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QTL analysis of bread-making quality in wheat using a doubled haploid population

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Abstract A set of 187 doubled haploid lines derived from the cross between cvs. Courtot and Chinese Spring was explored for QTLs for three bread-making quality tests: hardness, protein content and strength of the dough (W of alveograph). The scores of the parental lines were quite different except for protein content, and the population showed a wide range of variation. About 350 molecular and biochemical markers were used to establish the genetic map, and technological criteria were evaluated in 1 to 3 years. QTL detection was performed by the "marker regression" method. The most significant unlinked markers were used in the model as covariates, and the results were tested by bootstrap resampling. For hardness, we confirmed a previously tagged major QTL on chromosome 5DS, and two additional minor QTLs were found on chromosome 1A and 6D, respectively. For protein content two main QTLs were identified on chromosomes 1B and 6A, respectively. For W, three consistent QTLs were detected: two at the same location as those for hardness, on chromosomes 1A and 5D; the third one on chromosome 3B. Therefore, it appeared that except for the Glu-1A locus, storage protein loci were not clearly involved in the genetic control of the criteria studied in the present work. Despite the reasonable size of the population no QTL with interactive effects could be substantially established as measured. All computations were carried out using home-made programmes in Splus language, and these are available upon request.

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Introduction

The end-use quality of bread wheat (*Triticum aestivum L.*) is a complex character influenced by both genetic factors and plant growth conditions (Rousset et al. 1992; Peterson et al. 1998). With respect to this characteristic, a range of small-scale tests are available, particularly for predicting bread-making quality in early generations of breeding. Most of these tests are correlated to each other and allow the prediction of a significant part of the bread-making score (Branlard et al. 1991). The gluten proteins and the polymorphism of their coding alleles have been well-described (Payne and Lawrence 1983; Payne et al. 1987; Rogers et al. 1989; Gupta and Shepherd 1990; Carillo et al. 1990; Metakovsky 1991) and these are known to account for a part of the range in bread-making ability, depending of the fraction involved (Gupta et al. 1989; Khelifi and Branlard 1992; Nieto-Taladriz et al. 1994). Nevertheless, other components of the kernel, such as pentosans and enzymes (Roels et al. 1993; Poutanen 1997), and therefore other genetic factors, are likely to be involved in various aspects of wheat end-use quality and its genetics, as indicated by Zemetra et al. (1987) and Mansur et al. (1990) in studies on substitution lines sets. Quantitative trait loci (QTLs) analyses of end-use quality traits have been reported from crosses between wheat and related species (Zanetti et al. 1998) or in tetraploids wheats (Blanco et al. 1998). Zanetti et al. (1998) described three major QTLs on chromosome 5A for protein content, Zeleny and falling number and mentioned others on chromosome 1A [low-molecular-weight (LMW) glutenin subunit] and on the homoeologous group 6 and 7 chromosomes. Blanco et al. (1998) found a major effect of the Gli-B1/Glu-B3 loci and significant effects of loci located on chromosomes 1AL, 3AS, 3BL, 5AL, 6AL and 7BS on sedimentation volume. QTLs for soluble pentosans and associated components were recently mapped in two progenies of hexaploid wheats (Martinant et al. 1998). However no QTL analysis has yet been reported for rheological dough properties. The aim of the study reported here was to map and characterise QTL associated with small-scale tests usually performed in bread-wheat breeding.

Materials and methods

Genetic materials and field experiment

A total of 187 doubled haploid (DH) lines (Félix et al. 1996) derived by anther culture from the cross between the French bread wheat cv. Courtot and cv. Chinese Spring were used in our study. In 1993 and 1994, 85 and 165 DH lines, respectively, were grown in a randomised trial without replication at the INRA station in Clermont-Ferrand, France. Each plot consisted of three rows, 1.5-m-long, with 30 plants per row and 25 cm between rows. In autumn 1995, 143 DH lines were sown in a replicated randomised complete block design trial at the same location with two replicates. Each plot consisted of six rows as described above. Parental lines were included in each trial. As the lines evaluated in 1994, 1995 and 1996 were overlapping subsets, a total set of 169 DH lines was evaluated in at least 1 year. Missing data for 18 lines were caused by poor agronomic adaptation (mainly lodging and sterility). Plants were grown under normal field conditions with fungicide applications to control rusts and powdery mildew. Grain was harvested at maturity, and technological analyses (described below) were performed during the following winters.

Molecular and biochemical markers – mapping analysis

The genetic linkage map was developed using mainly restriction fragment length polymorphism (RFLP) markers (Cadalen et al. 1997) but also included storage protein loci, glutenins and gliadins (Félix et al. 1996), microsatellites and amplified fragment length polymorphism (AFLP) markers (Tixier et al. 1998). According to the method described by Singh et al. (1991), five polymorphic markers, either single or complex, corresponding to the five storage protein loci Glu-A1, Gli-A5, Gli-A1, Gli-B1 and Gli-D1 were detected. The map covered nearly 2900 cM, and most of the 21 chromosomes were saturated except for chromosomes from the D genome, in particular chromosomes 1D and 4D. Linkage analysis was performed using MAPMAKER/EXP 3.06 (Lander et al. 1987), and linkage groups were assigned to chromosomes using monosomic hybrids obtained from the cross between reciprocal monosomic lines of cvs. Courtot and Chinese Spring.

