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ORIGINAL PAPER

Mapping QTLs with main and epistatic effects underlying grain yield and heading time in soft winter wheat

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Abstract There is increasing awareness that epistasis plays a role for the determination of complex traits. This study employed an association mapping approach in a large panel of 455 diverse European elite soft winter wheat lines. The genotypes were evaluated in multi-environment trials and fingerprinted with SSR markers to dissect the underlying genetic architecture of grain yield and heading time. A linear mixed model was applied to assess marker-trait associations incorporating information of covariance among relatives. Our findings indicate that main effects dominate the control of grain yield in wheat. In contrast, the genetic architecture underlying heading time is controlled by main and epistatic effects. Consequently, for heading time it is important to consider epistatic effects towards an increased selection gain in marker-assisted breeding.

Introduction

Understanding the impact of epistasis on quantitative traits remains a major challenge in genetics of complex traits (Le Rouzic and Alvarez-Castro 2008). Classical approaches of quantitative genetics to elucidate the role of main effects and epistasis include generation means analyses and variance components reflecting different types of genetic effects (Hallauer and Miranda 1981). Detection of epistasis by generation means analysis is conservative, because they reflect only net effects over the entire genome and QTL effects with positive and negative sign may cancel in the sum (Melchinger et al. 2007). Moreover, many designs to estimate the relative importance of main versus epistatic effects from second-moment statistics have serious limitations with respect to unbiased estimation of genetic effects (Kearsey and Jinks 1968). Despite these restrictions, several studies in soft winter wheat based on either first- or second-moment statistics have reported a significant contribution of epistasis for grain yield and flowering time (for review see Goldringer et al. 1997).

Since the late 1980s, linkage mapping was applied in wheat in numerous studies to dissect the underlying genetic architecture of complex traits such as grain yield and flowering time. Several important main effect QTL involved in the expression of flowering time through their control of vernalization, photoperiod response, and earliness per se have been detected (Distelfeld et al. 2009; Griffiths et al. 2009). In contrast to studies based on first- or second-moment statistics, linkage mapping revealed that epistatic interactions contributed to a low extent to the genotypic variance for flowering time (Griffiths et al. 2009). Similarly, for grain yield, epistasis was also of minor importance compared to main effects (e.g., Kumar et al. 2007).

Major limitations of linkage mapping are a poor resolution in detecting QTL and the fact that with biparental crosses of inbred lines only two alleles at any given locus can be studied simultaneously (Flint-Garcia et al. 2003).

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Association mapping methods promise to overcome these limitations (Kraakman et al. 2004). In a first pioneering candidate region association mapping study in soft winter wheat, Breseghello and Sorrells (2006) found significant main effect QTL for kernel traits. For grain yield, Crossa et al. (2007) reported in a genome-wide association mapping study based on 94 and 76 historical spring wheat lines, that 88% of the markers exhibited significant additive and/or additive × additive interaction effects. Computer simulations in the context of linkage mapping (Beavis 1998; Göring et al. 2001; Allison et al. 2002) demonstrated that small population sizes can lead to a severe bias in QTL detection, which may explain the large proportion of markers significantly associated with grain yield in the study of Crossa et al. (2007).

Here, data from a large association mapping panel composed of 455 wheat lines were used to: (1) estimate the resolution of association mapping in a diverse panel of European elite soft winter wheat lines, (2) unravel the relative magnitude of main effect QTL versus epistatic interactions for grain yield and heading time using association mapping approach, and (3) dissect the genetic architecture of grain yield and heading time.

Materials and methods

Plant materials and field trials

A total of 455 soft winter wheat (*Triticum aestivum* L.) lines adapted to Central European conditions were used for this study. All 455 entries were evaluated for grain yield and heading time in a series of seven breeding trials in Germany (Table 1). Heading time was recorded as the developmental stage (BBCH; Hack et al. 1992) at that time when ears of approximately half of the genotypes were fully visible. Data for grain yield was recorded at four to eight environments with an average of six. Data for heading time was recorded at four to seven environments with an average of five. The experimental design for each trial was a lattice design with two replications per location with the number of entries per trial ranging from 56 to 110. Two of the 455 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.

