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ORIGINAL PAPER

Detection of epistatic interactions between exotic alleles introgressed from wild barley (*H. vulgare* ssp. *spontaneum*)

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Abstract The expression of a quantitative phenotype can be controlled through genotype, environment and genotype by environment interaction effects. Further, genotype effects can be attributed to major genes, quantitative trait loci (QTL) and gene by gene interactions, which are also termed epistatic interactions. The present study demonstrates that two-way epistatic interactions can play an important role for the expression of domestication-related traits like heading date, plant height and yield. In the BC_2DH population S42, carrying wild barley introgressions in the genetic background of the spring barley cultivar Scarlett, 13, 8 and 12 marker by marker interaction effects could be detected for the traits heading date, plant height and yield, respectively. Significant allelic combinations at interacting loci coincided for heading date, plant height and yield suggesting the presence of pleiotropic effects rather than several linked QTL. The mode of epistasis observed was primarily characterised by either (1) compensatory effects, where allelic combinations from the same genotype buffered the phenotype, or (2) augmented effects, where only the combination of the exotic allele at both interacting loci caused an altered phenotype. The present study shows that estimates of main effects of QTL can be confounded by interactions with background loci, suggesting that the identification of epistatic effects is important for gene cloning and marker-assisted selection. Furthermore, interaction effects between loci and putative candidate genes detected in the present study reveal potential functional relationships, which can be used to further elucidate gene networks in barley.

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Introduction

Epistasis occurs when the combined effect of two or more genes on a phenotype does not correspond to the sum of their separate effects (Fisher 1918). The significance of epistasis has already been suggested from classic quantitative genetic studies (Spickett and Thoday 1966; Pooni et al. 1987; Allard 1988). Even before the advent of molecular marker techniques, Fasoulas and Allard (1962) have demonstrated the existence of epistatic interactions in barley populations. Subsequent analyses using molecular markers in different plant species have shown that epistatic interactions play an important role for the expression of quantitative traits (Doebley et al. 1995; Lark et al. 1995; Li et al. 1997; Yu et al. 1997; Xing et al. 2002). In addition, recent studies have demonstrated the relevance of genetic interactions for the regulation of metabolic pathways. Rowe et al. (2008) identified a large number of epistatic interactions controlling the majority of the metabolic variation in an Arabidopsis RIL population. Similarly, Wentzell et al. (2008) found that the genetic architecture of an adaptive trait, glucosinolate activation, in *Arabidopsis thaliana* centred around large epistatic networks. These

studies demonstrate that the detection of genetic interactions may reveal functional relationships and networks among genes or their corresponding gene products.

Epistasis is also discussed as one of the possible mechanisms at the basis of hybrid performance encompassing a spectrum of important genetic phenomena, from positive heterosis or hybrid vigour to negative heterosis or hybrid sterility and lethality. A number of studies have demonstrated that epistasis is an important factor contributing to heterosis in rice (Luo et al. 2001; Hua et al. 2003; Li et al. 2008). On the other hand, epistasis may also cause post-zygotic reproductive isolation in intra and interspecific plant crosses. Kubo et al. (2008) demonstrated that hybrid male sterility was caused by two epistatic loci in rice. Two studies in Arabidopsis found that epistatic interactions were at the basis of autoimmune responses leading to hybrid necrosis and conditioning certain Dobzhansky-Muller type genetic incompatibilities (Bomblies et al. 2007; Alcázar et al. 2009). Earlier studies in evolutionary genetics have already shown that epistasis plays an important role for adaptation, population differentiation and speciation (Malmberg and Mauricio 2005). Quantitative trait loci (QTL) analyses for fitness or fitness components in field-grown or greenhouse-grown Arabidopsis populations have identified strong epistatic effects (Weinig et al. 2003; Malmberg et al. 2005). Similarly, genetic dissection of a 1-cM/210-kb interval in an Arabidopsis isogenic line revealed two QTLs with a significant epistatic effect of 34% on the total biomass depending upon the parental background (Kroymann and Mitchell-Olds 2005). Studies in crops have suggested that epistasis was also an important factor driving adaptation and domestication. Lukens and Doebley (1999) found that two chromosomal segments of teosinte introgressed into cultivated maize showed strong epistatic interactions shaping the wild type staminate flowers while every segment alone had only a small phenotypic effect. The two chromosomal segments together 'produce the nearly complete conversion of the ear on the tip of the branch into a tassel' (Lukens and Doebley 1999). Imtiaz et al. (2008) found that pre-harvest sprouting introgressed from *Aegilops tauschii*, a wild relative of wheat into cultivated durum wheat was affected by epistatic interactions. Likewise, Eshed and Zamir (1996) detected epistatic effects for yield-related traits in crosses between individual *Lycopersicon pennellii* chromosome segments introgressed into the genetic background of *L. esculentum* (cv. M82). The majority of these interaction effects were less-than-additive and the authors argued that this diminishing additivity of QTL effects may be amplified when more loci are involved and provide an important factor in phenotype canalisation and in breeding.

