Theor Appl Genet (2001) 102:591–599

M. Séne · C. Thévenot · D. Hoffmann · F. Bénétrix
M. Causse · J.-L. Prioul

QTLs for grain dry milling properties, composition and vitreousness in maize recombinant inbred lines

Received: 27 April 2000 / Accepted: 3 July 2000

Abstract Vitreousness and kernel hardness are important properties for maize processing and end-product quality. In order to examine the genetic basis of these traits, a recombinant inbred line population resulting from a cross between a flint line (F-2) and a semident line (Io) was used to search for vitreousness and kernel composition QTLs. Vitreousness was measured by image processing from a kernel section, while NIR spectroscopy was used to estimate starch, protein, cellulose, lipid and semolina yield. In addition, thousand-grain weight and grain weight per ear were measured. The MQTL method was used to map the QTLs for the different traits. An additional program allowed for the detection of interaction QTLs between markers. The total number of main-effect and interaction QTLs was similar. The QTLs were not evenly distributed but tended to cluster. Such clusters, mixing main-effect and interaction QTLs, were observed at six positions : on chromosomes 1, 2, 3, 6, 8 and 9. Two of them, on chromosomes 6 and 9, concerned both QTLs for kernel-weight traits and QTLs for kernel-composition traits (protein and cellulose). Technological-trait QTLs (vitreousness or semolina yield) were located less than 16 cM from a protein-content QTL on chromosome 2, and were co-located with lipid- and starch-content QTLs on chromosome 8. The co-location of a vitreousness and a semolina-yield QTL at the telomeric end of the chromosome 2 (Bin 2.02) is likely to be meaningful since measurement of these related traits, made by completely different methods (NIRS vs image processing), yielded very close QTLs. A similar location was previously reported independently for a kernel-friability QTL. Comparing the map location of the numerous loci for known-function genes it was shown that three zein loci were closely linked to QTLs for vitreousness on chromosome 3, for semolina yield and starch on chromosome 4, and for protein, cellulose and grain weight on chromosome 9. Some other candidate genes linked to starch precursor metabolism were also suggested on chromosomes 6 and 8.

Keywords Lipid · Maize-grain · Protein · Quantitative trait loci · Semolina yield · Starch · Vitreousness

Communicated by H.F. Linskens

M. Séne · C. Thévenot · J.-L. Prioul (✉)
Laboratoire Structure et Métabolisme des Plantes, IBP,
Bâtiment 630 (UMR CNRS 8618), Université Paris-Sud,
91405 Orsay Cedex, France
e-mail: prioul@ibp.u-psud.fr, Fax (+33)-1-6933 6424

M. Causse
Station de Génétique Végétale (INRA, CNRS, UPS),
La ferme du Moulon, 91190 Gif-sur-Yvette, France

D. Hoffmann · F. Bénétrix
Association Générale des Producteurs de Maïs (AGPM),
21 Chemin de Pau, 64121 Montardon, France

Present address:
M. Causse, Station de Génétique et d'Amélioration des Fruits et Légumes, INRA, Domaine St Maurice, BP 94, 84143 Monfavet, France

Introduction

Maize is widely used for food and feed. In developing countries, it is mainly transformed into semolina for traditional paste preparation, while in European countries 25% of the total consumed maize is used for starch or semolina production by the wet or dry milling industries. Industrial processors, cooperatives and producers are trying to define common quality criteria. In this context, many breeding programs have focused attention on grain hardness. Several definitions and measuring methods have been proposed for this parameter. Vitreousness, defined as the ratio of vitreous to floury endosperm, is one of them. It is measured on individual kernels and is highly correlated to the yield of fine semolina, which is a direct measure of the milling ability of maize (Louis-Alexandre et al. 1991).

Most agronomic traits, like grain weight and hardness, are quantitative traits, and their expression is governed by several genes, each of them contributed by a fraction of the genetic variance, and is influenced by en-

vironmental factors. Little is known about the biological nature and the function of the genes underlying the observed continuous variation. When a large number of molecular markers became available, the search for associations between genotypes at molecular-marker loci and quantitative-trait variations became an efficient way to detect quantitative trait loci (QTLs). This was facilitated by the construction of dense linkage maps in several crop species (Paterson et al. 1988; Causse et al. 1996). This methodology has been successfully applied to several traits of agricultural importance, such as yield, yield components (Edwards et al. 1992; Schon et al. 1994; Ajmon-Marsan et al. 1995; Stuber 1995), kernel quality (Goldman et al. 1993; Azanza et al. 1996; Rami et al. 1998), response to abiotic stress (Champoux et al. 1995; Teulat et al. 1998), and carbon metabolism (Causse et al. 1995, Séne et al. 2000).

Several studies have been devoted to the analysis of the phenotypic correlations between the biochemical composition and mechanical properties of maize grains. Mestres and Matencio (1996) outlined the ambivalent role played by endosperm proteins in grain hardness. Total protein content was positively correlated with grain hardness, but the peculiar class of proteins involved (zein or globulin) is still a matter of debate. Albumin and globulin proteins were likely to be associated with endosperm softness. QTLs for kernel hardness have been previously detected by Sourdille et al. (1996) in wheat.

In the present study, we analyzed a population of maize recombinant inbred lines for milling ability and kernel biochemical composition as determined by Near Infra-red Reflectance Spectroscopy (NIRS), and vitreousness as determined by image processing. Our aim was to map QTLs for starch, lipid and protein content and to relate them to QTLs detected for semolina yield or grain vitreousness. The use of a map mainly constituted of markers corresponding to genes functionally involved in starch and carbohydrate metabolism allowed a discussion of the biochemical bases of maize technological traits.

