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ORIGINAL PAPER

Association mapping for quality traits in soft winter wheat

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Abstract Improvement of end-use quality in bread wheat (*Triticum aestivum* L.) depends on a thorough understanding of the genetic basis of important quality traits. The main goal of our study was to investigate the genetic basis of 1,000-kernel weight, protein content, sedimentation volume, test weight, and starch concentration using an association mapping approach. We fingerprinted 207 diverse European elite soft winter wheat lines with 115 SSR markers and evaluated the genotypes in multi-environment trials. The principal coordinate analysis revealed absence of a clear population but presence of a family structure. Therefore, we used linear mixed models and marker-based kinship matrices to correct for family structure. In genome-wide scans, we detected main effect QTL for all five traits. In contrast, epistatic QTL were only observed for sedimentation volume and test weight explaining a small proportion of the genotypic variation. Consequently, our findings suggested that integrating epistasis in marker-assisted breeding will not lead to substantially increased selection gain for quality traits in soft winter wheat.

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Introduction

The development of high yielding varieties with good bread-making quality is a major focus in wheat breeding programs. Knowledge of the genetic architecture of agronomic traits is important to optimize marker-assisted selection programs. Although increasing evidence for the existence of epistasis has been provided at the molecular level for several plant and animal species (for review see Carlborg and Haley 2004), its importance for quality traits in bread wheat has received little attention.

Classical linkage mapping based on biparental populations was frequently used to dissect the genetic architecture of wheat quality traits. Several main effect QTL have been identified for 1,000-kernel weight (Groos et al. 2003; Huang et al. 2006; Sun et al. 2010; Tsilo et al. 2010), grain protein content (Prasad et al. 2003; Groos et al. 2003, 2004; Breseghello et al. 2005; Kuchel et al. 2006; Mann et al. 2009; Sun et al. 2010; Tsilo et al. 2010), test weight (Campbell et al. 1999; Galande et al. 2001; Huang et al. 2006; Kunert et al. 2007; Sun et al. 2010), sedimentation volume (Blanco et al. 1998; Rousset et al. 2001; Kunert et al. 2007; Li et al. 2009), and starch concentration (Ma et al. 2005; McCartney et al. 2006; Yang et al. 2007). Nevertheless, only a few studies investigated the role of epistasis for quality traits in soft winter wheat (Kolster et al. 1991; Kulwal et al. 2005; Ma et al. 2005; Patil et al. 2009; Zhao et al. 2010).

Association mapping was suggested as a promising alternative strategy to linkage mapping to elucidate the genetic basis of complex traits (Yu et al. 2006). In a first pioneering candidate region association mapping study in soft winter wheat, Breseghello and Sorrells (2006) found significant main effect QTL for kernel weight, length and width of the kernels. Neumann et al. (2010) used a world-wide panel of 96

bread wheat accessions and detected main effect QTL for several agronomic and quality traits such as 1,000-kernel weight, protein content, and sedimentation volume. Nevertheless, a genome-wide association mapping study of quality traits based on elite soft winter wheat germplasm is still lacking. Moreover, epistatic interactions for quality traits in wheat have not been investigated until now based on association mapping designs.

A major problem of association mapping is that any nonfunctional association between the trait under consideration and the underlying population structure can also be detected as a QTL (Zhao et al. 2007). To correct for this population stratification, information on the relatedness among genotypes is commonly incorporated as population effect and/or as kinship matrix (Yu et al. 2006). A critical issue in the use of marker-based kinship matrixes is the estimation of the conditional probability that marker alleles are alike in state, given that they are not identical by descent (further denoted as T value) (Lynch 1988). Yu et al. (2006) determined the T value as the average proportion of marker loci with shared variants among inbreds of the association mapping panel. In contrast, Zhao et al. (2007) used the minimum value of the proportion of marker loci with shared variants among inbreds of the association mapping panel as T value. Stich et al. (2008) suggested a restricted maximum likelihood (REML) method to estimate the optimum value of T and validated this REML approach using experimental data in wheat. Optimum value of T may vary depending on the crop, the association mapping panel underlying the study, the marker system, and the trait under consideration. Therefore, a detailed knowledge of the influence of the estimated T value on the number and consistency of QTL is of utmost importance.

