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Molecular breeding for grain yield in barley: an evaluation of QTL effects in a spring barley cross

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Abstract We report results from a breeding strategy designed to accumulate favorable QTL alleles for grain yield identified in the Steptoe × 'Morex' (SM) barley germplasm. Two map lines (SM73 and SM145) from the original mapping population were selected based on their marker genotype and QTL structure. When crossed, these lines would be expected to produce progeny with most favorable QTL alleles. One hundred doubled haploid (DH) lines from the F_1 hybrid of this cross were genotyped with ten RFLP markers and one morphological marker defining grain yield to monitor QTL segregation. A subset of 24 lines representing various combinations of putatively favorable and unfavorable QTL alleles, together with Steptoe, 'Morex', SM73, and SM145, were phenotyped for grain yield in five environments. Multiple regression procedures were used to explore phenotype and genotype relationships. Most target QTLs showed significant effects. However, significance and magnitude of QTL effects and favorable QTL allele phase varied across environments. All target QTLs showed significant QTL-by-environment interaction (QTL × E), and the QTL on chromosome 2 expressed alternative favorable QTL alleles in different environments. Digenic epistatic effects were also detected between some QTL loci. For traits such as grain yield, marker-assisted selection efforts may be better targeted at determining optimum combinations of QTL alleles rather than pyramiding alleles detected in a reference mapping population.

Key words Barley · Yield · Marker-assisted selection · QTL · QTL × E

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Introduction

The development of molecular marker techniques has allowed the construction of high-density genome linkage maps for a range of crops including barley (*Hordeum vulgare* L.) (Graner et al. 1991; Kleinhofs et al. 1993; Kasha et al. 1995). Coupled with statistical procedures, these maps have been employed to identify, locate and estimate the phenotypic effects of quantitative trait loci (QTLs) that determine economically important traits such as grain yield (Hayes et al. 1993; Tinker et al. 1996), malting quality (Hayes et al. 1993; Mather et al. 1997) and disease resistance (Chen et al. 1994; Steffenson et al. 1996). There are, as yet, few examples of QTL information applied to the development of crop varieties.

Two general strategies have been proposed to use marker-QTL associations for marker-assisted selection (MAS) in crop improvement. One involves introgression of a limited number of QTLs via marker-assisted backcrossing (Dudley 1993). Through this process, molecular markers can minimize linkage drag and expedite the transfer of target genome blocks from exotic

germplasm into a desired background (Young and Tanksley 1989; Tanksley and Nelson 1996). In barley, this approach has been used to introgress QTLs conferring adult plant resistance to stripe rust (*Puccinia striiformis f.* sp. *hordei*) into a genetic background unrelated to the mapping population (Toojinda et al. 1998). Another strategy, suitable for larger number of QTLs and for multiple-trait selection, is to use QTL information to design matings that will maximize the probability of pyramiding most, if not all, favorable QTL alleles in a single genotype (Dudley 1993; Hayes et al. 1996).

We attempted to accumulate favorable QTL alleles for grain yield in a single genotype derived from the 'Steptoe' × 'Morex' (SM) germplasm. The doubled-haploid (DH) population from Steptoe × Morex has been the subject of intensive efforts for genome mapping and QTL analysis by the North American Barley Genome Mapping Project (Hayes et al. 1996). Steptoe is a high-yielding, broadly-adapted six-rowed feed barley. Morex is a six-rowed cultivar used as the American malting quality standard. We reported QTLs that determine grain yield using interval mapping procedures and phenotypic data from multiple trials conducted throughout the USA and Canada over a 2-year period (Hayes et al. 1993, 1996). This provided a detailed assessment of the inheritance for this trait, including estimates of QTL number, location and effect (Table 1). Grain yield QTLs mapped to all chromosomes, and Steptoe was the primary contributor of favorable alleles. The most consistently detected QTLs were on chromosomes 2 and 3. The chromosome 3 QTL was significant in 10 of 16 environments. The significance and magnitude of other QTL effects varied with environments. The QTL on chromosome 2 was significant in 11 of 16 environments, and this QTL showed alternative favorable alleles in different environments. No genotype in the original mapping population had all favorable QTL alleles for grain yield. However, based on marker-QTL associations, we identified 2 DH lines, SM73 and SM145, that, when crossed, would be expected to produce progeny with a maximum number of favorable alleles both for grain yield and malt extract (a malting quality trait). When data are available, the results of the malting quality selection experiment will be reported. For grain yield, some favorable QTL alleles with large effects were already fixed in this mating. Therefore, in the progeny of the SM73 × SM145 cross, we could evaluate those QTLs that had small effects or that showed QTL-by-environment interaction (QTL × E). DH lines were developed from the F_1 hybrid of SM73 × SM145. These were then genotyped and phenotyped for grain yield in 5 environments. The objectives of this research were to validate QTL effects for grain yield in the Steptoe × Morex mapping population and to assess the utility of a MAS strategy for grain yield improvement in barley.