Technological analyses

Kernels from each DH line at each replication were milled in a Chopin Dubois mill (CD1). Flour of each sample (50 g) was used for the microalveograph test, performed according to AACC method 54–30 (American Association of Cereal Chemists 1995) adapted to the micro-test, which allowed the measurement of tenacity (PMAX, in mm), extensibility (L, in mm), P/L and strength (W, in J 10^{-4}).

SDS sedimentation volume, (SDS, in ml) was evaluated on whole flour according to the method described by Axford et al. (1978) adapted to a mechanical shaker.

Protein content (PROT, in percentage) and hardness (HARD, in percentage) were evaluated by near-infrared reflectance spectroscopy (NIR) using an Inframatic 8620 system (Perten, France). For protein content, the NIR instrument was calibrated with a set of at least 60 cultivars, as described in the AACC method 39–10; the reference values of nitrogen content were obtained by the Kjeldhal method. For kernel hardness, reflectance was measured at two wavelengths (1680 and 2230 nm), and the NIR instrument was calibrated according to the manufacturer's indications with two sets of hard and soft samples.

Statistical analysis

Because of our unbalanced design, we first performed a statistical analysis to justify using the whole data set. A two-way (genotype and block) analysis of variance performed on data obtained from the 1996 harvest provided an estimate of the true residual variance, which was further used to test genotype × year interactions. As genotype × year interactions were not significant at the 0.05 threshold for PMAX, L, W and PROT, and at the 0.01 threshold for HARD, we used the adjusted least-square genotype means provided by the GLM procedure, option LSMEANS, from SAS (SAS Institute 1991). Hence a data set of 169 DH lines from the 3-year trial was available for stable QTL detection. A principal component analysis was performed to identify a subset of nearly uncorrelated variables.

Associations between markers and kernel hardness, protein content and W value of alveograph were first investigated through a one-way ANOVA. The locations and effects of every putative QTL were then evaluated on the whole genome using the ‘‘marker regression’’ method as proposed by Kearsey and Hyne (1994) for a one-QTL model and Hyne and Kearsey (1995) for two-QTLs models. These methods were slightly modified to use the reverse of the Kosambi mapping function (Kosambi 1944) in the prediction of QTL effects along the linkage group in order to better fit the actual recombination frequency between markers. Moreover, when scanning a given chromosome, we included the most significant (at $P = 0.005$), unlinked markers from the other linkage groups in the model as covariates in order to reduce the residual variance and thus increase detection power and accuracy, as suggested by Jansen and Stam (1994). All computations were carried out using specialised programmes in the Splus language (Splus 1993). Owing to the coding of genotypic data, QTLs additive values were positive if the favourable allele came from cv. Courtot and negative if it came from cv. Chinese Spring. Confidence intervals of QTL location and effect were estimated through 500 bootstrap resamplings for the one-QTL model and 100 bootstrap resamplings for the two-QTLs model (Vissher et al. 1996).

Significant interactions between pairs of markers were also explored systematically through ANOVA. As there were so many correlated statistical tests, a threshold could not be fixed *a priori* in order to keep a global type-I error below 5%. Instead, we plotted the ordered, observed interaction F values against a set of simulated values from a Fisher law with appropriate degrees of freedom. A bi-dimensional scan of the 2 chromosomes where interactive markers had been identified was then performed using our adaptation of the marker regression approach (Charmet et al. 1998), and confidence intervals for QTLs location and effect were constructed from 100 bootstrap resamplings.

Results

The results of the principal component analysis (PCA) identified two groups of variables: W, hardness and SDS were highly correlated to PC1, while PROT and P/L were highly correlated to PC2 (Fig. 1). Consequently, we focused our QTL approach on W and protein content, i.e. one trait of each independent component. However, as hardness is known to affect W (Branlard et al. 1997), it was also included in the analysis. Figure 2 shows the distributions of the DH lines as well as parental scores with respect to energy for dough deformation (W of alveograph), kernel protein content and kernel hardness. The bimodal shape of the distribution of kernel hardness

