Theor Appl Genet (2010) 121:1465–1482
DOI 10.1007/s00122-010-1402-x

ORIGINAL PAPER

Meta-analysis of QTL involved in silage quality of maize and comparison with the position of candidate genes

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Received: 24 December 2009 / Accepted: 5 July 2010 / Published online: 25 July 2010

Abstract A meta-analysis of quantitative trait loci (QTL) associated with plant digestibility and cell wall composition in maize was carried out using results from 11 different mapping experiments. Statistical methods implemented in "MetaQTL" software were used to build a consensus map, project QTL positions and perform meta-analysis. Fifty-nine QTL for traits associated with digestibility and 150 QTL for traits associated with cell wall composition were included in the analysis. We identified 26 and 42 metaQTL for digestibility and cell wall composition traits, respectively. Fifteen metaQTL with confidence interval (CI) smaller than 10 cM were identified. As expected from trait correlations, 42% of metaQTL for digestibility displayed overlapping CIs with metaQTL for cell wall composition traits. Coincidences were particularly strong on chromosomes 1 and 3. In a second step, 356 genes selected from the MAIZEWALL database as candidates for the cell wall biosynthesis pathway were positioned on our consensus map. Colocalizations between candidate genes and meta-QTL positions appeared globally significant based on χ^2 tests. This study contributed in identifying key chromosomal regions involved in silage quality and potentially associated genes for most of these regions. These genes deserve further investigation, in particular through association mapping.

Communicated by T. Luebberstedt.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-010-1402-x) contains supplementary material, which is available to authorized users.

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Introduction

Grasses (*Poaceae*) are the major source of nutrients for wild and domesticated herbivores. Among grasses, maize (*Zea mays* L.) is the most important annual forage crop in Europe. 3.5 million hectares of maize are ensiled every year, mainly in Northern Europe (Thomas et al. 2010). Until the 1990s, maize varieties used for forage production had been selected for grain production. Breeding efforts and germplasm development have resulted in remarkable genetic improvements in yield, lodging resistance, and biotic and abiotic stress tolerance during the past 25 years. However, a significant drift towards lower cell wall digestibility values has been observed in hybrids registered between 1975 and 2000 (Barrière et al. 2004). It, therefore, appeared increasingly important to take into account silage quality traits and select for silage maize varieties more suitable for cattle feeding. Thus, forage quality was introduced as a criterion for variety registration in several countries [in 1998 in France, in 1986 in the Netherlands (Argillier and Barrière 1996)].

Digestibility is defined as the percentage of silage that is absorbed in the animal digestive tract (Barrière et al. 2003). The whole plant can be divided into cell content and cell wall. Cell content (mainly starch) is highly digestible. On the contrary, cell wall is hardly digested by cattle rumen and is, therefore, the main limiting factor of maize feeding value. It is consequently the main target for silage maize

energy value improvement (Barrière et al. 2003). The digestibility of cell wall is determined by its composition and by the organization and cross-linkages of wall constituents. The grass cell wall is composed of cellulose (nearly 45%), hemicelluloses (mostly arabinoxylan, nearly 45%) and phenolics (nearly 10%). Phenolics include lignins and *p*-hydroxycinnamic acids. Lignins, which are resistant to fungal and bacterial degradation in animal rumen, are non-digestible. They form a complex polymer of guaiacyl (G) and syringyl (S) units together with minor amounts of *p*-hydroxyphenyl (H) units. When oxidized, these units yield *p*-hydroxybenzaldehyde, vanillin (Va) and syringaldehyde (Sg). Participation of *p*-hydroxycinnamates in cell wall composition and organization of the lignified tissues is the most specific feature of grass lignification. Among them, *p*-coumaric acid (pCA) is mainly esterified to the phenylpropane side chain of S lignin units. Ferulic acids (FA) are primarily esterified to arabinoxylans (esterified ferulic acid, ESFA). Lignins and arabinoxylans are secondarily bridged through FA ether-linkages to G units (etherified ferulic acid, ETFA). Over 50% of wall ferulates can undergo dehydrodimerization, and arabinoxylans are thus extensively cross-linked by ferulate dimerization [5-5-diFA (DFA) and 8-O-4-diFA (ODFA) dimmers] in mature cell wall (Grabber et al. 2004). The proportion of the different lignin units affects the physical organization of the cell wall and consequently cell wall digestibility. However, in vivo measurements of digestibility on animals are too complex to perform and also too costly to be used routinely in breeding programs. Digestibility is, therefore, mainly measured in vitro either directly, using enzymes that degrade organic matter, or more often indirectly, using prediction equations based on near infra red reflectance spectroscopy (NIRS). Knowing this, cell wall composition can be estimated using chemical analyses according to the "three steps method" proposed by Goering and Van Soest (1970). After cell soluble content elimination, the residual, called neutral detergent fibre (NDF) corresponds to the cell wall. After acid detergent treatment, hemicelluloses are solubilized leaving the residual, called acid detergent fibre (ADF) that mainly contains cellulose and lignins. The hemicelluloses content can thus be estimated by the difference between NDF and ADF content. After another acid treatment, cellulose and the acido-soluble part of the lignins are solubilized, yielding acid detergent lignin (ADL). The cellulose content is thus estimated as the difference between ADF and ADL. Alternatively the whole lignin content in the NDF can be estimated directly after one acid treatment as Klason lignin (KL) (Dence and Lin 1992). The degree of lignification is often expressed as the percentage of lignin (ADL or KL) in the cell wall (NDF) (Méchin et al. 2001; Roussel et al. 2002). NIRS calibrations have been developed to estimate all these cell wall components and avoid chemical analyses.

All the above described traits related to cell wall composition have shown significant correlations with whole plant and cell wall digestibility. Several specific measures of digestibility have been proposed on this basis. Measures of in vitro digestibility of organic matter (IVDOM) in maize were used by Lübberstedt et al. (1997a, b) and Papst et al. (2001). In vitro NDF digestibility (IVNDFD) is an estimate of cell wall digestibility assuming that the non-NDF part of plant material is completely digestible (Méchin et al. 2000). In vitro digestibility of the "non-starch, non-soluble carbohydrates and non-crude protein" (DINAG) is estimated by assuming that starch and soluble carbohydrates are completely digestible (Argillier et al. 1996; Barrière et al. 2001). A modified DINAG criterion named DINAGZ (Barrière et al. 2003) was defined in the same way as DINAG, but here crude proteins were added as they were also supposed to be completely digestible. Barrière et al. (2003) showed that more than 50% of the in vitro cell wall digestibility variation among commercial hybrids was explained by lignin content, which appeared to be the most important cell wall component related to digestibility. However, in another study, Argillier et al. (2000) showed that among normal breeding lines of diverse origins a large part (approximately 75%) of digestibility (measured using DINAGZ) variation remained unexplained by lignin content. This unexplained part of digestibility was attributed to the variation in lignin structure and effects of other cell wall components. For this reason, it is necessary to study both digestibility traits and cell wall composition traits.

These traits display a large genetic variation due to both major mutations and quantitative trait loci (QTL) of small effects generating a continuous variation amenable to QTL mapping experiments. QTL for cell wall composition traits have been mapped in many studies (Barrière et al. 2001; Lübberstedt et al. 1997a, b; Roussel et al. 2002; Krakowsky et al. 2003, 2005, 2006; Méchin et al. 2001; Riboulet et al. 2008). Several studies have been carried out to detect QTL for digestibility traits (Barrière et al. 2007; Bohn et al. 2000; Fontaine et al. 2003; Lübberstedt et al. 1997a; Méchin et al. 2001; Papst et al. 2001; Riboulet et al. 2008; Roussel et al. 2002). In total, QTL related to digestibility and cell wall composition were found all over the genome (Barrière et al. 2008). In order to identify key regions that have a role in lignin biosynthesis and cell wall digestibility, Barrière et al. (2008) performed a first synthesis of QTL detected for ADL/NDF-related traits detected in silage maize. By projecting QTL on a reference map, the IBM2 Neighbours map (Sen et al. 2009), using the software Biomercator (Arcade et al. 2004), they compared the QTL confidence intervals (CIs) and concluded to 43 different

loci based on 58 individually observed QTL. Hot-spots of three or more QTL were found on bins 2.08, 5.03, 6.04 and 9.06.