Phenotypic data analyses

The phenotypic data of each environment were first analyzed separately based on the statistical model:

$$y_{ikno} = \mu + g_i + t_k + r_{nk} + b_{onk} + e_{ikno},$$

where y_{ikno} was the phenotypic observation for the ith wheat line in the oth incomplete block of the nth replication of the kth trial, μ was an intercept term, g_i was the genetic effect of the ith genotype, t_k was the effect of the kth trial, r_{nk} was the effect of the nth replication of the kth trial, b_{onk} was the effect of the oth incomplete block of the nth replication of the kth trial, and e_{ikno} was the residual. Except b_{onk}, all effects were regarded as fixed. We used estimates of variance of the residuals of single environments and calculated the average across environments, which was denoted in the following as σ_e^2.

A combined analysis across locations was performed using the following statistical model:

$$y_{ip} = \mu + g_i + \mathrm{env}_p + d_{ip},$$

where y_{ip} was the best linear unbiased estimate (BLUE) for the ith wheat line in the pth environment, μ was an intercept term, g_i was the genetic effect of the ith genotype, env_p was the effect of the pth environment, and d_{ip} was the residual, which is the sum of genotype × environment interaction effects and single environmental residuals. Variance components were determined by the restricted

Table 1 Description of the series of seven wheat breeding trials conducted in Germany

Trial name	Number of genotypes	Location (year)
AP1_2008	56	Bernburg (2008), Seligenstadt (2008), Wetze (2008), Wohlde (2008)
G01_2008	56	Bernburg (2008), Lautzschen (2008), Schmoel (2008), Seligenstadt (2008), STW (2008), Toisdorf (2008), Wetze (2008), Wohlde (2008)
G02_2008	56	Bernburg (2008), Lautzschen (2008), Schmoel (2008), Seligenstadt (2008), STW (2008), Troisdorf (2008), Wetze (2008), Wohlde (2008)
G02_2009	56	Bernburg (2009), Lautzschen (2009), Schmoel (2009), Seligenstadt (2009), STW (2009), Toisdorf (2009), Wetze (2009), Wohlde (2009)
G03_2009	110	Bernburg (2009), Kondratowice (2009), Seligenstadt (2009), Wetze (2009), Wohlde (2009)
G04_2009	100	Bernburg (2009), Kondratowice (2009), Seligenstadt (2009), Wetze (2009), Wohlde (2009)
G05_2009	64	Bernburg (2009), Kondratowice (2009), Seligenstadt (2009), Wetze (2009), Wohlde (2009)

maximum likelihood (REML) method assuming a random model. The estimate of σ_d^2 reflects the sum of $\sigma_{G\times E}^2$ and σ_e^2 divided by the number of replications, which was 2 in our study. Variance component due to genotype × environment interactions was therefore calculated as $\sigma_{G\times E}^2 = \sigma_d^2 - \sigma_e^2/2$ following standard procedure (Cochran and Cox 1957). Heritability on an entry-mean basis was calculated as the ratio of genotypic to phenotypic variance according to Melchinger et al. (1998). In addition, BLUEs across environments were estimated by assuming fixed genetic effects.

Molecular data analyses

The 455 wheat lines were fingerprinted following standard protocol with 115 simple sequence repeat (SSR) markers (Supplementary Table S1). 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). Moreover, extent of linkage disequilibrium (LD) between all pairs of loci was determined estimating D' as described by Hedrick (1987). LD analyses and PCoA were performed using software Plabsoft (Maurer et al. 2008).