Materials and methods

Plant material

A population of recombinant inbred lines was derived from a cross between two contrasting genotypes, a late dent line from the American Iodent group (encoded Io) and a French early flint line (encoded F-2) (Causse et al. 1996). One-hundred recombinant inbred lines (RILs) from the 6th generation of selfing were planted in the field at Gif-sur-Yvette (20 km SW of Paris, France), during the summer of 1994, in a randomised complete block design with two replications. Ten plants from each line were planted in a row. Ears from three plants in each block were collected at maturity and a composite sample was made by pooling six kernels per ear for chemical composition. Grain weight per ear was measured on the remaining part of the ear, taking into account the sampled ones.

Grain composition and physical properties

Samples were ground in a ball grinder, and stored at 4°C until use. Dry weight was determined on a 500-mg aliquot placed at 85°C until constant weight. The powder for NIRS analysis was obtained by a MILL 3100 Perten grinder equipped with a grid having 1 mm-round holes. The water content in samples was standardized to <16% by drying at 40°C for 1 night. The measurements were performed at the analysis laboratory of the "Association Générale des Producteurs de Maïs" (AGPM, Pau, France) using a NIRS system (Lytical-Foss Electric, Nanterre, France) in the range of 1100 to 2500 nm at a 2-nm path. The spectrum was calibrated for starch, protein, lipid content and semolina yield with a series of genotypes covering variability in French national production since 1986. Each year the database was enriched with 24 samples from the main production areas. Protein content was evaluated as total nitrogen (N)×6.25. The references of the normalized chemical/biochemical methods were: NF V03708 for water content, NF V03050 for protein, NF V03040 for cellulose, NF V03713 for lipid, EC "directive 72/199" for starch contents. Those reference values were fitted to IR values in order to derive prediction equations. The validation tests for the prediction equations are given in Table 1. Semolina yield in the RILs was estimated by the same NIRS method, but samples for validation were different (Table 1).

Table 1 Validation tests for the prediction equations used to determine grain composition and technological properties from NIRS measurements. SD represents the standard deviation of the values for the reference population; SE the standard error of the predicted values. Semolina yield calibration was obtained from the 1990–1992 samples of field trials under various conditions (AGPM data base). The presented R^2 values were calculated in the 54–76% range; under these conditions the residual SD of the prediction is only equal to 1.5-times the reference-method SD

Trait	Sample number	SD reference	SE validation	R^2
Water content	25	1.01	0.28	0.88
Starch content	25	0.91	0.35	0.85
Lipid content	25	0.32	0.16	0.83
Protein content	25	0.54	0.11	0.92
Cellulose content	25	0.20	0.12	0.77
Semolina yield	105	4.02	0.51	0.88

TGW represents 1000-grain weight and GWE the total kernel weight per ear. Weights were determined on grain samples with a final water content adjusted to 15%±0.5, by drying in a dessicator at 30°C, under partial vacuum (300 mbar) for 2 weeks.

Vitreousness

The vitreousness of RIL grains was determined in ten individual kernels taken from the pool originating from six ears. The procedure was derived from the method described by Louis-Alexandre et al. (1991). Grains were cut longitudinally in a plane parallel to the germ, through the center of the largest kernel diameter, using a buffing wheel. Kernel sections were scanned using a Canon color scanner, interfaced to a MacIntosh Quadra 950 L. Outlines of the vitreous, floury and total endosperm areas were drawn by a magnetic pen. The Adobe Photoshop software allowed the calculation of the vitreousness index as mean percentage of vitreous area/total endosperm area.

Maize genetic map

The details of RFLP analysis and maize genetic-map construction have been reported for the IoxF-2 family (Causse et al. 1996); 145 RILs were genotyped with 152 markers. A large part of the markers have a known function (encoded gsy). Among them, functions related to carbohydrate metabolism (hexoses, sucrose and starch) and kernel-protein genes are well represented. The remaining

Table 2 Variation of the kernel biochemical composition, semolina yield and physical traits in the parental lines, and in the 100 recombinant inbred lines. Statistics from two independent measurements, in duplicate. SD standard deviation; CV coefficient of variation; max maximal value; min minimal value; TGW 1000-grain weight; GWE grain weight per ear

Traits	Io	F-2	RILs			Range (max–min)
			Mean	SD	CV	
Starch content	74.8	73.6	73.5	1.02	1.4	76.2–70.9
Lipid content	3.8	4.1	3.9	0.1	3.5	4.3–3.7
Protein content	11.9	12.8	12.1	0.9	7.4	14.8–9.4
Cellulose content	2.60	2.87	2.98	0.14	4.6	3.29–2.61
Vitreousness	0.66	1.6	1.35	0.53	39	2.63–0.27
Semolina yield	67.2	74.6	71.6	3.1	4.3	77.8–63.1
TGW	226	255	246	53.4	22	385–132
GWE	63	59	56.5	15.8	28	103–19

Table 3 Linear correlation (Pearson's) between traits in the recombinant inbred lines. Semolina Y: semolina yield; TGW: 1000-grain weight; GWE: grain weight per ear

Trait	Starch	Lipid	Protein	Cellulose	Vitreousness	Semolina Y	TGW
Starch content	1						
Lipid content	–0.22*	1					
Protein content	–0.85***	–0.08	1				
Cellulose	–0.87***	0.25*	0.91***	1			
Vitreousness	–0.30**	0.19	0.30**	0.31**	1		
Semolina Y	–0.81***	0.45**	0.77***	0.83***	0.50**	1	
TGW	–0.05	0.38*	0.08	0.06	0.19	0.09	1
GWE	0.01	0.17	0.05	0.01	0.26*	0.01	0.65***

* Significant (P=0.05); ** very significant (P=0.01); *** highly significant (P=0.001)

anonymous probes (encoded umc and bnl) served as anchors to compare with the already published maps.

