

K. Pillen · A. Zacharias · J. Léon

Advanced backcross QTL analysis in barley (*Hordeum vulgare* L.)

Received: 18 October 2001 / Accepted: 29 November 2002 / Published online: 2 April 2003
© Springer-Verlag 2003

Abstract This paper reports on the first advanced backcross-QTL (quantitative trait locus) project which utilizes spring barley as a model. A BC₂F₂ population was derived from the initial cross Apex (*Hordeum vulgare* ssp. *vulgare*, hereafter abbreviated with *Hv*) × ISR101-23 (*H. v.* ssp. *spontaneum*, hereafter abbreviated with *Hsp*). Altogether 136 BC₂F₂ individuals were genotyped with 45 SSR (simple sequence repeat) markers. Subsequently, field data for 136 BC₂F₂ families were collected for 13 quantitative traits measured in a maximum of six environments. QTLs were detected by means of a two-factorial ANOVA with a significance level of $P < 0.01$ for a marker main effect and a marker × environment (M × E) interaction, respectively. Among 585 marker × trait combinations tested, 86 putative QTLs were identified. At 64 putative QTLs, the marker main effect and at 27 putative QTLs, the M × E interaction were significant. In five cases, both effects were significant. Among the putative QTLs, 29 (34%) favorable effects were identified from the exotic parent. At these marker loci the homozygous *Hsp* genotype was associated with an improvement of the trait compared to the homozygous *Hv* genotype. In one case, the *Hsp* allele was associated with a yield increase of 7.7% averaged across the six environments tested. A yield QTL in the same chromosomal region was already reported in earlier barley QTL studies.

Keywords Molecular breeding · Microsatellite/Simple sequence repeat · Advanced backcross-quantitative trait locus · Introgression · *Hordeum spontaneum*

Communicated by G. Wenzel

K. Pillen (✉) · J. Léon
Department of Crop Science and Plant Breeding,
University of Bonn, Katzenburgweg 5, 53115 Bonn, Germany
e-mail: k.pillen@uni-bonn.de
Fax: +49-228-732045

A. Zacharias
KWS, Kleinwanzlebener Saatzucht AG, Grimsehlstr. 31,
37555 Einbeck, Germany

Introduction

The agronomic performance of crop varieties is mainly influenced by complex quantitative traits, for example, yield and quality components. Since the development of molecular markers, it has become feasible to identify and genetically localize the underlying polygenes as quantitative trait loci (QTLs) and to utilize these QTLs for crop improvement. Subsequently, a still increasing number of QTL studies, involving all agronomic important crop species, have been conducted (e.g. Paterson et al. 1988).

Despite all efforts to map QTLs, their relevance for breeding new varieties is still low. Two reasons for this phenomenon might be important: (1) since most QTL projects have been conducted within the elite pool of a crop species, the identified favorable QTL alleles could not be utilized as a new genetic resource for elite improvement; (2) almost all QTL studies used early generations (F₂, F₃ and BC₁) for mapping and QTL detection. Unfortunately, the favorable QTL alleles often lost their effects after they were purified into elite lines. This phenomenon might be explained with the relatively high level of epistatic interactions between QTLs and other donor genes in early generations. These interactions might be fixed in advanced generations possibly, leading to silencing of the measured QTL effects.

A potential solution to the problem was presented by Tanksley and Nelson (1996). The authors integrated the mapping of favorable QTL alleles and the introgression of these alleles into one process. In order to achieve this goal, they utilized exotic germplasm as the genetic donor for the improvement of quantitative agronomic traits and conducted the marker and phenotype analysis in advanced backcross generations like BC₂ or BC₃. It is expected that through the introgression of new exotic QTL alleles, the AB (advanced backcross – QTL) strategy will contribute to an increased level of genetic diversity in our modern crop varieties.

To date, several reports on the application of the AB-QTL strategy are available for tomato and rice. In all cases, favorable exotic QTL alleles for important agro-

onomic traits have been identified. For instance, fruit yield could be improved in tomato through the introgression of wild-species alleles from *Lycopersicon pimpinellifolium* and *L. peruvianum* by 17% and 34%, respectively (Tanksley et al. 1996; Fulton et al. 1997). A further AB-QTL study, which used *L. hirsutum* as the donor species, revealed 25 favorable wild-species QTL alleles out of 121 detected QTLs (Bernacchi et al. 1998a). Again, the authors detected wild-species alleles which increased yield by 15%. The most recent AB-QTL study in tomato was published by Fulton et al. (2000). As in other tomato wild species, the authors could localize favorable exotic QTL alleles from *L. parviflorum* which, for instance, increased yield by 27%. Similar results could be extracted from AB-QTL studies in rice. Here, two wild-species QTL alleles have been associated with an increase of yield by 17% and 18% on rice chromosomes 1 and 11, respectively (Xiao et al. 1996, 1998). Subsequently, the yield QTL effect on chromosome 1 was validated in a second cross using the same *Oryza rufipogon* donor accession (Moncada et al. 2001).

The favorable wild-species QTL alleles are useful as a breeding resource after they have been fixed in nearly isogenic lines (QTL-NILs) and after the superior performance of a QTL-NIL has been confirmed in comparison to the recurrent elite line. Bernacchi et al. (1998b) have already validated the effects of exotic tomato QTLs in QTL-NILs. In field evaluations at five locations worldwide, 22 QTL-NILs out of 25 tested (88%) exhibited phenotypic improvement compared to the recurrent parent, as had been predicted in the previous AB-QTL analysis. For instance, a QTL-NIL possessing an exotic QTL allele for a 15% yield increase did, indeed, outperform the control line by 12%. These reports clearly illustrate that the AB-QTL strategy is a powerful tool for the improvement of quantitative agronomic traits in elite varieties.

In this paper, we report on the first AB-QTL project which utilizes spring barley as a model. Our goal was: (1) to localize QTLs for the expression of quantitative traits in spring barley and (2) to identify favorable QTL alleles from the wild barley donor which improve the respective traits.

Materials and methods

Plant material

For QTL analysis, a BC₂F₂ population originating from the cross of the German spring barley var. *Apex* and the Israeli *Hordeum vulgare* ssp. *spontaneum* (*Hsp*) wild barley accession ISR101-23 (A×101) was generated via two cycles of backcrossing with *Apex* followed by one cycle of selfing. The BC₂F₂ population consisted of 136 randomly chosen individuals.

Simple sequence repeat markers

The following prefixes of SSR names indicate the published sources from which the primer sequences were taken: *HVM*, Liu et

al. (1996); *BMS*, Russell et al. (1997); *GMS*, Struss and Plieske (1998); *WMS*, Röder et al. (1998); *Bmac*, *Bmag*, *Ebmag* and *Ebmac*, Ramsay et al. (2000); *Hv*, Becker and Heun (1995) and Pillen et al. (2000). A suffix with the chromosomal identifier in brackets was added to each SSR name as a simple reference. Linkage distances between SSR markers were inferred from Ramsay et al. (2000) and Pillen et al. (2000) or estimated after linkage analysis of 96 F₂ individuals from the cross A×101 using the MAPMAKER software (Lander et al. 1987).