Association mapping

Following Stich et al. (2008a), we applied for each of the 115 SSR markers, a two-step association mapping approach based on y_{ip}, the BLUE for the ith wheat line in the pth environment, using the following statistical model:

$$y_{ip} = \mu + m_v + g_i + \mathrm{env}_p + d_{ip},$$

where m_v denotes the effect of the vth marker genotype. All effects except m_v were regarded as random. Note that the applied two-step approach possesses only a slightly reduced power for detection of marker-phenotype associations than one-step approaches following Stich et al. (2008a). The variance of the random effects $g^*\{g_1,\ldots, g_{455}^*\}$ was assumed to be $\mathrm{Var}(g^*) = 2\,K\sigma_G^2{}^*$, where $\sigma_G^2{}^*$ refers to the genetic variance estimated by REML and K was a 455 × 455 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. In practice, the value of T_{ij} is unknown. We estimated one optimum value of T for all pairs of inbreds using a REML method suggested by Stich et al. (2008b) setting negative kinship values between inbreds to zero. Based on the Wald F statistic, we performed tests for the presence of marker-phenotype associations with a significant ($P < 0.05$) effect on grain yield and heading time applying the Bonferroni–Holm procedure (Holm 1979). For significant main effects, QTL × environment interactions have been tested extending the above statistical model with a term m_v:env_p (Stich et al. 2008a). The proportion of the phenotypic variance explained by all QTL was determined by the estimator R_{adj}^2 as described by Utz et al. (2000). The proportion of the genotypic variance explained by all detected QTL was estimated from the ratio $p_G = R_{\mathrm{adj}}^2/h^2$.

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

$$y_{ip} = \mu + m_v + m_w + m_v : m_w + g_i + \mathrm{env}_p + d_{ip},$$

where m_v and m_w denote the effect of the vth and wth marker genotype and m_v:m_w refers to the interaction effect of the vth and wth marker genotype. Based on the Wald F statistic, we performed a test for the presence of significant ($P < 0.05$) pairwise interaction effects for grain yield and heading time 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 variance component analysis across environments revealed significant variances for environments, genotypes and genotype × environment interactions for both traits (Table 2). Heritability was substantially higher for heading time compared to grain yield. Grain yield averaged 9.8 Mg ha^{-1}. Heading time ranged from 49.8 to 63.6 at BBCH stage. This difference of 14 BBCH stages corresponds to around 10 days. Both traits, grain yield and heading time, showed no linear relationship with a correlation coefficient of 0.00 (Supplementary Figure S3).

The average number of alleles per locus was 6.4, with the number of alleles per marker locus ranging from 2 to 20. The proportion of marker loci with shared variants between inbreds i and j, S_{ij}, averaged 0.51 (Supplementary Figure S1). The extent of LD decreased with increasing genetic map distance between marker loci (Fig. 1). In total, we observed 88% of adjacent marker loci pairs were in

Table 2 Means, ranges, environmental variance components (σ^2_E), genotypic variance components (σ^2_G), genotype × environment interaction variances ($\sigma^2_{G\times E}$), error variances (σ^2_e), and broad sense heritabilities (h^2) of 455 wheat lines evaluated for grain yield (Mg ha^{-1}) and heading time (BBCH stage) in five (heading time) to six environments (grain yield)

Parameter	Grain yield	Heading time
Mean	9.8	55.9
Min	8.1	49.8
Max	11.1	63.6
σ^2_E	1.17**	2.42**
σ^2_G	0.12**	6.56**
$\sigma^2_{G\times E}$	0.17**	0.94**
σ^2_e	0.07	0.82
h^2	0.78	0.96

** Significant at $P < 0.01$

significant ($P < 0.05$) LD. The first principal coordinate revealed two subgroups of genotypes (Fig. 2). A closer look at the pedigree information revealed that the smaller group consisted mainly of British varieties or varieties derived from one British parent.

The optimum identity-by-state probability for estimation of the K matrix, which was calculated for the current data set using a REML approach, was 0.75 for both traits (data not shown). The genome-wide scan revealed 10 significant ($P < 0.05$) marker-phenotype associations for grain yield and 12 for heading time (Fig. 3). The proportion of the genotypic variance explained simultaneously by all markers with significant main effect was the same for heading time (46%) and grain yield (46%). The proportion of the genotypic variance explained by the individual markers ranged for grain yield from 2.0 to 14.9% and for heading time from 0.1 to 19.3% (Table 3).