Regarding major mutations, naturally occurring brown-midrib maize mutants (*bm1–bm4*) display strong effects on lignin content and/or cell wall digestibility (Barrière et al. 2004). These have permitted insights in genes involved in these mutations and/or the expression of which is controlled by mutations. Brown midrib 3 (*bm3*) mutant shows alterations in the expression of the caffeic acid *O*-methyltransferase gene (COMT) involved in the biosynthesis of S units in lignin. This results in less lignin, a decrease in S units and improved digestibility. *Bm3* has been mapped to bin 4.05 (Emerson 1935), but recent advances in maize sequencing have shown that the *COMT* gene is located in bin 4.04. The *bm1* mutant is affected by the activity of the *cinnamyl alcohol dehydrogenase* gene (*ZmCAD2*), involved in the conversion of cinnamic aldehydes into monolignols (Halpin et al. 1998). This mutation leads to a decrease in lignin content although less important than in the case of the *bm3* mutant. *ZmCAD2* is located closely to the *bm1* locus in bin 5.04 (Guillaumie et al. 2007a; Provan et al. 1997). Although no information is yet available on the genes responsible for the *bm2* and *bm4* mutations, these mutants also show a decrease in lignin content. They have been mapped to bins 1.11 and 9.07, respectively.

Beyond *bm* mutations, genes involved in monolignols or *p*-hydroxycinnamates biosynthesis have been identified in maize, even if the pathway towards the synthesis of ferulic acid (FA) remains partially hypothetic (Barrière et al. 2009). Genes involved in cell wall biosynthesis have also been extensively studied in woody plants (poplar, eucalyptus and pine) (Du and Groover 2010) or in the model plants *Arabidopsis thaliana* and *Zinnia* (Jung and Park 2007). These genes have been used as references to search for homologies with maize gene sequences or EST contigs originating from different tissues. These maize sequences, corresponding to genes potentially involved in the biosynthesis of the cell wall, have been organized in a database "Maizewall" (http://www.polebio.scsv.ups-tlse.fr/MAIZEWALL/) by Guillaumie et al. (2007b). A maize macro-array containing specifically cell wall biosynthesis candidate gene families (154 in total) was designed based on a list of nearly 650 genes. Transcriptional levels of cell wall genes were evaluated in roots, leaves and young stems of plants at the 4–5-leaf stage and on the adult plant at silking stage (Guillaumie et al. 2007a, b, 2008). These expression levels were made available online via the "Maizewall" database in order to facilitate the identification of candidate genes. Independently, Shi et al. (2007) developed a "forage quality array" which contained 439 candidate ESTs for cell wall digestibility that were shown to be strongly differentially expressed in (1) three sets of brown-midrib isogenic lines (Shi et al. 2006) and (2) inbred lines from different populations and isogenic lines displaying extreme values for digestibility. Using this array, Shi et al. (2007) detected eQTL in a flint × flint mapping population of 40 inbred lines showing contrasting phenotypes for digestibility. These eQTL were located on chromosomal bins 1.07, 1.12, 3.05, 8.03 and 9.04.

It is difficult from the results of individual QTL experiments that have been carried out in different populations, using different maps, to have a clear synthetic picture of the regions involved in the variation of traits related to silage quality. Meta-analysis of QTL results has been proposed as a way to synthesize information, infer the number of consensus chromosome regions (called meta-QTL) involved in trait variation and estimate the position of these metaQTL with an increased accuracy compared to position estimates of individual QTL (Goffinet and Gerber 2000). This type of approach enables one to determine the minimal number of actual QTL that can explain the QTL results observed in different studies and highlights chromosome regions that are the most likely to carry the causal polymorphisms underlying trait variation.

Two software packages presently allow meta-analysis of QTL experiments. Biomercator, which is based on the method proposed by Goffinet and Gerber (2000) was first used by Chardon et al. (2004) to study QTL related to flowering time in maize. This software was also used to study earliness traits (Hanocq et al. 2007) and ear emergence (Griffiths et al. 2009) in wheat, drought-tolerance in rice (Khowaja et al. 2009) and yield in rapeseed (Shi et al. 2009). Veyrieras et al. (2007) developed new algorithms for the approach of meta-analysis that have been implemented in "MetaQTL" software. This software uses a weighted least squares strategy to build the consensus map from the maps of individual studies, and provides criteria to test the consistency between individual maps. After QTL projection on the consensus map, a meta-analysis is carried out to identify the number of underlying metaQTL which best explain the observed distribution of QTL positions in the mapping experiments. This meta-analysis approach is based on a Gaussian mixture model and performs a quantitative clustering of the individual QTL to the different metaQTL. MetaQTL provides statistical criteria to determine the most likely underlying number of metaQTL on each chromosome. This software has been recently used on wheat for Fusarium head blight resistance (Löffler et al. 2009) and on apricot for Plum pox virus resistance (Marandel et al. 2009).

The studies published to date in maize show that many genes are likely to be involved in cell wall biosynthesis, and many QTL affect the variation of silage quality and cell wall-related traits. There is, therefore, a clear need to synthesize all this information. The objectives of this study

were (1) to identify, using MetaQTL analysis, chromosomal regions where metaQTL involved in traits related to whole plant and cell wall digestibility are located; (2) to project the position of potential candidate genes for digestibility and cell wall components-related traits on our consensus genetic map in order to test whether their positions would be correlated with metaQTL regions; and (3) to define a set of promising candidate genes for further investigation.

Materials and methods

Investigated traits and synthesis of QTL results

To date, QTL analysis of maize plant digestibility and related traits has been reported in 15 publications (see Table 1; Supplementary Table 1). Although Lübberstedt et al. (1997b) characterized four testcrossed populations for IVDOM, we unfortunately could not consider the QTL detected in this study in our analysis. The genetic map as well as the QTL positions (based on the closest RFLP marker) was indeed only partially available in this study. We therefore considered 14 experiments addressing a total of 26 traits.

These 26 traits (Table 2) were divided into two groups for the meta-analysis: (1) traits corresponding to the different in vitro estimates of digestibility further referred to as "digestibility" group and (2) traits corresponding to the composition of the cell wall, further referred to as "cell wall". Traits related to different cell wall components were jointly analysed since they are often correlated to each other (Barrière et al. 2008).

Synthesis of QTL results

Table 1 provides a summary of the genetic material that was used for each of the 14 publications mentioned above. The 14 publications correspond to 11 independent populations. QTL analyses were mostly conducted on testcross values, except for Méchin et al. (2001) and Roussel et al. (2002) for which they were conducted on both per se and testcross values. QTL detection conducted on a same population will be further referred to as an "experiment". Three experiments over 11 studied the two groups of traits simultaneously (Table 1). Within each group of traits ("digestibility" or "cell wall"), QTL detected for different traits within a given experiment were sometimes positioned at similar locations with overlapping confidence intervals. The method implemented in MetaQTL to search for metaQTL assumes that all the individual QTL within each group of traits are detected independently from each other (so in independent populations). Therefore, when QTL detected for different traits in a given experiment had overlapping CIs, only the QTL with the highest contribution to phenotypic variation (R^2), and the best precision was kept for the meta-analysis.

Table 1 Studies involving digestibility and/or cell wall component traits on maize used for the meta-analysis

No.	QTL studies	Parents	Type of population	Population size	Nb of QTL	Nb of markers	Map reference	Trait groups
1	Barrière et al. (2001)	F288 × F271	RIL	135	0	108	Barriere_2001	–
2	Barrière et al. (2007)	F286 × F838	RIL	242	34	101	Barriere_2007	CW, DIG
3	Bohn et al. (2000)	DO6 × D408	F2	226	6	95	Bohn_2000	DIG
4	Cardinal et al. (2003)	B73 × B52	RIL	200	26	152	Cardinal_2003	CW
5	Fontaine et al. (2003)	F288 × F271	RIL	135	10	108	Barriere_2001	CW, DIG
6	Krakowsky et al. (2003)	B73 × DE811	F3	150	17	88	Krakowsky_2003	CW
7	Krakowsky et al. (2005)	B73 × DE811	RIL	200	13	226	Krakowsky_2005	CW
8	Krakowsky et al. (2006)	B73 × DE811	RIL	200	24	226	Krakowsky_2005	CW
9	Lübberstedt et al. (1997a, b)	KW1265 × D146	F2	380	11	89	Lubberstedt_1997	DIG
10	Méchin et al. (2001)	F2 × MBS847	F5	100	13	152	Mechin_2001	CW, DIG
11	Papst et al. (2001)	1396A × F478	F2	230	3	78	Papst_2001	DIG
12	Riboulet_unpublished	F7025 × F4	RIL	231	9	94	Riboulet_unpub	CW
13	Riboulet et al. (2008)	Fl1 × F2	RIL	140	6	189	Riboulet_2007	DIG
14	Roussel et al. (2002)	F288 × F271	RIL	135	37	108	Barriere_2001	CW, DIG

For each study, the parental lines of the population, the type (recombinant inbred line, RIL or cross Fx) and size of population, the number of detected QTL and the number of markers genotyped are listed. The map reference is also mentioned as some experiments used the same genetic map. Trait group studied in each experiment: CW for cell wall traits, DIG for digestibility traits. "–" provides the map of the populations used in QTL studies 5 and 14