Materials and methods

Two map lines, SM73 and SM145, were selected from the Steptoe × Morex mapping population (Hayes et al. 1993) based on their marker genotypes and QTL structure. One hundred DH lines, derived from the F_1 hybrid of this cross by the *Hordeum bulbosum* method (Chen and Hayes 1989), were genotyped with markers bracketing segregating yield QTLs. Of these lines 24 were selected based on their marker genotypes to represent various combinations of desirable QTL alleles. These lines, together with Steptoe and Morex (grandparents), SM73 and SM145 (parents), were phenotyped for grain yield in 5 environments.

The selected DH lines and their genotypes are presented in Table 2. Four RFLP markers, spanning a total length of 46.3 cM according to the original map (Hayes et al. 1993), were selected to genotype the chromosome 2 QTL. Intervals between flanking markers ranged from 7.9 to 19.6 cM. A large segment was chosen at this region because of position ambiguity for this QTL in different environments. This was the only QTL showing a change of favorable allele type QTL × E in the original mapping population (Hayes et al. 1996). The chromosome 4 QTL was significant only at

Table 1 Distribution of grain yield QTLs in the Steptoe × Morex (SM) population and their status in the SM73 × SM145 ideal genotype (IG) population

Chromosome	Marker interval	Favorable allele[a]	Genotype[a]		Status in IG population[b]	Number of environments[c]
			SM73	SM145		
1	*Plc-ABG380*	S	S	S	F	2/16
2	*ABC311-CDO064-ABC454-ABC162*	S/M[c]	S	M	Seg	11/16[d]
3	*ABC171-His4b*	S	S	S	F	10/16
4	*ABG472-ABG397*	S	M	S	Seg	1/16
5	*His3b-ABG387A*	S	S	S	F	2/16
6	*ABG458-ABG47*	M	M	S	Seg	2/16
7	*Ale-ABC302-mSrh*	M	S	M	Seg	2/16

[a] S and M indicate the parents (Steptoe or Morex) contributing the favorable QTL allele, as measured in the SM population. Superscript C following S/M indicates QTL allele conflicts, where alternative favorable alleles were detected in the SM population

[b] F indicates that the favorable allele is fixed, and seg indicates that the QTL alleles will segregate among the progeny of the IG population

[c] The number of environments where the QTL was significant in the SM mapping population

[d] Steptoe contributed the favorable allele in 6 environments and 'Morex' contributed the favorable allele in 5 environments

Table 2 Genotypes and grain yield phenotypes of parental lines and DH progeny from the QTL validation population