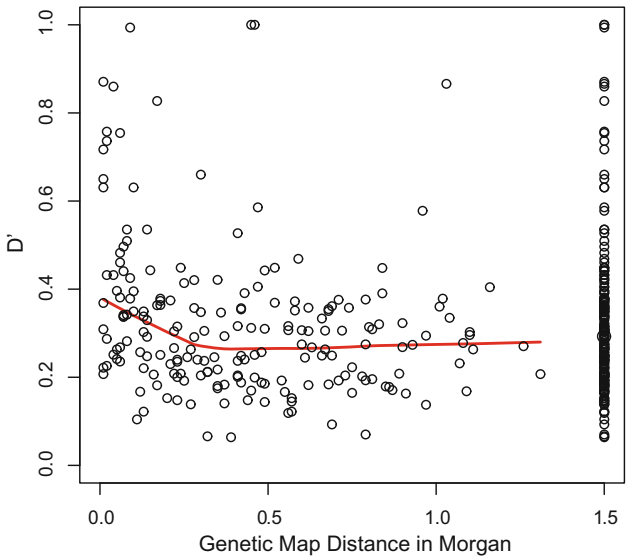

Fig. 1 Linkage disequilibrium (D′) between SSR markers as a function of genetic map distance. A genetic map distance of 1.5 M was chosen to represent unlinked loci on different chromosomes. Curve was fitted by robust locally weighted regression

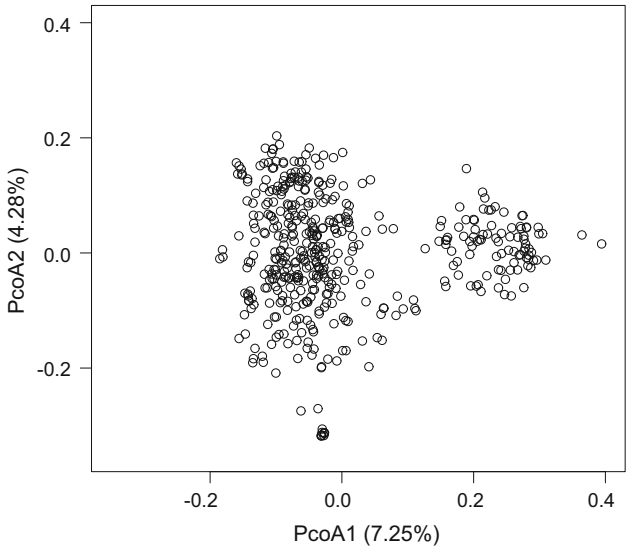

Fig. 2 Principal coordinate analysis of the 455 entries based on modified Rogers' distance estimates. Percentages in parentheses refer to the proportion of variance explained by the principal coordinate

The two-dimensional scan revealed 5 significant ($P < 0.05$) digenic epistatic interactions for grain yield and 7 for heading time (Fig. 4). The proportion of the genotypic variance explained simultaneously by all main and epistatic marker effects was substantially higher for heading time (93%) compared to grain yield (58%). The proportion of the genotypic variance explained by the individual digenic epistatic interactions ranged for grain yield from 0.2 to 2.2% and for heading time from 0.4 to 7.7% (Table 4).

Discussion

The relevance, magnitude and sign of epistatic effects are important for understanding gene function and interaction (Boone et al. 2007; Phillips 2008), speciation (Coyne 1992), evolution of sex and recombination (Barton and Charlesworth 1998), the dynamic of evolving populations (Cheverud and Routman 1996), and changes of genetic variances caused by long-term selection (Carlborg et al. 2006) or by a population bottleneck (Goodnight 1987). Unraveling epistasis based on association mapping designs allows estimating effects in populations with allele frequencies, which are of direct relevance for breeding. Moreover, detection of epistatic QTL with association compared to linkage mapping may be more promising,