Genotyping BC₂F₂ individuals

The genotypes of 136 BC₂F₂ individuals were determined by means of SSR analysis. For this, plant DNA was extracted, polymerase chain reaction (PCR) analyses conducted and SSRs surveyed after silver staining as reported in Pillen et al. (2000). At each informative SSR locus, homozygous *H. vulgare* ssp. *vulgare* (*Hv*), heterozygous and homozygous *Hsp* genotypes were distinguished. The genetic measure P[*Hsp*] as the percentage of *Hsp* alleles present in a single BC₂F₂ line was calculated from 40 co-dominantly scored SSRs according to the formula:

$$P[Hsp] = \frac{2[aa] + [Aa]}{2([AA] + [Aa] + [aa])} \quad (1)$$

where [AA], [Aa] and [aa] correspond to the frequencies of the homozygous *Hv*, heterozygous and the homozygous *Hsp* genotypes, respectively, calculated from all investigated marker loci.

Phenotyping BC₂F₂-derived families

For field testing, each BC₂F₂ individual was bulk propagated until BC₂F_{2.5} (1999) and BC₂F_{2.6} (2000), respectively. During the seasons 1999 and 2000, the 136 backcross lines were grown at the three locations Dikopshof (University of Bonn), Gudow (Nordsaat Saatzeit) and Irlbach (Dr. J. Ackermann Saatzeit). These experimental stations are located in the west, north and south of Germany, respectively. At each location, a randomized complete block design with two replications was applied, except at the Dikopshof 1999, where three replications were taken. As a control, the recurrent parent was tested with ten replications per block. Plot size (3.75–5.5 m²), seeding rate (300 kernels/m²) and field management were in accordance with the local practice. At each plot, up to 13 quantitative traits were evaluated (Table 1).

QTL analysis

The QTL detection from BC₂F₂ genetic data and field data from multiple environments was conducted using the procedure GLM from the SAS software (SAS Institute 1999). In each environment, the field data of all replications were averaged by the least square means option in SAS. At each marker locus, only the homozygous genotypes (*Hv* or *Hsp*) were included in the calculation since the repeated selfing of heterozygous BC₂F₂ individuals lead to a mix of both homozygous genotypes in the derived BC₂F_{2.5} and BC₂F_{2.6} field plots, resulting in a false estimate of the performance of true heterozygotes. The model used to detect QTLs included the effects marker genotype (M), environment (E) and M × E interaction. Under the assumption of a mixed model with the marker as a fixed effect and the environment as a random effect, the mean square of M × E was used as the error term for the computation of the *F*-statistic for a marker main effect. The mean square of the residual variance was used as the error term for the computation of the *F*-statistic for a M × E effect.

Following Stuber et al. (1992) and Xiao (1998), the presence of a stable QTL in the vicinity of a marker locus was accepted, if the marker main effect was significant at *P* < 0.01. In addition, the presence of an environment-dependent QTL was accepted, if the M × E interaction was significant at *P* < 0.01.

Table 1 List of 13 quantitative traits measured in up to six environments

Trait	Abbreviation	Method of measurement	Value ^a	Environment ^b
Ears per square meter	EAR	Number of ears, collected from a row of 50 cm at maturity	+	D99, D00
Days until heading	HEA	Number of days from sowing to complete emergence of the ear	-	D99, G99, I99, D00, G00, I00
Height	HEI	Plant height up to the tip of the ear (excluding awns) at maturity	-	D99, I99, D00, I00
Harvest index	HI	Ratio of grain yield to above ground biomass, collected from a row of 50 cm at maturity	+	D99, D00
Kernels per ear	KER	Number of kernels, collected from a row of 50 cm at maturity divided by the number of ears	+	D99, D00
Lodging at flowering	LOF	Visual rating (1–9) of the severity of lodging at flowering	-	G99, I99, D00
Lodging at harvest	LOH	Visual rating (1–9) of the severity of lodging at harvest	-	G99, I99, I00
Above ground biomass	MAS	Total dried biomass above ground, collected from a row of 50 cm at maturity	+	D99, D00
Malt tenderness	MT	Percentage of soft malt, measured from micro malting 50 g of barley	+	D99, D00
Protein content	PRO	Protein content, measured with NIRS from 20 g of ground barley	-	D99, D00
Thousand-grain weight	TGW	Mass of 1,000 kernels, harvested from the plot	+	D99, G99, D00, G00, I00
Water absorption	WA	Relative water absorption after soaking 50 g of barley	+	D99, D00
Yield	YLD	Plot yield, measured after harvesting with a combine and purifying with a stationary threshing device	+	D99, G99, I99, D00, G00, I00

^a The value of the trait should be increased (+) or reduced (-) with respect to the breeding goal

^b Combination of the location [Dikopshof (D), Gudow (G); Irlbach (I)] and the year [1999 (99), 2000 (00)]

The explained phenotypic variances as a measure of the strength of a QTL main effect (R^2_M) and a QTL \times environment interaction (R^2_I), respectively, were calculated as follows:

$$R^2_M = SS_M / SS_{Total} \quad (2)$$

$$R^2_I = SS_{M \times E} / SS_{Total} \quad (3)$$

where SS_M , $SS_{M \times E}$ and SS_{Total} are the sum of squares of the factors marker and $M \times E$ interaction and the total sum of squares, respectively, which were obtained from the GLM procedure.

The relative performance of the homozygous *Hsp* genotype (RP[*Hsp*]) as a measure of the improvement of a trait by replacing both *Hv* elite alleles with the exotic *Hsp* alleles was calculated as follows:

$$RP[Hsp] = \frac{aa - AA}{AA} * 100 \quad (4)$$

where, for each trait, *aa* and *AA* are the least square means of the homozygous *Hv* and the homozygous *Hsp* genotypes, respectively, calculated across all environments.

Table 2 Least square means of 13 traits calculated across all environments for the backcross population and the recurrent parent *Apex*

Trait ^a	Backcross	Apex	LSD test ^b
EAR	636.0	625.3	
HEA	62.15	64.36	***
HEI	81.99	81.36	
HI	0.45	0.48	***
KER	18.48	20.53	***
LOF	2.25	2.00	
LOH	4.00	3.62	
MAS	42.16	41.68	
MT	66.09	71.68	**
PRO	11.55	11.64	
TGW	46.94	47.00	
WA	43.77	43.06	***
YLD	58.42	65.68	***

^a The trait abbreviations are listed in Table 1

^b The significance thresholds for the LSD tests are: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; no asterisks: not significant

Results and discussion

Traits

In order to assess the value of the backcross population, we calculated the least square means of all traits for the backcross population and for the recurrent parent *Apex* (Table 2). The comparison of the means revealed that the backcross significantly ($P < 0.01$) excelled the recurrent parent *Apex* in the two traits HEA and WA. On the other hand, the backcross was significantly ($P < 0.01$) inferior to *Apex* in traits HI, KER, MT, YLD (Table 2). The inferior performance of the backcross for most traits can

be explained by the absence of any positive selection during the process of backcrossing. Our goal was to keep the available genetic contrast – for instance, with respect to yield – in order to maximize the chance of detecting QTLs. Nonetheless, we could always identify individual backcross lines which outperformed the recurrent parent (data not shown).

