PROC VARCOMP (SAS Institute Inc., Cary, NC, USA), fitting locations, lines, G×E, and blocks as random effects. Least square means were calculated from two blocks for each location and over both locations with random block and G×E effects to account for missing values using SAS PROC MIXED. Phenotypic and genotypic correlations of traits were calculated using SAS PROC MIXED according to Holland [23]. Means of parents B73 and Mo17 were compared with two-sided Student’s *t* tests at 5 % significance level, assuming unequal variances. Coefficient of variation (CV) was calculated as standard deviation divided by the population mean.

Path Analysis

Path analyses are used to fit proposed path models according to covariance structure of the underlying data [24–26]. In path analysis, correlations can be used to estimate total, direct, and indirect effects of observed variables (exogenous and intervening causal variables) on other observed variables (endogenous dependents and endogenous intervening variables) as an extension of the regression model. Calculated path coefficients are often given as standardized regression coefficients for direct effects in the model. Indirect effects and total effects are calculated based on the product and sum of those direct effects. Standardization is implemented by expressing effects in standard deviations.

Two similar models (A and B; Fig. 2) to explain cob biomass (weight) have been tested for statistical fit based on Akaike’s information criterion (AIC), root mean square error of approximation (RMSEA), comparative fit index (CFI), chi square (likelihood ratio chi-square test), and HoelterN using IBM SPSS Amos 20 [24]. The best fit was defined by a chi square>0.05, RMSEA<0.05, CFI>0.95, a HoelterN indicator of sufficient sample size of >40, and ultimately a minimum AIC value. To investigate the double

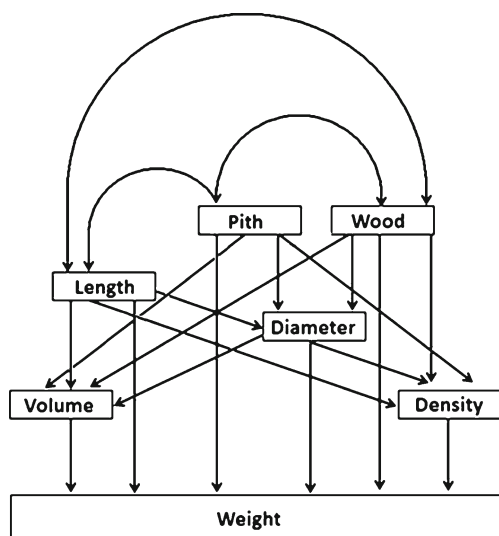


Fig. 2 Proposed model B with diameter fitted as intervening variable. *Pith* Diameter of pith, *wood* thickness of wooden part, *length* cob length, *diameter* cob diameter, *volume* cob volume, *density* DEN2 values for cob tissue density, *weight* average single cob weight. *Arrows* indicate fitted direct and indirect effects in the initial model

role of different cob tissue fraction by diameter and density on cob weight and because insufficient grain yield components were collected, only single cob-based traits downstream of cob weight were included. Model A was based on the hypothesis that pith diameter, thickness of the wooden ring, total diameter, and cob length have an effect on the three dependent variables, volume, density, and ultimately weight, where weight was also dependent on volume and density. Then, all direct and indirect effects were fitted with the exception of direct relations between volume and density, which are independent based on correlations. Since density is calculated from volume and weight, one might argue that density rather than weight should be the resulting variable of highest order. However, density is influenced by the density and fraction of different cob tissues of the cob and can be understood as a weighted average of those. Model B (Fig. 2) was modified compared to model A in such a way, that total diameter was fitted as intervening dependent variable for pith and wood. Both models were further optimized towards a minimum AIC by deleting insignificant effects from the model (A-AIC and B-AIC).

Quantitative Trait Locus Mapping

Genotypic data for a subset of 184 IBM RILs was obtained for all 1,339 markers from the IBM1 framework map from MaizeGDB.org based on data for all 302 lines from the Maize Mapping Project (<http://www.maizegdb.org/qtl-data.php>, verified 3 October 2012). The total genetic distance of this

map adds up to 6,242.7 cM, leading to an average marker interval size of 4.66 cM.

QTL analysis was carried out with QTL Win Cartographer version 2.5 [27] using composite interval mapping (CIM; Zmap model 6) with forward and backward regression for identification of the ten most significant cofactors. Test positions occurred at 1-cM intervals and at each marker. During CIM, cofactor effects originating from positions mapping within 10 cM of the test position were excluded from the model. In order to limit the type II error rate, comparison-wise thresholds (CWTs) based on 1,000 permutations of the phenotype data were determined at significance levels of $\alpha=0.001$ for each trait by using scripts updated for CIM to include cofactor reselection for each permuted data set [28]. Additional runs were performed using a default empirical experiment-wise threshold after 1,000 permutations each, without reselecting cofactors (EWT) as implemented in Win Cartographer 2.5 [27]. EWT was also used for analyses of data from each single environment. The same settings were used as for CWT. Results were compared with the method described above. In both cases, a distance of >10 cM was used to separate significant regions into different QTL. For each QTL, support intervals based on 1.5 logarithm of odds (LOD) drops from each likelihood peak are reported [29]. In addition, intervals of significant marker intervals ($p=0.001$) around peaks according to CWT are reported as indicator for mapping precision. Only results for CWT are shown in detail and compared to results based on EWT. As a final step, all detected QTL of a trait were analyzed simultaneously using their most likely position based on CWT analysis. For this analysis, multiple interval mapping (MIM) implemented in QTL Win Cartographer was used to calculate single R^2 for each QTL and sum r^2 of all QTL for a given trait.

Results

Trait Characteristics and Correlations

B73 outperformed Mo17 significantly ($p=0.05$) for increased single cob weight (131 %), diameter of pith (193 %) and wooden part (118 %), cob diameter (112 %), TD3 (137.3 %), cob volume, and all cob and grain yield-related traits, but displayed reduced cob length and CL3 (81.5 %; Table 1). No significant difference between the parents was observed for the two density traits.