In the present study, we demonstrate that epistatic interactions play an important role for the expression of the domestication-related traits heading date, plant height and yield in a BC_2DH population carrying wild barley introgressions in the genetic background of a spring barley cultivar.

Materials and methods

Plant material

The development of the barley advanced backcross population S42 with 301 BC_2DH lines originating from the cross of the German spring barley variety Scarlett and the Israeli wild barley accession ISR42-8 is described in detail in von Korff et al. (2004).

Molecular characterisation

The BC_2DH population was genotyped with 98 SSR markers and ten flowering time candidate genes from the photoperiod and vernalisation pathways as described in von Korff et al. (2004) and Wang et al. (2010). At each locus, a homozygous elite barley genotype (*Hv*) and a homozygous exotic barley genotype (*Hsp*) could be distinguished.

Cultivation of the DH population

For phenotypic evaluation, the population S42 was grown in eight different environments in Germany, at four different locations (Dikopshof, Gudow, Irlbach and Morgenrot) and for two consecutive growing seasons (2003 and 2004). Details on the growing conditions and the evaluation of the traits heading date (HEA), plant height (HEI) and plot yield (YLD) are presented in von Korff et al. (2006) and Wang et al. (2010).

Testing for epistatic interactions

Epistatic interactions between all SSR marker pairs were tested with SAS procedure Mixed (SAS 2006) using the following mixed hierarchical model:

$$y_{ijkmno} = \mu + E_i + B1_j + B2_k(B1_j) + L_m + M1_n + M2_o + M1_n \times M2_o + E_i \times M1_n + E_i \times M2_o + \varepsilon_{ijkmno} \quad \text{(Model 1)}$$

where μ is the general mean and E_i is the random effect of the ith environment. $B1_j$ is the random effect of jth BC_1 plant, $B2_k(B1_j)$ is the random effect of kth BC_2 plant derived from jth BC_1 plant, and L_m is the random effect of the mth BC_2DH line. The effect $E_i \times L_m$ was not included, because the BC_2DH lines were measured in one replication per environment. $M1_n$, $M2_o$ are the fixed effects of the nth first marker (M1) genotype and the oth second marker ($M2$)

genotype, respectively. $M1_n \times M2_o$ is the fixed interaction effect of the nth $M1$ genotype with oth $M2$ genotype. $M1_i \times E_k$, $M2_j \times E_k$ are the interaction effects of the nth $M1$ genotype and the oth $M2$ genotype with the kth environment, respectively. ε_{ijkmno} is the error of y_{ijkmno}. The random effects E_i, $B1_j$, $B2_k(B1_j)$, L_m, $E_i \times M1_n$ and $E_i \times M2_o$ were declared in the random statement of the SAS procedure MIXED using the "solution" option to estimate the random effect parameters. Significant $M1 \times M2$ interactions were determined with a false discovery rate of FDR < 0.1 (Benjamini and Yekutieli 2005).

In addition, to remove interactions due to the background genetic effects, significant $M1 \times M2$ interactions from model 1 were recalculated in the following mixed model using all QTL for the respective trait as cofactors, which were identified in the single marker analysis of von Korff et al. (2006):

$$y_{ijkmnopq} = \mu + E_i + B1_j + B2_k(B1_j) + L_m + M1_n + M2_o + \sum Q_{pq} + M1_n \times M2_o + E_i \times M1_n + E_i \times M2_o + \varepsilon_{ijkmnopq} \quad \text{(Model 2)}$$

where $\sum Q_{pq}$ are the effects of the qth genotype at the pth QTL from von Korff et al. (2006). Each of the p trait QTL was represented by the closest flanking SSR marker. $M1 \times M2$ interaction effects significant in both model 1 (FDR < 0.1) and model 2 ($P < 0.01$) were considered to be due to epistasis and used for further analyses.

Significant ($P < 0.05$) differences of least squares means (Lsmeans) were calculated with the PDIFF=CONTROL option in the model 2 of the Mixed procedure (SAS Institute 2006). For significant $M1 \times M2$ effects, the Lsmeans of the three genotype combinations *Hv*/*Hsp*, *Hsp*/*Hv* and *Hsp*/*Hsp* were compared to the Lsmeans of the *Hv*/*Hv* double elite genotype as a control using a Dunnett adjustment for multiple comparison (Dunnett 1955) in order to identify a positive or negative change of phenotype due to the introgression of exotic alleles.