A calculation of correlations between the 13 traits was conducted across all environments (Table 3). Significant correlations ($P < 0.05$) were found for 56 trait combinations. From these, LOF and LOH revealed the strongest correlation ($r = 0.83$), which was expected since both

Table 3 Pearson's correlation coefficients^a (r) between 14 quantitative traits^b

	HEA	HEI	HEI	HI	KER	LOF	LOH	MAS	MT	PRO	TGW	WA	YLD	P[<i>Hsp</i>] ^c
EAR	-0.31***	-0.45***	0.34***	0.34***	-0.13	-0.27**	-0.29***	0.16	0.08	-0.26**	-0.19*	-0.03	0.21*	-0.19*
HEA		0.64***	-0.30***	-0.30***	0.43***	0.46***	0.59***	0.62***	-0.20*	0.00	-0.39***	-0.03	-0.04	0.22**
HEI			-0.50***	-0.50***	0.30***	0.66***	0.65***	0.67***	-0.24**	0.31***	0.02	0.15	-0.29***	0.32***
HI					0.30***	-0.34***	-0.28**	-0.36***	0.40***	-0.54***	0.01	-0.29***	0.49***	-0.47***
KER						0.11	0.28**	0.28***	0.25***	-0.41***	-0.20*	-0.19*	0.47***	-0.21*
LOF							0.83***	0.45***	-0.33***	0.19*	-0.07	0.19*	-0.35***	0.36***
LOH								0.47***	-0.34***	0.07	-0.15	0.05	-0.23**	0.36***
MAS									-0.29***	0.16	-0.12	0.16	-0.18*	0.29***
MT										0.16	-0.12	0.16	0.47***	-0.41***
PRO										-0.51***	-0.03	-0.12	-0.64***	0.31***
TGW											0.17*	0.34***	-0.64***	-0.41***
WA												0.12	-0.12	-0.06
YLD													-0.59***	0.13
														-0.46***

^a For calculating correlations, the trait performance of each single backcross line was averaged across all environments. The significance thresholds for r values are: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; no asterisks: not significant

^b The trait abbreviations are listed in Table 1

^c The trait P[*Hsp*] is defined as the percentage of *Hsp* alleles present in a BC₂F₂ line (see Materials and methods)

traits are related. HEI displayed medium positive correlations with HEA, LOF, LOH and MAS, where r values ranged from 0.64 to 0.67. YLD displayed medium negative correlations with WA and PRO ($r = -0.59$ and -0.64 , respectively) and medium positive correlations with KER, MT and HI, where r values ranged from 0.47 to 0.49. Both the negative correlation of YLD with PRO and the positive correlations with the yield-related traits KER and HI have been frequently observed in other studies.

One could expect that the presence of large chromosomal segments from wild barley would exhibit negative effects on agronomic traits. In order to test this hypothesis, the percentage of *Hsp* alleles (P[*Hsp*]) present in a BC₂F₂ line was estimated from all co-dominantly scored SSRs. No strong correlations could be measured between P[*Hsp*] and other traits. However, P[*Hsp*] revealed significant negative correlations with YLD ($r = -0.46$), HI ($r = -0.47$) and MT ($r = -0.41$). The negative correlations, in accordance with the significantly reduced trait mean values of the backcross population, confirm that without selection the introgression of wild barley germplasm would not lead to an improvement of agronomic traits. The same tendency holds true for the traits HEA, HEI, LOF, LOH, MAS and PRO. These traits, which should be reduced in regard to the breeding goals, show significant but weak positive correlations with P[*Hsp*], indicating that there is an overall negative influence of *Hsp* alleles on most agronomic traits. Despite the aforementioned correlations, the goal of the AB-QTL analysis was to identify those rare positive effects which exotic alleles can exert on agronomically important quantitative traits.

Marker data

Altogether, more than 200 SSRs were tested for polymorphism between the parents *Apex* and ISR101-23. Sixty-seven SSRs turned out to be polymorphic. From these, 22 (33%) failed to exhibit the *Hsp* allele in the BC₂F₂ population. Thus, the 136 BC₂F₂ lines were successfully genotyped with 45 SSRs. They were distributed over all seven barley chromosomes (Fig. 1). The chromosomal location of the SSRs were inferred from Ramsay et al. (2000) and Pillen et al. (2000) or, if necessary, from linkage analysis in a reference F₂-population from the A×101 cross (data not shown). The complete set of 67 mapped SSRs covers 852 cM of the barley genome; the mean SSR density is equal to 12.7 cM. The first almost complete SSR map for barley includes 299 SSRs and covers 1,173 cM (Ramsay et al. 2000). Thus, the backcross map represents 73% of the Ramsay map. The A×101 map possesses nine gaps with a marker distance of more than 30 cM. Large regions, which are not covered by any polymorphic SSR, can be found, for instance, on chromosomes 1H and 6H (Fig. 1). The low coverage of the A×101 map can be explained with the occurrence of genetic drift during the backcrossing