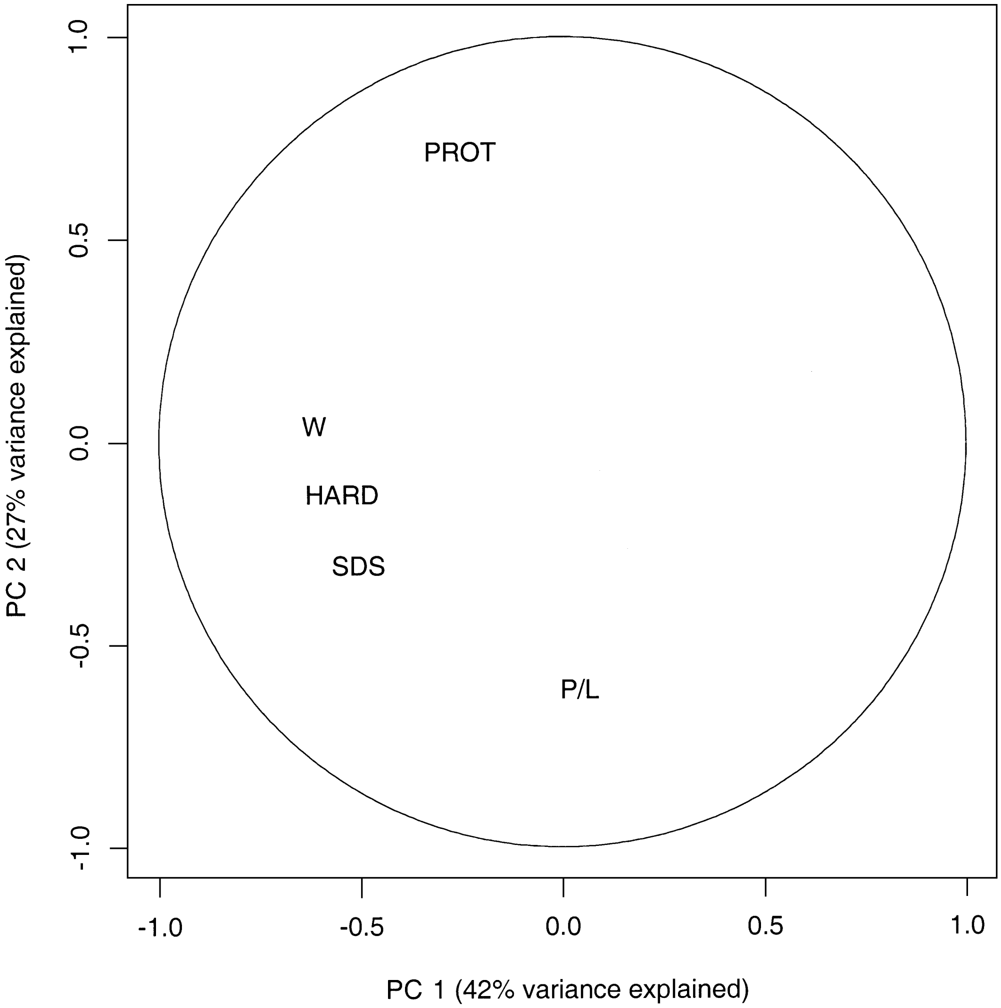

Fig. 1 Graphical display of the first two axes of the principal component analysis of five quality traits

Table 1 Parental values, range of DH lines values for eight quality-related traits[a] (least significant means of 3-year experiments, see text)

	Range	Courtot	Chinese Spring
Pmax (mm)	33.1–147.6	89.3	82.9
L (mm)	31.4–138.2	95.1	47.2
P/L	0.02–4.21	0.99	1.92
W (J 10^{-4})	75.2–360.2	237.9	130.5
Prot (%)	11.3–17.1	14.0	14.4
Hard (%)	4.3–84.7	72.5	34.0
SDS (ml)	29.0–64.4	51.3	42.2
Pel (mn)	25.2–140.9	128.7	45.7

[a] See Materials and methods (Technological analysis)

[b] Pelshenke swelling text

clearly indicated the presence of the major gene that had been identified earlier (Symes 1965). Distribution of Prot does not significantly deviate from a Gaussian distribution, while that of crude values of W is slightly skewed. Parental scores and the range of the recombinant lines for each quality trait are reported in Table 1.

QTLs with an additive effect

Results with respect to QTLs significant for quality-related traits are given in Table 2, and a graphical representation of the chromosomes involved is given on Fig. 3. For kernel hardness, scanning of the whole genome was carried out using two marker loci as covariates: *Xmta10* (chromosome 5D) and *Xgwm55* (chromosome 6D), with *Xmta10* being omitted when scanning chromosome 5D, and *Xgwm55* when scanning chromosome 6D. Three chromosomal regions on three different chromosomes showed a significant QTL effect. The most important one was on chromosome 5DS close to locus *Xmta10*, with an additive value AV = 17.0 (confidence interval 14.7;19.5) and an heritability $h^2 = 0.67$. Two minor but significant QTLs were detected on chromosome 1A (locus *Xfba92)* and 6D (*Xgwm55*), with AV= 4.0(2.1;6.5) and –4.8(–7.3;–2.5) and $h^2 = 0.03$ and 0.06 respectively.