Table 2 List of traits considered for the meta-analysis. The traits are divided into two groups (digestibility and cell wall)

Trait_group	Trait	Definition	Articles
Digestibility	DINAGZ	In vitro digestibility of non-starch and non-soluble carbohydrate parts	5, 10, 13, 14
Digestibility	IVDMD	In vitro dry matter digestibility	5, 10, 14
Digestibility	IVDOM	In vitro digestible organic matter	3, 9, 11
Digestibility	IVNDFD	In vitro NDF digestibility	2, 5, 10, 14
Cell wall	ADL	Acid detergent lignin over NDF	2, 5, 10, 12, 13, 14
Cell wall	agNDF	Sheath NDF adjusted for ADF	7, 8
Cell wall	atADF	Stalk ADF adjusted for ADL	7, 8
Cell wall	atNDF	Stalk NDF adjusted for ADF	7, 8
Cell wall	Cell	Cellulose over NDF	5, 14
Cell wall	DFA	5-5-diferulic acid content	2
Cell wall	EFA	Ester FA	2
Cell wall	ESFA	Esterified ferulic acid content	2
Cell wall	ETFA	Etherified ferulic acid content	2
Cell wall	gADF	Sheath acid detergent fibre	4, 6, 7, 8
Cell wall	gADL	Sheath acid detergent lignin	4, 7, 8
Cell wall	gNDF	Sheath neutral detergent fibre	4, 6, 7, 8
Cell wall	Hcell	Hemicellulose over NDF	5, 14
Cell wall	KL	Klason lignin content over NDF	5, 14
Cell wall	NDF	Neutral detergent fibre	5, 10, 14
Cell wall	ODFA	*β*-*O*-4-diferulic acid content	2
Cell wall	PCA	Ester pCA	2, 5, 14
Cell wall	SG	Syringaldehyde content	2
Cell wall	tADF	Stalk acid detergent fibre	4, 6, 7, 8
Cell wall	tADL	Stalk acid detergent lignin	4, 6, 7, 8
Cell wall	tNDF	Stalk neutral detergent fibre	4, 6, 7, 8
Cell wall	VA	Vanillin content	2

For each trait, we indicated the papers where QTL for this trait have been detected (see Table 1 for the correspondence between the number attributed to the article and its complete reference)

Consensus map

Markers used in the different experiments were microsatellites and RFLP. Before being able to build the consensus map, we had to determine the correspondences between the names given to the marker loci in the different individual maps. RFLP probes often revealed different loci in a given experiment, usually labelled "a", "b", etc. by the authors. For instance, the RFLP markers umc32b in Lübberstedt et al. (1997a) and umc32g in Méchin et al. (2001) had similar mapping positions on their respective genetic maps. We assumed that they correspond to the same locus. A preliminary analysis (using the "InfoMap" command of the MetaQTL software) was carried out in order to list markers whose orders were not consistent in the different individual maps. Six markers with inconsistent position were removed from the analysis.

The command "ConsMap" was then used to create a consensus marker map. This is based on a weighted least squares method that takes into account the individual distances between markers in each individual map, rescaled if needed by the software in Haldane unit, and their accuracy determined from the population size and type. The method assesses the goodness-of-fit parameter λ of the consensus model. This parameter is calculated per chromosome as:

$$\lambda = \sum_{i=1}^{n} \sum_{jk} \frac{\left[d_{i,jk} - (\gamma_k - \gamma_j)\right]^2}{\gamma_{i,jk}^2}$$

where n is the number of mapping experiments (11 here), $d_{i,jk}$ is the estimate in the ith map of the distance between the adjacent markers j and k in cM, $\gamma_{i,jk}^2$ is the corresponding expected variance of the distance estimate based on the population type and its size following Veyrieras et al. (2007), and γ_k and γ_j are the positions (in cM) of the markers j and k on the consensus map, respectively.

This parameter evaluates the consistency between the marker distances in the consensus map and in the different individual mapping populations. A high value of λ corresponds to a low level of consistency between the marker distances in the different individual maps. Under the null

hypothesis (same actual maps for all experiments), the goodness-of-fit value of the consensus map for each linkage group is affected only by sampling errors in individual maps and follows a χ^2 distribution $\lambda \sim \chi^2_{q-M+1,}$ where q is the total number of marker intervals over the 11 individual maps, and M the total number of markers for the chromosome (Veyrieras et al. 2007). This makes it possible to build a statistical test, using a usual 5% type I risk level.

We then compared the marker order on our consensus-map with the marker order on the IBM2 FPC0507 neighbour map (Sen et al. 2009). This map is used as a reference map within the maize community and was used to anchor the physical maize map.

QTL projection and overview

The projection of the 209 QTL positions and their CIs was based on a scaling rule between the positions of the flanking markers of QTL on their original map and the positions of these markers on the consensus map. The variance (σ^2_q) associated with the position of a given QTL was determined from the 95% CI of QTL position, considering $CI = 2 \times 1.96 \times \sigma_q$. For the CI, we used the option in MetaQTL software that considers the maximum between (1) the value reported directly in the studies when available and (2) the value derived from the percentage of variance explained by the QTL (R^2) using the empirical formula proposed by Darvasi and Soller (1997): $CI = \frac{530}{N \times R^2}$ where N is the population size. This formula usually gives larger CI (more conservative) than those reported in the studies.

To visualize the importance of a given chromosome region for each group of traits, we also used the "overview" statistics developed by Chardon et al. (2004). Briefly, we assumed that the true position of a given QTL in an initial experiment follows a normal distribution $N(\mu_q, \sigma_q)$ where μ_q is the estimated position of the QTL and σ_q its standard deviation (obtained with MetaQTL from the CI, see above). For a given QTL q detected on a given linkage group, the probability P_{qx} that the true position of q lies between positions x and $x + 0.5$ cM was calculated by integrating the probability density function of q over this interval. The overview for this interval was obtained as the sum over all the detected QTL of the probabilities P_{qx} that each QTL was positioned in this interval, and then divided by the total number of experiments (nbE). Its unit is, therefore, a number of QTL per 0.5 cM segment per experiment. The overview value in a given interval increases with (1) the number of QTL detected close to this interval and (2) the mapping accuracy of these QTL (small CI or high R^2). The overview was compared to the value $U_{0.5}$ which corresponds to the expected value of the overview on a segment of 0.5 cM long, knowing the average number of QTL detected per experiment (nbQTL/nbE), and under the hypothesis that QTL are allocated at random over the map:

$$U_{0.5} = \frac{\text{nbQTL/nbE}}{\text{Total length of map}} \times 0.5.$$

This aims at providing an empirical reference value for the overview criterion to facilitate the identification of the main chromosome regions where QTL have been detected in different studies. Note that this does not correspond to a statistical test (achieved below using meta-analysis).

QTL clusters with MetaQTL

We then used the QTLClust command of MetaQTL software (Veyrieras et al. 2007) to determine the most likely number of metaQTL on a given chromosome, estimate their positions and the corresponding CIs. Briefly summarized, MetaQTL considers QTL positions and corresponding CIs in individual experiments, after projection on the consensus map. For a given number of hypothetic meta-QTL, a Gaussian mixture model is applied to jointly (1) perform a quantitative clustering of the individual QTL to the different metaQTL and (2) estimate metaQTL positions and CIs, by maximizing the likelihood of the initial QTL positions. On each chromosome, MetaQTL considers several models, corresponding each to a different number of metaQTL. Several penalized likelihood criteria are computed by MetaQTL to choose between the different proposed models: AIC (Akaike information criterion), AICc, AIC3, BIC (Bayesian information criterion) and AWE (average weight of evidence) (Veyrieras et al. 2007).

The QTLClust command creates a "model" file output, which gives for each criterion and each number of meta-QTL, the criterion value and its "weight of evidence", ω_K, calculated as:

$$\omega_k = \frac{e^{\frac{-\Delta_k}{2}}}{\sum_{j=1}^{K\max} e^{\frac{-\Delta_j}{2}}},$$

where Δ_k is the difference between the criterion value for the model with k metaQTL and that of the model with the best criterion value. It can be interpreted as the probability that model K is in fact the best model for the data according to the criterion used. The reliability of the number of metaQTL was checked by comparing the number of metaQTL determined with the different criteria. In general, we chose the number of metaQTL given by the AIC criterion. However, when this number was different from that given by most of the other criteria, we considered the most frequent K value. In rare cases where results appeared variable among criteria, we considered the results

of the QTLtree command that performs a hierarchical clustering of individual QTL based on a Ward's method (Veyrieras et al. 2007).