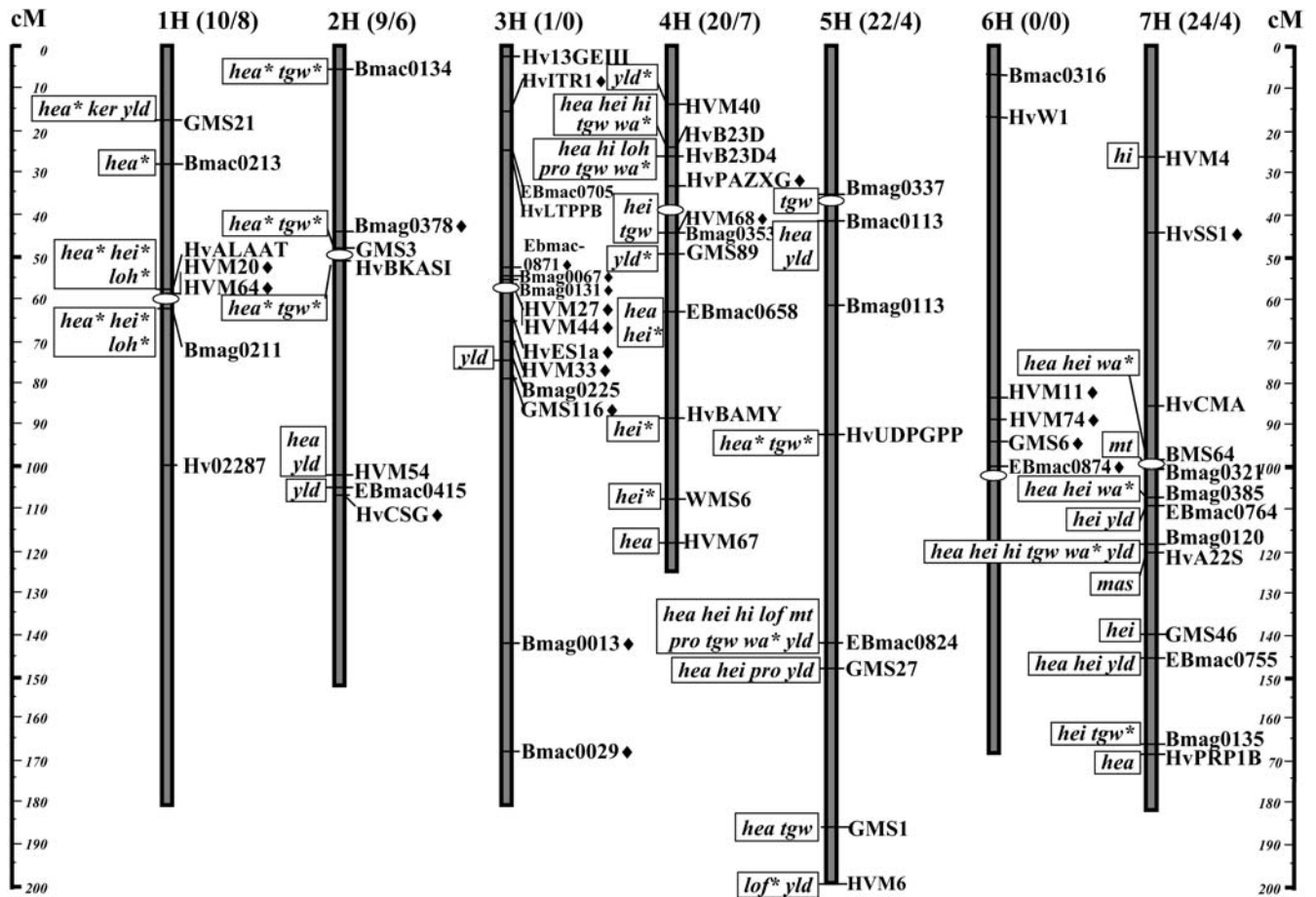


Fig. 1 Barley SSR map containing 86 putative QTLs with 29 favorable *Hsp* alleles detected from the BC₂F₂ cross Apex × 101-23. The SSR marker order is based on Ramsay et al. (2000). Twenty-two SSR marker markers that were polymorphic between the parents Apex and ISR101-23 but failed to show the ISR101-23 allele in the BC₂F₂ population are indicated by ◊. The centromere positions, which were taken from the current Steptoe × Morex BIN map (<http://barleygenomics.wsu.edu/databases/#databases.html>),

are indicated by open ovals. Putative QTLs which revealed either a significant ($P < 0.01$) marker main effect or M × E interaction are written to the left of the SSR locus. The abbreviations of the quantitative traits follows Table 1. A favorable effect of the *Hsp* allele at a putative QTL is indicated by *. Each chromosome name is followed in brackets by the number of detected putative QTLs and the number of favorable *Hsp* alleles

process. Presumably because only four BC₁ individuals were used for the production of BC₂ families, several chromosomal *Hsp* segments were lost until the BC₂F₂ generation was reached. This is particularly true for chromosomes 3H and 6H where 11 and four polymorphic SSRs, respectively, failed to segregate in the BC₂F₂ population (Fig. 1). The occurrence of marker gaps was also observed in other QTL analyses. Backes et al. (1995), for instance, could map only a single marker to barley chromosome 1H using a set of 50 informative restriction fragment length polymorphism (RFLPs). Their map included nine gaps encompassing more than 30 cM.

From 45 informative SSRs genotyped, 40 (89%) could be scored co-dominantly. Three dominantly scored SSRs did not amplify a *Hsp* allele and two failed to amplify a *Hv* allele. The theoretical frequency of the three possible co-dominant marker genotypes is equal to 13:2:1 (AA:Aa:aa) in BC₂F₂. Thus, the expected mean portion of the *Hsp* genome ($P[Hsp]$) would be 0.125. Based on

genotype data from the 40 co-dominantly scored SSRs, the $P[Hsp]$ value was estimated for each BC₂F₂ line. The average $P[Hsp]$ value of the BC₂F₂ lines was equal to 0.127 with a range from 0.093 to 0.296 and a standard deviation of 0.066. This finding confirms that the investigated A×101 population corresponds indeed to a BC₂F₂ generation. Furthermore, the good accordance of the expected and observed mean $P[Hsp]$ values proved that during backcrossing no serious selection in favor of one parent was exercised in the population. Nevertheless, a distorted segregation was observed for single SSR loci. Altogether 16 SSRs (36%) revealed a significant distorted segregation at $P < 0.01$. However, no general prevalence of either *Hv* or *Hsp* alleles was detected. At eight loci, the population showed an excess of *Hsp* alleles and at the remaining eight loci the *Hsp* alleles were underrepresented.

QTL detection

A single-point marker analysis by means of a two-factorial ANOVA rather than an interval mapping was preferred for QTL analysis because of the low marker coverage and the presence of gaps in the A×101 map. It should be noted that QTL effects simultaneously detected at two or more adjacent marker loci by ANOVA are presumably due to the existence of a single, linked genetic factor which exerts the QTL effect. However, for two reasons we kept all putative QTLs in the records. First, the alternative hypothesis, that there are two or more QTL factors present on a single chromosomal segment, cannot be ruled out, and second, the ANOVA gives no clear indication which of the significant markers is located nearest to the true QTL factor.

Among 585 marker × trait combinations tested, 86 putative QTLs were detected. At 64 putative QTLs, the marker main effect and at 27 putative QTLs, the M × E interaction were significant at $P < 0.01$ (Fig. 1, Table 4). In five cases, both effects were significant. Altogether, 29 (34%) favorable QTL effects were detected. At these loci, the homozygous *Hsp* genotype was associated with an improvement of the trait compared to the homozygous *Hv* genotype (Fig. 1, Table 4). The putative QTLs were unevenly distributed over the chromosomes (Fig. 1). Whereas at least 20 QTLs were located on chromosomes 4H, 5H and 7H, only one or zero QTLs were detected on chromosomes 3H and 6H, respectively. However, on the latter chromosomes only four and two SSRs, respectively, were genotyped. Most of the favorable QTLs were located on chromosomes 1H, 2H and 4H (8, 6 and 7, respectively). No favorable QTLs were detected on chromosomes 3H and 6H. The distribution of putative QTLs among the 45 genotyped SSR markers was also uneven. Eight SSRs were not associated with any QTL effect, whereas marker EBmac0824_[5H] showed putative QTL effects on nine traits, simultaneously. In the following, the detected putative QTLs are described for each trait (see also Table 4).