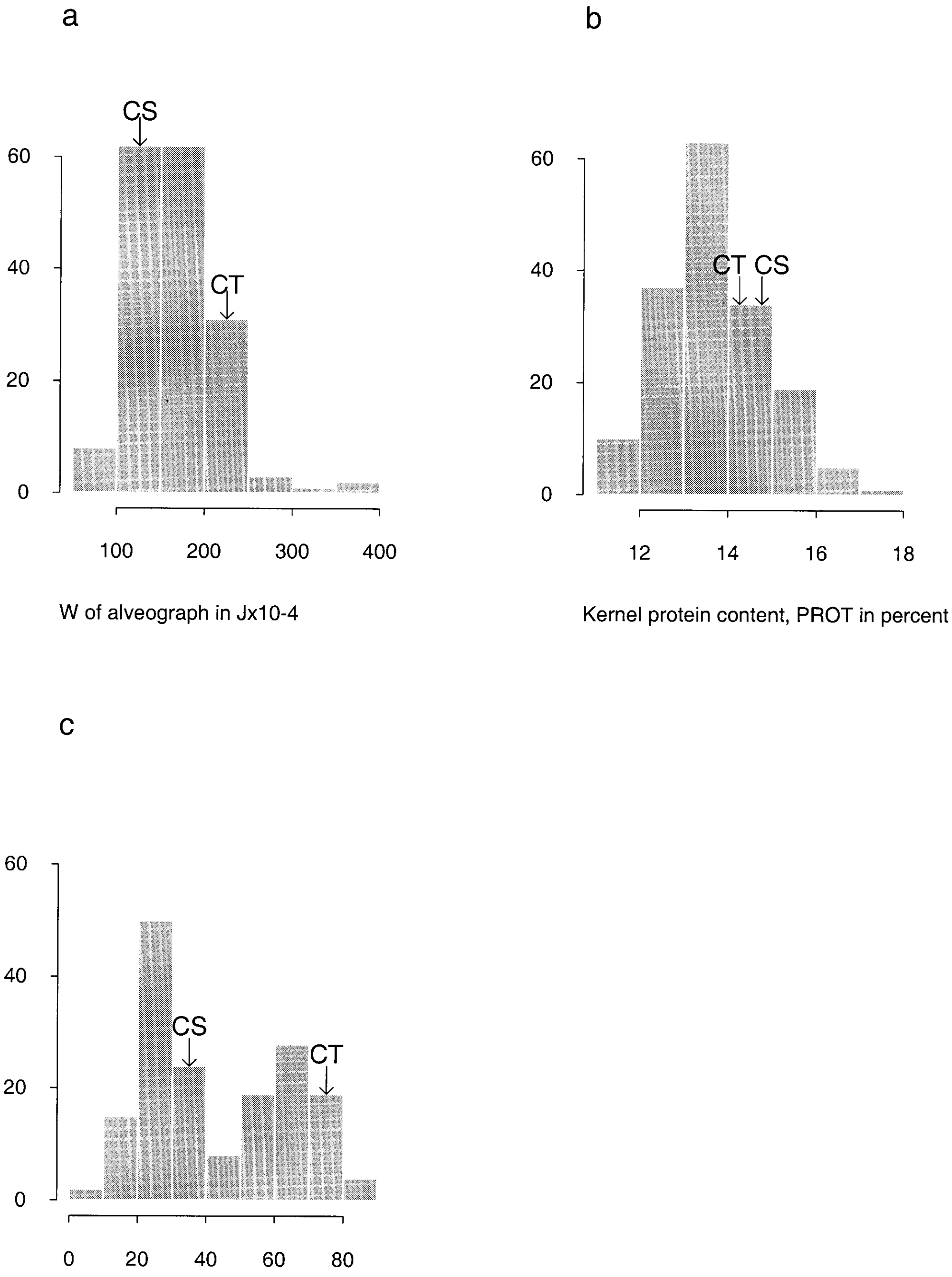

Fig. 2a–c Distribution of the quality traits among the 187 DH lines derived from the cross between Courtot (*CT*) and Chinese Spring (*CS*)

For kernel protein content, the marker locus *XE38M60*$_{200}$ (chromosome 6A) was used as a covariate in the QTL mapping. Significant QTLs were detected at two chromosomal locations on two different chromosomes. The most important one was on chromosome 6 A, close to marker *XE38M60*$_{200}$ with AV = –0.50(–0.76; –0.24) and $h^2 = 0.17$, the other was on chromosome 1B, close to locus *Xcdo1188* with AV = –0.31(–0.10;–0.52) and $h^2 = 0.07$.