Here, data from an association mapping panel composed of 207 wheat lines fingerprinted with 115 simple sequence repeat (SSR) markers and evaluated for 1,000-kernel weight, protein content, sedimentation volume, test weight, and starch concentration were used to: (1) determine the underlying population structure to select an appropriate statistical model for association mapping, (2) investigate the influence of different T values on QTL detection, (3) identify the chromosomal regions affecting quality traits in European wheat, and (4) unravel the relative contribution of main effect QTL versus epistatic interactions for quality traits using an association mapping approach.

Materials and methods

Plant materials and field trials

A total of 207 soft winter wheat (*Triticum aestivum* L.) lines were used for this study. Genotypes were advanced breeding lines complemented with a set of registered varieties. The entries were divided in a series of four trials. The experimental design for each trial was a lattice design with one replication per environment with 56 entries per trial. Two of the 207 entries were evaluated as common entries in each lattice. Sowing density was 350 grains m^{-2} and plot size ranged from 5.5 to 15.0 m^2. The 207 lines were evaluated for protein and starch content (%) both determined by near infrared transmittance (Infratec 1241, calibrations by FOSS), 1,000-kernel weight (g), Zeleny sedimentation volume (ml) and test weight (kg hL^{-1}). Data for 1,000-kernel weight, protein content, sedimentation volume, and test weight were recorded for each line in five environments. Data for starch concentration were recorded for each line in four environments.

Molecular data analyses

The molecular analyses were previously described in detail by Reif et al. (2010). Briefly, the 207 wheat lines were fingerprinted following standard protocols with 115 SSR markers. These markers were randomly distributed across the wheat genome. Map positions of markers were based on the linkage map published by Somers et al. (2004). Associations among the inbred lines were analyzed by applying principal coordinate analysis (PCoA) (Gower 1966) based on the modified Rogers' distances (Wright 1978).

Phenotypic data analyses

The phenotypic data were analyzed based on the following statistical model:

$$y_{ijko} = \mu + g_i + l_j + t_{kj} + b_{ojk} + e_{ijko},$$

where y_{ijko} was the phenotypic observation for the ith wheat line at the jth environment of the kth trial in the oth incomplete block, μ was an intercept term, g_i was the genetic effect of the ith genotype, l_j was the effect of the jth environment, t_{kj} was the effect of the kth trial at the jth environment, b_{ojk} was the effect of the oth incomplete block at the jth environment of the kth trial, and e_{ijko} was the residual. Environments and trials were regarded as fixed, the other effects as random. Error and block variances were assumed to be heterogeneous among environments. Variance components were determined by the REML method. Heritability on an entry-mean basis was calculated as the ratio of genotypic to phenotypic variance according to Melchinger et al. (1998). In addition, best linear unbiased estimates (BLUEs) were estimated assuming fixed genetic effects.

Association mapping

We applied for each of the 115 SSR markers a one-step association mapping approach using following statistical model:

$$y_{ijko} = \mu + m_v + g_i + l_j + t_{kj} + b_{ojk} + e_{ijko},$$

where m_v denotes the effect of the v*th* marker genotype. We regarded g_i and b_{ojk} as random and all other effects as fixed. The variance of the random effects $g^*\{g_1,\ldots, g_{207}^*\}$ was assumed to be $\mathrm{Var}(g^*) = 2\,\mathrm{K}\sigma^2{}_\mathrm{G}^*$, where $\sigma^2{}_\mathrm{G}^*$ refers to the genetic variance estimated by REML and K was a 207 × 207 matrix of kinship coefficients that define the degree of genetic covariance between all pairs of entries. We followed the suggestion of Bernardo (1993) and calculated the kinship coefficient K_{ij} between inbreds i and j on the basis of marker data as $K_{ij} = 1 + (S_{ij} - 1)/(1 - T_{ij})$, where S_{ij} is the proportion of marker loci with shared variants between inbreds i and j and T_{ij} is the average probability that a variant from one parent of inbred i and a variant from one parent of inbred j are alike in state, given that they are not identical by descent. The coefficient T_{ij} was estimated using a REML method setting negative kinship values between inbreds to zero (Stich et al. 2008). Based on the Wald F statistic, we performed tests for the presence of marker-phenotype associations with a significant ($P < 0.01$) effect on the five traits under consideration. To correct for co-linearity, the selected markers were simultaneously fitted in a final model in the order of their P values. Markers with significant ($P < 0.05$) association in the final model were declared as main effect QTL. The proportion of the phenotypic variance explained by all QTL was determined by the estimator $R^2{}_\mathrm{adj}$ as described by Utz et al. (2000). The proportion of the genotypic variance explained by all detected QTL was estimated from the ratio $p_G = R^2{}_\mathrm{adj}/h^2$.