After determining the number of metaQTL for each group of traits and chromosome, the software provides the position and CI of each metaQTL. It also provides, for each initial QTL, the probability that it corresponds to a given metaQTL located on the same chromosome. As a result, each metaQTL position and CI correspond to the consensus position of all the initial QTL attributed to this metaQTL, weighted by their individual accuracies and probability of being attributed to the metaQTL.

Finally, we computed the percentage of genome covered by (1) the metaQTL for each group of traits and (2) overlapping metaQTL between the two groups. A χ^2 test was then performed in order to compare (2) to the value expected by chance under the hypothesis of independence between the two sets of metaQTLs (determined as the product of the percentages of genome covered by each set of metaQTL).

Mapping of candidate genes

Sequences for candidate genes were retrieved from "Maizewall" database (http://www.polebio.scsv.ups-tlse.fr/MAIZEWALL/). Among the 154 different gene families represented in the database, 90 were selected based on their putative importance for digestibility (Barrière, personal communication). The candidate gene families were ranked in three categories, based on the knowledge of the cell wall biosynthesis pathway, to give a prior on the probability that they were candidates for the trait (Barrière, personal communication)

- Rank 1: High evidence candidate genes (103 sequences from 17 gene families).
- Rank 2: Medium evidence candidate genes (315 sequences from 46 gene families).
- Rank 3: Low evidence candidate genes (42 sequences corresponding to 14 gene families).

The majority of these 77 gene families were multigene families. They were represented by 460 different sequences in the Maizewall database. Sequences were blasted against the maize sequence database (Sen et al. 2009) version available in April 2009. Genome sequences with a Blast e-value above 1×10^{-50} were discarded. The remaining sequences were then positioned onto our consensus map based on the positions of the flanking markers. Molecular markers from the IBM2 FPC0507 neighbour map were used as a bridge between the physical map and our consensus map. When it was not possible to find a genetic marker on the BAC where the sequence of the gene had been positioned, we considered the genetic marker located on the closest BAC of the physical map.

Statistics on coincidence between metaQTL and candidate genes positions

The proportion of the genome covered by the metaQTL was used to perform χ^2 tests of whether candidate genes were located more often in metaQTL CIs than would have been expected simply by chance (if they were randomly distributed in the genome). Those tests were performed globally for all candidate genes and then for each group of candidate genes in function of the prior "rank" that they were given.

The χ^2 value was calculated as:

$$\chi^2 = \frac{(f_o - f_e)^2}{f_e}$$

where f_o is the observed number of genes that are falling into the CIs of the metaQTL. f_e is the expected number of genes positioned in the CI of the metaQTL under the null hypothesis, based on the percentage of the genome covered by the metaQTL.

Results

Consensus map

The consensus map was 2,157 cM long (Supplementary Table 2) and included a total of 905 markers, with a minimum of 63 markers for chromosome 10 and a maximum of 126 markers for chromosome 1. The average distance between two flanking markers was 2.4 cM. 71% of the markers were mapped in only one experiment, 17% were common to two experiments and 12% were common to three or more (up to seven) individual maps. The goodness-of-fit values of the consensus map ranged from $\lambda = 55.6$ for chromosome 7 ($\lambda \sim \chi^2_{125,}\ \chi^2_{125,5\%} = 152$) to $\lambda = 277.5$ for chromosome 2 ($\lambda \sim \chi^2_{98,}\ \chi^2_{98,5\%} = 115$). λ values generally exceeded the 5% probability value of the χ^2 test except for chromosomes 5, 7 and 8.

The consensus map showed several marker order inversions compared to the IBM neighbour map, but most of these inversions concerned markers mapped at close positions (maximum length of 10 cM).

QTL projection on the consensus map

For the four digestibility traits (DINAGZ, IVDMD, IVDOM and IVNDFD), over 102 QTL were considered: 43 QTL appeared redundant with another digestibility QTL

detected in the same experiment. The remaining 59 QTL were kept for the meta-analysis, ranging from two QTL for Riboulet et al. (2008) to 20 for Barrière et al. (2001) per initial experiment. Similarly, from the 376 cell wall QTL, 226 QTL appeared redundant and 150 QTL were kept for the meta-analysis, ranging from four QTL for Riboulet et al. (2008) to 37 for Krakowsky et al. (2005).

These QTL were located on all chromosomes. For digestibility traits, the number of QTL per chromosome ranged from 2 on chromosome 10 to 16 for chromosome 1. For cell wall traits the number of detected QTL ranged from 2 for chromosome 10 to 25 for chromosome 1. After projection on the consensus map the CI of the selected QTL ranged from 10 to 66 cM, with an average of 29 cM.

58% of the genome was covered by digestibility QTL and 77% was covered by cell wall QTL.

Meta-analysis

Digestibility QTL

We determined the number of metaQTL per chromosome using the AIC criterion, except for chromosomes 2 and 4 (Table 3). For these chromosomes, the different criteria did not give consistent results, and we used the QTLtree clustering to determine the number of metaQTL. The number of metaQTL per chromosome varied from one (chromosome 7) to six (chromosome 1), yielding a total of 26 metaQTL (Table 4). Note that with our definition of metaQTL, a metaQTL may correspond to a single initial QTL. On average, one metaQTL corresponded to 2.3 QTL coming from different initial experiments. Five metaQTL corresponded to more than three initial QTL and five metaQTL corresponded to only one initial QTL [metaQTL (MQTL): 10, 13, 16, 26 and 27 on Table 4].

MetaQTL displayed an average CI length of 24.5 cM. The CI of metaQTL was smaller than that of individual QTL by a factor of 1.2. The metaQTL covered 30% of the genome, whereas the original QTL covered in total 58% of the genome. The meta-analysis, therefore, was able to decrease the percentage of genome potentially harbouring genetic factors controlling digestibility by a factor 1.9.

Nine metaQTL showed CIs smaller than 15 cM. Among them, five metaQTL appeared particularly interesting because they had small CIs and/or gathered initial QTL with high R^2. MQTL 4 on bin 1.08 had a CI of 11.9 cM and corresponded to four QTL with R^2 ranging from 7 to 21%. MQTL 12 on bin 3.07 had a CI of 7.7 cM and corresponded to two individual QTL out of which one had a high R^2 of 21%. MQTL 14 on bin 4.07 had a CI of 13.6 cM and corresponded to two QTL experiments with R^2 of 6 and 14%. MQTL 22 on bin 8.05 had a CI of 12.6 cM and gathered three QTL experiments, one having a R^2 of 17%. MQTL 24 on bin 9.03 showed a CI of 13.9 cM and corresponded to three QTL with R^2 from 5 to 16%.

Cell wall QTL

Model choice was based on the AIC criterion for chromosomes 4–9. For the other chromosomes, we chose the model that was the most frequently determined by the different criteria (Table 3). This yielded a total of 42 metaQTL (Table 4), evenly balanced over the chromosomes. On average, one metaQTL corresponded to 3.6 QTL coming from different initial experiments. This number varied from one QTL for MQTL 10 on bins 2.08–2.09 and MQTL 24 on bins 5.07–5.08 to seven QTL for MQTL 19 on bin 4.07 and MQTL 23 on bin 5.06.

The CI of MetaQTL was 13.4 cM long on average. Use of meta-analysis decreased the size of CI by a factor of 1.9 compared to the CIs of individual QTL. 26% of the genome was covered by the cell wall metaQTL, whereas the original QTL covered 76% of the genome. The meta-analysis, therefore, decreased the percentage of genome potentially harbouring genetic factors controlling cell wall traits by a factor of 2.9. Three metaQTL (MQTL 8, 19 and 23) had CI smaller than 13 cM. They all corresponded to six or more initial QTL. The CI of MQTL 8 on bin 2.05 was particularly low (7 cM) and corresponded to six individual QTL with large effects (with R^2 varying from 12 to 28%). MQTL 19 on bin 4.07 had a CI of 11.4 cM and corresponded to seven QTL, one of which reaching a R^2 of 17%. On bin 5.06, MQTL 23 had a CI of 12.7 cM, and corresponded to seven QTL with R^2 ranging from 9 to 13%.

From a total of 42 metaQTL, 16 metaQTL were specific to lignin traits, and one metaQTL was specific to *p*-hydroxycinnamic acids (metaQTL 10 on bin 2.08–2.09). The other 25 metaQTL comprised QTL from different cell wall traits.

Colocalization between digestibility and cell wall metaQTL

Digestibility and cell wall metaQTL CI covered in total 47% of the genome, which is 1,020 cM. From this, 181 cM were common for the digestibility metaQTL and cell wall metaQTL, which represents 18% of the total genome covered by metaQTL. A χ^2 test showed that this level of colocalization is significant at a 5% risk level (results not shown).