Results

The importance of epistatic interactions was tested for the three traits heading date (HEA), plant height (HEI) and yield (YLD) in the population S42. Of 5,778 two-way interactions tested between 98 SSR markers and 10 candidate genes with model 1, 32, 17 and 22 significant interactions were detected for the traits HEA, HEI and YLD, respectively. After re-analysing significant interactions using all single marker QTL from von Korff et al. (2006) as cofactors (model 2), 13, 8 and 12 effects remained significant for HEA, HEI and YLD, respectively. This adjustment was performed, because the sample size

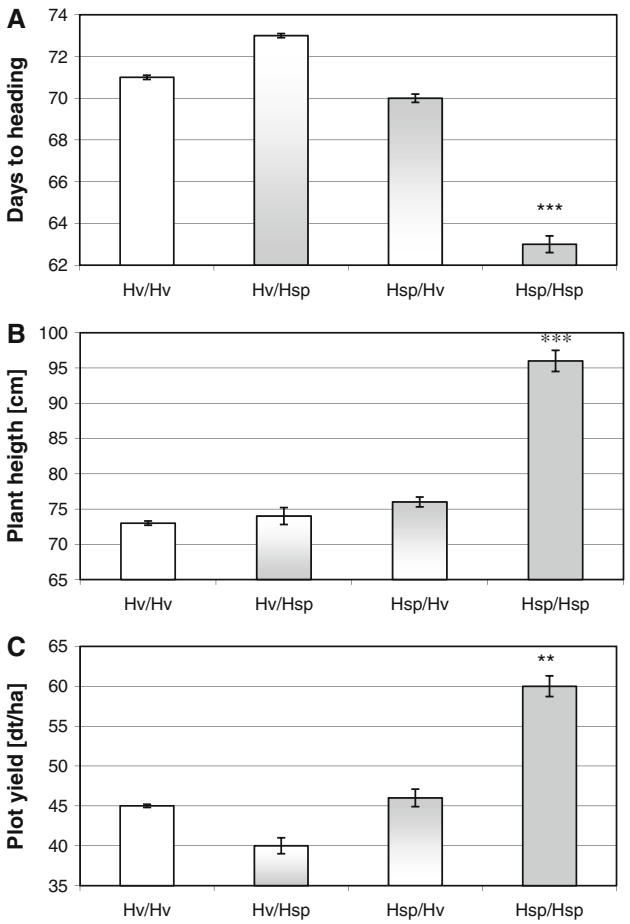

Fig. 1 Effect of epistatic interactions between **a** GBM1035$_{[2HS]}$ and Ebmac415$_{[2HL]}$ on heading date, **b** EBMAC705$_{[3HS]}$ and HVA22S$_{[7HL]}$ on plant height and **c** HVPAZXG$_{[4HS]}$ and GMS61$_{[5HL]}$ on plot yield. Mean phenotypic performance and standard errors are shown for genotype combinations *Hv*/*Hv*, *Hv*/*Hsp*, *Hsp*/*Hv* and *Hsp*/*Hsp* (*Hv* = homozygous elite genotype, *Hsp* = homozygous exotic genotype). Genotype combinations with significantly different least squares means when compared with the control genotype *Hv*/*Hv* are marked with *asterisks* (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

for each of the pairwise genotype combination was relatively small, and there were a number of QTL segregating for each of the traits. Significant interactions may thus have arisen from the non-random sampling of segregating QTL. The final number of interactions was determined after removing interactions that were due to linkage effects. In the following, the detected epistatic effects are presented for each trait separately. An example for each trait is also given in Fig. 1.

Heading date

Using HEA as an example, Fig. 2 illustrates the interaction effects for HEA relative to each other and to the genomic locations of flowering time candidate genes and main effects (von Korff et al. 2006; Wang et al. 2010).