Genotype						Grain yield (kg/ha)					
Line[a]	Chr 2 *ABC311-ABG162*	Chr 4 *ABG472-ABG397*	Chr 6 *ABG478-ABG47*	Chr 7		Klamath Falls, 1995	Klamath Falls, 1996	Pendleton, 1996	Pullman, 1996	Kimberly, 1996	Overall
				Ale-ABC302	*mSrh*						
Steptoe	S	S	S	S	S	6055	4179	4749	2007	3680	4133
Morex	M	M	M	M	M	5270	3752	3689	1517	2900	3426
SM73	S	M	M	S	S	5547	3266	3771	1350	3627	3518
SM145a	M	S	S	M	M	5195	3562	4086	1734	3134	3542
1[b]	S	S	M	S	S	5698	4048	4450	1738	3668	3119
2b	S	M	S	M	M	4878	4342	4252	1507	3713	3738
3c	S	M	M	M	S	4863	3645	3728	1691	3215	3428
4	S	S	S	S	M	5488	4409	4056	1711	3513	3835
5	S	S	S	S	S	6968	4319	4236	1652	3508	4137
6d	S	S	S	M	S	6752	4036	4059	1354	3361	3912
7e	S	M	S	S	S	7400	4497	4156	1550	3476	4216
8f	S	M	M	S	M	5078	3655	3778	1716	3534	3552
9	M	S	M	M	S	5671	3336	4031	1475	3245	3552
10	M	S	M	S	S	7050	3592	3851	1846	3712	4010
11g	M	S	M	M	M	5648	3686	3566	2049	3754	3741
12c	S	M	M	M	S	5564	3616	3914	1491	3652	3647
13d	S	S	S	M	S	6244	4184	4559	1765	3718	4094
14b	S	M	S	M	M	4973	3400	3734	1568	3279	3391
15	M	M	M	S	M	6325	4902	3899	1696	3756	4098
16a	M	S	S	M	M	6246	3930	3832	1694	3618	3864
17	M	M	S	S	S	6150	3395	4154	2101	3474	3855
18f	S	M	M	S	M	4925	4189	4355	1629	3524	3724
19e	S	M	S	S	S	5640	4490	4201	1359	3316	3801
20g	M	S	M	M	M	5388	3579	3435	1840	3490	3546
21	M	M	S	M	S	6091	4042	3657	1632	3565	3797
22	S	S	M	M	M	5496	3028	4294	1663	3489	3594
23	S	M	M	M	M	4288	3544	3505	1269	3764	3274
24	S	M	S	S	M	5239	3915	4447	1744	3610	3791
				Mean		5680	3865	3989	1646	3257	3562
				SD		744	423	258	178	271	391
				Range		3112	1875	1124	832	630	1097
				Heritability (h^2%)		59	64	74	80	43	53

[a] DH lines followed by the same letter have the same genotype at the target QTL loci
[b] Lines 1–24 are the DH progeny of SM73/SM145

Bozeman, Montana in the 1992 dryland data set with Steptoe contributing the favorable allele. Two markers, spanning 34.5 cM, were selected to genotype this region. On chromosome 6, a yield QTL was identified in 2 of the 16 environments. Morex contributed the favorable allele. Two markers defining a 12.6-cM interval were used to genotype this QTL. On chromosome 7, grain yield QTLs were detected in 2 of the 16 environments. Morex contributed the favorable allele. Two restriction fragment length polymorphism (RFLP) markers (*Ale* and *ABC302*, 11.5 cM apart) and *mSrh*, a morphological marker controlling rachilla hair length, were used to monitor the segregation of this QTL. RFLP genotypes were assayed following standard protocols (Kleinhofs et al. 1993). The morphological marker *mSrh* was scored under a dissecting microscope.

The 24 DH lines, Steptoe, Morex, SM73 and SM145 were evaluated in field experiments in 5 environments: Klamath Falls, Oregon (1995 and 1996); Pendleton, Oregon (1996); Pullman, Washington (1996); and Kimberly, Idaho (1996). At each location, plot size and management were in accordance with local practice. The Pullman and Pendleton experiments were grown under dryland conditions (without irrigation). The Klamath Falls and Kimberly experiments were irrigated. A randomized complete block design with three or four replications was employed at each location. Grain yield and agronomic traits were measured as described by Hayes et al. (1993).

The selected DH lines, SM73 and SM145 were included in the following analyses. Analysis of variance (ANOVA) was performed on data from each environment and on the combined data from 5 environments. Because the selected lines are not a random sample of the reference population, approximate heritability estimates were calculated for each environment, on a plot basis, as $\hat{h}^2 = \hat{\sigma}_g^2/(\hat{\sigma}_g^2 + \hat{\sigma}_e^2/r)$ where $\hat{\sigma}_g^2$ and $\hat{\sigma}_e^2$ are the sample genotypic and error variances, respectively, and r is the number of replications in a single environment. Approximate heritability across environments was estimated as $\hat{h}^2 = \hat{\sigma}_g^2/(\hat{\sigma}_g^2 + \hat{\sigma}_{g\times e}^2/n + \hat{\sigma}_e^2/nr)$, where $\hat{\sigma}_{g\times e}^2 = G \times E$ variance, and n = number of environments. Variance components were computed by equating mean squares to their expectations.