Statistical calculations and QTL detection

Correlations between traits were computed using the PROC CORR procedure of SAS (SAS Institute 1988). Associations between genotypes at marker loci and trait values were first assessed by single-factor analysis of variance for protein, lipid and cellulose contents, in addition to vitreousness and semolina yield, using the SAS software (SAS Institute 1988). In addition, QTLs were detected by composite interval mapping (MQTL-CIM by Tinker and Mather 1995). MQTL-CIM used markers as co-factors, selected on the basis of their partial R^2 value in a stepwise regression, to account for variability in genome regions external to the region tested for the presence of a QTL. This enabled a greater power for detection and a better resolution. The number of co-factors must be limited in order to minimize false-positive detection. Simulations showed that a maximum of ten co-factors with a partial R^2 above 0.03 appeared to be convenient for a RIL population of about 100 individuals (Leonardi, in preparation). We chose 5–9 co-factors, the cut off being placed just before a drop in the partial R^2 values in the stepwise regression. A test statistic was computed every 2 cM as a function of the ratio of the residual sums of squares of the full model to the residual sums of squares of the model without the effect being tested. The distribution of this statistic was empirically assessed using a permutation test (1000 permutations), and a threshold corresponding to a 5% type-I error for the whole genome was determined (Churchill and Doerge 1994). The initial value of the statistics was then divided by this threshold, and the ratio was plotted along the chromosomes. Maxima with peaks over 1 were considered as the most-probable location of QTLs. Confidence intervals for QTL position cannot easily be determined (Mangin and Goffinet 1997); however, simulation studies (Leonardi, personal communication) showed that 10–20 cM is a likely range. Each QTL was also characterized by its effect (% variation of the trait explained, R^2) and by the parental allele leading to the highest value of the trait.

Epistasis was checked using a home-made program based on ANOVA, fitting a model including main-effect QTLs and interactions between all pairs of markers (Leonardi, personal communication). A stringent type-I error risk (P<0.0005) was chosen to test every interaction, in order to minimize the number of false positives over all the interactions tested. A significant interaction between markers revealed two interaction QTLs in the vicinity of these markers. The model combining the main-effect QTLs and the combination of interaction QTLs that yielded the highest R^2 was considered as providing the best set of genetic factors accounting for the trait variability. Due the method used for interaction detection (ANOVA), interaction QTLs are placed at marker loci and not in-between them as for MQTL.

Results

Trait variation and correlation

The two parental lines were different for all the measured traits (Table 2). Lipid, protein and cellulose contents, vitreousness, semolina yield and TGW, were higher in F-2 than Io, but starch content and GWE were lower in F-2 than in Io. For all traits examined in the RILs, one-way analysis of variance showed a significant genetic effect. The frequency distribution of every trait fitted with a normal distribution. The extreme phenotype values of some individual lines were higher than the highest parent value or lower than the lowest parent value, revealing transgression. Such variation has been well-documented in many species for several traits. It results from the inheritance of complementary allelic effects from the two parents (de Vicente and Tanksley 1993). Segregants with a high phenotype value generally accumulated most of the favorable alleles at the detected QTLs, while individuals with a low phenotype value inherited most of the unfavorable ones.

Examination of phenotypic correlations between traits (Table 3) showed that starch content was negatively corre-