Days until heading (HEA)

Twenty-two putative QTLs for HEA were located on five chromosomes. In all cases, the marker main effect was significant at $P < 0.01$. In addition, the loci *HvB23D4*_[4H], *HvB23D4*_[4H], and (EBmac0824_[5H]) exhibited a significant M × E interaction. For eight QTLs, a favorable effect of the *Hsp* allele on HEA was observed. At these loci, which were located on chromosomes 1H, 2H and 5H, the presence of the *Hsp* allele was associated with a reduced heading time of up to 9.7% (GMS3_[2H]). At the remaining QTLs, the *Hsp* allele was associated with an increased heading time of up to 7.4% (*HvB23D4*_[4H]). The maximum explained phenotypic variance was found at *HvB23D4*_[4H] with 6.8%.

Height (HEI)

Seventeen putative QTLs for HEI were located on four chromosomes. All loci exhibited significant marker main effects. For five QTLs, a favorable effect of the *Hsp* allele on HEI was observed. At these loci, the presence of the *Hsp* allele led to a reduction in plant height of up to 10.4% (GMS3_[2H]). Most QTL alleles from *Hsp* resulted, however, in an increased plant height of up to 13.4% (EBmac0824_[5H]). The phenotypic variance explained by a QTL reached its maximum with 14.1% at Bmag0120_[7H].

Lodging at flowering (LOF)

Only two QTLs for LOF were located on chromosome 5H. The unlinked SSRs HVM6_[5H] and EBmac0824_[5H] exhibited a significant marker main effect and a significant M × E interaction, respectively. The two QTLs showed effects of opposite direction. Whereas the *Hsp* allele was associated with a 33.5% reduction of lodging at HVM6_[5H], it increased the tendency to lodge by 106.2% at EBmac0824_[5H]. The maximum explained phenotypic variance was reached with 2.6% at EBmac0824_[5H].

Lodging at harvest (LOH)

As in the case of LOF, two QTLs were located for LOH. However, these were mapped in close proximity on chromosome 1H. Both QTLs exhibited significant marker main effects with favorable *Hsp* alleles. The stronger effect was measured at locus Bmag0211_[1H]. Here, the *Hsp* allele resulted in a lodging tendency at harvest which was reduced by 27.2%. The explained phenotypic variance for Bmag0211_[1H] amounted to 1.1%.

Ears per square meter (EAR)

No QTLs were detected for EAR in this study.

Kernels per ear (KER)

Only one QTL was detected for KER. Marker GMS21_[1H] exhibited a significant main effect. The negative effect of the *Hsp* allele resulted in a 6.5% reduction of kernels per ear. The explained phenotypic variance for GMS21_[1H] amounted to 3.1%.

Thousand grain weight (TGW)

A total of 12 putative QTLs were located for TGW on four chromosomes. While six loci exhibited a significant marker main effect, the other six loci showed a significant M × E interaction. Favorable effects of the *Hsp* alleles on

Table 4 List of 86 putative QTLs detected from the BC₂F₂ cross Apex × 101-23^a

Trait	Marker	Chromosome ^b	Positive ^c	QTL ^d	S _M ^e	R ² _M ^f (%)	S _I ^e	R ² _I ^f (%)	LSM-[Hsp] ^g	RP[Hsp] ^h (%)	P/N ⁱ
HEA	GMS21	1H	17	M	**	0.3		0.1	61.11	-1.51	+
	Bmac0213	1H	28	M	**	2.7		0.4	59.20	-4.55	+
	<i>HvALAAT</i>	1H	57	M	**	2.9		0.4	58.16	-6.34	+
	Bmag0211	1H	62	M	**	3.2		0.4	58.28	-6.32	+
	Bmac0134	2H	5	M	**	4.5		0.8	60.07	-3.86	+
	GMS3	2H	48	M	**	1.3		0.4	56.19	-9.65	+
	<i>HvBKASI</i>	2H	50	M	**	1.2		0.3	56.19	-9.25	+
	HVM54	2H	103	M	**	3.3	*	1.0	64.33	4.48	-
	<i>HvB23D</i>	4H	24	M+I	**	5.7	***	1.7	65.84	7.37	-
	<i>HvB23D4</i>	4H	26	M+I	**	6.8	***	2.1	65.82	7.25	-
	EBmac0658	4H	62	M	**	0.2		0.1	62.59	1.39	-
	HVM67	4H	118	M	**	0.5		0.1	62.67	1.84	-
	Bmac0113	5H	41	M	**	0.4		0.1	63.25	2.50	-
	<i>HvUDPGPP</i>	5H	91	M	**	0.2		0.1	60.83	-1.76	+
	EBmac0824	5H	141	M+I	**	3.7	**	1.1	65.80	6.85	-
	GMS27	5H	148	M	**	2.4		0.5	64.52	4.88	-
	GMS1	5H	187	M	**	0.9		0.1	65.14	5.54	-
	BMS64	7H	98	M	***	0.8		0.1	64.24	4.03	-
	Bmag0385	7H	107	M	**	0.4		0.1	63.00	2.01	-
	Bmag0120	7H	118	M	**	1.8		0.4	63.42	3.07	-
	EBmac0755	7H	145	M	**	0.3		0.1	62.73	1.59	-
	<i>HvPRP1B</i>	7H	167	M	**	1.2		0.2	63.03	2.14	-
	HEI	<i>HvALAAT</i>	1H	57	M	**	1.3		0.1	77.65	-5.86
Bmag0211		1H	62	M	**	2.3		0.1	76.78	-7.16	+
<i>HvB23D</i>		4H	24	M	**	4.3		0.1	88.83	9.06	-
<i>HvB23D4</i>		4H	26	M	**	5.7		0.2	89.23	9.11	-
Bmag0353		4H	45	M	***	1.6		0.0	85.33	4.60	-
EBmac0658		4H	62	M	**	0.2		0.0	80.35	-2.00	+
<i>HvBAMY</i>		4H	88	M	**	5.4		0.2	74.05	-10.39	+
WMS6		4H	108	M	**	3.6		0.3	77.62	-6.32	+
EBmac0824		5H	141	M	**	7.4		0.5	92.21	13.38	-
GMS27		5H	148	M	**	8.3		0.4	90.84	12.29	-
BMS64		7H	98	M	**	1.9		0.0	88.68	8.42	-
Bmag0385		7H	107	M	***	4.9		0.1	88.05	8.69	-
EBmac0764		7H	109	M	**	2.4		0.2	87.70	8.45	-
Bmag0120		7H	118	M	***	14.1		0.1	90.46	12.00	-
GMS46		7H	139	M	**	2.7		0.1	85.99	6.36	-
EBmac0755		7H	145	M	**	6.2		0.2	89.68	10.21	-
Bmag0135		7H	165	M	**	1.4		0.1	85.06	3.92	-
HI	<i>HvB23D</i>	4H	24	I		4.9	**	3.6	0.41	-8.89	-
	<i>HvB23D4</i>	4H	26	I		6.1	***	4.5	0.41	-8.89	-
	EBmac0824	5H	141	I		14.2	**	2.0	0.38	-15.56	-
	HVM4	7H	27	M	**	5.0		0.0	0.42	-6.67	-
	Bmag0120	7H	118	I		13.8	**	1.8	0.41	-8.89	-
KER	GMS21	1H	17	M	**	3.1		0.0	17.33	-6.48	-
	EBmac0824	5H	141	I		10.3	**	2.6	4.31	106.22	-
LOF	HVM6	5H	201	M	**	2.3		0.0	1.55	-33.48	+
	<i>HvALAAT</i>	1H	57	M	**	0.4		0.0	3.37	-17.20	+
LOH	Bmag0211	1H	62	M	**	1.1		0.0	3.00	-27.18	+
	<i>HvA22S</i>	7H	120	M	**	0.6		0.0	38.99	-5.11	-
MAS	EBmac0824	5H	141	M	**	1.7		0.0	61.08	-8.16	-
	Bmag0321	7H	100	M	**	0.6		0.0	64.00	-3.96	-
PRO	<i>HvB23D4</i>	4H	26	I		0.3	**	0.6	11.81	2.07	-
	EBmac0824	5H	141	I		1.2	***	0.9	12.14	5.47	-
	GMS27	5H	148	I		1.1	***	1.2	12.03	4.61	-
TGW	Bmac0134	2H	5	I		0.9	**	1.5	47.70	1.51	+
	GMS3	2H	48	M	**	0.1		0.0	48.35	2.63	+
	<i>HvBKASI</i>	2H	50	M	**	0.1		0.0	48.35	2.46	+
	<i>HvB23D</i>	4H	24	I		1.3	***	1.3	45.93	-3.10	-
	<i>HvB23D4</i>	4H	26	I		2.6	***	2.0	45.76	-3.72	-
	Bmag0353	4H	45	I		0.0	***	1.6	47.04	-0.47	-
	Bmag0337	5H	35	M	**	1.5		0.1	46.03	-2.93	-
	<i>HvUDPGPP</i>	5H	91	M	***	1.8		0.1	49.18	4.55	+
	EBmac0824	5H	141	I		0.3	**	1.1	46.48	-1.61	-