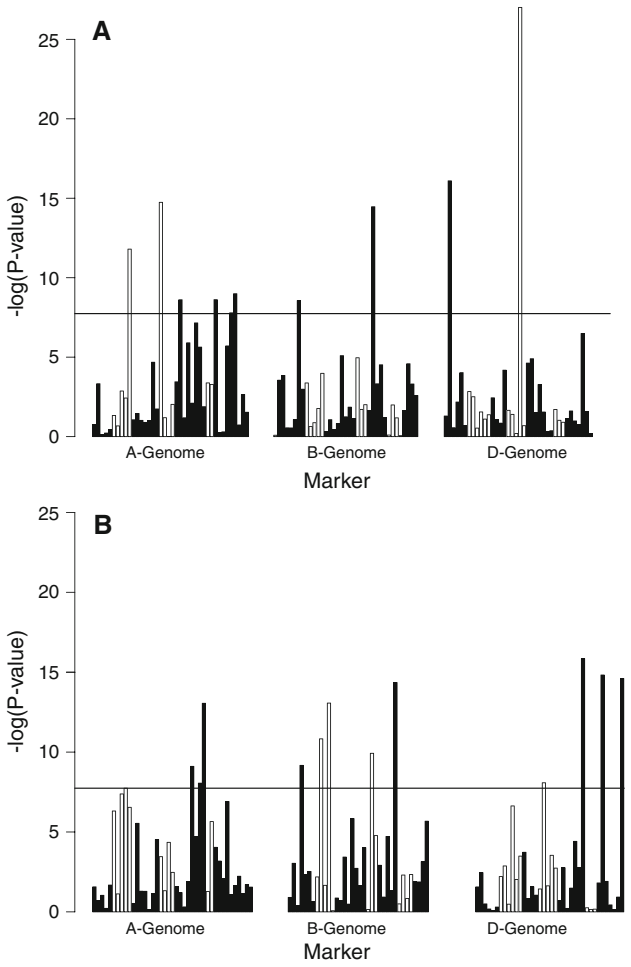

Fig. 3 Plot of *P* values of the 115 markers associated with (**a**) grain yield and (**b**) heading time. The *horizontal line* refers to a threshold of $P < 0.05$ applying a Bonferroni–Holm correction for multiple tests. *Black or no filling* of the bars were used to differentiate the different chromosomes

because fixation of QTL alleles is less likely. Therefore, we investigated the extent of epistasis in grain yield and heading time in soft winter wheat on the basis of an association mapping design.

Applicability of genome-wide association mapping in soft winter wheat

The power to detect QTL greatly depends on the sample size, the heritability of the traits under consideration, and the applied marker density. Our study was based on a large experimental data set of 455 wheat elite lines. Lines are adapted to Central European conditions as underlined by the absence of a positive association between grain yield and flowering time (Supplementary Figure S3). Genotypic effects were estimated with high accuracy reflected by heritabilities of 0.78 for grain yield and 0.96 for flowering time (Table 2). Therefore, the experimental setup should allow to elucidate the genetic architecture of grain yield and heading time in soft winter wheat.

Association mapping is expected to enable a higher mapping resolution than traditional linkage mapping methods, as it employs LD based on historical recombinations (Myles et al. 2009). For our dataset we found that LD stretched over a distance of up to 20 cM (Fig. 1). This is in accordance with a survey of US wheat lines (Chao et al. 2007) as well as with a study based on CIMMYT wheat lines (Crossa et al. 2007) and corroborates results from breeding populations of other crops such as maize (Stich et al. 2005) and barley (Kraakman et al. 2004). In contrast, Breseghello and Sorrells (2006) observed in US soft winter wheat that LD extended in centromeric regions of chromosome 5A for 5 cM and observed an even faster decline on chromosome 2D within <1 cM. The differences in the extent of LD between the studies can be explained by varying population histories of the underlying germplasm with differences in the number of effective meiosis, population stratification, degree of relatedness, and presence of selection as well as genetic drift (Stich et al. 2007).

The extent of LD due to linkage of up to 20 cM suggests that the applied marker density with an average of one marker per 22 cM represents the lower limit for genome-wide association mapping in this soft winter wheat elite breeding population. Although utmost emphasis was given to select equally spaced markers, some chromosomal regions were not covered with markers (Supplementary Table S1). These gaps must be kept in mind when interpreting the QTL results from our study.