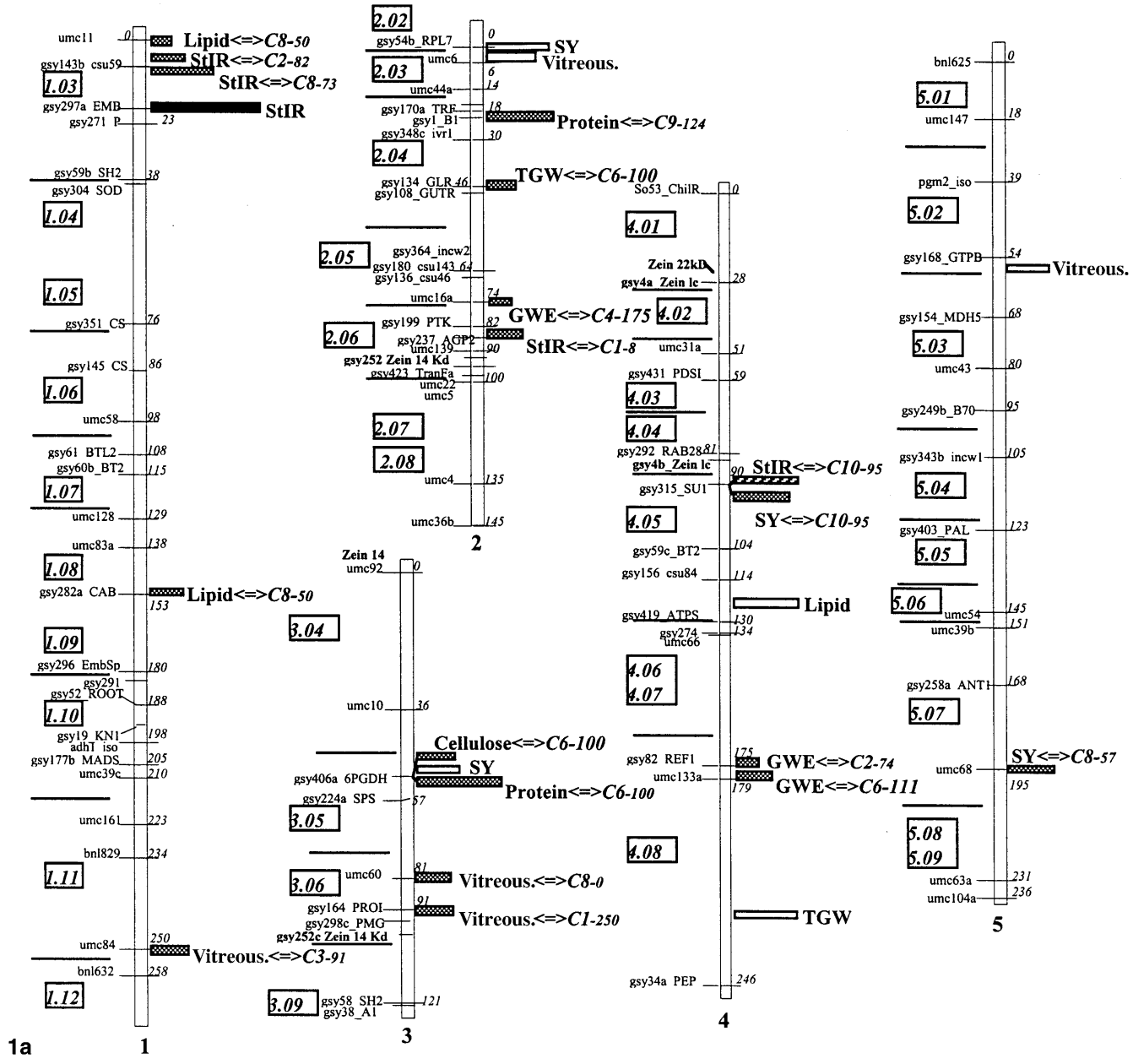
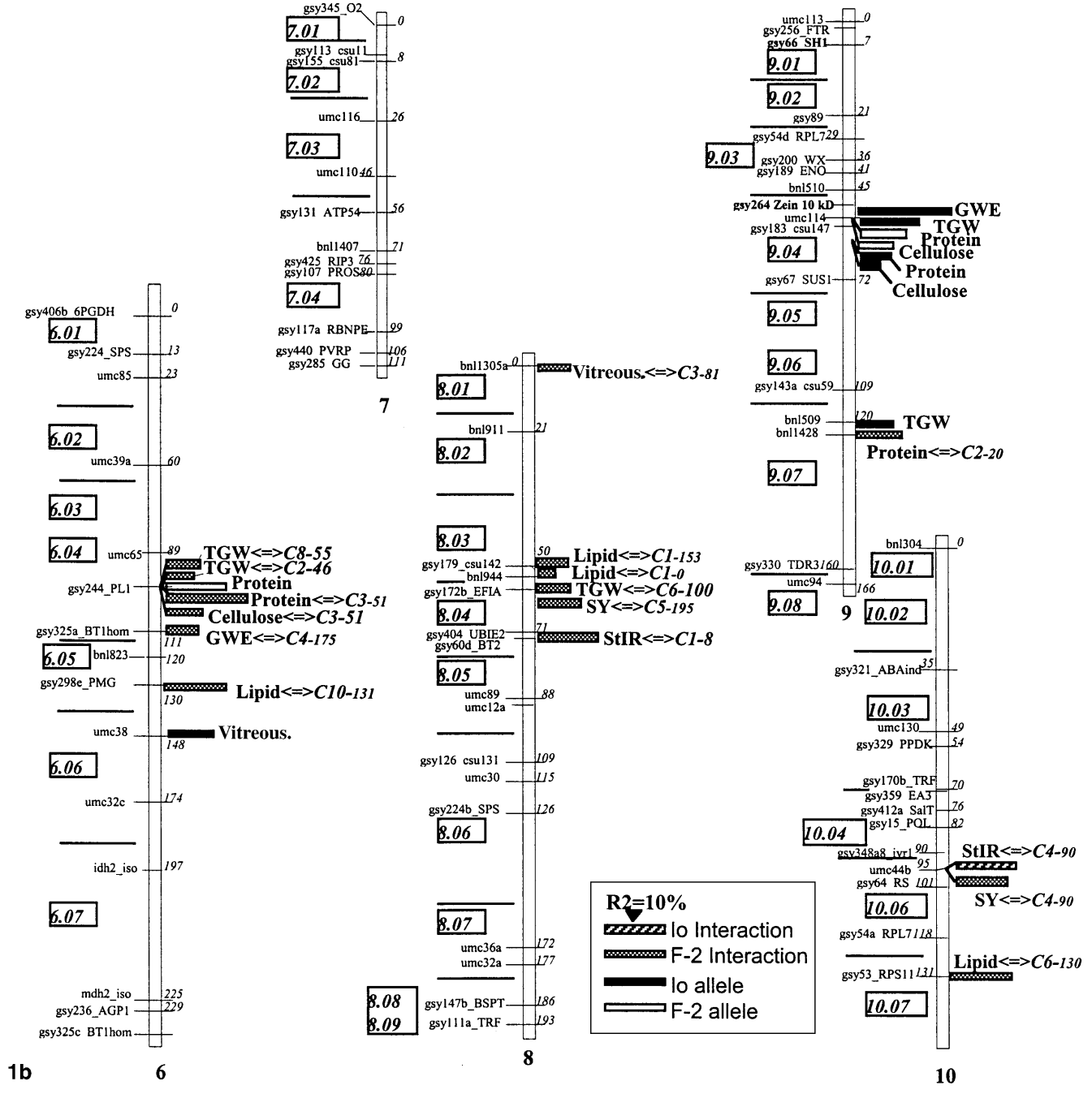