Table 4 (continued)

Trait	Marker	Chromosome ^b	Positive ^c	QTL ^d	S _M ^e	R _M ^{2f} (%)	S _I ^e	R _I ^{2f} (%)	LSM-[<i>Hsp</i>] ^g	RP[<i>Hsp</i>] ^h (%)	P/N ⁱ
WA	GMS1	5H	187	M	**	0.8		0.1	44.97	-4.70	-
	Bmag0120	7H	118	I		0.0	**	0.9	47.13	-0.15	-
	Bmag0135	7H	165	M	**	0.2		0.0	47.55	0.85	+
	<i>HvB23D</i>	4H	24	I		0.1	**	3.8	43.89	0.21	+
	<i>HvB23D4</i>	4H	26	I		0.0	***	6.1	43.83	0.00	+
	EBmac0824	5H	141	I		4.2	**	2.1	44.52	1.83	+
	BMS64	7H	98	I		0.0	**	2.9	43.80	0.07	+
	Bmag0385	7H	107	I		0.0	***	4.1	43.77	0.05	+
Bmag0120	7H	118	I		2.3	***	3.7	44.11	0.87	+	
YLD	GMS21	1H	17	M	***	0.3		0.0	56.81	-3.43	-
	HVM54	2H	103	I	*	1.7	***	1.2	55.35	-7.13	-
	EBmac0415	2H	105	I		1.5	**	1.1	55.62	-6.43	-
	Bmag0225	3H	74	M	***	1.4		0.1	55.48	-6.52	-
	HVM40	4H	14	M	**	0.5		0.1	61.40	5.63	+
	GMS89	4H	50	M	***	0.8		0.1	62.55	7.66	+
	Bmac0113	5H	41	M	**	1.4		0.2	52.55	-10.20	-
	EBmac0824	5H	141	M+I	**	6.9	**	0.8	46.78	-20.99	-
	GMS27	5H	148	M+I	**	6.2	**	1.0	48.79	-17.99	-
	HVM6	5H	201	M	**	3.0		0.5	53.21	-9.83	-
	EBmac0764	7H	109	M	***	1.5		0.2	52.77	-10.98	-
	Bmag0120	7H	118	M	**	3.6	*	0.7	53.58	-9.60	-
	EBmac0755	7H	145	M	**	2.5		0.4	52.71	-10.46	-

^a The quantitative traits are defined in Table 1

^b Chromosomal assignment of SSRs

^c Chromosomal position of SSRs deduced from Ramsay et al. (2000), and Pillen et al. (2000), and from a reference F₂ map of the cross A×101

^d A QTL was assumed within the vicinity of a marker locus if the marker main effect or the M × E interaction was significant in the two-factorial ANOVA at $P < 0.01$

^e S_M and S_I: level of significance of the marker main effect and the M × E interaction, respectively, with: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; no asterisks: not significant

^f R_M² (%), R_I² (%): the portion of the phenotypic variance which is explained by the marker main effect and the M × E interaction, respectively

^g LSM [*Hsp*], The least square mean across all tested environments for homozygous *Hsp* genotypes at the given marker locus

^h RP[*Hsp*], The relative performance of homozygous *Hsp* genotypes compared to homozygous *Hv* genotypes at the given marker locus

ⁱ P/N, Positive (+) or negative (-) effect of the QTL-associated *Hsp* allele

TGW were observed for five QTLs on chromosomes 2H, 5H and 7H. At these loci, the presence of the *Hsp* allele resulted in a TGW increase of up to 4.6% (*HvUDPGPP*_[5H]). Contrasting negative effects of the *Hsp* allele were detected on chromosomes 4H, 5H and 7H, which resulted in reduced TGW of up to 4.7% (GMS1_[5H]). The highest portion of the phenotypic variance was explained by *HvB23D4*_[4H] (2.6%).

Yield (YLD)

Altogether, 13 putative QTLs for YLD were located on all barley chromosomes except for 6H. Whereas 11 loci exhibited a significant marker main effect, four loci showed a significant M × E interaction. Both, the main effect as well as the interaction were significant at the linked loci EBmac0824_[5H] and GMS27_[5H]. Most QTL alleles from *Hsp* resulted in yield reductions with a maximum of 21.0% (EBmac0824_[5H]). However, in two cases a favorable wild species effect on plot yield could be detected. The *Hsp* alleles of the two loosely linked SSRs, HVM40_[4H] and GMS89_[4H], were associated with a yield increase of 5.6% and 7.7%, respectively. The yield

increasing main effect of both *Hsp* alleles was detected in all six environments measured. A maximum explained phenotypic variance was found at EBmac0824_[5H] with 6.9%.