In addition, we performed a two-dimensional scan for pairwise interaction effects among the 115 SSR markers extending the above model to:

$$y_{ijko} = \mu + m_v + m_w + m_v : m_w + g_i + l_j + t_{kj} + b_{ojk} + e_{ijko},$$

where m_v and m_w denote the effect of the v*th* and w*th* marker genotype and m_v:m_w refers to the interaction effect of the *v*th and *w*th marker genotype. Based on the Wald F statistic, we performed a test for the presence of significant ($P < 0.05$) pairwise interaction effects for the five traits under consideration correcting for multiple testing with the Bonferroni–Holm procedure (Holm 1979). All mixed-model calculations were performed with ASReml release 2.0 (Gilmour et al. 2006).

Results

The ANOVA across environments revealed significant genotypic variance for all five traits (Table 1). Heritability was high for all traits and ranged from 87% for protein content to 98% for sedimentation volume. Absolute values of phenotypic correlations among the five traits were minimum ($r = 0.04$) between 1,000-kernel weight and test weight and maximum ($r = 0.60$, $P < 0.01$) between protein content and sedimentation volume (Fig. 1).

The PCoA revealed a slight population structure with respect to principal coordinates 1, 2, 4, and 7 (Fig. 2a). Moreover, each of the first ten principal coordinates explained only a small proportion of the total variation with a range from 2.1 to 6.5% (Fig. 2b). The proportion of marker loci with shared variants between inbreds i and j, S_{ij}, averaged 0.51 (Supplementary Figure S1). The optimum value of T, which was calculated for the current data set using a REML approach, ranged from 0.35 for sedimentation volume and starch concentration to 0.75 for protein content. It is important to note, that in the kinship matrix K, all negative kinship values K_{ij} between inbreds i and j, i.e. $S_{ij} < T$, were set to zero.

The genome-wide scan for marker-trait associations revealed for all five traits QTL distributed throughout the genome (Table 2). The number of QTL varied from 3 for

Table 1 Means, ranges, genotypic variance components ($\sigma^2{}_\mathrm{G}$), error variances ($\sigma^2{}_\mathrm{E}$), and broad sense heritabilities (H^2) of 207 wheat lines evaluated for 1,000-kernel weight (g), protein content (%), sedimentation volume (ml), test weight (kg hL^{-1}), and starch concentration (%) in multi-environment field trials

Parameter	1,000-kernel weight	Protein content	Sedimentation volume	Test weight	Starch concentration
Mean	47.4	12.1	46.9	79.2	67.2
Min	37.7	10.5	25.5	74.7	69.6
Max	58.4	14.1	71.2	83.1	71.4
$\sigma^2{}_\mathrm{G}$	12.03**	0.32**	106.12**	1.58**	0.44**
$\sigma^2{}_\mathrm{E}$	5.53	0.23	12.63	0.74	0.24
H^2	0.92	0.87	0.98	0.91	0.88