Multiple regression procedures were used to detect the relationships between phenotype and genotype for each environment. Marker genotypes, whose alleles from Steptoe and Morex were scored as 1 and −1, respectively, were considered as independent variables. Yield data were considered as dependent variables. When consecutive marker alleles defining a QTL were from the same parent, that is, they did not show recombination, they were treated as a single variable. All QTLs were analyzed in this fashion except the QTL on chromosome 7, where there were lines showing crossovers between *ABC302* and *mSrh*. In this report, the QTL defined by *Ale-ABC302* will be referred to as QTL7a, and the QTL linked to *mSrh* will be referred to as QTL7b. Two steps were carried out for multi-locus regression analysis. First, only QTL main effects were included in the model. Second, both QTL main effects and their digenic interaction terms were included to detect the presence of epistasis. Backward elimination procedures were used to eliminate non-significant variables until all variables left were significant at the 0.05 level. The sign of the estimates was used to identify the favorable alleles, or allele combination, contributed by each parent. For QTL main effects, positive and negative signs of the estimates indicate that Steptoe (S) and Morex (M), respectively, contributed the higher value alleles for grain yield. For two-locus epistasis, a positive sign means that, on average, the combination of QTL alleles from the same parent was favorable compared with alleles from different parents; a negative sign indicates that a combination QTL alleles from two different parents was superior. The phenotypic coefficient of determination (R_p^2), computed from multiple regression models with replicated observations (plot basis), was used as a measure of the total phenotypic variation accounted for by a set of markers. The genotypic coefficient of determination (R_g^2), calculated as R_p^2/h^2 (where h^2 = heritability as described above), was used to describe the total genotypic variation accounted for by a set of markers (Schön et al. 1994). Partial R^2 values were used to evaluate the proportion of variation that could be explained by individual QTLs and/or their epistatic effects.

To address questions regarding G × E and QTL × E, we performed two steps of ANOVA analysis on the combined data from 5 environments, following the procedures of Sari-Gorla et al. (1997). First, we used the model containing environmental effects (E), genotypic effects (G, line-based), replicate effects nested within the environments and G × E. Second, we conducted the analysis using a model containing environmental effects, replicate effects nested within environments, QTL main effects, digenic epistatic effects and QTL × E. The proportion of G × E that could be explained by individual QTL × E was estimated by $\Sigma(SS_{QTL\times E})/SS_{G\times E}$, where $SS_{G\times E}$ (from step 1) and $SS_{QTL\times E}$ (from step 2) are the sums of squares for G × E and individual significant QTL × E, respectively. The percentage of genotypic variation accounted for by the individual significant QTLs and/or their digenic epistatic interactions was computed by $\Sigma(SS_{QTL} + SS_{epistasis})/SS_G$, where SS_G (from step 1), SS_{QTL} and/or $SS_{epistasis}$ (from step 2) were the sums of squares for genotypes (line-based), individual QTLs and/or their digenic epistatic interactions, respectively. All analyses were performed using SAS software (SAS Institute 1989).

Results

Grain yield phenotypes are shown in Table 2. There were large differences in mean performance among the 5 environments. The two parent lines, SM73 and SM145, were of average to lower yield in all environments. Phenotypic ranges were greater than four standard deviations except the data from Kimberly. The genotypic variation among DH lines was significant within each environment. There were both positive and negative transgressive segregants. The approximate estimates of heritability (h^2) ranged from 0.43 to 0.80. Across the environments, the h^2 was estimated to be 0.53.