For the energy required for dough deformation W, a set of 6 marker loci from six different chromosomes was used as covariates in QTL mapping: *Xfba92* (chromosome 1 A), *Xmta14* (chromosome 1B), *XksuE3* (chromosome 3B), *Xfbb166* (chromosome 5 A), *Xmta10* (chromosome 5D), *Xfbb250* (chromosome 6B). Three QTLs on 3 different chromosomes were detected at $P < 0.01$. The most important one was on chromosome 5DS close to locus *Xmta10*, the same location as the QTL identified for kernel hardness, but its h^2 value was much smaller than that of Hard – $h^2 = 0.20$ and the additive value was 21(15; 29). One QTL was detected on each of chromosomes 1 A and 3B. On chromosome 1 A, the closest locus to the QTL was *Xfba92*, AV = 17(10; 25) and $h^2 = 0.11$. On chromosome 3B, the closest locus was *XksuE3*, AV = 15(6; 24) and $h^2 = 0.09$. It should be noted that for Hard and Prot, additional QTL locations were detected

Table 2 List of significant QTLs for three quality-related traits. Chromosome location is in centiMorgans (cM) based on origin and additive effects, with their confidence intervals (in brackets from 500 bootstrap resamplings). QTL heritability is estimated as the ratio of the squared additive effect to the phenotypic variance

QTL location			Kernel hardness (%)		Kernel protein content (%)		W of alveograph (J 10^{-4})	
Chromosome	Closest locus	Chromosome location (cM)	QTL additive value	QTL heritability	QTL additive value	QTL heritability	QTL additive value	QTL heritability
1A	*Xfba92*	84(64;97)					17(10;25)	0.109
	Xfba92	83(56;103)	4.0 (2.1; 6.5)	0.031				
1B	*Xcdo1188*	65(39;103)			–0.31(-0.52; -0.10)	0.065		
3B	*XksuE3*	54(12;120)					15(6;24)	0.094
5D	*Xmta10*	1(0;5)	17.0(14.7; 19.5)	0.669				
	mta10	4(0;13)					21(15;29)	0.195
6A	*XE38M60*$_{200}$	59(36;86)			–0.50(-0.76; -0.24)	0.171		
6D	*Xgwm55*	53(34;70)	-4.8(-7.3; -2.5)	0.055				