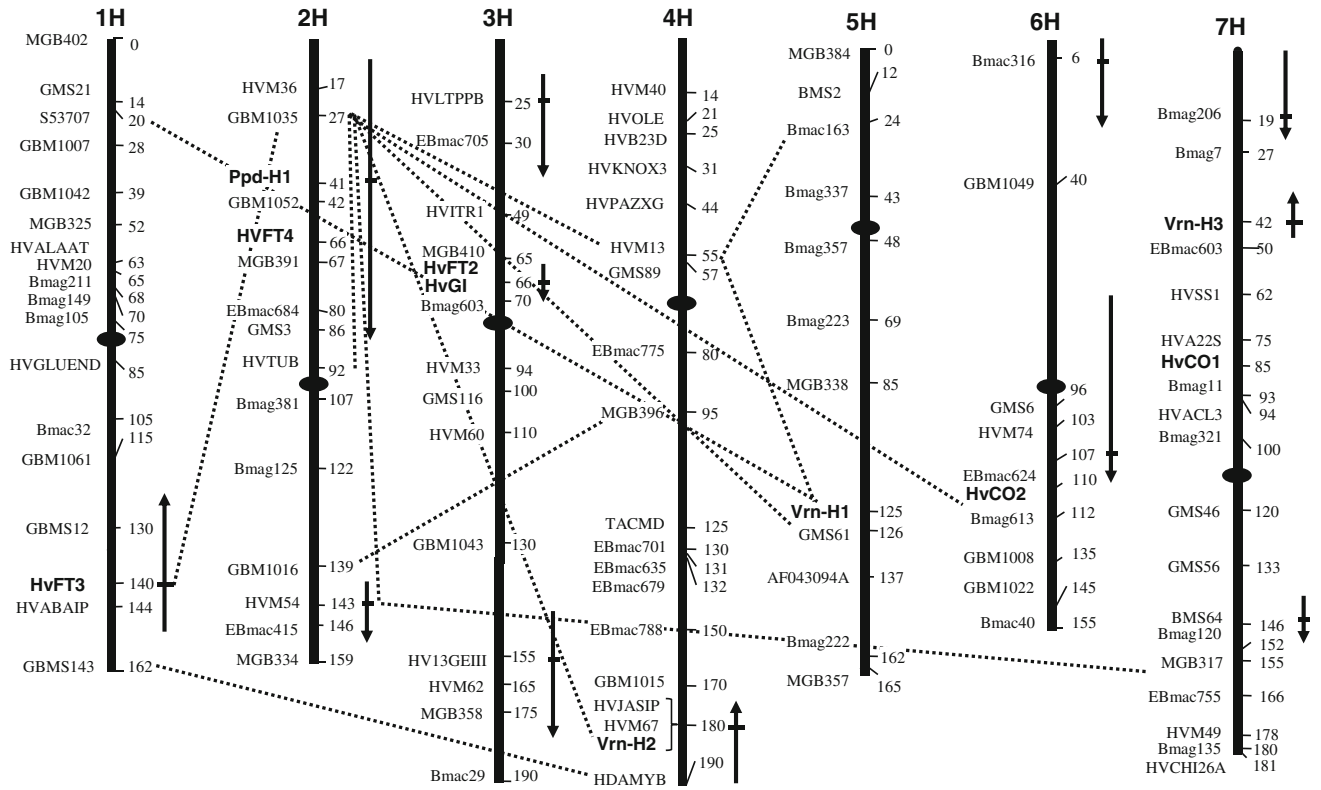

Fig. 2 QTL map for trait heading (HEA) in the barley advanced backcross population S42. QTL main effects as detected by von Korff et al. (2006) and Wang et al. (2010) are indicated by *arrows* extending from the half-intervals flanking the first and the last significant marker in a linkage group. The *horizontal dashes in the arrows* indicate the marker with the highest *F* value in the ANOVA. The *direction of the arrows* indicates the allelic effect: *upward* the *Hsp* genotype is increasing the trait value, *downward* the *Hsp* genotype is decreasing the trait value. Epistatic effects between marker pairs are indicated by a *dashed line*. Candidate genes have been mapped as described by Wang et al. (2010). The *number* to the right of the marker name represents the map position in cM taken from von Korff et al. (2004)

GBM1035$_{[2HS]}$ and markers on 4HS exhibited multiple interaction effects. The strongest effect was identified between the markers GBM1035$_{[2HS]}$ and Ebmac415$_{[2HL]}$ and between HVM13$_{[4HS]}$ and Vrn-H1$_{[5HL]}$ (Table 1, Fig. 1a), where lines carrying the exotic allele (*Hsp*) at both marker pairs flowered 8 days earlier than lines with the elite allele (*Hv*) at both loci. In contrast, BC_2DH lines with the genotype combination *Hsp*/*Hv* and *Hv*/*Hsp* at both marker combinations exhibited the same heading time as lines with *Hv*/*Hv* genotype. Similarly, interactions between the exotic alleles at marker pairs GBM1035$_{[2HS]}$/GMS61$_{[5HL]}$ and GBM1035$_{[2HS]}$/Bmag613$_{[6HL]}$ caused earlier flowering by on average 6 days when compared with the elite allele at both loci. For interaction effects of GBM1035$_{[2HS]}$ with HVM13$_{[4HS]}$ and Vrn-H2$_{[4HL]}$, respectively, the allelic combinations of *Hsp*/*Hv* triggered earlier flowering. A delay in heading date was caused by the *Hsp*/*Hsp* combination at four marker pairs. For example, epistatic interactions between exotic alleles at S53707$_{[1HS]}$/Vrn-H1$_{[5HL]}$, and GBM1016$_{[2HL]}$/MGB396$_{[4HL]}$ delayed heading by 4 days on average.

Plant height

The allelic combination of *Hsp*/*Hsp* at four interacting loci increased plant height significantly as compared to the combination *Hv*/*Hv*. For example, BC_2DH lines carrying the *Hsp*/*Hsp* genotype at Ebmac705$_{[3HS]}$ and HVA22S$_{[7HS]}$ were on average 23 cm taller than lines with the allelic combination *Hv*/*Hv* (Fig. 1b). In addition, the genotype combinations of *Hv*/*Hsp* and *Hsp*/*Hv* increased height significantly as compared to the *Hv*/*Hv* genotype at four out of eight interaction effects, where the combination of Hsp/Hsp did not change height. The combination of *Hv*/*Hsp* at Bmag125$_{[2HL]}$ and Ebmac705$_{[3HS]}$, for example, increased plant height by 14 cm on average. The only *Hv*/*Hsp* combination which decreased plant height was found at MGB402$_{[1HS]}$ and Ebmac684$_{[2HS]}$.