Fourteen from 26 metaQTL for digestibility traits showed overlapping CIs with metaQTL for cell wall traits. Reciprocally, 15 from 42 metaQTL for cell wall traits showed overlapping CIs with metaQTL for digestibility. Note that MQTL 23 for digestibility traits overlapped with

Table 3 Meta-analysis results

Chr	Number of QTL	Average CI (cM)	Number of metaQTL	Average CI of metaQTL (cM)	Chosen criterion
Meta-analysis for digestibility-related QTL					
1	16	37.3	6	21.3	AIC
2	7	33.6	2	17.7	AICc
3	6	25.2	4	15.4	AIC
4	5	24.8	4	29.1	–[a]
5	5	66.9	2	46.8	AIC
6	5	28.8	2	21.9	AIC
7	4	39	1	22.4	AIC
8	5	30	2	30.6	AIC
9	4	47	2	36.2	AIC
10	2	10	2	10	AIC
Meta-analysis for cell wall-related QTL					
1	25	24.1	6	9.9	AICc
2	15	27.9	4	15.1	AICc
3	17	30.3	5	15.3	AICc
4	20	35.9	5	15	AIC
5	13	31.5	4	15.2	AIC
6	13	26.2	4	12.4	AIC
7	11	87.7	3	13.2	AIC
8	13	34.9	4	15	AIC
9	13	26.3	4	11.5	AIC
10	10	26.7	3	11.7	AIC3

For each chromosome and group of traits are given the number of QTL, the average confidence interval (CI) of the individual QTL, the number of metaQTL chosen, the average CI of these metaQTL and the criterion used to determine the number of metaQTL

[a] As no consensus model could be drawn from the different criteria, model choice was based on the QTLtree display (see "QTL clusters with MetaQTL")

two metaQTL (MQTL 34 and 35) for cell wall traits on bins 8.07–8.08.

Considering that three experiments out of 11 (Table 1) jointly studied the two groups of traits we checked their contribution to the colocalizations observed between metaQTL. Out of the 14 colocalizations, one comprised QTL from experiments where one single group of traits was studied (digestibility or cell wall traits). For the remaining 13, seven corresponded to colocalizations detected in at least one of the experiments that studied both groups of traits. The six others corresponded to situations where a QTL for digestibility did not colocalize with any QTL detected for cell wall traits in the same experiment, but to at least one QTL detected for these traits in a different experiment. Absence of colocalization in the same experiment may be partly explained in this case by power detection issues.

QTL overview

The density curve exceeded 31 times the average value $U_{0.5}$ for digestibility traits, and 49 times for cell wall traits, illustrating that many regions in the maize genome are involved in the genetic variation of cell wall components and/or digestibility traits (see Fig. 1).

As expected, the overview curve was generally high in the vicinity of metaQTL which corresponded to several QTL, whereas it was low for metaQTL which corresponded to only one QTL or metaQTL with large CIs. For cell wall traits, QTL overview did not reach the average value for MQTL 15 on bin 3.08 and MQTL 16 on bin 4.02 that correspond to several individual QTL but with large confidence intervals, yielding a "flat" profile.

Candidate genes and correspondence with metaQTL

Candidate genes are listed in Supplementary Table 3 with their position on the consensus map and correspondences on the IBM2 neighbour map (Sen et al. 2009). Gene families showed between 1 and 17 copies on the genome with an average of four. Among the 488 corresponding genes, a total of 356 could be projected on the consensus map. The number of genes per chromosome ranged from 18 on chromosome 7 to 62 on chromosome 1.

Fifty-five candidate genes were ranked as "good" candidate genes (ranked 1), 258 were putative candidates (ranked 2) and 43 were possibly candidate genes (ranked 3).

For digestibility traits out of 356 candidate genes, 140 (39%) were located within metaQTL CIs (30% of the genome). For cell wall traits, 136 candidate genes (38%)

Table 4 List of the metaQTL found in the meta-analysis

Chr	MQTL Nb	Position	Bin	95% CI	Nb QTL/metaQTL	Nb CG	Class 1 candidates	Colocalization
(a) MetaQTL for digestibility traits								
1	1	66.8	1.03	21.1	1.8	6	4CL_6	×
1	2	107.6	1.04	17.8	2.6	6	L_1	
1	3	134.6	1.05–1.06	24.9	1.8	9	P_2	
1	4	203.1	1.08	11.9	3.8	4	4CL_5; F5H	×
1	5	230.0	1.1	18.2	3.0	3	–	
1	6	288.4	1.12	33.7	3.2	3	–	
2	7	66.1	2.04	14.6	5.0	4	HCT_2	
2	8	149.4	2.08	20.9	2.0	1	–	
3	9	80.3	3.04	26.3	2.0	2	–	×
3	10	117.7	3.05	14.0	1.0	10	P_1	
3	11	134.9	3.06	13.8	1.2	6	–	×
3	12	166.0	3.07	7.7	1.8	2	C3H	×
4	13	105.5	4.04	15.1	1.0	6	COMT; PAL_TAL_1; PAL_TAL4	
4	14	168.9	4.07	13.7	2.0	4	L_3	×
4	15	206.8	4.08–4.09	42.7	1.0	10	CCR2; CCOAOMT; L_4	×
4	16	250.0	4.1	44.8	1.0	0	–	
5	17	94.3	5.03–5.04	64.1	1.4	17	4CL_2; 4CL_3; P_3	×
5	18	165.4	5.06	29.5	3.6	5	HCT_1	×
6	19	27.8	6.01	15.7	3.0	8	–	×
6	20	140.1	6.06–6.07	28.1	2.0	2	–	×
7	21	81.0	7.04	22.4	4.0	6	CAD2_1	
8	22	69.4	8.05	12.6	3.0	4	–	
8	23	126.9	8.07–8.08	48.6	2.0	7	C4H_2	×
9	24	48.0	9.03	13.9	2.3	1	–	
9	25	123.7	9.05–9.06	58.6	1.7	5	–	×
10	26	78.2	10.03	10.0	1.0	1	–	
10	27	96.5	10.04	10.0	1.0	1	–	×
(b) MetaQTL for cell wall traits								
1	1	57.0	1.03	15.2	3.0	5	4CL_6	×
1	2	94.5	1.04	6.4	4.8	1	–	
1	3	118.6	1.05	12.5	2.3	5	L_1	
1	4	158.7	1.06	6.6	5.0	0	–	
1	5	198.9	1.08	9.0	5.0	1	F5H	×
1	6	250.6	1.10–1.11	9.8	5.0	1	–	
2	7	46.5	2.03	23.5	2.0	6	CAD2_4	
2	8	83.1	2.05	7.2	6.0	1	–	
2	9	130.9	2.07	12.3	5.9	2	CCoAOMT_6	
2	10	172.6	2.08–2.09	17.6	1.1	4	CCR_1	
3	11	39.4	3.02–3.03	17.4	1.9	1	4CL_1	
3	12	71.5	3.04	15.3	3.1	0	–	×
3	13	107.8	3.05	9.5	3.1	4	–	
3	14	126.8	3.06	8.9	4.6	3	–	×
3	15	181.2	3.08	25.7	4.4	6	C3H	×
4	16	52.1	4.02	27.4	2.0	0	–	
4	17	117.6	4.05	11.2	5.4	8	REF1_REF2_3	
4	18	152.0	4.06	16.4	1.4	3	REF1_REF2_4	
4	19	172.6	4.07	11.4	6.8	3	L_3	×

Table 4 continued

Chr	MQTL Nb	Position	Bin	95% CI	Nb QTL/metaQTL	Nb CG	Class 1 candidates	Colocalization
4	20	213.2	4.09	8.7	4.4	2	CCR_2; L_4	×
5	21	83.5	5.03	15.9	2.1	2	–	×
5	22	118.3	5.04	17.9	3.0	8	P_3	
5	23	170.5	5.06	12.8	6.9	3	HCT_1	×
5	24	201.6	5.07–5.08	14.2	1.0	4	–	
6	25	21.1	6.01	15.0	4.0	8	–	×
6	26	78.3	6.04	17.6	2.1	8	REF1_REF2_5; P_5	
6	27	100.1	6.05	9.7	4.3	0	–	
6	28	136.2	6.07	7.2	2.7	0	–	×
7	29	66.6	7.03	19.2	4.7	3	CAD2_1	
7	30	118.8	7.04	13.7	1.7	0	–	
7	31	139.4	7.05	6.7	4.5	0	–	
8	32	30.3	8.02–8.03	21.8	2.0	2	–	
8	33	59.6	8.04	10.5	4.0	5	C4H_1	
8	34	108.4	8.07	17.0	2.0	4	–	×
8	35	129.7	8.08	10.8	5.1	3	C4H_2	×
9	36	17.6	9.01	11.8	3.4	0	–	
9	37	61.5	9.03	11.2	4.2	7	–	
9	38	81.2	9.04	13.4	3.5	11	4CL_4; CCR_5; CCR_7	
9	39	112.0	9.06	9.5	2.1	1	–	×
10	40	92.8	10.04	9.0	4.0	12	CAD2_3; CCR_8; CCR_9	×
10	41	124.7	10.06	15.8	2.9	5	–	
10	42	148.2	10.07	10.4	3.1	3	–	

The position, confidence interval, number of QTL attributed to the metaQTL, the number of candidate genes (Nb CG) positioned in the confidence interval of the metaQTL as well as the list of class 1 candidates are indicated for each metaQTL. Candidate genes names are here abbreviated, list of complete names can be found in Supplementary Table 3. Colocalization indicates overlapping with metaQTL for the other group of traits

were located within metaQTL CIs (26% of the genome). The total number of candidate genes observed in the metaQTL CIs was significantly higher than expected by chance (P value was 2.81×10^{-3} for digestibility traits and 3.24×10^{-10} for cell wall traits), showing that globally there was a significant coincidence between the location of metaQTL and candidate genes (Table 5). The level of significance was higher for cell wall than for digestibility traits.