For all traits, maximum and minimum values among 184 lines transgressed those of the two parents. B73 was among the highest ranking genotypes for diameter (rank 2), cob yield (rank 3), and cob weight based on 10 plants (rank 7),

Table 1 Statistics for all measured traits

Variable	Trait	N	Unit	Mean	B73	Mo17	SD	CV	Min	Max	h^2
Length	Length	708	cm	11.41	10.79	13.27	1.79	15.67	5.6	17.27	0.76
Weight	Single cob weight (3 cobs)	708	g	11.04	14.44	11.07	3.68	33.36	1.7	25.04	0.75
Pith	Pith diameter	708	cm	0.62	0.81	0.42	0.14	22.69	0.3	1.1	0.91
Wood	Wooden part thickness	708	cm	0.34	0.40	0.34	0.06	18.95	0.1	0.5	0.78
Diameter	Diameter	708	cm	2.29	2.72	2.02	0.23	10.18	1.2	2.9	0.77
Volume	Volume	708	cm ³	48.16	62.79	43.59	13.25	27.51	8.82	87.6	0.77
DEN1	Density 1 from cone ass.	708	g/100 cm ³	22.89	23.02	25.25	4.00	17.48	11.6	38.2	0.77
DEN2	Density 2 from piece	708	g/100 cm ³	26.61	26.73	26.88	4.73	17.78	11.2	42.8	0.76
CWC	Weight/cobs of 10 plants	724	g	9.83	13.92	8.51	3.66	37.24	0.5	23.07	0.71
GWC	Grain wt/plant*10	719	g	41.57	44.08	15.30	23.53	56.60	0.01	145.63	0.64
Cob yield	Cob yield/plot	724	g	278.22	527.45	91.89	141.58	50.89	1.7	815.48	0.72
Grain yield	Grain yield/plot	719	g	1199.00	1676.07	168.86	816.27	68.08	0.36	3863	0.66
Including Ames location											
CL3	Length over 3 location	1022	cm	12.87	12.14	15.02	15.02	22.14	22.14	5.6	0.68
TD3	Diameter over 3 location	1022	cm	2.41	2.85	2.09	0.29	11.93	1.2	1.2	0.75

Values are given for population mean, mean performance of each parent, population standard deviation (SD), coefficient of variation (CV), minimum and maximum line averages, and entry mean heritabilities for Wisconsin locations and including Ames location (CL3, TD3)

while Mo17 was among genotypes with the longest cobs (rank 15). On average, pith represented 27 % (16–39 %) of total cob diameter and the wooden part 30 % (20–40 %), leaving 43 % (36–55 %) to chaff. Together, the two low density fractions pith and chaff represent 60–80 % of total cob diameter.

Single cob weight, grain and cob yield showed the highest coefficients of variation (CV; 33.4–68.1), and total diameter the lowest CV (10.2; Table 1). For all traits, significant effects of genotype were found ($p=0.001$). For most traits, the location effect was significant ($p=0.05$), except for length ($p=0.27$), pith ($p=0.91$), and DEN2 ($p=0.34$). $G \times E$ interactions were significant for all traits at $p=0.05$. However, at $p=0.01$, wood ($p=0.024$), and total diameter ($p=0.026$) did not show significant $G \times E$ interactions. Heritabilities were estimated on an entry-mean basis and intermediate to high for all traits ranging from 0.64 for GWC to 0.91 for pith (Table 1).

Correlations

Cob length, total diameter, volume, and weight were all positively correlated with each other (Table 2). Cob weight was positively correlated with all traits. Cob volume was closely correlated with both, cob length ($r_g=0.71$), and total diameter ($r_g=0.85$). Cob volume showed positive correlations with all traits (Table 2), but was not significantly correlated with any of the two density traits. DEN1 and DEN2 were closely correlated ($r_g=0.94$, $r_p=0.88$). While variance components and heritabilities did not differ considerably, absolute

differences were found between the two methods for nearly all parameters shown in Table 1. Cob tissue densities (DEN1 and DEN2) were negatively correlated with pith ($r_g=-0.26$ and -0.30 , respectively), but positively correlated with wood ($r_g=0.37$ and 0.43 , respectively), cob weight ($r_g=0.31-0.55$, respectively), and cob yield ($r_g=0.44$). No other trait showed a significant correlation ($p=0.01$) with DEN2. However, at $p=0.05$, DEN1 showed a weak positive phenotypic correlation with length ($r_p=0.13$). While pith was significantly positively correlated with total diameter ($r_g=0.61$, $r_p=0.58$), pith showed a significant negative correlation with wood ($r_g=-0.26$, $r_p=-0.13$).

Path Analysis

All models showed good fit for all observed criteria. Both models and their optimized daughter models showed exactly matching results for all criteria, respectively (see Supplementary Table 4). We, therefore, focus on reporting result of model B because diameter as intervening variable for pith and wood appears reasonable (see Supplementary Table 5).

Total variance of 94, 21, 98, and 72 % in weight, density, volume, and diameter were explained in model B, respectively (Supplementary Table 5). All total effects on cob weight were positive in model B. Most other significant total effects were also positive with the exception of effects of pith, length, and diameter on density. The strongest direct (and total) effect was found between volume and weight with 1.13 standardized