Plot yield

For YLD, at 4 out of 12 interaction effects the allelic combination exotic by exotic caused a strong decrease in

Table 1 List of significant *M*1 × *M*2 interactions for heading date (HEA), plant height (HEI) and plot yield (YLD) located in the barley advanced backcross population S42

Locus1[a]	Chr1[b]	Pos1[c]	Bin[d]	Locus2[a]	Chr2[b]	Pos 2[c]	Bin[d]	Hv/Hv[e]	Hv/Hsp[e]	Hsp/Hv[e]	Hsp/Hsp[e]	Main effect 1[f]	Main effect 2[f]
Heading date													
S53707	1H	20–28	3	VRNH1	5H	125.1	10	70	68	71	74***		
HVFT3	1H	130–144		GBM1035	2H	17–27	3	71	68**	72	72	QHea.S42–1H.a	QHea.S42-2H.a
GBMS143	1H	162	14	HDAMYB	4H	190	13	68	70**	70**	67		QHea.S42-4H.a
GBM1035	2H	27	3	HVTUB	2H	92	8	72	71	69**	71	QHea.S42-2H.a	
GBM1035	2H	27	3	EBMAC415	2H	146	13	71	73	70	63***	QHea.S42-2H.a	QHea.S42-2H.b
GBM1035	2H	17–27	2	HVM13	4H	44–57	6	72	71	69**	72	QHea.S42-2H.a	
GBM1035	2H	17–27	3	VRNH2	4H	180–190	13	71	72	69**	73	QHea.S42-2H.a	QHea.S42-4H.a
GBM1035	2H	17–27	2	GMS61	5H	126	10	72	73	70	66***	QHea.S42-2H.a	
GBM1035	2H	17–27	3	BMAG613	6H	96–112	9	72	72	72	66***	QHea.S42-2H.a	QHea.S42-6H.b
GBM1016	2H	139	12	MGB396	4H	95	8	69	70	70	73**		
EBMAC415	2H	122–146	13	EBMAC755	7H	146–166	11	70	69	68**	72**	QHea.S42-2H.b	QHea.S42-7H.b
HVM13	4H	55–57	6	BMAC163	5H	12–24	4	69	71	70	66***		
HVM13	4H	55–57	6	VRNH1	5H	125.1	10	70	71	70	62***		
Plant height													
MGB402	1H	0	1	EBMAC684	2H	80	7	77	70***	75	93***		QHei.S42-2H.b
GBMS12	1H	130	13	BMAC163	5H	24	4	84	82	83	93**		
BMAC32	1H	105	10	HVA22S	7H	75	5	77	84**	84**	76		
BMAG125	2H	122	10	EBMAC705	3H	25–30	3	77	91**	77	74		QHei.S42-3H.a
BMAG125	2H	122	10	GBM1043	3H	130	10	79	87**	77	97***		QHei.S42-3H.b
MGB410	3H	65	5	BMAC29	3H	190	16	77	80	83**	70*	QHei.S42-3H.a	QHei.S42-3H.b
EBMAC705	3H	25–30	3	HVA22S	7H	75	5	73	74	76	96***	QHei.S42-3H.a	
BMS2	5H	12	3	HVACL3	7H	75–100	7	77	79	71	95***		
Yield													
S53707	1H	20–39	3	BMAG603	3H	67–70	6	50	42**	49	52		QYld.S42-3H.a
BMAG105	1H	75	8	VRNH3	7H	42.5	3	48	43	36**	50		
HVABAIP	1H	144	13	BMAC163	5H	12–24	4	44	45	38**	27***		
HVABAIP	1H	144	13	GBM1008	6H	135	10	46	42	37**	42		
BMAG125	2H	122	10	HVFT2	3H	67	5	46	32***	43	41	QYld.S42-2H.c	QYld.S42-3H.a
HVITR1	3H	49	4	GBM1043	3H	130	10	50	44	30***	46	QYld.S42-3H.a	
HVFT2	3H	65–67		BMAC29	3H	190	16	50	48	37**	50	QYld.S42-3H.a	QYld.S42-3H.b
HVFT2	3H	67		HVM74	6H	103–107	6	48	44	34***	44	QYld.S42-3H.a	QYld.S42-6H.a
HVGI	3H	66.5		HVCO1	7H	75–120	5	50	50	41**	24***	QYld.S42-3H.a	