** Significant at $P < 0.01$

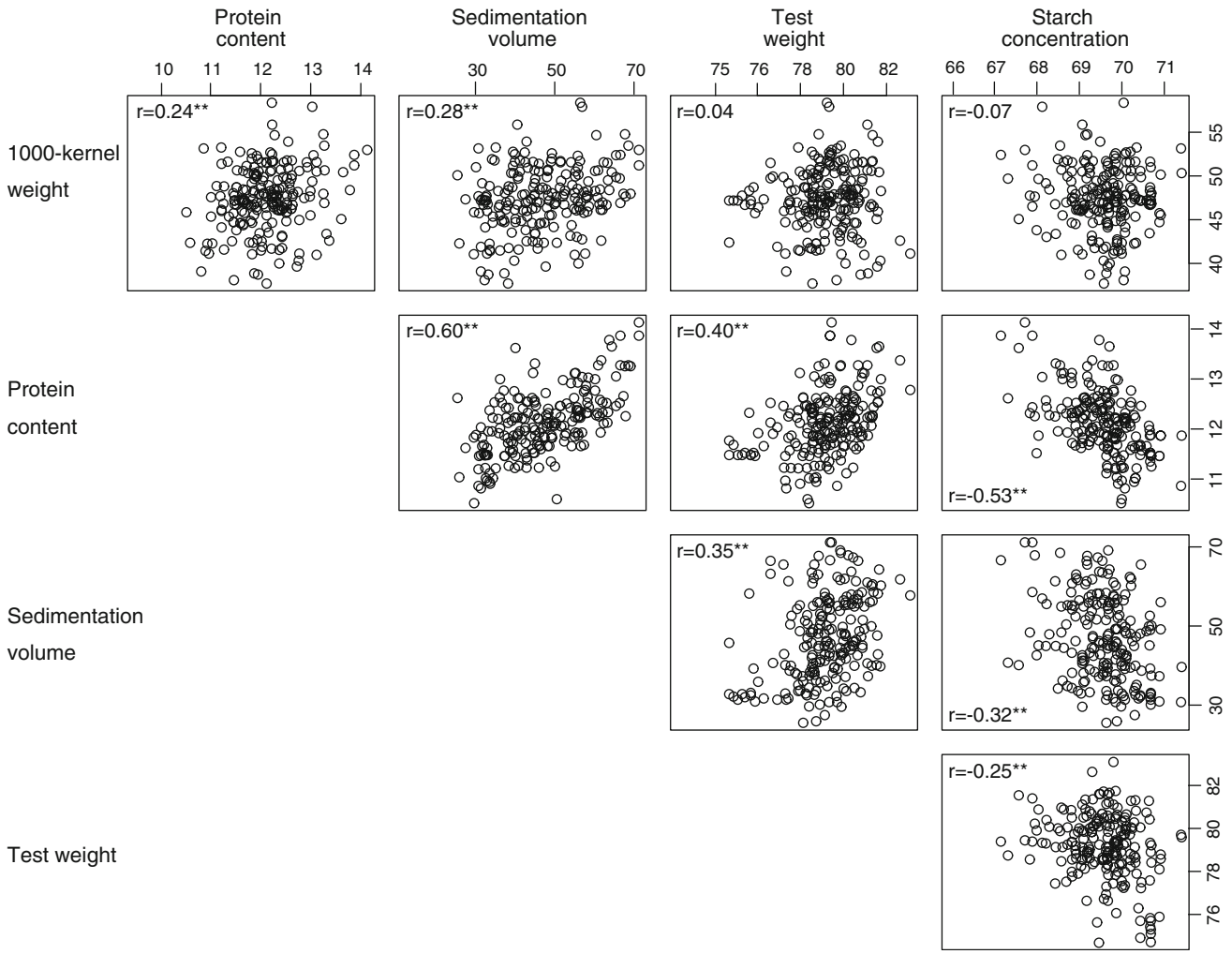

Fig. 1 Associations among best linear unbiased estimates of 207 wheat lines evaluated for the five traits 1,000-kernel weight (g), protein content (%), sedimentation volume (ml), test weight (kg hL^{-1}), and starch concentration (%) in multi- environment field trials. **Significant at $P < 0.01$

starch concentration to 12 for test weight. The proportion of genotypic variance explained simultaneously by all markers with significant ($P < 0.01$) main effect ranged from 14% for starch concentration to 79% for test weight. The maximum range of the proportion of the genotypic variance explained by the individual markers was observed for test weight (0.7–34.1%).