Table 2 Phenotypic and genotypic correlations

	Length	Weight	Pith	Wood	Diameter	Volume	DEN1	DEN2	CWC	GWC	Cob yield	Grain yield
Length		0.68 ^a	0.08	0.21 ^a	0.31 ^a	0.76 ^a	0.13 ^b	0.01	0.64 ^a	0.51 ^a	0.58 ^a	0.49 ^a
Weight	0.6 ^a		0.27 ^a	0.52 ^a	0.65 ^a	0.83 ^a	0.56 ^a	0.46 ^a	0.89 ^a	0.56 ^a	0.76 ^a	0.53 ^a
Pith	0.05	0.24 ^a		-0.13 ^b	0.58 ^a	0.45 ^a	-0.18 ^a	-0.18 ^a	0.26 ^a	0.25 ^a	0.2 ^a	0.2 ^a
Wood	0.11	0.51	-0.26 ^a		0.53 ^a	0.46 ^a	0.28 ^a	0.34 ^a	0.47 ^a	0.22 ^a	0.34 ^a	0.16 ^a
Diameter	0.24 ^a	0.66 ^a	0.61 ^a	0.41 ^a		0.83 ^a	-0.04	0.00	0.63 ^a	0.48 ^a	0.5 ^a	0.37 ^a
Volume	0.71 ^a	0.81	0.47 ^a	0.35 ^a	0.85 ^a		0.04	-0.01	0.79 ^a	0.6 ^a	0.66 ^a	0.51 ^a
DEN1	0.04	0.55 ^a	-0.26 ^a	0.37 ^a	-0.09	-0.04		0.88 ^a	0.43 ^a	0.14 ^a	0.39 ^a	0.17 ^a
DEN2	-0.06	0.48 ^a	-0.03 ^a	0.43 ^a	-0.08	-0.09	0.94 ^a		0.32 ^a	0.02	0.29 ^a	0.05
CWC	0.59 ^a	0.96 ^a	0.29 ^a	0.54 ^a	0.72 ^a	0.84 ^a	0.44 ^a	0.34 ^a		0.72 ^a	0.82 ^a	0.65 ^a
GWC	0.4 ^a	0.48 ^a	0.31 ^a	0.18	0.57 ^a	0.59 ^a	-0.02	-0.14	0.6 ^a		0.65 ^a	0.9 ^a
Cob yield	0.59 ^a	0.82 ^a	0.19 ^a	0.35 ^a	0.54 ^a	0.07 ^a	0.44 ^a	0.32 ^a	0.88 ^a	0.62 ^a		0.82 ^a
Grain yield	0.47 ^a	0.49 ^a	0.22 ^a	0.12	0.43 ^a	0.52 ^a	0.08	-0.05	0.61 ^a	0.94 ^a	0.77 ^a	

Genotypic correlations are shown below the diagonal, phenotypic correlation above

^a 99 % significant CI not including 0

^b 95 % CI not including 0

regression coefficients in standard deviations (SD). Changes in density had an effect on weight, which was about half as strong as the effect of volume on weight with 0.52 SD. Total effect of diameter on weight was 0.32 SD in model B. Total effects on weight and volume were positive for pith (0.31 and 0.50), length (0.55 and 0.64), wood (0.49 and 0.40), and diameter (0.32 and 0.67, respectively) (Supplementary Table 5). Effects of pith (0.71), length (0.13), and wood (0.59) on diameter were also positive. Length and diameter showed negative direct effects on density (-0.01 and -0.36, only significant for diameter) but a positive effect on volume (0.55 and 0.67, respectively). Density (0.52) and volume (1.13) were positively affecting weight.

Total effects of pith (-0.17), length (-0.06), and diameter (-0.36) on density were negative, whereas total effect of wood on density was positive (0.36). The positive total effect of pith on weight of 0.31 was small and positive, due to its positive effect on diameter, while the direct effect of pith on weight was not significant. The same is true for wood, which showed nonsignificant direct effects on volume (0.01) and weight (-0.004), but positive effects on diameter (0.59) and density (0.57; Supplementary Table 5). Therefore, the effect of enlarged wood diameter on density by increasing volume outweighs its negative effect of volume on density.

Quantitative Trait Locus Analysis

Analyses across locations yielded total 57 QTL in 40 QTL regions at $p=0.001$ CWT levels for all eight traits using ten

cofactors. These are described in detail below (Table 3, Fig. 3). Twenty-nine QTL were confirmed within 10 cM peak-to-peak distance in at least one of the single environment analyses, whereof six were detected in at least two single environment analyses (Table 3). Fifteen QTLs detected using CWT with reselection were not detected using EWT (Table 3, indicated by a star). In a simultaneous fit using MIM six of the 57 QTL found with CWT were not confirmed (Table 3, missing values for R^2_{MIM}). Four additional QTLs (two volume, one DEN1, and one wood QTL) found with EWT ($p=0.05$, without reselection of cofactors) were not significant using CWT (data not shown). Additional analyses were carried out for across location data with EWT using five and 20 cofactors. Increasing cofactors to 20 yielded a total of 139 QTL, while reduction to five cofactors resulted in a drop to 13 QTL of the 43 QTLs found with ten cofactors.

No QTL explained phenotypic variance larger than $R^2_{MIM}=13.4$ % (DEN2). The average explained variance over all QTL was $R^2_{MIM}=6.5$ %. The average 1.5 LOD confidence interval size was 18 cM (max. 37.1 cM, min. 4.6 cM). The average interval size based on CWT with a standardized LR>1 was 9.2 cM (max. 28.9 cM, min. 0 cM).

Six QTLs were found for cob length over all three locations on chromosomes 1, 5, 8, and 9. QTLs on chromosomes 1 (QTL 2) and 9 were found for both cob length and CL3 (QTLs 2 and 6, Table 3). The sum of R^2_{MIM} over all CL3 QTL was 25.0 %, for cob length 21.9 %. For pith diameter, six QTLs were found on chromosomes 1, 2, 7, and 9. Altogether, these QTL accounted for 41.0 % of the phenotypic variation (R^2_{MIM}). Except for QTL 1 and 5, all B73