Fig 1a,b Mapping of QTLs, by MQTL, for Protein, Starch (StIR), Lipid, Cellulose and semolina yield (SY) determined by NIRS, vitreousness (Vitreous.) determined by flint/floury ratio, 1000-grain weight (TGW) and grain weight by ear (GWE) in maize grains grown in 1994. All composition values are expressed as % kernel dry weight. The recombinant inbred lines originated from the Io and F-2 parent lines (8th generation). Main-effect QTLs are displayed by *black bars* when the favorable effect originated from the Io parent, and by *open bars* for the F-2 parent. Interaction QTLs are represented by *stippled bars* for the Io allele effect, and *dense dotted bars* for the F-2 allele effect. For each locus, the position of the other pair member is given, after the double arrow, in *italics* by chromosome # and distance in cM from the top. Whatever the QTL type the bar length is proportional to the R^2 value. The full description of marker names placed along each chromosome is reported in Causse et al. (1996). Genetic distances are given in centiMorgans (cM) from the top of each chromosome. Bin position has been placed using anchor markers from the University of Missouri Core map

lated to most of the traits, especially to protein and cellulose contents, and semolina yield, with r values ranging from –0.81 to –0.87. Protein and lipid contents were not correlated. Vitreousness measured as the flint/floury endosperm ratio is highly significantly correlated to semolina yield (r=0.50), and slightly correlated to protein amount (r=0.3). By contrast, semolina yield is negatively correlated to starch (r=–0.81) and cellulose (r=–0.83) contents and positively to lipid (r=0.45) and protein (r=0.77) contents.

The grain-weight per ear (GWE) is an essential yield component; it depends on two factors, grain weight and grain-number per ear. In the RIL population, a positive highly significant correlation (r=0.65) was found between GWE and TGW (1000-grain weight), and showed the importance of grain size for yield (Table 3). However, this correlation explained only a part (R^2=42%) of the GWE



variability, which could explain why elevated grain weight is not always associated with a higher grain yield per ear. None of these two yield-parameters were correlated with either protein, starch or cellulose amounts in RIL grains.

QTL detection

For each trait, the main-effect QTLs, which have been detected by MQTL, and the interaction QTLs, which have been detected by subsequent ANOVA, are represented on the genetic map (Fig. 1); the length of each bar is proportional to the percentage of total variation explanation and allele effects are represented by solid or empty bars depending on parent origin.

For each trait, 1–3 main-effect QTLs and a similar number of significant interactions were detected. The number of main-effect QTLs was lower than that obtained by one-way ANOVA detection at a 0.01 threshold. Apart from possible threshold differences between both detection methods, the reduction in the number of QTLs also originated from the limited co-factor number used in MQTL that minimizes false positive detection (Leonardi, in preparation). However, part of the QTLs undetected by MQTL re-appeared when taking interactions into account (data not shown).

Starch content

Only one main-effect QTL was detected near the end of the short arm of chromosome 1, explaining 17% of the total variation (Fig. 1). Close to this position, two starch-interaction QTLs were associated with either a locus in the medial part of chromosome 2 (gsy199_PTK) or a locus on chromosome 8 (gsy60d_BT2). A third pair of interaction QTLs was detected between chromosomes 4 and 10 (gsy315_SU1 and umc44b loci). The Io genotype provided the favorable alleles at the main-effect QTL (Fig. 1) and at the interaction QTLs on chromosomes 4 and 10, while it was the F-2 genotype at the chromosomes 1–2 and 1–8 interaction QTLs.

Lipid content

As for starch content, one main-effect QTL and three pairs of interaction QTLs were detected, with R^2 values ranging from 10 to 3%. The main-effect QTL was located in the middle of chromosome 4, near gsy419_ATPS, in a zone where no other QTL was noted (Fig. 1). By contrast, interaction QTLs between chromosomes 1 and 8 (couples C1-umc11_C8-gsy179 and C1-gsy282a_C8-gsy179), and between chromosomes 6 and 10 (C6-gsy298e_C10-gsy53), clustered with QTLs for other traits (see chromosomes 1, 6 and 8). The favorable alleles for high oil content all came from the F-2 genotype (Fig. 1).

Protein and cellulose contents

The two main-effect QTLs for protein content accounted for 7.6 and 5.1% of the total variation. They were located very close to each other on chromosome 9 (umc114 and gsy183) but presented opposite allele effects (Fig. 1). Such detection of dual opposite QTLs is specifically allowed by MQTL, and several occurrences were noted previously with other traits (Séne et al. 2000). Two pairs of interaction QTLs were observed, the first one between the short-arm end of chromosome 2 (gsy1_B1) and the second third-part of chromosome 9 (bnl1428), and the second one between the medial zone of chromosome 3 (gsy406a_6PGDH) and the second third of chromosome 6 (gsy244). The R^2 values for the interaction QTLs were relatively high (10.5 and 13.3%), respectively. The favorable alleles for both came from F-2 (Fig. 1).

The two cellulose-content main-effect QTLs (R^2=5.8 and 8.3%) were mapped at the same loci as the protein-content QTLs on chromosome 9, with the same opposite allele effect (Fig. 1). One cellulose-content interaction QTL (R^2=6%) was observed between chromosomes 3 (gsy406a_6PGDH) and 6 (gsy244_PL1), also co-locating with interaction QTLs for protein content. These tight co-locations and identical allele effects were consistent with the high value of the correlation between these two traits.

Technological properties

The variability in predicted semolina yield (SY) was accounted for by two main-effect (R^2=10.0 and 6.4%) and two pairs of interaction QTLs (R^2=7.3 and 8.6%) explaining 32.3% of the total variation (Fig. 1). The main-effect QTLs were located at the end of chromosome 2 (gsy54b_RPL7) and in the middle of chromosome 3 (gsy406a_6PGDH). Interaction QTLs were detected: (1) between umc68 (chromosome 5) and gsy172b_EFIA (chromosome 8, first third), and (2) between gsy315_SU1 (chromosome 4, medium) and umc44b (chromosome 10, last third). The locus on chromosome 8 (gsy179b) was shared by two other interaction QTLs for lipid content and grain weight, respectively. The favorable alleles were provided by the F-2 genotype for all the QTLs.