Multiple regression models containing only QTL main effects were used to explore genotype and phenotype relationships. As shown in Table 3 (Model 1), significant QTL effects were detected at target regions. However, the significance and magnitude of QTL effects, as well as favorable allele phase, varied with environments. At Klamath Falls (1995), Steptoe contributed favorable alleles on chromosomes 4 and 7, while Morex contributed the favorable allele on chromosome 2. Together, these QTLs gave a multi-locus phenotypic coefficient of determination (R_p^2) of 27%. The heritability for this environment was 0.59, giving a genotypic coefficient of determination (R_g^2) of 46%. QTLs on chromosome 7 explained the largest proportion of variation in this environment. For 1996, the data from the same environment gave somewhat different information about QTL effects. Steptoe contributed favorable alleles on chromosomes 6 and 7, which accounted for 19% of the phenotypic variation and 29% of the genotypic variation. The QTL on chromosome 2, however, did not show a significant main effect. At Pendleton, Oregon (1996), all target QTLs, except QTL7b, were significant, and Steptoe contributed the favorable alleles at all loci. These QTLs explained 28% of the phenotypic variation and 39% of the genotypic variation. For Pullman, Washington (1996), the only

Table 3 Multiple regression analysis of QTL effects and their contribution to phenotypic and genotypic variation in the QTL validation population (*NA* not available)

Environment	QTL[a]	Model 1 (Main effect model)			Model 2 (epistatic model)		
		Effect	Favorable allele	Partial R_p^2 (R_g^2%)	Effect	Favorable allele	Partial R_p^2 (R_g^2%)
Klamath Falls, 1995	QTL2	−211	M	6(10)	−251	M	5(9)
	QTL4	233	S	5(8)	–	–	–
	QTL7a	256	S	4(7)	–	–	–
	QTL7b	317	S	12(21)	334	S	12(21)
	QTL2 × QTL4	NA	NA	NA	223	M/M; S/S[b]	4(7)
	QTL2 × QTL6	NA	NA	NA	238	M/M; S/S	7(11)
		Multi-locus R^2	27(46)			Multi-locus R^2	28(48)
Klamath Falls, 1996	QTL6	155	S	8(12)	119	S	4(7)
	QTL7a	179	S	11(17)	166	S	7(11)
	QTL2 × QTL6	NA	NA	NA	128	M/M; S/S	12(19)
	QTL2 × QTL7b	NA	NA	NA	129	M/M; S/S	5(8)
		Multi-locus R^2	19(29)			Multi-locus R^2	28(45)
Pendleton, 1996	QTL2	132	S	11(15)	120	S	11(15)
	QTL4	97	S	5(7)	–	–	–
	QTL6	92	S	7(10)	73	S	4(5)
	QTL7a	88	S	5(7)	–	–	–
	QTL2 × QTL4	NA	NA	NA	112	M/M; S/S	12(16)
		Multi-locus R^2	28(39)			Multi-locus R^2	27(36)
Pullman, 1996	QTL2	−106	M	16(20)	−107	M	16(20)
	QTL2 × QTL4	NA	NA	NA	52	M/M; S/S	3(4)
		Multi-locus R^2	16(20)			Multi-locus R^2	19(24)

[a] Individual QTLs are designated with the chromosome number; 7a denotes the interval defined by *Ale-ABC302*, and 7b denotes QTL linked to marker *mSrh*

[b] Homogeneous combinations of alleles were consistently favorable at interacting loci

significant QTL effect was on chromosome 2, with Morex contributing the favorable allele. This QTL explained 16% of the phenotypic variation and 20% of the genotypic variation. For Kimberly, Idaho (1996), no variation could be attributed to the target QTLs. The only significant QTL detected in the SM reference population at a nearby site (Aberdeen) was on chromosome 3 (Hayes et al. 1993), and this QTL was fixed in this population.

In order to detect epistatic interactions among QTL loci, the multiple regression models composed of QTL main effects and their digenic interaction terms were used. As shown in Table 3 (Model 2), all significant epistatic effects were related to the QTL on chromosome 2. For Klamath Falls (1995), significant epistatic interactions between the QTL on chromosome 2 and QTLs on chromosomes 4 and 6 were detected, even though the QTL main effects on chromosome 4 and 6 were not significant. For data from Klamath Falls (1996), the QTL main effect on chromosome 2 was not significant. However, there was a significant epistatic interaction between the QTL on chromosome 2 and QTLs on chromosomes 6 and 7. These two interaction effects accounted for 18% of the phenotypic variation and 27% of the genotypic variation. For Pendleton, there was a significant epistatic effect between the QTL on chromosome 2 and the QTL on chromosome 4 which explained 12% and 16% of the phenotypic and genotypic variation, respectively. There was also significant epistasis between QTLs on chromosome 2 and chromosome 4 at Pullman, but it did not account for a large proportion of the phenotypic and genotypic variation. No variation could be attributed to the target QTL loci at Kimberly. As in the Model 1 analysis, we attribute this to the fixation of favorable alleles at the chromosome 3 QTL. All significant epistatic effects are positive, showing that a homogenous (uniparental) combination of QTL alleles was favorable for higher yield, regardless of the sign of the QTL main effect.