The two-dimensional scan revealed a total of 12 significant ($P < 0.05$ applying a Bonferroni–Holm correction for multiple tests) digenic epistatic interactions for sedimentation volume and test weight (Table 3). The proportion of the genotypic variance explained by epistatic effects was 13.2% for sedimentation volume and 14.1% for test weight. The proportion of the genotypic variance explained by the individual digenic epistatic interactions ranged from 0.1% for test weight to 5.1% for sedimentation volume.

We investigated the relationship among T values and the number of significant main effect ($P < 0.01$) and epistatic QTL (Fig. 3; Supplementary Figure S2). No monotonic trends were observed for all five traits, but the number of main effect QTL was strongly associated with the model fit of the phenotypic data with the respective K matrix. The number of epistatic QTL was also strongly associated with the model fit of the phenotypic data with the respective K matrix for T values of up to 0.5–0.6, but for the most traits no epistatic QTL were identified for T values of above 0.5–0.6. The overlap of significant ($P < 0.01$) main effect QTL based on optimum value of T with QTL detected based on non-optimum T values was high and ranged on average from 62% for 1,000-kernel weight to 85% for test weight (Fig. 4).

Discussion

A potential problem in association mapping is that population stratification can lead to the discovery of many false positive QTL (Zhao et al. 2007). Therefore, several

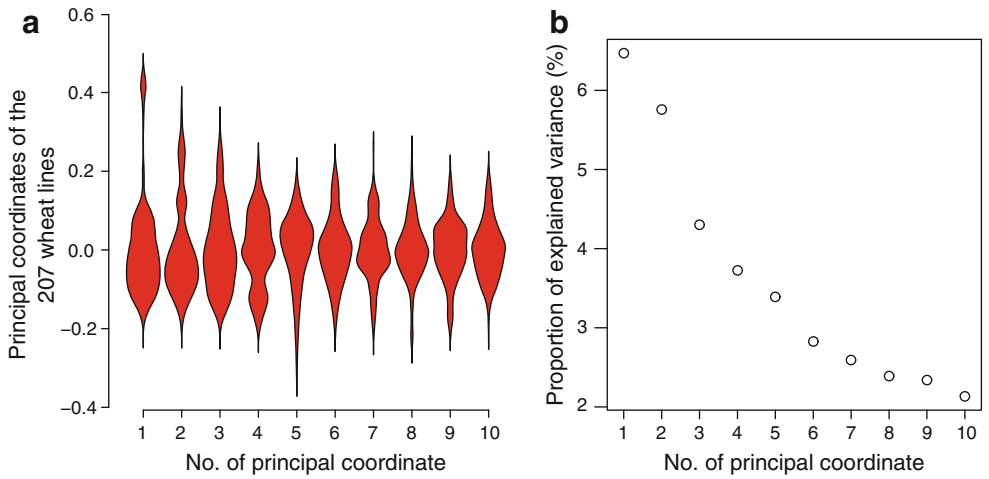

Fig. 2 **a** Violin plot showing the density distribution of the values of the first ten principal coordinates for the 207 wheat lines and **b** proportion of explained variance of the first ten principal coordinates

biometrical models have been developed, which correct for population stratification (for review see Zhu et al. 2008). One popular method consists in modeling population effects estimated within a Bayesian framework is implemented in the software package STRUCTURE (Yu et al. 2006). Alternatively, population structure can be modeled using classical multivariate statistical techniques such as principal component analysis (Price et al. 2006). Stratification due to the family relatedness is commonly controlled using a kinship matrix (Yu et al. 2006). Because population effects and kinship matrix are estimated with marker data, overcorrection for population stratification can result in a low power to detect QTL (Würschum et al. 2010). Therefore, a careful choice of the biometrical model is pivotal in association mapping and greatly depends on the underlying population structure of the germplasm used in the study.

Underlying population structure and consequences for association mapping

The PCoA of the 207 wheat lines revealed a slight population structure with respect to principal coordinates 1, 2, 4, and 7 (Fig. 2a). Consequently, a population effect could be considered in the association mapping analysis. However, the proportion of explained variance of these principal coordinates was very low with a maximum of 6.5% (Fig. 2b), which suggests that no major population structure is present in the wheat association mapping panel underlying our study. This finding is expected in elite breeding germplasm of selfing species and can be explained by the germplasm handling in intra-population improvement with a lack of distinct genetic pools, which is in contrast to hybrid breeding, with clearly defined heterotic groups (Reif et al. 2005). Nevertheless, the high S_{ij} values observed between several pairs of wheat lines (Supplementary Figure S1) clearly points to the presence of family structures, which can be explained by the intensive use of elite lines as parents of several crosses. Consequently, we solely corrected for stratification due to family structure using a kinship matrix.