Table 3 QTL position and effects

Trait	QTL	Chr	Position (cM)	Closest marker	Distance	LOD	R ² (%)	R ² _{MIM} (%)	α (B73)	αMIM (B73)	PCWT	1.5 LOD interval		PCWT interval		AME	ARL	MAD (pos.)
												left pos	interval	left pos.	interval			
1	Length	1 ^a	357.01	bnlg2295	3.39	3.17	5.6	6.5	-0.347	-0.37	1.13	348.61	25.6	355.01	6.4	x	x	x
1	CL3	2	742.21	chrom7	0.19	4.28	6.5	5.2	0.367	0.371	1.54	737.21	11.2	739.21	6.2	x	x	x
1	Length	2	749.71	phi011	-1.01	3.63	6.2	5.1	0.3745	0.358	1.54	737.21	18.1	744.41	7.3	x	x	x
1	Length	3	168.81	bnlg1879	-7.01	2.74	5.1	6.8	-0.334	-0.39	1.08	161.51	37.1	167.81	4	x	x	x
1	CL3	4	259.91	mmp58	0.79	5.18	8.6	7.2	-0.599	-0.38	1.62	257.91	8.3	257.91	15.4	x	x	x
1	CL3	5	479.21	php20793	-0.01	7.58	12	7.3	0.4714	0.388	1.6	473.21	9	470.11	18.9	x	x	x
1	CL3	6	39.81q	lim343	6.39	5.26	11	5.2	0.4704	0.376	1.86	33.81	16.4	33.81	14.4	x	x	x
1	Length	6	46.21	lim343	-0.01	3.29	5.1	3.4	0.3463	0.296	1.41	29.81	20.4	37.81	9.4	x	x	x
2	Pith	1 ^a	100.81	umc1685	0.89	3.48	4.6	4.5	-2.791	-2.54	1.59	88.11	22	96.11	7.8	x	x	x
2	Pith	2	404.91	umc1676	-0.01	3.95	5.1	3.9	3.1856	2.421	1.08	400.61	7.2	404.81	0.1	x	x	x
2	Pith	3 ^a	958.81	phi064	-1.01	2.86	3.9	7.1	2.5817	3.141	1.26	957.11	17.8	957.81	8	x	x	x
2	Pith	4	280.31	php10012	-0.01	4.69	6.1	10	3.5512	4.493	1.45	277.01	10.3	273.71	13.6	x	x	x
2	Pith	5	211.51	umc1393	0.49	4.89	6.7	9.6	-3.791	-3.97	1.64	197.91	18.2	203.71	11.4	x	x	x
2	Pith	6	216.81	umc1191	0.19	4.57	6.2	6	3.3099	3.119	1.34	213.31	19.8	215.81	1	x	x	x
3	Wood	1	236.51	umc1465	0.09	6.56	10	2.9	-2.023	-1.05	2.44	231.41	6.2	232.41	13.8	x	x	x
3	Wood	2	429.11	mmp3	-0.01	4.46	6.2	7.9	1.2816	1.393	1.05	424.61	14.7	429.11	0	x	x	x
3	Wood	3	234.91	umc1935	0.39	10.2	6	10.2	2.0225	1.71	4.1	232.71	5.4	223.71	21.8	x	x	x
3	Wood	4	201.61	rz244a	-0.01	4.06	5.6	5.3	-1.271	-1.05	1.37	197.21	6.3	199.01	3.5	x	x	x
3	Wood	5 ^a	173.41	bnlg1712	-0.01	2.96	4	5.5	-1.053	-1.2	1.08	168.91	16	173.41	0	x	x	x
4	Diameter	1	404.91	umc1676	-0.01	3.87	5.8	6.6	4.6541	4.68	1.61	401.71	12.8	403.813	1	x	x	x
4	TD3	1	405.91	umc1676	-1.01	5.43	8.2	7.4	6.6011	4.751	1.3	402.81	11.1	404.81	7.1	x	x	(x)
4	Diameter	2 ^a	477.71	mmp34	0.59	3.56	5.9	5.4	5.698	4.488	1.28	473.71	4.6	476.71	1	x	x	(x)
4	TD3	2 ^a	478.31	mmp34	-0.01	2.75	3.7	5.7	3.4016	4.337	1.03	467.41	17.8	476.71	1.6	x	x	(x)
4	TD3	3 ^a	516.51	umc1140	9.79	3.14	7.1	4	4.6756	3.848	1.09	505.51	35.3	512.51	6	x	x	(x)
4	TD3	4	397.81	mmp115	-4.01	4.77	8.4	-	5.0717	-	1.58	393.41	29	393.81	28.6	x	x	(x)
4	Diameter	5	410.71	asg33	-0.01	5.44	8.3	8	5.3719	5.199	1.82	404.41	14	401.81	14.6	x	x	(x)
4	TD3	5	417.41	umc1775	-1.01	6.12	9.7	9.3	5.4883	5.252	3.44	396.81	23.6	393.81	28.6	x	x	(x)
4	Diameter	6	552.01	umc1999	-6.01	2.95	7	-	5.3488	-	1.25	545.01	32.3	550.01	5	x	x	(x)
4	TD3	7	239.91	umc114	-1.71	5.21	7.2	4.9	4.8275	3.998	1.6	231.51	12.1	233.51	8.5	x	x	(x)
5	Volume	1	131.01	bnlg1447	-3.01	3.89	7.3	8.5	-2.863	-2.99	1.16	127.11	24	129.01	5	x	x	(144)
6	Weight	1 ^a	358.01	bnlg2295	2.39	2.46	5	5	-0.664	-0.64	1.08	339.91	24.2	355.01	3	x	x	(341)
6	Weight	2	548.71	ufg50	0.69	2.28	4.2	-	-0.619	-	1.06	541.11	16.4	547.71	4.1	x	x	(341)
6	CY	2	551.41	uazi147b	0.39	3.97	7.3	5.4	-30.47	-27.8	1.96	541.51	13.3	544.51	11	x	x	(341)
6	CWC	3	764.81	umc197a	-0.01	2.5	4.4	4.3	0.6344	0.617	1.26	750.71	20.1	764.81	2	x	x	(341)

Table 3 (continued)