Table 1 continued

Locus1[a]	Chr1[b]	Pos1[c]	Bin[d]	Locus2[a]	Chr2[b]	Pos 2[c]	Bin[d]	Hv/Hv[e]	Hv/Hsp[e]	Hsp/Hv[e]	Hsp/Hsp[e]	Main effect 1[f]	Main effect 2[f]
HVPAZXG	4H	44	5	GMS61	5H	126	10	45	40	46	60**		QYld.S42-5H.c
HVKNOX3	4H	31	4	HVCO2	6H	110	7	43	43	45	37***		QYld.S42-6H.a
GBM1008	6H	135	10	BMS64	7H	146	8	47	43	45	29**		

[a] First and second interacting locus, respectively

[b] Chromosomal location of interacting loci

[c] Position (in cM) of locus derived from von Korff et al. (2004)

[d] Barley bin group of locus taken from von Korff et al. (2004)

[e] The four possible genotype combinations at the two interacting loci, *Hv/Hv*, *Hv/Hsp*, *Hsp/Hv* and *Hsp/Hsp*, where *Hv* represents the elite genotype and *Hsp* the exotic genotype in the order of the loci. Genotype combinations with significantly different least squares means compared to the control genotype *Hv/Hv* are marked with asterisks (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$)

[f] Coincidence of significant marker interaction effects with main effects detected in von Korff et al. (2006)

grain yield. The strongest reduction in yield of on average 26 dt/ha was exhibited by lines carrying the exotic alleles at the markers, $HvGI_{[3HS]}$ and $HVCO1_{[7H]}$. At 9 out of 12 interactions, the allelic combination of exotic and elite reduced yield. For example, the allelic interaction between *Hv/Hsp* at the markers $S53707_{[1H]}$ and $Bmag603_{[3H]}$ was associated with a yield reduced by 8 dt/ha. However, generally the allelic combination *Hv/Hsp* or *Hsp/Hv* caused a smaller reduction of yield than the allelic combinations *Hsp/Hsp*. The interaction between two exotic alleles increased yield only between $HVPAZXG_{[4HS]}$ and $GMS61_{[5HL]}$ (Fig. 1c). BC_2DH lines with the genotype combination *Hsp/Hsp* at this marker pair yielded on average 15 dt/ha more than lines with the allelic combination of *Hv/Hv*.

Interactions between four locus pairs overlapped between plant height and yield (Table 1). The exotic allele at $GBMS12/HVABAIP_{[1HL]}$, for example, increased plant height and reduced yield in combination with the exotic allele at $Bmac163_{[5HS]}$. Likewise, the combination of exotic by exotic allele at the loci $Ebmac705/HvGI_{[3HS]}$ and $HVA22S/HvCO1_{[7H]}$ increased plant height by on average 23 cm and reduced yield by 26 dt/ha. When the loci involved in the two-way interactions were classified into two categories: (1) markers with a QTL effect in the single marker analysis, (2) markers not significant in the single marker analysis, the majority of markers involved in interaction effects for all three traits belonged to category 2. Thirty-four interacting loci were not significant in the single marker analysis, while 31 loci were also detected as marker main effects in von Korff et al. (2006).

Discussion

In the present study, a model testing all pairwise marker combinations was used for the detection of two-way epistatic interactions. The model was applied to phenotype data of the traits HEA, HEI and YLD in the population S42. The analysis demonstrated that epistatic interactions play an important role in shaping agronomic performance in barley. This is in contrast to recent findings of Xu and Jia (2007) who demonstrated that the contribution of epistatic interactions to genetic variation of quantitative characters in barley was negligible. Differences in the detection and estimation of epistatic effects may be due to different statistical procedures employed. In the present study, a mixed model approach including markers, marker pairs, population structure, environments and possible interactions was used for testing of two-way epistatic effects with multiple main effects at a time (model 2, Holland 1998; Malmberg et al. 2005). Alternative statistical procedures employ model selection strategies that search for multiple

epistatic effects simultaneously (Yi et al. 2003, 2005). Likewise, Xu and Jia (2007) employed an empirical Bayes method for simultaneous estimation of main effects of all individual markers and epistatic effects of all pairs of markers, which allows detecting interactions with a higher power. Therefore, the larger number of epistatic effects detected in the present study may rather be explained by the choice of germplasm analysed; Xu and Jia (2007) used a doubled haploid population derived from cultivated parents, while the present study used a BC_2DH population derived from a cross between cultivated and wild barley. Studies of epistatic interactions in interspecific crosses of maize–teosinte (Lukens and Doebley 1999), durum wheat, *A. tauschii* (Imtiaz et al. 2008) and *L. esculentum*, *L. pennellii* (Eshed and Zamir 1996) suggested that epistatic interactions play a larger role in crosses involving exotic germplasm than in elite by elite crosses. This may be due to the selection and conservation of different allele combinations in wild and elite barley as an adaptation to natural and agricultural environments, respectively; and hence large phenotypic differences in domestication related traits, such as heading date, growth behaviour and yield.