The vitreousness was measured independent of the previous trait since it was estimated from the ratio of areas of flint to floury endosperm on kernel sections. Three main-effect QTLs were detected: at the end of chromosome 2 (gsy54b_RPL7), the first quarter of chromosome 5 (gsy168_GTPB) and the middle of chromosome 6 (umc38); R^2 values were 7–8%. In addition, two pairs of interaction QTLs, between chromosomes 1 and 3 (C1-umc84_C3-gsy164), and between chromosomes 3 and 8 (C3-umc60_C8-bnl1305a), accounted for 5.5 and 6% of the variation. Taken together, all vitreousness QTLs accounted for 40.6% of the total phenotypic variation. Alleles associated with high vitreousness were carried by the F-2 genotype for all QTLs, except the main-effect QTL on chromosome 6. This result is consistent with the fact that F-2 is a flint genotype, compared to Io which is a dent genotype, and with the transgression observed in the RILs.

Kernel agronomic traits

Grain-weight per ear (GWE) variability was explained by one main-effect QTL on chromosome 9 (umc114) accounting for 15% of the variation (Fig. 1). This QTL clustered with protein and cellulose content, and 1000-grain weight (TGW), QTLs. The two pairs of interaction QTLs shared a locus on chromosome 4 (gsy82_REF1) which interacted with either chromosome 6 (gsy325a_BT1) or chromosome 2 (umc16a), each interaction accounting for 5.5 and 3.3%, respectively. The gsy 325a_BT1 locus is close to the gsy244 locus where a cluster of composition- and agronomic-trait QTLs was observed. The favorable alleles originated from the Io genotype for the main-effect QTL whereas they came from the F-2 genotype for the two pairs of interaction QTLs (data not shown).

For 1000-grain weight (TGW), three main-effect QTLs, with R^2 values ranging from 6 to 9%, were detected on chromosome 4 between the umc133a and gsy 34a_PEP markers (at 215 cM) and on chromosome 9 at two distant loci (umc114 and bnl509). The favorable alleles for the main-effect QTLs originated from the F-2 genotype for the QTL on chromosome 4, whereas they

originated from the Io genotype for those on chromosome 9. The QTL at the umc114 locus on chromosome 9 was associated with other QTLs for GWE, protein and cellulose contents. The two pairs of interaction QTLs shared a locus (gsy244_PL1) on chromosome 6, which was associated with either chromosome 2 at gsy134_GLR or chromosome 8 at bnl944.

Finally, on chromosome 6 an important QTL cluster was observed for agronomic traits and grain composition at the locus gsy244_PL1, with another one for agronomic traits and lipid content and semolina yield on chromosome 8.

Discussion

Number of QTLs and the percentage of phenotypic variation explained

The number of main-effect QTLs detected by MQTL-CIM ranged from 1 to 3 per trait as did the number of pairs of interaction QTLs, and in such a way that the total number of main-effect QTLs was about half that for interactions: 15 vs 34, respectively. The percentage of phenotypic variation explained per trait ranged from 21 to 41%, the interaction QTLs accounting for more than half of the total. Such a high number of interactions was not obtained using ANOVA detection. Less QTLs were detected by MQTL-CIM, and comparisons showed that undetected QTLs frequently re-appeared as interaction QTLs. For most of the traits favorable alleles originated mainly from the F-2 genotype, with the exception of grain-weight QTLs (TGW, GWE) which originated from the Io genotype. However, alleles from both genotypes frequently contributed to trait QTLs, which is consistent with the transgression observed in RILs (Table 2).

QTL co-locations and correlations between traits

The observed QTLs were not evenly distributed over the map and they constituted several apparent clusters, as also noted for developmental traits by Khavkin and Coe (1997). Assuming a 10–20 cM confidence interval for QTL position, we considered that sets of QTLs for different traits mapped within this interval as clusters. Using that threshold, the marker composition of these clusters was examined in a search for possible candidates, located at the same loci as the QTLs, and being able to participate in the control of trait expression. Such clusters, associating more than three QTLs for different traits, were evident at six map positions, namely on chromosomes 1, 2, 3, 6, 8 and 9. Frequently (4/6 instances), main-effect QTLs and interaction QTLs were intermixed. These QTL co-locations should be first discussed in the light of phenotypic correlations since common QTL locations have been widely reported for correlated traits (Velboom and Lee 1994; Xiao et al. 1996). QTL co-location may correspond either to the pleiotropic effect of a single gene or to a tight linkage of several genes involved in regulation of the traits.

TGW and GWE are complementary and positively correlated components of grain yield (Table 3). They shared two QTLs, on chromosome 6 at 100 cM, close to gsy244, and on chromosome 9 close to umc114. It is noteworthy that close to the same marker positions (at C6-umc65 and at C9-umc114), Austin and Lee (1998) observed a kernel-weight QTL. Otherwise, the TGW QTL was also co-located with a lipid-content QTL on chromosome 8 (57 cM) and with a protein-content QTL on chromosome 9 (at 53 and 122 cM); while TGW and lipid content were correlated (r=0.38) TGW and protein content were not.

The negative correlation between starch, protein and lipid contents was consistent with previous observations on lines selected for high vs low protein content or high vs low oil content, which presented opposite alterations in starch content (IHP/ILP and IHO/ILO strains in Dudley and Lambert 1992; Goldman et al. 1993; Berke and Rocheford 1995). Starch also appeared negatively correlated to cellulose content and semolina yield (Table 3). Despite these correlations, few co-locations appeared between the main-effect QTLs for starch and other traits. One of the reasons may be that only one main-effect QTL was detected for starch, possibly because of the low variability of this trait (Table 2). More co-locations showed up between interaction QTLs for starch and other traits. The most-significant ones were on chromosome 1 with a lipid-content QTL, on chromosome 2 with a GWE QTL, and on chromosomes 4 and 10 with semolina yield QTLs.