For both groups of traits, the rank 2 candidate genes showed a significant colocalization with metaQTL at a 5% level: P-value of 2.27×10^{-2} for digestibility traits and 8.24×10^{-11} for cell wall traits. Rank 3 genes had a P-value of 1.19×10^{-4} for cell wall traits but the P-value for digestibility traits was not significant. Rank 1 genes did not show significant correlation with the metaQTL positions (P-values of 1.52×10^{-1} and 7.66×10^{-2} for digestibility and cell wall traits, respectively).

A large variation in the number of candidate genes underlying a given metaQTL was observed. Seventeen candidate genes were mapped in digestibility MQTL 17 CI (bins 5.03–5.04), but this CI was clearly larger than others (62 cM) (Table 4). Excluding this MetaQTL, the number of candidate genes underlying a given metaQTL was four on average, ranging from none (eight metaQTL for cell wall traits and two metaQTL for digestibility traits) to 12 candidate genes (for cell wall MQTL 40 on bin 10.04; Table 4). 12 metaQTL for digestibility traits and 13 metaQTL for cell wall traits colocalized with five or more candidate genes.

Fourteen metaQTL for digestibility traits and 20 metaQTL for cell wall traits colocalized with at least one rank 1 candidate gene. At least one member of each rank 1 gene-family colocalized with a metaQTL. Out of the 14 Rank 1 gene families, 10 present multiple gene copies. In some cases, genes from a same family colocalized with two or more metaQTL. For example, 36 genes were found for *expansin* (gene family coding for a protein responsible for acid-induced wall expansion and considered as being a very good candidate), out of which 25 sequences gave a BLAST result with value superior to 1×10^{-50}. Over the corresponding 25 mapped positions, 15 colocalized with

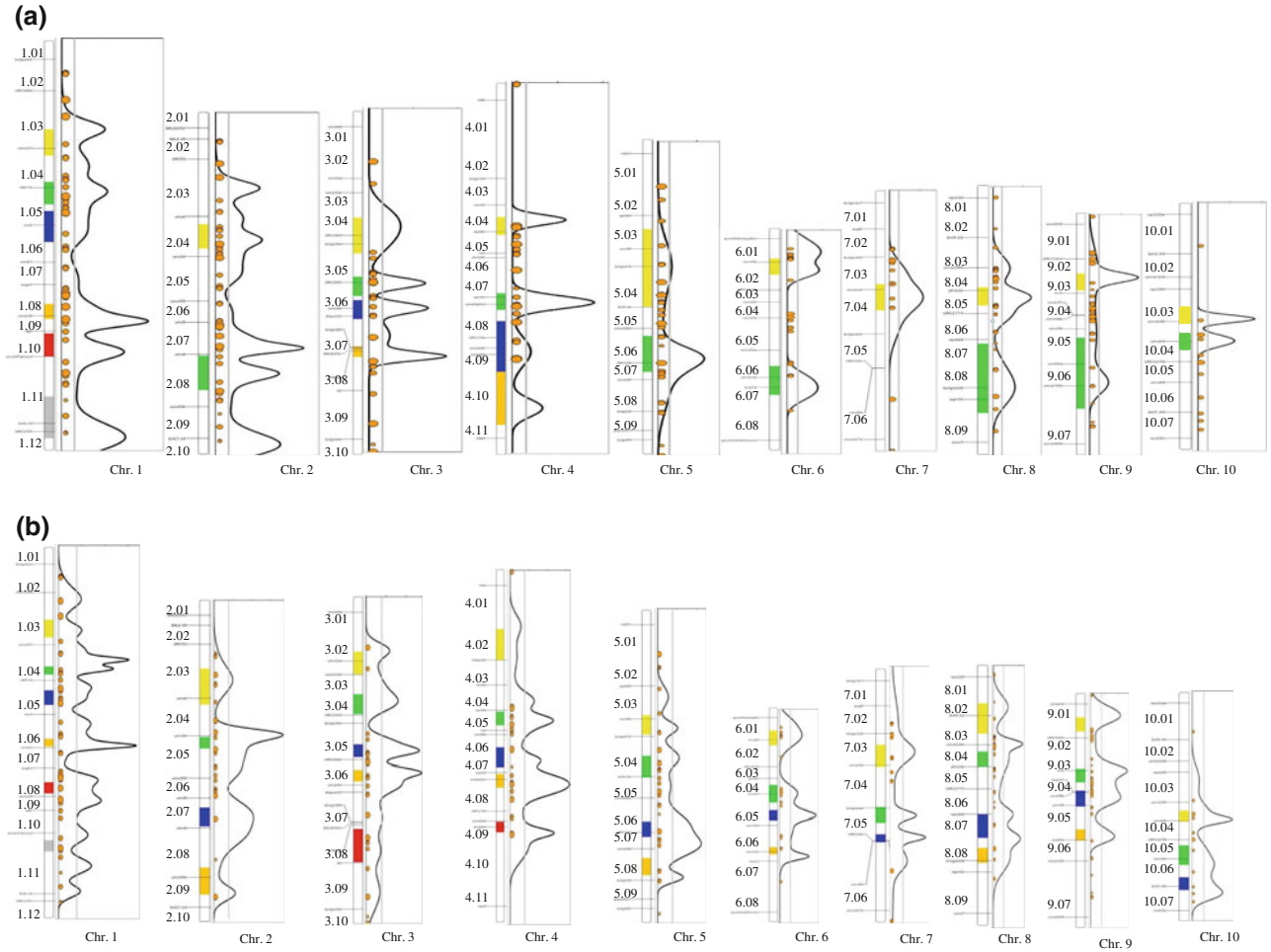

Fig. 1 Meta-analysis results for the ten chromosomes: **a** for digestibility traits and **b** for cell wall components traits. The bins are displayed on the *left side* of each chromosome, metaQTL confidence intervals are displayed as *thick coloured bars* on the chromosomes; the candidate genes are represented as *yellow dots with different sizes* depending on the class of candidate genes they belong to, class 1 genes being plotted with the *bigger-sized dots*). QTL overview is shown on the *right* of each chromosome; the *horizontal line* corresponds to the average overview $U_{0.5}$ value (see text)

metaQTL. For this gene family, several copies often mapped at close positions. For instance, four sequences of *expansin* were positioned around position 75.9 cM on chromosome 9. This position lies in the confidence interval of MQTL 38 for cell wall traits.