Trait	QTL	Chr	Position (cM)	Closest marker	Distance	LOD	R ² (%)	R ² _{MIM} (%)	α (B73) (B73)	αMIM (B73)	PCWT	1.5 LOD interval		PCWT interval		AME	ARL	MAD (pos.)
												left pos	interval	left pos.	interval			
6 s	CY	4 ^a	916.21	umc84a	-0.01	3.21	5.5	4.6	-26.42	-23.5	1.46	902.21	29.7	914.11	12.8			
6	CY	5 ^a	220.21	umc1963	-1.01	2.56	4.5	3.4	24.092	21.31	1.18	203.01	26.7	216.91	6			
6	CWC	6	240.91	bnl5.04	-1.01	4.23	7.4	-	1.0734	-	1.12	235.71	10.9	239.91	2.1			
7	DEN2	1	369.21	esu3	-2.01	6.2	8	8.9	-1.124	-1.08	1.85	356.01	18.2	359.01	19.9		x	
7	DEN2	2	244.21	mmp167	-0.01	5.48	6.4	6.1	-1.023	-1.11	1.87	239.81	7.4	233.51	12.7			x
7	DEN1	3 ^a	249.91	psr628	-0.01	3.13	3.9	3.7	0.6964	0.625	1.06	245.91	18	249.91	0			x
7	DEN2	4 ^a	428.81	umc1752	0.19	3.09	3.5	4.5	0.7804	0.794	1.05	417.21	17.5	426.81	2.2			x
7	DEN1	5 ^a	354.61	umc1859	-0.01	2.55	3.2	3	-0.597	-0.63	1.02	349.01	29.9	354.61	0			x
7	DEN2	6	367.61	bcd738a	-4.01	3.3	4.5	4	-0.85	-0.84	1.02	351.91	27	366.61	2			x
7	DEN2	7	212.01	umc1393	-0.01	8.94	11	13.4	1.4162	1.464	2.99	208.51	6.5	206.51	18.6			x
7	DEN1	7	217.11	isu086	-1.01	5.66	8.1	8.4	0.9683	0.972	2.25	208.51	15.6	209.51	17.6			x
7	DEN2	8	444.81	umc1768	-4.01	3.32	4.2	3	-0.83	-0.76	1.15	423.91	30	434.51	12.3			x
7	DEN1	9	214.31	umc1910	0.29	3.23	4.3	11.4	-0.759	-1.12	1.15	201.61	22.2	212.71	7.6			(229)
7	DEN2	9	220.31	mmp195	-2.01	6.78	8.1	10.6	-1.16	-1.15	2.38	215.61	13.3	214.61	25			x
8	GY	1 ^a	557.51	umc1925	0.19	2.42	3.9	-	-129.8	-	1.23	536.11	21.6	543.51	14			x
8	GWC	2	140.11	mmp79	-2.01	6.61	12	9.6	-6.378	-5.61	2.15	131.01	18.1	128.01	28.9			x
8	GY	2 ^a	143.11	mmp186	0.99	3.46	6.1	5.7	-160	-153	1.13	128.01	22.1	140.11	5			x
8	GWC	3	500.81	umc1842	-0.01	3.68	5.7	3.6	4.5332	4.088	1.17	486.31	18.8	496.01	4.8			x
8	GY	4 ^a	569.81	mmp94	-0.01	3.02	4.8	3.6	148.28	132.5	1.66	547.01	27.3	566.91	4.9			
8	GWC	5	15.01	npi253a	-1.01	2.88	4.8	4	4.1291	3.892	1.04	4.01	22.3	15.01	0			
8	GWC	6	403.61	mmp181	-0.01	5.54	8.8	9	-5.517	-5.93	1.24	398.81	8.5	400.81	5.5			
8	GY	6	403.61	mmp181	-0.01	6.23	10	10.2	-217.2	-212	2.83	398.81	7.5	393.81	22			
8	GWC	7	418.81	bnlg1450	3.79	2.33	4.7	-	-3.96	-	1.19	391.21	31.4	411.81	8			

QTL were numbered the same if QTL positions from different phenotyping methods or different number of used cofactors were less than 10 cM apart. Positions (pos.) are reported in centimorgans. Additive effects for B73 allele (α) are reported in centimeters for length, in tenth of millimeters for pith, wood, diameter, and TD3, in cubic centimeters for volume in grams for weight, cob yield, CWC, grain yield, and GWC and in grams/100 cm³ for DEN1 and DEN2. Subscript “MIM” indicates R² and α after simultaneous fit using MIM. P_{CWT} gives the standardized LR score (LR/LR_{CWT}), and P_{CWT} interval gives the left marker and interval in centimorgans where positions showed P_{CWT}>1. 1.5 LOD interval gives the left marker and the interval size in centimorgans for the 1.5 LOD drop zone of original LOD scores. “X” in columns AME, ARL, or MAD indicates QTL that were also found in the according analysis. Numbers in parentheses indicate the actual position in that analysis. For example, QTL 5 for length was found for phenotype CL3, which is for the mean over all three locations as well as in the separate environments AME and ARL

^a QTL not detected across locations using EWT

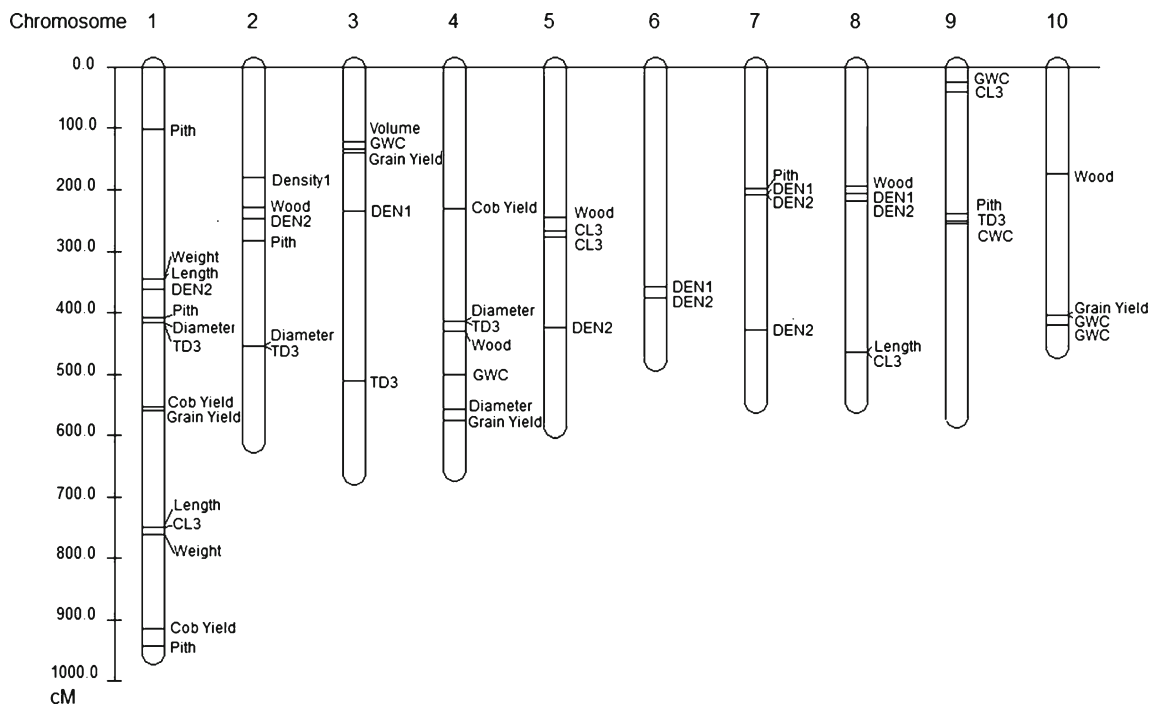


Fig. 3 Chromosome map. Lines mark most likely position of QTL indicated by the scale on the right side (cM). QTL for traits length, CL3, pith, wood, diameter, TD3, volume, DEN1, DEN2, weight, CWC, GWC, cob yield, and grain yield are shown

alleles increased pith diameter. Five QTL on chromosomes 2, 4, 5, 8, and 10 significantly ($p=0.001$) affected the thickness of the wooden part of the cob. 31.9 % (R^2_{MIM}) of the variation was accounted for by those five QTL. B73 alleles decreased the wooden part diameter on chromosomes 2 and 8.