Protein content was highly correlated with cellulose content, semolina yield and to a lesser extent with vitreousness (Table 3). Accordingly, numerous co-locations were observed between protein-content QTLs and QTLs for vitreousness and semolina yield on chromosome 2; for cellulose, protein contents and semolina yield on chromosome 3; for grain weight, protein and cellulose contents on chromosome 6; for lipid content, grain weight and semolina yield, on chromosome 8; for grain-weight traits and cellulose content, on chromosome 9. No chromosomal region was significantly associated with QTLs for both starch and protein content, or for starch, lipid and protein contents. This means that the regulation of the biosynthesis of these three components should be partly independent.

The two technological traits semolina yield and vitreousness were measured by two very different methods: IR estimation on the one hand, and direct measurement of the flint/floury area ratio on the other hand. These two traits were highly significantly correlated (r=0.50), and a QTL co-location was detected on chromosome 2. This locus seems very consistent with the observation of Rami (1999) who obtained a QTL for kernel friability (NIRS estimate) at the same umc6 locus, using a different population grown at two sites in the South of France. A kernel-friability QTL was located on chromosome 5 (Bin 5.06) at 55 cM from the present vitreousness QTL

(Bin 5.03) which is likely to be too far away to be significant. Another feature of the chromosome-2 cluster was the association of two technological QTLs with a protein-content QTL. Many workers have reported a tight correlation between protein amount and vitreousness or semolina yield, in non-parental lines (Mestres and Matencio 1996). In addition, the present study also showed a significant phenotypic negative correlation (r=–0.81) between starch content and semolina yield. This is consistent with the identification of genomic regions on chromosomes 4 and 10 where interaction QTLs for the two traits co-localized with inverse allele effects.

QTL mapping in the vicinity of candidate genes

The identification of a gene whose allelic variations allow QTL detection is an important step toward the understanding of complex-trait expression. It may also provide a more-precise marker for marker-assisted selection. One way to grasp this question is to look for co-locations between QTLs and genes known to be involved in the trait under study (Prioul et al. 1999). This approach is greatly facilitated when the map consists of known function genes, as in the present case (Causse et al. 1996). In addition these markers were placed on the comprehensive map provided by the University of Missouri (maize DB at www.agron.missouri.edu) so that other candidate genes, pertinent mutations or previously described QTLs, may be found at the same Bin position (Fig. 1). A main limitation is that the molecular bases for the presently analyzed traits are not well known, except for starch and protein amounts. In this view the kernel-deficient mutants (dek), especially those affecting kernel hardness or starch aspects, may like the floury (fl) mutants be meaningful (see Neuffer et al. 1997). In the present study, two GWE interaction-QTLs mapped on chromosome 4 (179 cM) at umc133a (Bin 4.08), in the vicinity of two mutation-loci (*dek31* and *opaque endospem1*, Bin 4.07). Furthermore, QTLs for kernel weight were also detected at similar location on chromosome 4 (qgrwt2 QTL, Bin 4.08) by Berke and Rocheford (1995) in a population derived from IHP×ILO.

Possible candidate genes for grain protein-content QTLs are those encoding zeins and globulins. Several zein genes were located on the map, on chromosomes 2 (95 cM), 3 (0 and 95 cM), 4 (28 and 85 cM) and 9 (55 cM). In addition, two globulin loci have been reported on chromosomes 1 and 2 (Bin 1.12 and 2.04). Three of the zein loci were closely linked to QTLs for vitreousness (chromosome 3, Bin 3.06), for semolina yield and starch content (chromosome 4, Bin 4.04), and for the large chromosome-9 cluster comprising grain-weight trait QTLs and protein- and cellulose-content QTLs (Bin 9.04). At these three loci, other co-locations with genes of interest were noted. At Bin 3.06, a kernel-weight QTL (q399k27) was reported by Austin and Lee (1996). At Bin 4.04, a *floury* mutation (*fl2*), leading to a soft and opaque endosperm, and a reduced zein production was described (Neuffer et al. 1997). Around the chromosome-9 QTL cluster two other genes, *waxy* and *Sus1*, mapping very close together, are possible candidates since they code for enzymes involved in starch metabolism, namely granule-bound starch synthase and sucrose synthase. Moreover, several grain-weight QTLs have been reported in the same 9.03–9.04 region (Schon et al. 1994 and maize DB). For the chromosome-6 and –8 QTL clusters several candidate genes may also be suggested: *BT1* (111 cM, chromosome 6), encoding an ADP glucose transporter in the amyloplast envelope, and *BT2* (73 cM, chromosome 8), encoding an ADP glucose pyrophosphorylase subunit. In addition, a delta zein gene (*dzs23*, 6.04) and a kernel-weight QTL (*q1000k5*, 6.05) co-located with protein-content and TGW QTLs, respectively, on chromosome 6. Similarly, on chromosome 8, the TGW interaction QTL was in the vicinity of a *dek29* locus (8.04) and a kernel-weight QTL (*q1000k7*, 8.03). On the chromosome-2 telomere, a few known function genes were also mapped. A globulin locus (*glb*, 2.04) was co-located with a protein-content interaction QTL (gsy1, 20 cM), but no obvious candidate showed up for the technological kernel-property QTLs although this region appeared consistent with Rami's work (1999). The mapping in the same region of several mutations leading to opaque, soft or etched endosperm or to kernel deficiency (*dek3*, *os1*, *sens5*, *et2*, *fl-N1426*, *dks8*, Bin 2.0–2.2, Neuffer et al. 1997 and maize DB) could provide a clue. A similar absence of obvious candidates occurred for lipid- and starch-content QTLs at the end of chromosome 1. A *dek1* mutation was also located in this region. A genomic analysis of these two regions would be worthwhile in order to identify new candidate genes.