Appropriate statistical model for association mapping

Correcting for population and/or family structure is essential for association mapping to decrease the number of false positive QTL (Yu et al. 2006; Zhao et al. 2007). The first two principal coordinates explained only 11.53% of the total variation (Fig. 2). This finding is expected in elite wheat breeding germplasm improved through intra-population selection and suggests absence of a clear population structure as for instance in maize (Reif et al. 2005). Therefore, we have not used a biometrical model with subpopulation effects, which could lead to an overcorrection for population stratification resulting in a low power to detect QTL (Würschum et al. 2011). For both traits, the REML estimate of the optimum identity-by-state probability for the calculation of the *K* matrix was 0.75, which is in accordance with findings from Stich et al. (2008b) using the same marker system in soft winter wheat. It is important to note, that all negative kinship values K_{ij} between inbreds *i* and *j*, i.e., $S_{ij} < 0.75$, were set to zero. Consequently, covariances between genotypes were only relevant for 622 pairs (0.6%) of inbred lines (Supplementary Figure S1).

Table 3 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*	Position (cM)	p_G (%)	Reported in the literature
Grain yield				
gwm047	2A	66	10.9	–
cfd071	4A	8	5.0	–
gwm205	5A	32	5.0	–
barc151	5A	13	11.9	–
barc108	7A	71	3.5	Quarrie et al. (2006)
wmc009	7A	72	2.0	Quarrie et al. (2006)
wmc694	1B	32	6.5	–
gwm213	5B	68	5.2	–
cfd072	1D	49	12.2	–
wmc457	4D	35	14.9	Quarrie et al. (2005)
Heading time				
gwm186	5A	62	2.7	–
barc232	5A	111	1.8	Kuchel et al. (2006)
gwm291	5A	163	6.3	Griffiths et al. (2009)
gwm018	1B	33	1.7	Griffiths et al. (2009)
barc018	2B	60	7.5	Hanocq et al. (2004)
barc101	2B	76	1.2	Hanocq et al. (2007)
wmc413	4B	30	0.1	Griffiths et al. (2009); Hanocq et al. (2007)
barc059	5B	154	4.6	–
sw4brht2	4D	20	0.2	Griffiths et al. (2009)
gwm272	5D	116	19.3	Hanocq et al. (2007)
barc184	7D	28	9.2	–
cfd175	7D	154	5.7	Hanocq et al. (2007)

* For markers mapping to multiple loci the first position was given

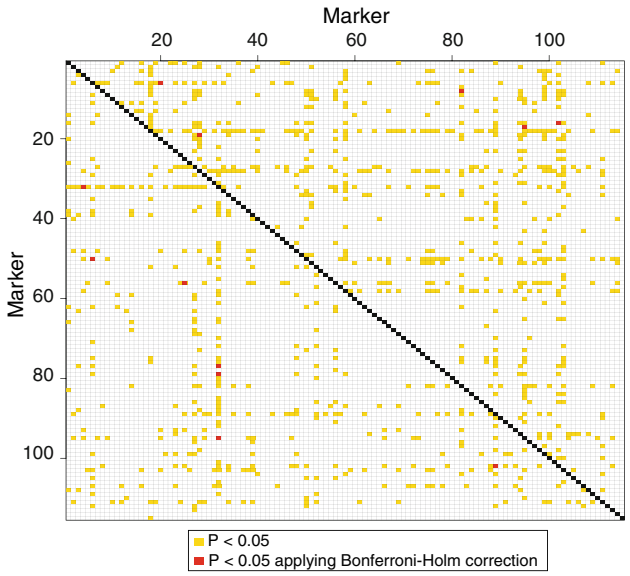

Fig. 4 Two-dimensional scan for epistatic markers associated with grain yield (*above diagonal*) and heading time (*below diagonal*)

Table 4 Trait-associated epistatic markers with their position on chromosomes, and the explained proportion of the genotypic variance p_G