Above ground biomass (MAS)

Only one QTL was detected for MAS. Marker *HvA22S*_[7H] exhibited a significant main effect. The negative effect of the *Hsp* allele resulted in a 5.1% reduction of the above ground biomass. The explained phenotypic variance for *HvA22S*_[7H] amounted to 0.6%.

Harvest index (HI)

Five putative QTLs were located for HI on chromosomes 4H, 5H and 7H. While four loci exhibited a significant M × E interaction, HVM4_[7H] showed a significant marker main effect. No favorable effects of the *Hsp* alleles on harvest index were observed. The presence of the *Hsp* allele resulted in a HI decrease of up to 15.6% (EBmac0824_[5H]). As in case of YLD, the highest portion

of the phenotypic variance was explained by (EBmac0824_[5H]) with 14.2%.

Protein content (PRO)

Three putative QTLs were located for the quality-related trait PRO on chromosomes 4H and 5H. All QTLs were detected as significant M × E interactions and exhibited, with respect to brewing, negative effects of the *Hsp* alleles. The highest increase of the protein content (5.5%) was associated with the *Hsp* allele from the EBmac0824_[5H] locus. At this locus, the explained phenotypic variance reached a maximum with 1.2%.

Water absorption (WA)

Six putative QTLs were located for the quality-related trait WA on chromosomes 4H, 5H and 7H. All QTLs were detected as significant M × E interactions and exhibited, with respect to brewing, favorable effects of the *Hsp* alleles. The highest increase in water absorption (1.8%) was associated with the *Hsp* allele from the EBmac0824_[5H] locus. The highest portion of the phenotypic variance was explained by *HvB23D4*_[4H] (6.1%).

Malt tenderness (MT)

Two putative QTLs were located for the quality related trait MT on chromosomes 5H and 7H. Both QTLs were detected as significant marker main effects. The effects of both *Hsp* alleles were negative in regard to brewing. The highest reduction in malt tenderness (8.2%) was associated with the *Hsp* allele from the EBmac0824_[5H] locus. At this QTL the explained phenotypic variance reached its maximum with 1.7%.

Comparison with other QTL analyses in barley

In the following, the AB-QTL analysis will be compared with other barley QTL analyses – first, with respect to the differences in methods and second, with respect to QTLs mapped in common.

To date, more than 60 publications are available which deal with yield- and quality-related QTL analyses in barley – for instance, Hayes et al. (1993), Han et al. (1995), Backes et al. (1995), Thomas et al. (1995, 1996), Tinker et al. (1996), Mather et al. (1997), Bezant et al. (1997a, 1997b), Kircherer et al. (2000), Marquez-Cedillo et al. (2000, 2001). For comparison with the AB-QTL analysis, the aforementioned studies will be termed "classical" QTL analyses. While classical QTL analyses were conducted in early, balanced generations like doubled haploid (DH) and F₂, our AB-QTL analysis was based on a BC₂F₂ population. This change was necessary since we used an exotic cross with the barley

progenitor *Hsp* as the donor of potential favorable QTL alleles. By means of backcrossing, the genome portion of *Hsp* was reduced to a mean P[*Hsp*] value of 0.127. It is assumed that masking negative side effects of linked or unlinked *Hsp* alleles on quantitative traits are reduced by this strategy. The high frequency of 33.7% of trait-improving *Hsp* alleles supports this assumption. However, it is still open if the identified favorable QTL alleles from *Hsp* are indeed unmatched in the elite gene pool of barley. Studies in *Oryza* and *Lycopersicon* have shown that the overwhelming majority of the genetic diversity in those taxa is present in the wild-species gene pool (Tanksley and McCouch 1997). Similar results for *Hordeum* have been reported by Powell and Russell (2000). Based on this findings, it is likely that at least a portion of the identified favorable QTL alleles from *Hsp* are new alleles, so far not present in the barley elite gene pool.

The number of genotyped DNA markers in classical QTL analyses range from 50 to 129, with the majority of markers being RFLPs and, to some extent, amplified AFLPs. In future, it can be expected that the majority of QTL analyses will be conducted with highly automatable markers like SSRs or single nucleotide polymorphism (SNPs). For barley, there are already more than 300 high quality SSRs available in the public domain (Becker and Heun 1995; Liu et al. 1996; Russell et al. 1997; Struss and Plieske 1998; Pillen et al. 2000; Ramsay et al. 2000; Kota et al. 2001). To our knowledge, our study represents the first example of a QTL study in barley which is exclusively based on SSR markers. However, the number of 45 SSRs still segregating in the BC₂F₂ population is relatively low. Our attempts to fill in gaps, for instance on chromosome 3H and 6H, failed since either no SSRs were available for that particular chromosomal region or an informative SSR did not segregate within the BC₂F₂. The latter phenomenon is most likely caused by the loss of *Hsp* alleles during the backcrossing process.

The number of 86 putative QTLs corresponds to classical QTL studies in barley which have detected between 11 and 159 QTLs. A variety of factors may affect the outcome of a QTL analysis – For example, the selection of the cross, population structure and size, number of measured replications and environments and type, number and density of markers. In addition, the selection of the statistical method exerts a major impact on the results of a QTL experiment. Examples of refined methods applied to classical QTL analyses are simple interval mapping (SIM, Haley and Knott 1992), composite interval mapping (CIM, Jansen and Stam 1994; Zeng 1994) and simplified CIM (sCIM, Tinker and Mather 1995). Several software programs which are based on these methods have been written for detection of QTLs, e.g. MAPMARKER/QTL (Lander and Botstein 1989), QTL-CARTOGRAPHER (Basten et al. 1994), MQTL (Tinker and Mather 1995) and PLAB-QTL (Utz and Melchinger 1996). Unfortunately, these programs are focused on the analysis of balanced populations which are used in classical QTL analyses. For unbalanced populations, which are used in AB-QTL studies, the program QGENE was written (Nelson

1997). QGENE operates with single marker regression as well as simple interval mapping for QTL detection. However, QGENE cannot handle multiple environments simultaneously. Since our AB-QTL study was conducted in six separate environments and since we wanted to include the $M \times E$ interaction as a measure of the environment stability of a QTL effect, we preferred to use a two-factorial ANOVA with the marker genotype and the environment as factors. By including the environment in the statistical model, we expected to reduce the residual variance of the experiment and, thus, to increase the probability of detecting a QTL effect. Simultaneously, a two-factorial model allowed us to differentiate between a QTL significant as a marker main effect, which is considered to be stable across the tested environments, and a QTL significant as a $M \times E$ interaction where the effect is considered to depend on a particular environment.