All QTLs studied in this experiment showed significant QTL-by-environment interaction (Table 4). The significant QTL × E interactions were due to both changes in magnitude and changes in sign of QTL effects (Table 3). The QTL on chromosome 2 showed an alternative favorable allele in different environments, as it had in the original mapping population (Hayes et al. 1996). Other QTLs showed changes in

magnitude but not in sign. The sum of the individual QTL × E variation could explain 41% of G × E averaged across the entire genome. Across the environments, we could still detect significant QTL main effects on chromosomes 6 and 7, and the epistatic interaction of the QTL on chromosome 2 with QTL on chromosomes 4 and 7. Together, these QTL effects could account for 24% of genotypic variation.

Discussion

If QTL information is to be useful in plant breeding, it must have predictive utility. Accurate estimates of QTL location, effect and allele phase are, of course, essential. A number of factors may lead to biased estimation of QTL effects, including experimental design (genetic and field), models used to detect and characterize the QTL, sample size, deficiency of recombinant gametes, G × E and underestimation of epistasis (Lee 1995; Cockerham and Zeng 1996; Li et al. 1997; Sari-Gorla et al. 1997). Accurate estimation of QTL parameters in a base mapping population may not guarantee successful QTL manipulation in a breeding program (1) if QTLs show different patterns of expression and interaction when they are introgressed into different genetic backgrounds, or (2) when alleles are reconfigured in a common genetic background. Crop production environments are variable, and it would be reasonable to expect that QTL parameters would not be static in the face of such variability. In this experiment, we validated the significance of QTLs detected in the source mapping population. However, we found differences in favorable allele phase at these QTLs, interaction between QTL alleles and interaction between QTL alleles and environments.

The grain yield phenotype represents the cumulative effects of a complex of interrelated pathways operating during crop growth and development. Therefore, multiallelic interactions (epistasis) could be important determinants of grain yield. However, most QTL mapping experiments have revealed little evidence of epistasis (Edwards et al. 1992; Paterson et al. 1991; Stuber et al. 1992; DeVincente and Tanksley 1993; Schön et al. 1994; Cockerham and Zeng 1996). This may be due to the experimental procedures and analysis methods employed rather than the underlying biology (Kearsey and Farquhar 1998). Large populations are recommended for detecting epistasis in QTL mapping studies because of the large number of possible multi-locus combinations and recombination between QTLs and marker loci (Lee 1995). In this experiment, we limited the number of target segregating QTLs by fixing the largest-effect QTL. Furthermore, we selected a subset of the total population that was composed of genotypes with limited or no recombination between QTL and marker loci. The existence of epistasis was detected between some QTL loci. In all cases, estimates of significant epistatic effects were positive, indicating that the epistatic interaction led to higher yield when alleles came from the same parent. The same phenomenon was reported in rice, leading Li et al. (1997) to suggest that the epistatic loci affecting complex quantitative traits act in a predominantly complementary manner. As discussed by Li et al. (1997), the classification of QTL alleles as "favorable" or "unfavorable" may be misleading. The effect of an allele may be positive, neutral or negative depending on interactions with other loci and with environments. Therefore, for traits such as grain yield, QTL mapping and selection experiments should place more emphasis on identifying the best multi-locus allelic combinations instead of pyramiding individual favorable QTL alleles.