Of particular interest was the identification of significant interactions between loci not identified as QTL in the single marker analysis. In many studies, only epistatic effects between loci with significant marker main effects are tested. Findings in this study, however, illustrate that a large number of significant interactions might escape discovery if not all possible marker interactions are examined. Similarly, Li et al. (1997), who analysed epistatic interactions among QTL affecting grain yield components in rice, found that most of these interactions would have remained undetected, had not all possible pairs of markers been tested for epistasis. A number of markers (48%) involved in two-way interactions were also detected as QTL. Thus, the usual estimates of QTL main effects can be confounded by interactions with background loci, as demonstrated by Eshed and Zamir (1996), Doebley et al. (1995), and Lark et al. (1995). Cockerham and Zeng (1996) even suggested that many of the QTL detectable by an ANOVA might actually represent groups of tightly linked epistatic genes.

In the present study, because the sample size for each of the pairwise genotype combination was relatively small (*Hsp*/*Hsp*), and because there were a number of QTL segregating for each of the traits, significant interactions may have arisen from a bias due to the sampling of segregating QTL. Consequently, inclusion of QTL as co-factors for the verification of significant interaction seems important, since several interactions were not significant anymore when re-analysed with QTL as cofactors. Some of the detected interactions were thus clearly spurious interaction effects, a problem, which may be particularly pronounced in unbalanced populations. For the same reason, however, a significant proportion of two-way interactions may also have gone undetected. In the following, the epistatic interactions are discussed in detail for each trait.

Heading date

In our study, the majority of significant interactions for HEA were detected for the marker $GBM1035_{[2H]}$ adjacent to the major photoperiod response gene *Ppd-H1* (Turner et al. 2005). This locus did also show the strongest effect on heading date in S42 identified in the single marker analysis (von Korff et al. 2006; Wang et al. 2010). The exotic allele promoted heading, while the elite allele delayed heading at this locus by on average 8 days across four locations in Germany and 2 years of spring plantings. Turner et al. (2005) demonstrated that a mutation in the CCT domain of this gene causes insensitivity to photoperiod in spring barley grown in Northern production areas. The non-functional mutation in *Ppd-H1* delays the circadian timing of the barley Constans orthologs and thus causes reduced expression during the light period and a concomitant decline in *Vrn-H3* (*HvFT1*) levels (Turner et al. 2005). The candidate gene *Ppd-H1* itself did not show significant interactions, presumably because only a small number of lines carried the exotic allele at this locus, so that even fewer lines carried the exotic allele at *Ppd-H1* in combination with the elite or exotic allele at the second locus. However, the direction of the effect of the exotic allele at *Ppd-H1* corresponded to the effect at the marker locus GBM1035, while the magnitude was generally greater.

For example, the exotic allele at GBM1035 (*Ppd-H1*) locus did accelerate heading only in interaction with the exotic allele at Bmag613/$HvCO2_{[6H]}$, while it did not show an effect on heading in combination with the elite allele at this locus. *HVCO2* is next to *HVCO1* the closest barley ortholog of the Arabidopsis Constans gene which promotes flowering in response to photoperiod (Griffiths et al. 2003).

In addition, the exotic allele at the locus on 2HS reduced time to heading in combination with the elite alleles at *HvFT3* and *Vrn-H2*. Several studies suggested that *HvFT3* is identical to *Ppd-H2*, which is a major QTL for heading time under SD conditions in barley (Faure et al. 2007; Kikuchi et al. 2009). The exotic allele at *HvFT3* seems to counterbalance the early induction of flowering by *Ppd-H2*, since only the combination of *Hv*/*Hsp* at *HvFT3*/*Ppd-H1* advanced heading significantly. The *Vrn-H2* region includes three similar ZCCT genes encoding proteins with a putative zinc finger and a CCT domain with no clear orthologs in Arabidopsis (Yan et al. 2004). These ZCCT genes, which repress heading under long day conditions, are downregulated by cold, and deletion of both genes is

associated with spring growth habit in barley (Dubcovsky et al. 2005). It is interesting to note that the exotic allele at 2HS only promoted flowering in a genetic background lacking the *Vrn-H2* genes (Scarlett). These results suggest that in wild barley the *Vrn-H2* gene delays flowering in the absence of vernalisation and thus counterweighs the induction of flowering through *Ppd-H1*. A locus on the short arm of chromosome 4H (HVM13) showed a similar effect on flowering in interaction with 2HS. The exotic allele on 2HS did accelerate flowering only in combination with the elite allele at $HVM13_{[4H]}$, suggesting that the exotic allele at HVM13 reduced the effect of the dominant *Ppd-H1* on the induction of flowering. The latter two interactions are examples for less-than additive interaction effects which may be important factors in phenotype compensation, domestication and breeding as described by Eshed and Zamir (1996). Furthermore, the interaction effects between loci and putative candidate genes detected in the present study reveal potential functional interactions, which can be used to further elucidate the floral network in barley.