Conclusion

A QTL approach, applied to grain composition and technical properties, yielded numerous loci, which tended to form clusters. Around cluster positions, loci of candidate genes, of mutations, or even QTLs for traits related to those analyzed in the present study, were identified. For example, three zein gene loci co-located with protein-content, semolina yield and kernel-weight QTLs, and the chromosome-9 cluster mapped near a region shown to be important for the regulation of starch accumulation in the maize kernel.

Acknowledgments We are grateful to Dr. Agnès Leonardi and to Dr. Catherine Damerval for their help in the use of QTL detection methods and their comments on the manuscript.

References

Ajmon-Marsan P, G, Ludwig WF, Melchinger AE, Franceschini P, Pagnotto G, Motto M (1995) In an elite cross of maize a major quantitative trait locus controls one-fourth of the genetic variation for grain yield. Theor Appl Genet 90:415–424

Austin DF, Lee M (1996) Comparative mapping in F-2:3 and F-6:7 generations of quantitative trait loci for grain yield and yield components in maize. Theor Appl Genet 92:817–26

Azanza F, Tadmor Y, Klein BP, Rocheford TR, Juvik JA (1996) Quantitative trait loci influencing chemical and sensory characteristics of eating quality in sweet corn. Genome 39:40–50

Berke TG, Rocheford TR (1995) Quantitative trait loci for flowering, plant and ear height, and kernel traits in maize. Crop Sci 35: 1542–1549

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

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

Champoux MC, Wang G, Sarkarung S, Mackill DJ, Otoole JC, Huang N, McCouch SR (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor Appl Genet 90:969–981

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

Dudley JW, Lambert RJ (1992) Ninety generations of selection for oil and protein in maize. Maydica 37:1–7

Edwards MD, Helentjaris T, Wright S, Stuber CW (1992) Molecular-marker-facilitated investigations of quantitative-trait loci in maize. 4. Analysis based on genome saturation with isozyme and restriction fragment length polymorphism markers. Theor Appl Genet 83:765–774

Goldman IL, Rocheford TR, Dudley JW (1993) Quantitative trait loci influencing protein and starch concentration in the Illinois long term selection maize strains. Theor Appl Genet 87:217–224

Khavkin E, Coe E (1997) Mapped genomic locations for developmental functions and QTLs reflect concerted groups in maize (*Zea mays*). Theor Appl Genet 95:343–352

Louis-Alexandre A, Mestres C, Faure J (1991) Measurements of endosperm vitreousness of corn: a quantitative method and its application to African cultivars. Cereal Chem 68:614–617

Mangin B, Goffinet B (1997) Comparison of several confidence intervals for QTL location. Heredity 78:345–353

Mestres C, Matencio F (1996) Biochemical basis of kernel milling characteristics and endosperm vitrousness of maize. J Cereal Sci 24:283–290

Neuffer MG, Coe EH, Wessler SR (1997) Mutants of maize. Cold Spring Harbor Laboratory Press, USA

Paterson AH, Lander ES, Hewitt JD, Paterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. Nature 33:721–726

Prioul JL, Pelleschi S, Séne M, Thevenot C, Causse M, de Vienne D, Leonardi A (1999) From QTLs for enzyme activity to candidate genes in maize. J Exp Bot 50:1281–1288

Rami JF (1999) Etude des facteurs génétiques impliquées dans la qualité technologique du grain chez le maïs et le sorgho. Thèse Université de Paris XI Orsay

Rami JF, Dufour P, Trouche G, Fliedel G, Mestres C, Davrieux F, Blanchard P, Hamon P (1998) Quantitative trait loci for grain quality, productivity, morphological and agronomical traits in sorghum (*Sorghum bicolor* L. Moench). Theor Appl Genet 97:605–616

SAS Institute (1988) SAS user's guide: statistics, version 6. SAS Institute, Cary, North Carolina

Schon CC, Melchinger AE, Boppenmaier J, Brunklaus-Jung E, Herrmann RG, Seitzer JF (1994) RFLP mapping in maize: quantitative trait loci affecting test-cross performance of elite European flint lines. Crop Sci 34:378–389

Séne M, Causse M, Damerval C, Thévenot C, Prioul JL (2000) Quantitative trait loci affecting amylose, amylopectin and starch content in maize recombinant inbred lines. Plant Physiol Biochem 38:459–472

Sourdille P, Perretant MR, Charmet G, Leroy P, Gautier MF, Joudrier P, Nelson Jc, Sorrells ME, Bernard M (1996) Linkage between RFLP markers and genes affecting kernel hardness in wheat. Theor Appl Genet 93:580–586

Stuber CW (1995) Mapping and manipulating quantitative traits in maize. Trends Genet 11:477–481

Teulat B, This D, Khairallah M, Borries C, Ragot C, Sourdille P, Leroy P, Monneveux P, Charrier A (1998) Several QTLs involved in osmotic adjustment trait variation in barley (*Hordeum vulgare* L.). Theor Appl Genet 96:688–698

Tinker NA, Mather DE (1995) MQTL: software for simplified composite interval mapping of QTLs in multiple environments. JQTL 1: 2 (Electronic Journal, access at: http://probe.nalusda.gov:8000/otherdocs/jqtl/)

Velboom LR, Lee M (1994) Molecular-marker facilitated studies of morphological traits in maize. 2. Determination of QTLs for grain yield and yield components. Theor Appl Genet 89: 451–458

Vicente de MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134: 585–596

Xiao J, Li J, Yuan L, Tanksley SD (1996) Identification of QTLs affecting traits of agronomic importance in a recombinant inbred population derived from a sub-specific rice cross. Theor Appl Genet 92:230–244