Impact of the choice of the *T* value on marker-trait associations

The REML approach revealed a large variation of optimum values of T across the five traits (Supplementary Figure S1). This large variation was not observed comparing previous studies in wheat (Stich et al. 2008; Reif et al. 2010; Miedaner et al. 2010) and can be explained by (1) differences in the relevance of additive versus epistatic variance among traits as reported in a previous study (Goldringer et al. 1997), (2) different fixation rates of positive alleles due to varying selection pressures for different traits which is very likely in multi-stage selection programs of wheat (Ebmeyer personal communication), and/or (3) a bias in the estimate of the kinship matrix. The number of required markers to correct for the genetic background in association mapping was investigated by Yu et al. (2009) in a simulation appraisal and their findings suggested that 100 polymorphic SSR markers should result in unbiased estimates of the kinship matrix. This finding and the fact that the markers used in our study have been mainly selected for maximizing the genome coverage, points towards a low probability of a bias in the estimate of the kinship matrix.

The number of QTL associated with the model fit of the phenotypic data analyzed using the kinship matrix: We detected a lower number of QTL with a better model fit (Fig. 3; Supplementary Figure S2). This is expected

Table 2 Trait-associated markers and their position on respective chromosomes, the explained proportion of the genotypic variance p_G, and QTL reported in the literature in the same region

Marker name	Chromosome[a]	Position (cM)	p_G (%)	Reported in the literature
1,000-kernel weight				
gwm160	4A	79.0	15.0	–
gwm408	5B	117.0	7.8	Groos et al. (2003)
wmc18	2D	64.0	5.9	Cuthbert et al. (2008)
wmc285	4D	10.0	8.1	Huang et al. (2006)
orw6	7D	152.0	21.5	Groos et al. (2003)
orw1	7D	151.0	2.9	Groos et al. (2003)
Total			42.4	
Protein content				
gwm82	3A	45.0	7.8	Groos et al. (2004); Li et al. (2009)
wmc419	1B	31.8	11.1	Mann et al. (2009); Suprayogi et al. (2009)
gwm190	5D	9.0	4.9	Zhang et al. (2009)
wmc18	2D	64.0	10.3	Zhao et al. (2010)
Total			28.4	
Sedimentation volume				
gwm312	2A	74.0	19.5	–
wmc419	1B	31.8	23.2	Li et al. (2009)
wmc73	5B	63.0	8.6	Mann et al. (2009)
cfd116	2D	65.8	3.4	–
sec99	1B	0.0	3.9	–
Total			43.9	
Test weight				
wmc336	1A	35.0	0.7	–
wmc11	3A	0.0	0.9	–
wmc415	5A	81.0	20.6	–
cfa2163	5A	104.0	4.5	–
barc174	7A	64.0	18.1	–
taglgap	1B	0.0	2.0	–
barc84	3B	97.0	1.1	Sun et al. (2009)
gwm219	6B	59.0	34.1	–
barc149	1D	14.0	4.1	–
barc71	3D	79.0	2.0	–
wmc285	4D	10.0	3.6	–
gwm44	7D	78.0	2.8	–
Total			79.3	
Starch concentration				
gwm186	5A	62.0	14.7	–
wmc73	5B	62.5	3.1	–
cfd72	1D	50.6	11.1	–
Total			14.0	

[a] For markers mapping to multiple loci the first position was given

because of a more stringent control of the genetic background with a better model fit. Moreover, a high proportion of QTL identified in the association mapping analyses at optimum value of T were also found in the genome-scans based on non-optimum values of T (Fig. 4). Consequently, association mapping based on optimum values of T estimated with a REML approach results in conservative and robust tests for main effect QTL.