Genotype-by-environment interaction (G × E) is an essential component of phenotypic performance in breeding programs (Stuber et al. 1992). Various statistical methods have been developed to characterize G × E averaged across the entire genome (Kang 1990), but little is known about the underlying genetic mechanisms responsible for this phenomenon. Molecular markers offer the opportunity to study the interaction of individual QTL with environments (QTL × E) (Paterson et al. 1991; Dudley 1993; Hayes et al. 1993; Beavis et al. 1994; Beavis and Keim 1996; Tinker et al. 1996; Sari-Gorla et al. 1997). In this experiment, the overall G × E variation was dissected into the interaction of environments with individual QTLs. The significance of QTL × E was due to: (1) changes in the presence and magnitude of significant QTL main effects and/or their epistatic interaction across environments, and (2) contrasting favorable alleles (change in sign) at a QTL in different environments. Paterson et al. (1991) suggested that pyramiding environment-specific QTLs in a single genotype might lead to the development of varieties which would have the genetic buffering capacity to adapt to changing environments. However, change-in-sign-type QTL × E interaction poses challenges for applying MAS in crop improvement. In this experiment, the QTL on chromosome 2 showed contrasting favorable alleles in different environments and/or the same environment in different years. This QTL was the major genetic determinant for heading date in the original mapping population, with Morex contributing the later heading allele (Hayes et al. 1993). In this experiment, we found that whenever Morex contributed the higher value allele for grain yield, yield and later heading were significantly and positively correlated. However, if Steptoe contributed the higher value allele for grain yield, then yield and heading date were negatively correlated. Even in this geographically restricted and modest sample of environments, there was not a consistent yield advantage to late- or early-maturity.

Positive fixation of a single largest-effect QTL detected in a base mapping population may not be sufficient

Table 4 Analysis of variance for G × E and QTL × E in the QTL validation population (only significant terms with a *P* value of less than 0.05 are included in the table)

Model (G × E)			Model (QTL × E)		
Source of variation	*df*	Sum of squares	Source of variation	*df*	Sum of squares
Environments (E)	4	719590138	Environments (E)	4	719590138
Replicates within E	12	19054631	Replicates within E	12	19054631
Lines (G)	25	21722948	QTL6	1	750091
			QTL7a	1	3000871
			QTL2 × QTL4	1	699341
			QTL2 × QTL7b	1	745862
G × E	100	42027625	QTL2 × E	4	4389229
			QTL4 × E	4	2462607
			QTL6 × E	4	2285974
			QTL7a × E	4	2335540
			QTL7b × E	4	5842931
Error	268	41033324	Error	369	69559551

Percentage of genotypic variation explained by individual QTLs: 24%
Percentage of G × E variation explained by individual QTL × Es: 41%

to ensure target levels of performance. For example, although the two parent lines, SM73 and SM145, both had the favorable QTL on chromosome 3, they were of average to lower phenotypic values (Table 2). The progeny of SM73 and SM145 showed significant genotypic variation, indicating that the parents contrasted for favorable QTLs elsewhere in the genome. In addition, there are significant yield differences between DH lines that share the same target QTL genotype (Table 2). These results could be due to the disruption of a unique assemblage of coupling linkages and the epistatic effects.

In summary, our findings validated the significance of certain yield QTLs detected in a reference population. However, due to epistasis and QTL × E, genotypes with putatively favorable QTL alleles did not always have the predicted phenotypic value. Our data suggest that this sample of barley germplasm may have multiple mechanisms for maximizing reproductive capacity in variable environments. These mechanisms include allelic interactions and alternation of favorable alleles in different environments. Is MAS for grain yield in barley a viable breeding strategy? In this sample of germplasm and environments, it appears that conventional doubled-haploid breeding with phenotypic selection would be as effective as MAS. However, two traits – grain yield and malt extract – were targeted when this experiment was designed. It is possible that the two traits are confounded and/or that MAS may prove to be a useful tool for multi-trait selection. These issues will be explored in subsequent investigations. We hypothesize that pyramiding QTL alleles determining a single phenotype may be more successful in situations where heterozygosity is maintained (e.g. in hybrids) and in cases where specific yield-limiting constraints – i.e. biotic and abiotic stresses – are addressed. QTL information can also be very useful for defining the allelic structure of critical germplasm, identifying the components of metabolic pathways, determining the basis of correlated responses and as a platform for gene isolation. We concur with Li et al. (1997) that selection for phenotypes such as grain yield should focus on allelic combinations rather than on individual QTLs and with Tanksley and Nelson (1996) that QTL analysis and cultivar development should be an integrated process. The challenge will be to achieve this integration and to identify combinations of alleles using a range of breeding strategies and mating designs.

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