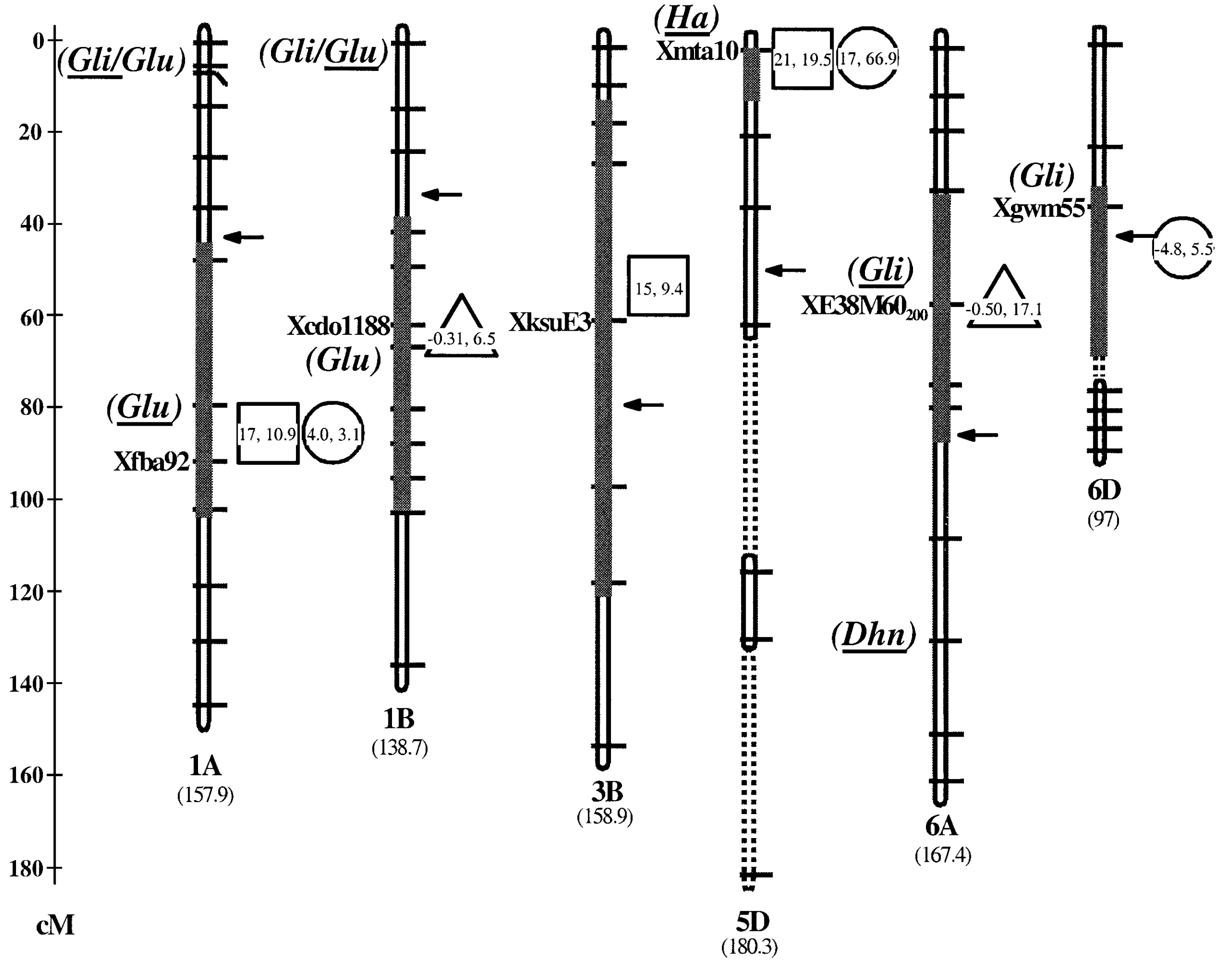

Fig. 3 Graphical representation of six significant QTL for quality traits. □ W of alveograph, ○ Hard, △ Prot. Additive effects and heritability are indicated *inside* the symbol. *Black filled* part of the chromosome gives the confidence region of each QTL. *Arrows* indicate the centromeric regions. The known functional gene loci influencing bread-making quality are indicated in *brackets*, with only the *underlined* ones are polymorphic

using this method compared to one-way ANOVA, which illustrates the gain in detection power achieved by including covariates in the regression model. This was obvious for Hard when a marker linked to *Ha*, the gene involved in the control of kernel hardness, accounting for 65% of the variation of the character was included in the model. This led to a considerable decrease in residual variance and thus to the detection of minor QTLs. The reverse was observed for W. Only three QTL regions re-