The number of epistatic QTL for T values below 0.5–0.6 was also strongly associated with the model fit of the phenotypic data with the respective K matrix (Fig. 3; Supplementary Figure S2). Consequently, association

Table 3 Trait-associated epistatic markers with their position on chromosomes, and the explained proportion of the genotypic variance p_G

Marker name	Chromosome/position (cM)[a]	Marker name	Chromosome/position (cM)[a]	p_G (%)
Sedimentation volume				
barc147	3B (7.0)	wmc694	1B (36.7)	5.1
barc147	3B (7.0)	gwm320	2D (101.0)	3.5
barc147	3B (7.0)	wmc419	1B (31.8)	2.4
cfd84	4D (67.0)	sw4brht2	4D (12.3)	2.2
Total				13.2
Test weight				
barc5	2A (63.0)	barc87	3B (14.0)	1.4
barc111	7D (115.0)	cfd188	6D (60.0)	2.0
barc173	6D (16.0)	cfa2028	7A (48.0)	1.2
cfd57	5D (55.0)	wmc415	5A (81.0)	3.1
sw4brht2	4D (12.3)	wmc553	6A (52.0)	*1.5*
wmc344	2B (60.0)	gwm320	2D (101.0)	1.4
wmc415	5A (81.0)	gwm320	2D (101.0)	0.6
gwm3	3D (43.0)	gwm320	2D (101.0)	0.1
gwm291	5A (163.0)	gwm320	2D (101.0)	2.2
gwm320	2D (101.0)	gwm389	3B (1.0)	0.7
Total				14.1

[a] For markers mapping to multiple loci the first position was given

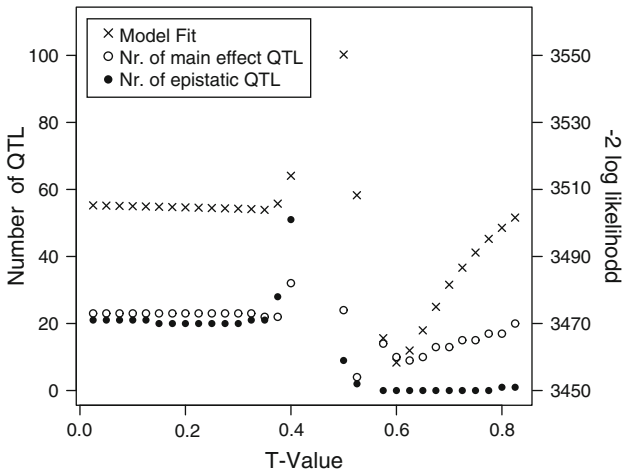

Fig. 3 Associations among the average probability that two alleles are alike in state, given that they are not identical by descent (T value) and the fit of the mixed model based on the phenotypic data for 1,000-kernel weight. Associations among T values and the number of (1) significant ($P < 0.01$) main effect QTL and (2) significant ($P < 0.05$ applying a Bonferroni–Holm correction for multiple tests) epistatic QTL detected in the genome-wide scan

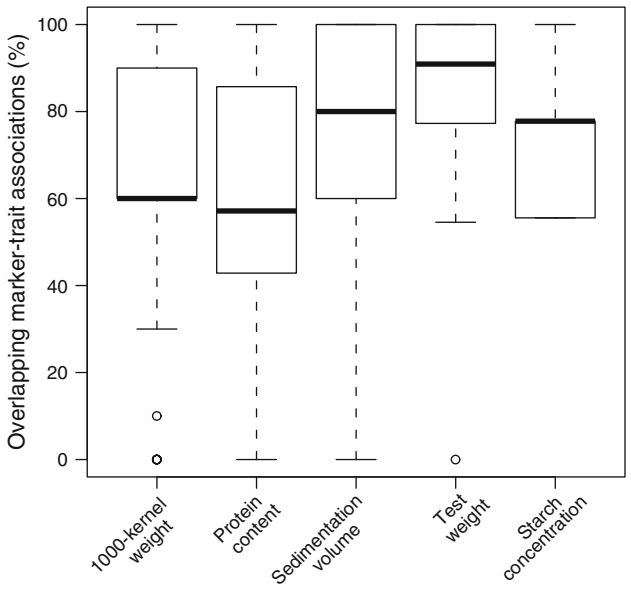

Fig. 4 Percentage of overlapping QTL for association mapping analyses based on optimum value of average probability that two alleles are alike in state, given that they are not identical by descent (T value) for each trait with those QTL detected in the analyses based on non-optimal values of T