Seven QTL for cob diameter (diameter and TD3) were found. QTLs 1, 2, and 5 were found for both diameter and TD3 on chromosomes 1, 2, and 4, respectively. For TD3, three additional QTLs were found, one close to QTL 5, the two other QTLs on chromosomes 3 and 9. For diameter, one additional QTL was located on chromosome 4, about 140 cM apart from another QTL on chromosome 4. However, not all QTLs were confirmed with MIM (Table 3). Sum of R^2_{MIM} was 20.0 % (31.3 %) for three diameter QTL (five TD3 QTL) after MIM. For all QTL, B73 additive allele effects were increasing cob diameter. For cob volume, one QTL was found ($p=0.001$) on chromosome 3 explaining 8.5 % (R^2_{MIM}) of the total variance. The B73 allele decreased cob volume by 2.9 cm³ (Table 3).

A total of six QTLs were found for cob weight, located on chromosomes 1 (weight, CWC, CY), 4 (CY), and 9 (CWC). For CWC (QTL 3 and 6) and CY (QTL 5), the B73 alleles showed a positive effect increasing weight, for all others the effect was negative. Two QTLs were not confirmed after MIM. The remaining QTL for CWC accounted for 4.3 % of the variation. Together, the QTL for cob yield accounted for 13.3 % (R^2_{MIM}) of the variation,

and the two QTL for cob weight per cob accounted for 5.0 % (R^2_{MIM}).

Two phenotypes were measured for cob density. Four QTLs were detected for DEN1 and seven QTLs for DEN2. On chromosomes 7 and 8, QTL were collocated for both traits. On chromosome 6, DEN1 and DEN2 QTL mapped 13 cM apart. Additional QTL were found for DEN1 on chromosomes 3 and for DEN2 on chromosomes 1, 2, 5, and 7. All DEN1 (DEN2) QTL taken together accounted for $R^2_{MIM}=26.5$ % (50.5 %) of the phenotypic variation.

For two grain weight traits, seven QTL were found on chromosomes 1, 3, 4, 9, and 10. On chromosomes 3 and 10, GWC and grain yield mapped to the same region (QTL 2 and 6). Additional QTL for grain yield were identified on chromosomes 1 and 4 and for GWC on chromosomes 3, 9, and 10. Three QTLs were not confirmed after MIM. For grain weight per cob, all five QTL together explained 26.2 % of the total variance (R^2_{MIM}). The four grain yield QTL explained 19.5 % of the variance (R^2_{MIM}).

In 15 regions, 1.5 LOD confidence intervals of multiple QTL overlapped, whereof five regions showed QTL from related traits, including the pairs of CL3 and cob length on chromosome 9 (39.8–46.2), GWC and grain yield on chromosome 10 (403.6–418.8), DEN1 and DEN2 on chromosome 6 (354.6–367.6), and TD3 and cob diameter on chromosome 4 (397.8–417.4). In addition, TD3 and cob

diameter mapped closely together with pith diameter on chromosome 1 (404.9–405.9). In a distance of 12.7 cM, wood diameter QTL 4 mapped near DEN1 on chromosome 8. Wood diameter QTL 9 mapped near DEN2 on chromosome 8 and 2 with 18.7 and 7.7 cM distance, respectively. Pith diameter QTL 5 mapped near density QTL 7 on chromosome 7 in a distance of 0.5 cM (DEN2) to 6.6 cM (DEN1). On chromosome 3, volume, CWC, and grain yield QTL mapped within 12.1 cM at positions 131.0, 140.1, and 143.1, respectively. On chromosome 9, pith, TD3, and CWC QTL overlapped (216.8–240.9). On chromosome 1, cob yield, cob weight, and grain yield QTL mapped within 8.8 cM. Also on chromosome 1, length, weight, and DEN2 QTL mapped within 12.2 cM (357–3.69.2), and at position 742.2 to 764.8, 1.5 LOD intervals of length, CL3 and CWC QTL overlapped. In all cases of colocated QTL, the B73 allele effects had the same direction for all colocated QTL, with the exception of the colocated pith and cob density QTL on chromosome 7, where the B73 allele for the pith QTL showed a negative effect and a positive effect for cob density.

Discussion

Heritabilities and QTL

The complexity of a trait is determined by the number of genes, their additive effects (G), their interaction (G × G, epistasis), their interaction with environmental factors (G × E), and the effects of environmental factors themselves (E). Under the assumptions of dominance and G × G effects being absent, narrow sense heritability and explained heritability by QTL can be estimated from genotypic and phenotypic variances and total R^2 of the modeled QTL effects. Heritabilities in this study were moderate to high (> 0.64) for all traits. The number of QTL detected ranged from one (cob volume) to seven for DEN2. However, the number of mapped QTL per trait is low in this study compared to other studies with equal population size. The average four QTL identified per trait in this study were substantially fewer than the 12, 14, and 22 QTL found for three different root complexity traits in the same population using 231 IBM RILs [30]. A different threshold was used in this analysis, and the choice of cofactors not reported plays a significant role in composite interval mapping. Fitting 20 cofactors in our study resulted in total 139 QTLs. Thus, differences between studies can result from different threshold levels as well as number of cofactors and must not represent actual different numbers QTL underlying a trait. More conservative parameters will likely result in lower number of detected QTL but control false positives.

In most QTL analyses, only a fraction of genes affecting a quantitative trait are detected as QTL [31, 32]. Two main reasons for this so-called missing heritability have been proposed: (1) overestimation of heritability due to incorrectly assuming absence of epistasis [32] and (2) a large number of hard to detect minor QTL [33]. Explained heritability as calculated from total $R^2/h^2 \times 100$ ranged from 9.6 % (volume) to 59.7 % (DEN2) leaving a missing heritability of 40–90 %.