Marker name	Chromosome*	Marker name	Chromosome	p_G (%)
Grain yield				
barc124	2A	gwm610	4A	0.3
gwm044	4A	barc232	5A	2.2
gwm312	2A	cfa2147	1D	1.9
gwm480	3A	cfd008	5D	0.2
gwm082	3A	sw4brht2	4D	1.5
Heading time				
barc151	5A	gwm577	7B	1.6
barc124	2A	barc018	2B	2.2
wmc415	5A	barc147	3B	0.4
barc151	5A	cfd072	1D	3.1
barc151	5A	sw4rht2	4D	0.8
taglut	1A	barc151	5A	7.7
gwm320	2D	cfd008	5D	0.4

* For markers mapping to multiple loci the first position was given

Genetic architecture of grain yield

A survey of the literature reporting QTL for grain yield in hexaploid wheat (Kuchel et al. 2007; Kumar et al. 2007; Li et al. 2007; Quarrie et al. 2005, 2006) was undertaken to validate the findings of our study. Three out of the ten QTL detected for grain yield collocated with QTL reported previously. Furthermore, we observed three major QTL

each explaining around 12% of the genotypic variance, which were not reported in other studies (Table 3). This shows that genome-wide association mapping holds the potential to detect previously unknown QTL and is well suited to unravel the genetic architecture of complex traits.

Besides the congruency with findings of previous linkage mapping studies, major candidate QTL have not been identified in our genome-scan. The *1B.1R* translocation has a positive effect on grain yield (Carver and Rayburn 1994), but was not identified as a QTL. This can be explained with the low frequency of the *1B.1R* translocation means only 7% of the 455 lines and the resulting reduced detection power of variants with low minor allele frequency (McCarthy et al. 2008). This reduced detection power has strong implications for plant and animal breeding, because marker-assisted selection is especially of interest for positive QTL alleles with minor frequency, which harbor a high risk of loss due to genetic drift in breeding populations with a commonly low effective population size. As a result, population sizes have to be extended in future association mapping studies to facilitate the detection of favorable low frequency QTL alleles.

Short-stature wheat varieties containing *Rht* genes have been reported to possess a positive effect on grain yield (Trethowan et al. 2007). Nevertheless, both *Rht-B1* (marker *sw4brht1*) and *Rht-D1* (marker *sw4brht1*) were not significantly associated with grain yield in our study (Table 3). As 18% of the lines carry neither *Rht-B1* nor *Rht-D1*, fixation of positive alleles cannot explain the lack of association, but rather point to an enhanced lodging resistance and harvest index through increased frequencies of positive alleles at several minor QTL which compensate for functional *Rht* alleles.

The variance of genotype × environment interaction was 1.4 times higher as compared to the genotypic variance (Table 2). Nevertheless, only 60% of QTL showed significant environmental interaction effects (data not shown). In addition, 80% of the QTL had larger genetic variation than QTL × environment effects (data not shown). These findings are in accordance with previous studies (Campbell et al. 2003; Kuchel et al. 2007) and suggest a stable genetic architecture of grain yield within the Central European mega-environment.

The two-dimensional scan revealed five significant digenic epistatic interaction effects (Fig. 4). Like previous studies in drosophila (Montooth et al. 2003), humans (Nyholt et al. 2008), mice (Leamy et al. 2005), rice (Li et al. 1997), and barley (Xu and Jia 2007), we found that the interacting loci have no significant main effects (Tables 3, 4). Consequently, any epistasis scan which is limited to testing only selected QTL with significant main effects will fail to detect the majority of epistatic interactions.

The proportion of the genotypic variance explained simultaneously by main and epistatic QTL effects was slightly higher (58%) compared to the fit considering only main effect QTL (46%). Moreover, genotypic variance explained by single epistatic QTL was very small with a maximum of 2.2% (Table 4). The low amount of epistasis observed for grain yield is surprising considering that we used a panel of adapted elite soft winter wheat lines. These lines have most often been developed based on second cycle breeding with the use of related inbreds, which would maintain favorable epistatic gene combinations, especially linked epistatic combinations (Lamkey et al. 1995). Fixation of alleles within elite breeding populations due to high selection intensity may be one explanation for the low amount of epistasis observed in our study.