mapping based on T values of below 0.5–0.6 also resulted in conservative and robust tests for epistatic QTL. In contrast, optimum T values of above 0.5–0.6 resulted in a drastic reduction in the number of detected epistatic QTL irrespective of the model fit of the phenotypic data. This can be explained by the fact that at high values of T random genetic effects used to correct for population stratification models consider additive and epistatic effects of lines. This finding has strong implications on the power to detect epistatic QTL in association mapping designs. Further research is warranted to investigate in more detail on role of the choice of T value on the probability to detect

epistatic QTL. Moreover, further research is needed for specifying optimal models to detect epistatic QTL using association mapping designs.

Main effect QTL

Many of the QTL detected for 1,000-kernel weight, protein content, and sedimentation volume were co-located with QTL reported previously (Groos et al. 2003, 2004; Huang et al. 2006; Li et al. 2009; Mann et al. 2009; Suprayogi et al. 2009; Sun et al. 2010; Zhao et al. 2010) (Table 2). This clearly underlines the potential of association mapping to investigate the genetic basis of complex traits. Moreover, we observed major QTL for sedimentation volume, test weight, and starch concentration, which were not reported elsewhere. This shows that association mapping holds the potential to detect previously unknown QTL.

As quality traits are important in wheat breeding programs, different groups of genes related to quality are well characterized and mapped (for review see Gupta et al. 2010; Liang et al. 2010). Among these glutenin and gliadins-related genes are most prominent. We identified the main effect QTL for protein content (*wmc419*), sedimentation volume (*wmc419*), and test weight (*barc149* and *taglgap*), which map closely to the chromosomal regions of glutenin and gliadin-related genes (Table 2). This clearly underlines the value of candidate genes for marker-assisted selection of quality traits in wheat.

For most of the traits, we observed L-shaped distribution of the explained genetic variance of the single QTL, with several QTL exhibiting small effects and some QTL with large effects (Table 2). This L-shaped distribution of genetic effect sizes may explain the high congruency with QTL detected previously in linkage mapping studies and points towards the potential of marker-assisted selection for quality traits in wheat. Nevertheless, results have to be interpreted carefully, because QTL effects might be overestimated as in linkage mapping studies. To realistically assess the prospects of marker-assisted selection based on the association mapping results, we suggest that validation of the observed QTL should be done in an independent sample (Würschum et al. 2010).

For 1,000-kernel weight, sedimentation volume, and test weight the explained genotypic variance of the detected QTL was above 40%. This is in accordance with the previous linkage mapping studies (Groos et al. 2003; Kunert et al. 2007; Patil et al. 2009). Nevertheless, for protein and starch concentration the genotypic variance explained by all detected QTL was substantially lower than previously reported in linkage mapping studies (Zanetti et al. 2001). Possible explanations for this discrepancy are (1) fixation of major QTL due to a high selection pressure in the underlying association mapping panel of elite lines or (2) a too low marker density for genome-wide association mapping. The marker density was estimated to be at the lower limit, because 88% of adjacent marker pairs are in significant linkage disequilibrium as shown in our companion study (Reif et al. 2010). Therefore, insufficient marker coverage can explain at least partially the low proportion of explained genetic variance of the detected QTL.

The role of epistasis

We detected significant epistatic QTL effects only for sedimentation volume and test weight (Table 3). Moreover, the proportion of the genotypic variance explained by epistatic QTL (Table 3) was substantially lower compared to the main effect QTL (Table 2). The genotypic variance explained by single epistatic QTL was very small for both sedimentation volume and test weight with a maximum of 5.1%. Our findings are in accordance with the results of previous linkage mapping studies (Carrillo et al. 1990; Patil et al. 2009) and indicate that integrating epistasis in marker-assisted breeding will not lead to a substantially increased selection gain for these traits.

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