Entry mean heritabilities were estimated assuming no genetic interaction. Overestimation of h^2 due to underestimation of epistasis directly affects explainable h^2 negatively in proportion to actually existing epistasis, following the general model mentioned above. Therefore, ignoring epistasis will affect estimation of explained heritability [32]. However, exploring epistasis will exponentially increase complexity of the analysis to the number of involved genes or QTL and was therefore not included in this analysis.

Moreover, population size affects power for QTL detection and even in large populations the majority of QTL with small effects will not be detected. Small population sizes have been shown to negatively affect power for QTL mapping in simulation [34] and field studies [35] also causing overestimation of QTL effects [36]. In addition, for traits that were subject to selection like grain yield or highly correlated with such traits under selection such as cob volume, cob weight, and cob yield, low numbers of QTL are expected because QTL with large effects are likely fixed [37]. In our study, where the QTL with the smallest R^2_{MIM} still explained 3 % of the phenotypic variation (DEN1), it is very likely that many other QTLs with smaller effects exist, but the statistical power of detecting those was not sufficient. The number of QTL for grain yield and correlated traits that were subject to selection was also low in other studies. Only three grain yield QTLs were found in a study of Mo17-topcross hybrids of 150 BC₁S₁ lines produced from Illinois High Oil and recurrent parent B73 [38]. Even at larger population size (302), only three QTLs were found for kernel weight analyzing the same IBM RIL population in four environments [39]. However, in the same study, >20 QTLs each were identified for oil and protein concentration, showing possible trait differences for number of QTL using the same method and experiment [39]. Absence of major QTL in our study, a small number of QTL per trait and high heritabilities support that the majority of QTL affecting those traits have minor effects, hard to detect even by very large population sizes [35–40].

QTL Consistency, Correlation, and Path Analysis Congruency

Consistency of QTL across studies can be expected if major genes are involved, and genetic complexity of traits is low

(oligogenic inheritance). Compared to two earlier $F_{2:3}$ studies in IBM [7] or involving parent Mo17 [12], only few QTLs (3 of 22) were consistent with our study. No QTLs were consistent for cob yield and cob diameter with a study employing 150 Illinois Low Protein \times B73 BC₁S₁ lines [11]. Similarly, no QTL for grain yield and cob length were in common with another QTL study based on 210 $F_{2:3}$ lines [14]. Taken together, these findings are in agreement with a complex inheritance of cob traits.

QTL colocating with known mutants indicate involvement of respective candidate genes in trait expression. Several mutants of genes involved in ear and cob formation have been found and described [41]. Examples include *ba2* (barren stalk 1), *ra1* (ramosa 1), or *ph1* (pith abscission 1) with cob disarticulation as one of the loci differentiating maize and teosinte [41]. Three genes are located nearby QTL locations of this study. Locus *td1* with the thick tassel dwarf phenotype with increased spikelet density in tassels maps within 1–2 IBM cM [between marker *umc1* (246.4 cM) and *umc1355* (246.6 cM)] within the CWT interval of wood diameter QTL 4. Even though this mutation affects primarily tassel spikelet density, it might affect densification in maize flower tissue and, consequently, cob tissue density. On chromosome 7, distal to pith QTL 5 and nearby density QTL 7 maps the *ra1* locus with effects on ear and tassel branching. The control of such second order meristems was proposed to be under selection for domestication where larger first order meristems were favored resulting in the modern cob architecture [42, 43]. Close to marker *umc259b*, *ba2* maps near wood QTL 1 at 236.5 IBM cM and DEN2 QTL 2 at 244.2 IBM cM. Mutants of *ba2*, encoding for a transcription factor, show severe ear phenotypes with no or poor ear development. Weaker expression of such severe phenotypes could affect cob architecture, tissue structure, branching pattern, and therefore cob density [44]. Not all colocated candidate genes will be causative for the according QTL. However, all those genes are involved in ear development especially with respect to branching and can, therefore, be considered as candidate genes with possible impact on agronomically important cob traits.

The proposed models for path analysis are based on the general architecture of cobs and correlations or covariance structure among traits. Due to trait hierarchy, colocalized QTL for component traits pith and wood diameter with QTL for cob diameter are most likely due to pleiotropy. B73 alleles increase both wood and cob diameter (chromosome 4) and pith and cob diameter (chromosomes 1 and 9). On chromosome 7 (206.01 cM), B73 alleles increased pith and decreased density at neighboring loci (212.01 cM). In addition, on chromosomes 2 and 8, B73 alleles decreased wood diameter and density for neighboring loci. These findings are in agreement with trait correlations and in agreement with expectations for pleiotropy of QTL affecting traits at different hierarchy levels.

Cob yield, cob weight, and CWC were positively correlated with all traits of lower order in the model including pith diameter. In the next lower order class, volume and density of single cobs directly explained single cob weight. It is important to note that density is only partly explained by pith and wood diameter and further depends on the actual density of all involved tissues including pith, wood, and chaff, which are not available. For pith, the (supposedly negative) indirect effect through density as opposed to the indirect effect through volume was minor because the total of indirect effects of pith on weight was positive. While the direct effects of wood and pith diameter on cob weight were not significant, the total effects as the sums of indirect and direct effects were positive and significant. Wood diameter has positive effects on cob diameter and weight. A reduced pith fraction will increase cob density by an increased wood fraction, as pith and wood diameter are negatively correlated. However, the ratio of pith and wood is independent from cob volume and thus offers optimization potential for higher cob biomass at a given volume.

Ideotype and Implementation

To satisfy future biomass demand for lignocellulosic energy production, larger cobs with denser tissue yielding more biomass being easier to transport and store would be desirable [2]. Porous cob tissue with large volume might only be favored for few applications such as animal bedding or absorption materials. Cob biomass could be maximized by increasing cob volume or cob density or a combination of both.