Summarizing, integrating epistatic QTL mapped using the framework of genome-wide association mapping in marker-assisted breeding will not lead to substantially increased selection gain for grain yield. Nevertheless, recent improvements in genomic selection (e.g. Habier et al. 2010) open new ways of improving marker-assisted breeding by accommodating epistasis also in the prediction models.

Genetic architecture of heading time

The life cycle of wheat is determined predominantly by three groups of genes (Worland et al. 1998; Sourdille et al. 2000): earliness per se, photoperiod sensitivity (*Ppd*), and vernalization (*Vrn*) genes. Two of these groups interact with the environment, namely those controlling vernalization (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-B3;* Law and Worland 1997) and photoperiod response (*Ppd-A1*, *Ppd-B1*, and *Ppd-D1*; Scarth and Law 1984). The *Vrn* and *Ppd* genes have profound effects on mega-environment adaptation but contribute only to a minor extent to the genotypic variation among varieties within a mega-environment (Worland et al. 1998; Griffiths et al. 2009). In accordance with this expectation, we identified no main effect QTL, which map closely to the chromosomal regions of *Vrn* or *Ppd* genes (Table 3).

To validate the findings of our study, we performed a literature review for QTL on heading and flowering time focusing on earliness per se genes (Hanocq et al. 2004, 2007; Kuchel et al. 2006; Griffiths et al. 2009). Approximately 60% of the QTL detected (Table 3) collocated with QTL regions reported in the two meta-QTL studies of Hanocq et al. (2007) and Griffiths et al. (2009). This clearly underlines the power of genome-wide association mapping for traits with medium complexity such as heading time. Moreover, we observed two minor QTL and one major QTL, which were not reported elsewhere (Table 3). The major QTL on chromosome 7D, associated with the marker

barc184, explained 9% of the genotypic variance and is, therefore, a promising candidate for further fine mapping approaches.

The genotypic variance observed for heading time was 7 times larger compared to the variance of genotype × environment effects (Table 2), which is also reflected at the molecular level with QTL main effects showing on average of 9 times larger genetic variation than QTL × environments effects (data not shown). These findings are in accordance with a study on the genetic architecture of flowering time in maize (Buckler et al. 2009) and clearly underline the stable genetic architecture of flowering time across environments.

The proportion of the genotypic variance explained simultaneously by main and epistatic QTL effects was substantially higher (93%) compared to the fit considering only main effect QTL (46%). Separating main and interaction effects for epistatic loci revealed that epistasis contributed 15.9% of the genotypic variance (Table 4). The marker *barc151*, located in close vicinity to *Vrn-A1*, was involved in four significant QTL interaction effects (Table 4). Moreover, 52% of the interactions involving marker *barc151* displayed at a comparison-wise significance level of $P < 0.05$ significant epistatic interaction effects (supplementary Figure S2). This clearly suggests that *Vrn-A1* plays a central role in the regulatory network controlling heading time in populations adapted to the Central European mega-environment.

Summarizing, in contrast to previous studies on the genetic basis of heading time in wheat based on linkage mapping (e.g. Griffiths et al. 2009), our results clearly suggest a significant contribution of epistasis to the genetic architecture of heading time. The significant role of epistasis is expected because heading time in plants results from interactive molecular pathways (Komeda 2004) and corroborates findings from *Arabidopsis* (El-Lithy et al. 2006) and rice (Uwatoko et al. 2008).

Implications

Our study clearly underlines that genome-wide association mapping possesses a high potential to unravel the contribution of main effect QTL as well as epistasis to the expression of complex traits. Main effects dominate the control of grain yield in wheat. In contrast, the genetic architecture underlying heading time is controlled by main but also epistatic effects. Consequently, exploitation of epistasis is central towards an increased selection gain in marker-assisted breeding.

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