Discussion

Consensus map

The majority of published QTL meta-analysis studies have been conducted on synthetic maps obtained by (1) iterative projections of individual maps (using for instance Biomercator software) or (2) by projecting individual maps on a reference map, often also involving projections from other maps for part of the markers (Löffler et al. 2009). Marker positions obtained after multiple projections of individual maps depend on the order used for the projections, and this can influence the meta-analysis process. We established here a consensus map for the individual maps used in QTL studies by the weighted least squares method implemented in MetaQTL software. This approach uses estimated distances between markers from each individual QTL map and takes into account their accuracy to estimate marker distances and marker order in a single-step analysis. MetaQTL provides a goodness-of-fit criterion to estimate the ability of the consensus map to synthesize individual maps. The values of the goodness-of-fit for our consensus map were relatively high and often exceeded the χ^2 threshold values except for chromosomes 5, 7 and 8 (data not shown). The fact that the goodness-of-fit criterion exceeded the χ^2 threshold showed that these heterogeneities cannot be only explained by sampling effects and might have a biological meaning. This suggests heterogeneity in recombination across populations, consistent with

Table 5 Chi-squared tests for correspondences between digestibility (DIG) or cell wall (CW) metaQTL and candidate genes positions

MetaQTL	Nb	Total size (cM)	% of the genome	
(a) Number of metaQTL per traits group and map size covered by their confidence intervals				
DIG	27	654.7	30	
CW	42	563.1	26	
	Class 1	Class 2	Class 3	Total
(b) Number of gene sequences underlying the metaQTL and *P* value of the χ^2 test for each class of candidate genes or in total				
DIG				
Nb	17 genes	95 genes	21 genes	133/356
P	1.52E–01	2.27E–02	1.62E–01	2.81E–03
CW				
Nb	16 genes	104 genes	25 genes	145/356
P	7.66E–02	8.24E–11	1.19E–04	3.24E–10

observations by Causse et al. (1996). Note that Veyrieras et al. (2007) constructed a consensus map of chromosome 8 of maize using 18 different individual maps and found a λ value of 365.31 for chromosome 8, with $\chi^2_{87,0.05} = 109.77$ which is higher than the value we observed on the same chromosome ($\lambda = 120.52$ with $\chi^2_{102,0.05} = 126.57$). This illustrates that heterogeneity in maps can vary across the sets of studies considered.

Despite these rather large λ values, our consensus map seems satisfactory. First, 29% of the markers were mapped in two or more experiments, which is more than in other studies using MetaQTL (e.g. Löffler et al. 2009). More importantly, marker orders in the different individual maps were consistent, which allowed us to obtain satisfactory projections of individual QTL onto our consensus map. Second, we checked the consistency between our consensus map and the IBM neighbour map (Schaeffer et al. 2006) which is the reference map in the maize community and that was used to anchor the physical map. We observed some marker inversions between the two maps, but these inversions were observed in groups of close markers. We checked that the positions of the markers used to place the candidate genes on our consensus map were consistent between our consensus map and the physical map. These elements support the relevancy of our consensus map to project QTL, perform the meta-analysis and to position candidate genes.

MetaQTL

Meta-analysis results in a simplified (consensus) model which provides a lower bound of the number of QTL that can underlie the QTL detected in the different studies considered. It provides estimates of QTL consensus positions and CI where the causative polymorphisms of the QTL are the most likely positioned. The number of metaQTL and the size of their CIs depend on the initial QTL number, position and CI estimates (Goffinet and Gerber 2000). Several metaQTL had a limited representation in the initial QTL mapping experiments (11% represented by a single QTL), suggesting that, compared to what had been observed for flowering time in maize (Chardon et al. 2004), additional QTL mapping studies for digestibility and cell wall traits with independent parents should probably lead to the discovery of new QTL. QTL meta-analysis enabled us to reduce CIs of metaQTL relative to those initial QTL by a factor of 1.2 for digestibility traits and 1.9 for cell wall-related traits. This is probably due to a higher number of initial QTL for this last group of traits, providing more information to refine the position of each given metaQTL. The CIs of these metaQTL can be considered as conservative. First, when two QTL from the same experiment and the same group of traits had overlapping CIs, we removed one of them because they cannot be considered as being statistically independent. We were very strict in our selection, and removed 60% of the initial QTL, thus reducing the risk of producing metaQTL with artificially small CI. Second, for computation of the variances of the QTL positions, we considered the maximum value between the variances inferred from the CI and from the R^2, in order to manage possible overoptimistic CIs reported in QTL mapping studies (Boitard et al. 2006). Considering the two groups of traits, individual QTL after projection on the consensus map covered in total 68% of the genome. The metaQTL CIs only covered 28% of the genome, which facilitated the search of colocalizations between candidate genes and QTL. Note that a given metaQTL might correspond to a single actual QTL with effects, possibly pleiotropic in the different studies considered, but it might also correspond to several actual closely linked QTL that cannot be distinguished in the meta-analysis process due to the poor accuracy of QTL

results in individual studies. So a given metaQTL might correspond to several genes.

MetaQTL positions were globally consistent with regions highlighted by Barrière et al. (2007) who carried out an empirical synthesis of QTL detected for ADL and NDF traits and found hotspots of QTL on bins 2.08, 5.03, 6.04 and 9.06. Indeed, we found cell wall metaQTL on bin 2.08 and bin 9.06, but metaQTL on bins 5.04 and 6.05 instead of bin 5.03 and 6.04, respectively. These two small differences might be due first to difference in methods. Barrière et al. (2008) used a reference map to simply project the QTL without really performing a meta-analysis. Second, our meta-analysis included QTL not only for ADL and NDF but also for additional cell wall component traits (hemicelluloses, acid contents, etc.). Colocalization between cell wall and digestibility metaQTL confirmed the link between digestibility and cell wall composition, consistent with genetic correlations between the two groups of traits (Argillier et al. 1996) and colocalizations reported in individual QTL studies addressing both groups of traits (e.g. Argillier et al. 1996; Guillaumie et al. 2007a, b; Méchin et al. 2001; Riboulet et al. 2008; Roussel et al. 2002). However, some metaQTL seemed specific to one group of traits, showing that they are partly controlled by different loci. In particular, our results suggest that approximately 60% of digestibility QTL are controlled by other factors than cell wall composition.

Finally, we compared our results with those of Shi et al. (2007) who performed an expression study using a forage quality array. They detected 5 hotspots of *trans* eQTL on bin 1.07, 1.11, 3.05, 8.03 and 9.04, affecting the expression of many other genes in the genome, which suggests that underlying genes might be regulators. They only reported colocalization with previous QTL for cell wall traits and/or digestibility results on bin 3.05. Interestingly, we found a metaQTL on bin 3.05, and also MetaQTL at additional positions corresponding to their eQTL hotspots on bins 1.11 (MQTL 6 for digestibility traits), 8.03 (MQTL 32 for cell wall traits) and 9.04 (MQTL 38 for cell wall traits) but none in the bin 1.07.

Colocalization between metaQTL and candidate genes

The list of candidate genes and related functional information constantly evolve, in particular thanks to intensive research addressing cell wall biosynthesis pathway. In particular, information from eQTL analysis (see above) should prove highly valuable to identify *cis*-regulatory factors controlling the expression of genes and to be considered as candidate genes for metaQTL. Using information available when starting this study, we could not conduct an exhaustive investigation of such factors but already found interesting colocalizations between metaQTL and candidate genes. χ^2 tests showed that candidate genes were located more frequently in metaQTL CIs than what could be expected just by chance. Coincidences were more significant for cell wall metaQTL than for digestibility metaQTL. This is not surprising since the candidate genes that we considered mainly correspond to genes involved in the cell wall biosynthetic pathway, which is better understood than the other genetic determinants of digestibility.

It also has to be noted that some candidate genes did not colocalize with metaQTL, for instance on bins 2.06 and 9.02. As most of the genes belong to multi-copy families, one explanation could be that some copies did not play a major role in cell wall or digestibility traits. As discussed above, another possible explanation could be that the number of QTL studies on traits related to cell wall or digestibility is not high enough yet to ensure that all chromosome regions involved in the variation of these traits have been already found.

Some metaQTL showed small CI (less than 20 cM) and colocalization with rank 1 candidate genes. Two metaQTL appear particularly interesting for further studies on candidate genes. MQTL 1 (bin 1.03) for digestibility traits encompassed five candidate genes. Among them, *4-coumarate coenzyme A ligase* (*4CL*) is a very good candidate. This enzyme catalyses the reaction from *p*-coumaric acid to *p*-coumaroyl-Coa, permitting the biosynthesis of the two major monolignols. *Xyloglucan galactosyltransferase* (*mur3*) is a membrane protein involved in the synthesis of xyloglucans, which have been shown to interlace with cellulose microfibrils in cotton (Madson et al. 2003). Chitinase-like enzymes are essential for normal plant growth and development in *Arabidopsis thaliana* (Zhong et al. 2002). Expansins (two copies in the CI of MQTL 1) are responsible for acid-induced wall expansion. GDP mannose pyrophosphorylase is involved in cell wall carbohydrate biosynthesis (Barrière et al. 2001).

MQTL 40 (bin 10.04) for cell wall traits comprised 12 candidate genes, including very good candidates such as one *CAD2* and one *CCR*. It has been shown that the copy of *CCR* in bin 1.07 is involved in monolignol biosynthesis (Lacombe et al. 1997) and the copy of CAD2-type enzyme on bin 5.04 in the conversion of cinnamic aldehydes into monolignols (Šamaj et al. 1998). However, the roles of the different copies of these genes localized within bin 10.04 need specific investigation. Xyloglucan endotransglycosylase/hydrolases (*XTH*, two copies here) are associated with the growth of fibres (Michailidis et al. 2009). RNA polymerase II subunit RPB10 is part of an enzyme involved in RNA production. Three copies of *glucosidases* were also found in the CI of MQTL 40. They are involved in the transport and storage of monolignols glucosides, precursors of monolignols (Barrière et al. 2009). Plasma membrane

protein, kobito1-2, is involved in the synthesis of cellulose during cell expansion (Pagant et al. 2002).