Plant height and plot yield

The majority of epistatic interactions detected for plant height involved markers, which were not significant in the single marker analysis (Table 1). The wild barley parent is significantly taller than Scarlett, and in the single marker analysis the exotic allele increased plant height at most loci (von Korff et al. 2006). Accordingly, the combination of *Hsp*/*Hsp* significantly increased plant height at five marker pairs. For example, the allelic combination *Hsp*/*Hsp* at the epistatic loci $Ebmac705_{[3H]}$ and $HVA22S_{[7H]}$ increased height by on average 23 cm as compared to *Hv*/*Hv* (Fig. 1b). As for several interactions detected for heading date, the phenotypic effects of the double introgression of the exotic genotype were of a higher magnitude than the effects of the combination of *Hsp* and *Hv* genotypes at two interacting loci.

Of all traits analysed in von Korff et al. (2006), plant height showed the strongest correlation to yield, where taller plant reduced grain yield. This correlation was reflected in a coincidence of loci affecting plant height and yield in the single marker analysis. Out of 12 interaction effects detected for yield, four were also significant for plant height, where an increase in plant height reduced yield. These genetic correlations may result from pleiotropic effects of single QTL or from linkage of several genes controlling the traits. However, the detection of the same interaction effects for more than one trait suggests pleiotropy of certain allele combinations affecting growth, and hence yield.

Markers on the short arm of chromosome 3H showed the largest number of interaction effects (Ebmac705, Bmac29). For example, the exotic allele at 3HS increased height and decreased yield in combination with the elite allele at $Bmac29_{[3H]}$. At $Bmac29_{[3H]}$, the strongest main effect on plant height in the single marker analysis was detected. This locus maps close to the semi-dwarf (*sdw1*) gene which controls plant height, but also yield and quality. A comparative genomic analysis revealed that the *sdw1* gene was located in the syntenic region of the rice semi-dwarf gene *sd1* on chromosome 1. The *sd1* gene encodes a gibberellic acid (GA)-20 oxidase enzyme which catalyzes the step to the biologically active plant hormone (Jia et al. 2009). Loci interacting with Bmac29 may therefore point to genes, which could be important to facilitate the GA 20-oxidase mediated growth phenotype, either by acting in the same or in a different interconnected pathway. Interestingly, one interaction between chromosomes 4H and 5H (HVPAZXG and GMS61) could be identified where the double exotic genotype *(Hsp/Hsp)* produced a significant increase of yield (+15 dt/ha = +33%). A main QTL effect was already identified for GMS61 where the exotic allele was associated with a reduction of yield (von Korff et al. 2006). Further studies are needed to verify the positive exotic interaction effect on yield. Individual introgression lines, derived from the present S42 population, are currently intercrossed to produce double introgression lines which simultaneously segregate at the two loci in question (see below).

Conclusions

The present analysis of epistatic interactions revealed differences in behaviour of alleles due to the effect of the interacting locus. This was also observed at loci, which were significant in the single marker analysis. The involvement of QTL in epistatic interactions demonstrates that the effects of the single-locus QTL are often dependent on the genotypes of other loci. The effect of a QTL can thus sometimes be increased or negated by the genotypes of a second locus.

Analysis of epistatic interactions may be used to place QTL or candidate genes into tentative pathways and networks and thus give further insight into their functional relationships. The majority of interactions we observed showed either (1) compensatory effects, where allelic combinations from the same genotype buffered the phenotype, or (2) augmented effects, where only the combination of the exotic allele at both interacting loci caused an altered phenotype. For continuously distributed traits, a large number of QTL affect a particular phenotype. Compensatory interactions among QTL ensure that the mutation

of an allele affecting a fitness trait will have a minimal effect on the phenotype. Multiple interactions between a large number of genes ensure that quantitative characters are developmentally buffered, such that the phenotype is kept within narrow boundaries, despite genetic and environmental disturbances.

On the other hand, cultivated spring barley and wild barley with very different life histories, i.e. biannual and annual growth, may have evolved distinct pathways in adaptation to different environments. This is indicated by the findings that certain phenotypes are only expressed when the exotic allele is present at both interacting loci.

In future, the detected epistatic effects in population S42 will be validated in double introgression lines (double ILs). For this, we have already developed and utilised a set of barley introgressions lines (S42ILs) which are derived through further backcrossing and marker-assisted selection from S42 (Schmalenbach et al. 2008, 2009). After crossing those S42ILs, sets of double ILs containing all four possible genotype combinations at two independent loci, *Hv*/*Hv*, *Hv*/*Hsp*, *Hsp*/*Hv* and *Hsp*/*Hsp*, can be selected with informative markers and then be used to validate the significant epistatic effects presented here. Thereupon, verified epistatic effects are the basis for both, map-based gene cloning projects of the underlying genes and for transferring the favourable interacting genotypes to new elite breeding germplasm, which may ultimately result in improved elite varieties.

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