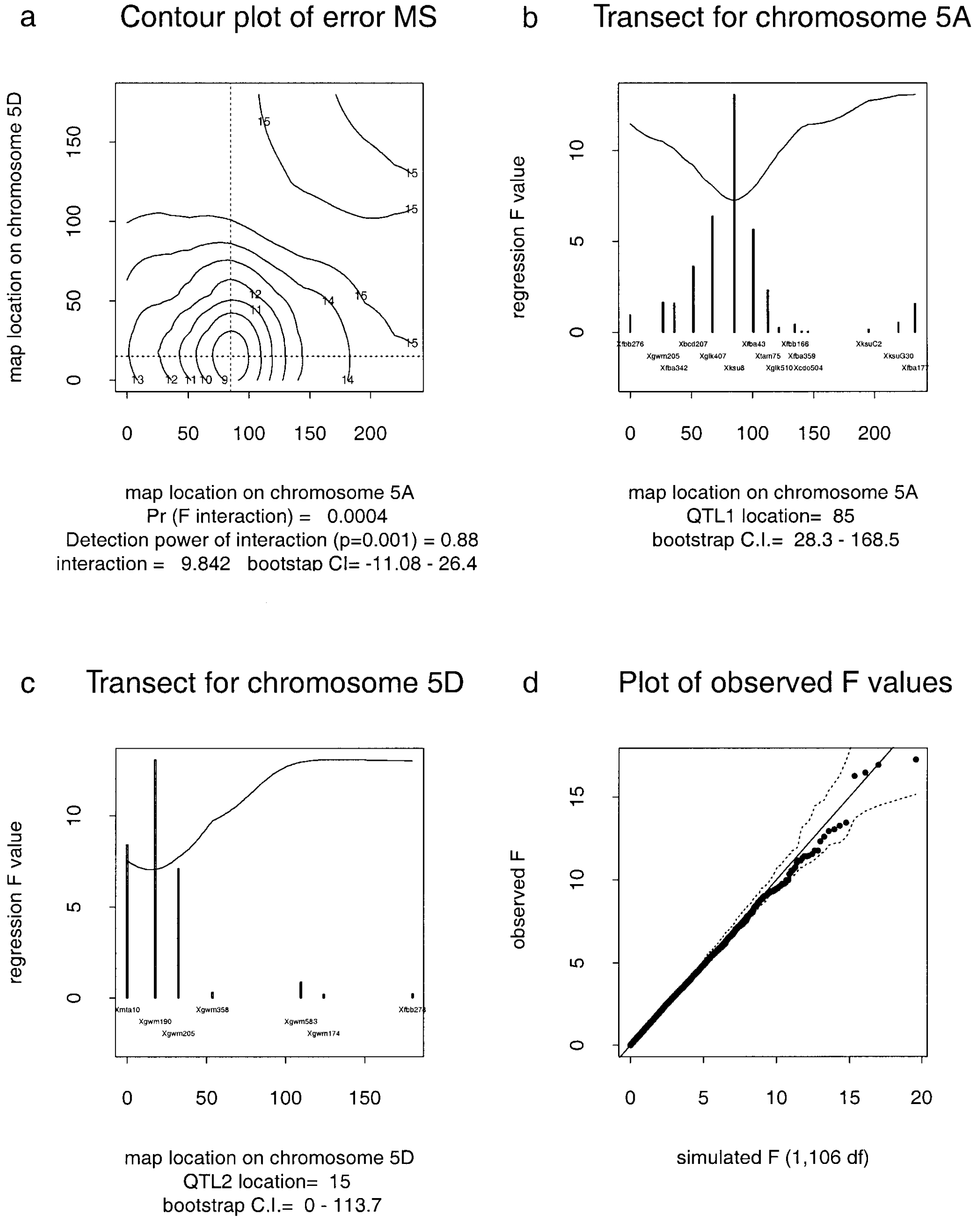

Fig. 4a–d Graphic display of putative interactive QTL for W. **a** Two-dimensional contour plot of regression error MS: the minimum indicates the most likely position of the two QTL. Bootstrap C.I. are computed from 100 resamplings. **b, c** Marker regression scans of chromosomes 5 A and 5D, respectively. **d** Plot of observed regression *F* values against simulated value from a Fisher distribution with (1, 106) *df. Dotted lines* roughly show the lower and upper bounds of the 95% confidence region

mained significant while six chromosome locations had been identified by ANOVA. It may be postulated that the other three locations were either false positives or true QTLs that were not robust enough through bootstrapping.

QTLs with an interactive effect

We identified six significant interactions at a threshold α = 0.001 involving six pairs of chromosome regions. However, when we plotted the observed *F* values for the 19 490 tested interactions against the ordered set of simulated values taken from the Fisher distribution with (1, 106) degrees of freedom, the points were inside the confidence region of the simulated values (parametric bootstrap). Thus, no interaction remained significant (e.g. Fig. 4d). This was confirmed by the bootstrap analysis of the joint scanning of chromosome 5 A and chromosome 5D for W, for which some observed values seemed to depart from the simulated ones. Although a significant interaction was detected in 88% of the samples (at P = 0.001), the confidence intervals were very large for both locations and the confidence interval of QTL effect even included zero, thus indicating a lack of significance (Fig. 4 a, b, c).

Discussion

We initially chose cvs. Courtot and Chinese Spring as parental lines to study a range of traits, including plant height, heading time and crossability, for which these lines differ for some major genes. They also differ for some quality traits – W, L and the Pelshenke swelling test, in spite of their both having the identical genes at the loci of all high-molecular weight sub-unit glutenins except for Glu-A1. In addition, a complete set of monosomic lines was available, which allowed the unambiguous assignation of markers to homoeologous chromosomes (Cadalen 1996). However, this choice had some drawbacks, in particular in agronomic behaviour and therefore for quality traits analyses. There were difficulties in growing some of the recombinant lines due to excessive height (either too short or too tall), low tillering capacity, lodging and susceptibility to spontaneous grain shedding. For these reasons and since agronomic data such as yield had not been recorded in a relevant design to be used as covariates, we only show results identifying QTLs that were strongly supported by bootstrap resampling.

Under our growing conditions, both parental lines displayed similar values for PMAX and for kernel protein content. In addition, the higher value of W shown by Courtot was correlated to a longer extensibility of the curve than that observed for Chinese Spring. This is not in agreement with the widespread idea that among most cultivated wheats, an increased hardness leads to an increased W due to a higher PMAX (Branlard et al. 1997), since Courtot is the hardest of the two parental varieties (Table 1).

Considering hardness, both parental lines showed highly contrasting values. We detected a major QTL on the distal part of chromosome 5DS, close to the *Xmta10* locus. This region is known to contain *Ha*, a gene involved in the control of kernel hardness. In a previous study carried out on a population derived from a cross between an amphihexaploid and a common wheat, Sourdille et al. (1996) also reported a close association between kernel hardness and the *Xmta9* locus. MTA9 and MTA10 are cDNA coding for puroindolines a and b, respectively (Gauthier et al. 1994). Puroindolines a and b are the two major polypeptide components of the 15-kDa marker protein for grain softness, also named friabilin. Giroux and Morris (1998) have recently provided further evidence on the function of puroindolines a and b in kernel hardness that supports our observations. In both our studies the puroindoline locus explained about 65% of the phenotypic variance of the trait. We also identified minor QTLs on chromosome 1 A and 6D that explained only 3% and 5.5%, respectively, of the phenotypic variance. Sourdille et al. (1996) also mentioned a QTL on chromosome 6D but we were unable to assert that the latter and the one we identified were the same because of the low density of markers in this region of our map. The hardest parental line, Courtot, had two out of three positive alleles for kernel hardness; the third one on chromosome 6D was from Chinese Spring. This explained partly the presence of many transgressive lines among the progeny. Indeed, the predicted range of progeny from QTL additive values is 50 (twice the absolute sum of additive values), while the predicted difference between the parental lines is 40 (twice the algebraic sum of additive values). For kernel protein content we also noticed a large transgressive segregation in the DH lines in spite of the similar scores of the parental lines. We were able to identify a major QTL on chromosome 6AS close to the AFLP locus $XE38M60_{200}$ that explained about 17% of the phenotypic variance of the trait. Blanco et al. (1996) also found a significant QTL ($r^2 = 13.6$) on the short arm of chromosome 6 A in a study using recombinants inbred lines (RILs) issued from a cross between tetraploid wheats. Based on the confidence interval of the QTL location, we suggest that these two QTLs are identical. The results of Joppa et al. (1997) also seem to be in in agreement with our own: They detected a QTL for high grain protein content near the centromere of chromosome 6B in a study using a population of recombinant inbred chromosome lines of tetraploid wheat cv. Langdon. This QTL may be homoeologous to those detected both by Blanco et al. (1996) and by us in the present study. Initially we thought that chromosome 6B was involved. In preliminary studies, we had identified one QTL on this chromosome, but the confidence interval was too large and the putative QTL was finally discarded from the presented results. Blanco et al. (1996) also mentioned a QTL on chromosome 4BS ($r^2 = 27.7$). In our study we also found two QTLs on this chromosome, however they were not robust enough after bootstrap resampling. We identified a significant QTL on chromosome 1BL in the vicinity of the known location of Glu-B1. Levy and Feldmann (1989) suggested earlier that genes controlling protein content might be located in the neighbourhood of high-molecular-weight loci. We postulate that structural genes with the same coding sequence may differ in the strength of the promoter or that a regulatory gene is closely linked to the structural one. Both hypotheses could lead to different levels of quantitative expression.