Other than the metaQTL discussed above, several metaQTL appeared particularly interesting because of their colocalization with brown midrib mutations. On bin 1.11 *bm2* colocalized with MQTL 6 for digestibility traits, which corresponds to QTL coming from three experiments. On bin 5.04 *bm1* colocalized with MQTL 17 for digestibility traits. Note that *ZmCAD1* and *ZmCAD2* genes were mapped in the same markers interval as *bm1*. *ZmCAD2* whose expression is altered in *bm1* mutant is a good candidate for this mutation. On bin 4.04, *bm3* colocalized with MQTL 17 for cell wall traits and MQTL 13 for digestibility traits. *COMT*, the expression of which is affected in *bm3* mutants, was mapped at a position corresponding to *bm3*. On bin 9.07, *bm4* colocalized with MQTL 39 for cell wall traits. There were only lignin-related QTL underlying this metaQTL, and five candidate genes mapped in the same marker interval. Only two have known functions: *Endo 1,3-1,4 beta D-glucanase*, which regulates plant growth and tissue elongation (Thomas et al. 2000) and *UDP-glucuronic acid decarboxylase*, which is involved in the transformation of UDP-D-glucose to hemicelluloses (Barrière et al. 2009). Since neither of these two genes is involved in lignin biosynthesis, the three others, which are potentially involved in regulation, deserve specific investigations. Meta-analysis confirmed that regions where important mutations have been mapped correspond to regions involved in quantitative variation of traits related to digestibility and cell wall components, suggesting allelic series with gradual effects (*bm* mutants being extreme) at these loci.

For MQTL 10, 13, 15, 16, 25 and 26 for digestibility traits, the MetaQTL software detected metaQTL based on only one QTL. In all cases (Barrière et al. 2001, 2007; Bohn et al. 2000; Lübberstedt et al. 1997a; Méchin et al. 2001) the parental lines were not used in any other study. This suggests that these QTL might be due to rare alleles that segregated in only one of the population studied so far. Nevertheless, these QTL are interesting to consider. For instance, the CI of MQTL 10 for cell wall traits on bins 2.08–2.09 encompassed several candidate genes of rank 1: two α-*mannosidases*, one *alpha-L-arabinofuranosidase* and one *CCR* (Lacombe et al. 1997). One α-*mannosidase* has been cloned in rice, but the function of this gene is as yet unclear (Wongvithoonyaporn et al. 1998). α-*L-arabinofuranosidase* in peach is responsible for modification of the cell wall architecture during fruit softening (Tateishi et al. 2005). As both enzymes are polysaccharide-modifying enzymes, they may be involved in the assembly of cell wall carbohydrates. However, although classed rank 1 in our study, very recent results suggest that genes involved in cell wall carbohydrate synthesis are less likely involved in the variation of digestibility than genes directly involved in lignin biosynthesis pathway (Penning et al. 2009). CCR is a key enzyme of the monolignol pathway (Barrière et al. 2009; Lacombe et al. 1997) and, therefore, a high priority candidate, but the other colocalizing genes are also good candidates for further analysis.

MQTL 13 for digestibility traits on bin 4.04 had six candidate genes positioned in its CI, out of which five are involved in cell wall carbohydrates synthesis: two copies of *cellulose synthase-like* (*CESA*), two copies of *phenylalanine ammonia lyase* (*PAL*) and *caffeic acid O-methyltransferase* (*COMT*). Cellulose synthase complexes are involved in the synthesis of hydrogen-bonded β-1,4-linked glucan chains at the plasma membrane, which are a main component of the cellulose. PAL is involved in the first step of monolignols biosynthesis, and COMT catalyses one reaction necessary for S unit biosynthesis (Barrière et al. 2009).

MQTL 15 for digestibility on bin 4.08 seems of great interest, as its CI encompasses *CCR* (see above), *laccase* and *caffeoyl-CoA O-methyltransferase* (*CCoAOMT*). Laccase is responsible of the dehydrogenative polymerization of *p*-hydroxycinnamyl alcohols to form lignins (Boerjan et al. 2003). CCoAOMT catalyses the biosynthesis of G units and contributes to S unit biosynthesis as well (Barrière et al. 2009). Genes in bin 4.08 are thus good candidates to further investigate this phenomenon as several candidate involved in cell wall biosynthesis are mapped at this position.

Perspectives

Even if meta-analysis is generally not accurate enough to discriminate between closely linked QTL and provides no information on allelic effects, it highlights chromosomal regions that are the most likely to carry the causal polymorphisms underlying trait variation. Hence, it enables one to focus research efforts on these regions in order to identify causal polymorphisms using higher resolution mapping strategies such as map-based cloning or association mapping.

Association mapping studies have already been conducted to investigate the relationship between traits related to digestibility and candidate gene polymorphisms. For example, three candidate genes for lignin biosynthesis pathways were investigated for their correlation to cell wall digestibility in 34 maize lines (Guillet-Claude et al. 2004). An association was highlighted for 18 bp-indel in *caffeoyl-CoA 3-O-methyltransferase* (*CCoAOMT2*) located on bin 9.01 within digestibility MQTL 24. A significant association was also observed for a single-base pair deletion in the intron of *5-hydroxyconiferaldehyde-O-methyltransferase* gene (*COMT*) located on bin 4.04, within digestibility

MQTL 13 and cell wall MQTL 17 in this study. Andersen et al. (2007) studied a panel of 40 elite European inbred lines for six candidate genes from the lignin biosynthesis pathway. Associations with forage quality traits were identified for several individual polymorphisms within the *4-coumarate CoA ligase* (*4CL1*), *p-coumarate 3-hydroxylase* (*C3H*) and *ferulate 5-hydroxylase* (*F5H*) genomic fragments. These three genes colocalized with digestibility MQTL 12 and cell wall MQTL 15 on bin 3.07–3.08 for *4CL1*, *C3H* and for *F5H* with digestibility MQTL4 and cell wall MQTL 5 on bin 1.08. This method, therefore, seems of great interest for studying other candidates reported above, including potential upstream and downstream regulatory regions.

For the nine metaQTL (one for digestibility traits and eight for cell wall traits) which displayed no colocalization with candidate genes or major mutations, and/or in case of failure to identify significant associations, two complementary methods can be considered to find causal polymorphisms underlying QTL. A first option is positional cloning (Bortiri et al. 2006; Salvi et al. 2007). This method requires the screening of large populations of individuals (several thousands) to identify recombinants, but development of markers to fine map regions of interest should now be facilitated to a large extent by the sequence of the B73 genome (Wei et al. 2009), that of Palomero (Vielle-Calzada et al. 2009) and an increasing body of sequence polymorphism information (Gore et al. 2009). The second option is to conduct LD mapping, i.e. association mapping with a priori neutral polymorphisms located at an appropriate density relative to linkage disequilibrium magnitude. This approach which could be used until now only in genetic materials with a "narrow" genetic basis (Beló et al. 2007) should now be more easily facilitated through advances made in the availability of information (see above) and major improvements in genotyping throughput and sequencing technologies.

Conclusion

This study used publicly available QTL mapping results to identify metaQTL for cell wall and digestibility traits in maize, yielding a total of 68 metaQTL. Many of these metaQTL had a limited representation in the initial QTL mapping experiments, suggesting that saturation has not been reached yet and that additional QTL mapping studies with independent parents should probably lead to the discovery of new QTL for these traits. Addressing globally a broad sampling of germplasm in a same study by means of nested association mapping (NAM, Yu et al. 2008) and/or linkage disequilibrium mapping in materials with a broad diversity should also be proved valuable to gain a representative picture of the true genetic complexity of cell wall in maize (Rong et al. 2007). In the meantime, our study highlights eight regions (localized on bins 1.08, 1.11, 2.08, 3.07, 4.04, 5.04, 9.01 and 9.07) that seem of particular interest. They present metaQTL for both digestibility and cell wall traits, and several candidate genes are found within their confidence intervals. The next step will be to further investigate these regions by conducting association mapping and/or positional cloning studies to identify polymorphisms correlated to cell wall components and digestibility traits. This should help better understand cell wall biosynthesis and provide markers to identify alleles of interest improving maize silage quality.

Acknowledgments We are grateful to Jean-Baptiste Veyrieras for his help regarding the use of MetaQTL software, Cedric Muller and Florence Servant for Blast of candidate genes from "Maizewall" to the maizesequence database, Olivier Sosnowski for help in informatics computations.

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