Considering grain yield and cob yield as economically most important traits and with cob yield as the trait easiest to score and closely correlated with grain yield ($r_g=0.77$) a selection index for grain yield based on grain and cob prices could be formulated. Based on prices of \$50–100 per ton for cobs [45, 46] and \$150–200 per ton grain [47], two contrasting indices could be created such as $index_{0.25}=4 \times \text{grain yield} + \text{cob yield}$ (grain four times as worth as cobs) or $index_{0.5}=2 \times \text{grain yield} + \text{cob yield}$ (grain worth twice as much as cob). While the 10 % (19 lines), 5 % (10 lines), and 1 % (4 lines) selected based on $index_{0.25}$ coincide with those selected only for grain yield, the $index_{0.5}$ would change the set of lines for the top 10 % (18/19 lines overlap) and 1 % (3/4 lines overlap) in favor for lines that rank high in cob yield.

Physiologically, a close correlation between cob and grain yield is likely due to the close correlation between grain yield and cob volume. Most studies are consistent with regard to positive correlations between ear length and grain yield [6, 7]. However, breeding for longer ears showed no positive impact on grain yield in a long term project by

Hallauer et al. [48]. In addition, extreme large cob types such as pipe corn or choclero types only show large cobs when planted at very low planting densities and do not perform accordingly for grain yield per area (Mike Blanco, personal communication, PIS Ames). Tissue density uncorrelated with grain yield and all other traits, but cob weight shows great potential for additional cob biomass increase and could also play a key role when transportation and storage costs of biomass are essential at high fuel cost. To capture the beneficial properties of higher density, positive correlation with cob yield and lack of negative effects due to its independence from grain yield is favorable. This enables for selection of denser cobs when selecting genotypes for dual purpose based on grain and cob yields. This hypothesis is supported by the genetic makeup of selected lines in this population. The five best lines for grain yield in this population are M0059, M0201, M0165, M0131, and M0017, sorted by grain yield performance over all environments ranking 5th, 52nd, 12th, 38th, and 1st for cob yield, respectively. M0017, best for cob yield, also ranked first for both density traits and carried all favorable alleles for cob yield, CWC, weight, DEN1, and DEN2 QTL detected, but unfavorable alleles for six of the eight grain yield, GWC, and volume-related QTL. M0059 showed all nine favorable grain yield, GWC, and volume alleles, but only eight of 12 genotyped QTLs for cob yield, CWC, weight, DEN1, and DEN2 QTL were unfavorable (two missing scores). Both

lines ranked top in their types showing that volume-cob types as well as density-volume-cob types can yield high grain and cob yield. However, all favorable alleles could be combined to develop superior genotypes.

However, when combining favorable QTL, not all QTL associated with diameter are equally useful to create more and denser biomass. To further investigate the potential for replacing pith by wood ratios for higher density at given volume, wood and chaff were sorted by fraction of pith for all lines (Fig. 4). Chaff fraction (in percent of total diameter) remains relatively constant in this population ($CV=0.08$), whereas the ratio of pith and wood account for the majority of differences in density between all lines of this population (Fig. 4). To increase density, the ratio of wood to pith diameter should be increased.

The simplest approach would be phenotypic selection improving traits that are easy to score such as cob weight and grain yield. A more compound but promising breeding strategy for complex inherited cob traits and grain yield appears to be genomic selection [9, 49, 50]. However, if pleiotropy between pith, wood and cob diameter, as well as density traits can be verified, selection of certain QTL that favor density and volume by a higher wood fraction will benefit from knowledge about gene function and the physiological basis of the underlying QTL. Here, known mutant genotypes could serve as a starting point for further investigations.

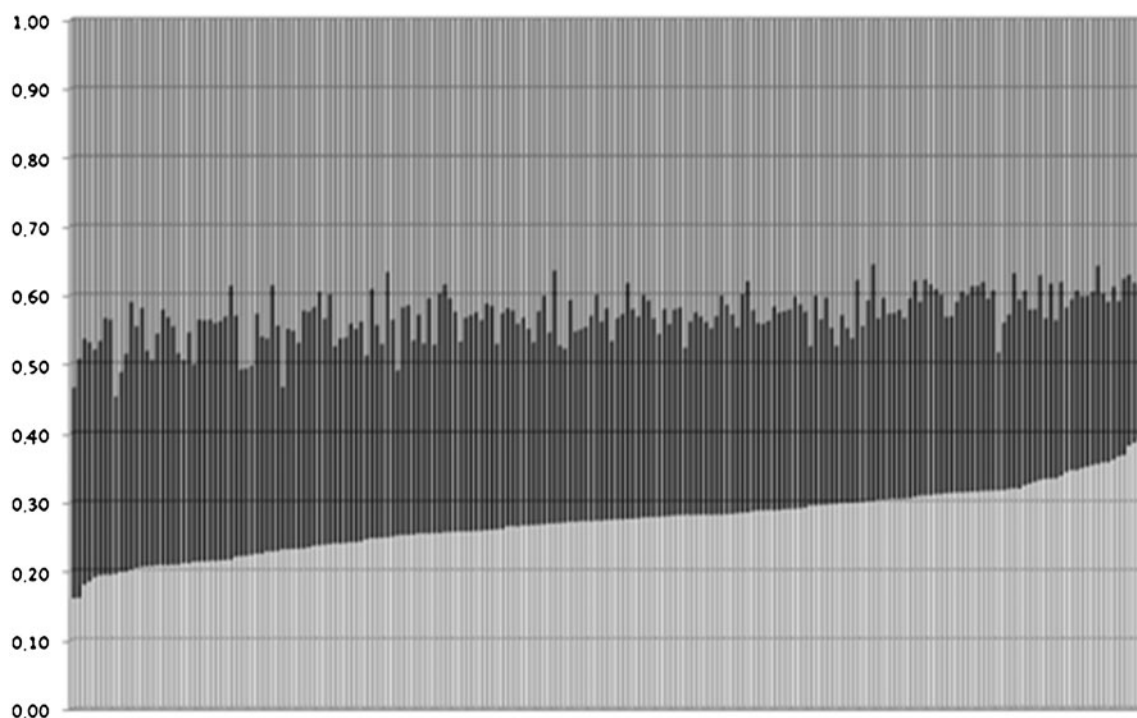


Fig. 4 Relationship of pith, wooden part and chaff fraction. Average fractions of pith (*gray on bottom*), wooden part (*black, middle*), and chaff (*gray on top*) are shown for 184 lines sorted ascending by pith fraction

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