For the energy required for dough deformation, W, the main QTL identified was on the short arm of chromosome 5D. As suggested previously from the distribution of this trait and the result of PCA, this QTL did not have a *per se* effect but rather an indirect effect through its impact on hardness. The effect of the QTL was much lower than the effect on hardness. This suggests that several loci are involved in the determinism of this trait, with a possible positive effect issued from the parental cv. Chinese Spring. Unfortunately, we were not able to identify many of them. The loci encoding the subunits of high-molecular-weight glutenins on group-1 chromosomes have been reported to be responsible for a part of the variability of the W score (Ruiz et al. 1995), but Glu-1B and Glu-1D were not polymorphic between the parental varieties. Similarly to the QTL on chromosome 5D, the QTL identified on chromosome 1 A was also coupled with a QTL for kernel hardness but its effect was higher for W than for hardness. However, even if both QTLs, hardness and W, were assigned to the same location, we could not conclude that they resulted from a pleiotropic effect or from the presence of two linked genes because of a large confidence interval for the location of each QTL. Nevertheless, since the confidence interval includes the Glu-A1 locus, this QTL is likely to result from the positive effect on W of the 2*-HMW subunit encoded by the Glu-1Ab allele that surprisingly did not have a major effect. Fine mapping of chromosomes 5DS and 1 A would be required to definitely confirm the functional role of puroindolines or glutenins in the genetic variation of W. Near-isogenic lines are currently being created by backcrossing appropriate DH lines to cv. Courtot to achieve this goal, as proposed by Paterson et al. (1990). In our study, QTLs identified on chromosomes 3B and 1 A nearly had the same additive effect. In a study on tetraploid wheats Blanco et al. (1998) found one QTL for SDS sedimentation volume on chromosome 3BL. Using substitution lines, Mansur et al. (1990) had already mentioned chromosome 3B with such an effect on loaf volume. An extensive search of candidate genes on this linkage group is thus required to further identify the possible functional gene.

A QTL for water extractable arabinoxylans of wheat endosperm has been reported on chromosome 1BL (Martinant et al. 1998). This trait might have an effect on the rheological properties of the dough. However no QTL for W was detected in this region.

We first thought that some of the 2 × 2 interactions between markers from different linkage groups were significant, given the *P* value of the *F*-test. However, in the case of multiple tests, the overall type-I error risk may be high and is difficult to compute analytically. Therefore, a comparison *a posteriori* of the observed values to a simulated set of *F*-values taken from a Fisher distribution with appropriate degrees of freedom was more informative. Such a comparison led us to discard the previously detected interaction, which was confirmed by the very large confidence intervals obtained through bootstrap resampling.

Conclusion

Energy required for dough deformation partly depends on kernel hardness, and it could be desirable to select lines in either the soft or the hard group of DH lines. This goal could be achieved with the aid of molecular markers. The use of molecular markers enables the early selection of elite plants, which can be interesting for shortening selection cycles, even for highly heritable traits such as grain softness. A crossing scheme is being carried out for combining QTLs with positive effects on W using selected genotypes from the present study. Obviously, QTLs detected in a single population may not be of general use for wheat improvement. Therefore, in order to confirm our results, a study will be carried out on RILs issued from crosses between modern cultivars of bread wheat. A multitrait QTL approach will be used on this material in order to set up a marker-assisted recurrent selection programme for the simultaneous improvement of yield, quality traits and other adaptations.

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