Next, we compared our AB-QTL analysis with classical QTL analyses with respect to common QTL effects. Unfortunately, a direct comparison of QTL effects is difficult. The classical barley studies have been conducted with RFLPs and, to a lesser extent, with AFLPs, whereas our AB-QTL study is exclusively based on SSR markers. Nonetheless, an indirect comparison of QTL effects is possible by means of the current Steptoe \times Morex map, published by A. Kleinhofs (<http://barleygenomics.wsu.edu/databases/databases.html>). The map integrates RFLP, AFLP and SSR markers, which are mapped in independent linkage studies, by allocating them to 99 evenly spaced BIN groups. Based on the Steptoe \times Morex BIN map, eight SSRs of our AB-QTL analysis – which are also placed in BIN groups and which have been associated with QTL effects – can be compared with classical barley QTL analyses. These markers are: *HvBKASI*_[2H], *HVM54*_[2H], *HVM40*_[4H], *WMS6*_[4H], *HVM67*_[4H], *HVM6*_[5H], *HVM4*_[7H] and *HvPRP1B*_[7H]. The QTL comparison was carried out by means of an up-to-date compilation of mapped barley QTLs from P. Hayes (<http://www.css.orst.edu/#barley/nabgmp/qlsum.htm>), which is based on the Steptoe \times Morex BIN classification. A possible common QTL was assumed, if the BIN groups of two independently detected QTLs were identical or, at least, overlapped.

The eight selected SSRs could be associated with 11 putative QTLs in the A \times 101 backcross. By comparison of BIN groups, four QTL effects (36%) could be verified in at least one classical barley QTL analysis. The first common QTL is the putative QTL for HEA associated with *HVM67*_[4H] on BIN 13. A classical QTL for HEA was also detected in the same or in overlapping BIN groups at locus *Bmy1* (Hackett et al. 1992) and in marker intervals *ABG397-ksuH11* and *ABG397-Bmy1* (Hayes et al. 1993, 1996). The second common QTL was again detected for HEA but associated with *HvPRP1B*_[7H] on BIN 12. This QTL was recovered at marker *BCD512A* (Laurie et al. 1995) and in marker interval *MWG539-MWG929* (Backes et al. 1995). The third common QTL was found for LAF associated with *HVM6*_[5H] on BIN 15.

This QTL was also detectable in marker interval *MWG650-MWG002* (Backes et al. 1995). The fourth common QTL was found for TGW associated with *HvBKASI*_[2H] on BIN 8. In this case, a classical QTL for kernel plumpness was also detected in the overlapping BIN groups for marker interval *HvBKASI-vrs1* (Marquez-Cedillo et al. 2001).

No classical QTL matched the favorable yield QTL which was associated with *HVM40*_[4H] on BIN 2. However, corresponding classical QTLs for the favorable yield QTL at marker locus *GMS89*_[4H] could be located, although a direct allocation of *GMS89*_[4H] onto the BIN map has not been made yet. Because *GMS89*_[4H] was placed 5 cM south of *HVM68* (Ramsay et al. 2000), we propose to allocate *GMS89*_[4H] to BIN group 7 or 8 on chromosome 4H. In that case, classical QTLs for grain yield have been reported in overlapping BIN groups at marker *Xpsb37(L)* (Bezant et al. 1997a) and in marker intervals *ABG472-ABG366* (Tinker et al. 1996) and *ABG472-ABG397* (Hayes et al. 1993). In addition, Ellis et al. (2002) also reported a QTL for grain yield in the region between *HVM68* and *HVM67* on chromosome 4H where *GMS89*_[4H] is placed.

The overall moderate conformity between the QTLs identified in our AB-QTL analysis and in classical QTL analyses can be regarded as a confirmation that most QTL effects from the exotic donor *Hsp* are unique. Thus, these QTLs can be exploited for improving and broadening the genetic basis of the barely elite gene pool. Nevertheless, it should be noted that there is also little conformity present between classical QTL studies. For example, Thomas et al. (1995) reported considerable differences in QTL identification between the Scottish cross *Blenheim* \times *E224/3* and the North American crosses *Steptoe* \times *Morex* and *Harrington* \times *TR306*. Likewise, Mather et al. (1997) reported that, when comparing the two aforementioned North American crosses, they found more differences than confirmations of QTL positions.

Comparison with AB-QTL analyses in tomato and rice

This report represents the first application of the AB-QTL strategy in barley. The goals of the AB-QTL analysis are the identification and simultaneous transfer of those exotic QTL alleles which have the potential to improve yield-related and quality-related agronomic traits. Within the A \times 101 population, a total of 29 favorable *Hsp* alleles (34%) were identified among 86 localized QTLs. These favorable *Hsp* alleles were detected for seven of the 13 traits investigated. The portion of favorable exotic alleles identified in A \times 101 is lower than, for instance, in rice where Xiao et al. (1998) reported a frequency of 51% favorable exotic alleles among 68 QTLs identified from an elite backcross with the wild rice *O. rufipogon*. On the other hand, Bernacchi et al. (1998a) found only 20% favorable alleles from the wild tomato species *L. hirsutum* among 121 QTLs localized.

The strength of the trait improvement can be taken as a further measure of the efficiency of the QTL detection. In all AB-QTL analyses published so far for tomato and rice, the total yield could be raised due to the presence of at least one favorable exotic QTL allele. The yield increases amounted to maximal values of 18% in rice (Xiao et al. 1996; Xiao et al. 1998) and of 17%, 34%, 15% and 27%, respectively, in four tomato studies (Tanksley et al. 1996; Fulton et al. 1997, 2000; Bernacchi et al. 1998a). In barley, the maximum yield increase was associated with the exotic *Hsp* allele at locus GMS89_[4H]. The increase of yield at locus GMS89_[4H] could be detected in all six environments and ranged from 2.7% to 10.9%, with an average of 7.7% across all environments. The failure to reach the level of yield increases observed in tomato and rice could be due to the wrong selection of the *Hsp* donor accession. However, the accession ISR101-23 was selected in a pre-test among ten candidate donor accessions due to its relatively high frequency of BC₁ and BC₂ lines revealing transgressive segregation for single plant grain yield (data not shown). Under this light, a stronger effect of other *Hsp* donor accessions on yield might be questionable.

An alternative explanation for the lower positive effect of *Hsp* alleles on yield compared with exotic alleles from tomato and rice could be the different breeding system. Whereas we have compared the performance of homozygous backcross lines derived from barley BC₂F₂ individuals, hybrids derived from BC₂ individuals were compared in tomato and rice. Thus, it cannot be ruled out that the stronger effects on yield detected in tomato and rice are due to heterosis effects occurring in hybrids between elite and exotic germplasm. The test of this hypothesis would be difficult to conduct in barley since an effective hybrid system, which can produce sufficient numbers of test cross seeds for field testing is, to date, not available. It would, thus, be more appropriate to compare the performance of hybrids and homozygous lines derived from an AB-QTL population in tomato or rice where both populations can be produced simultaneously.

Although the favorable QTL effects of the *Hsp* donor accession ISR101-23 are less pronounced than the effects from exotic donors in previous AB-QTL analyses in tomato and rice, the first application of the method to barley can be regarded as a success. In order to validate the significant effects presented in this study, we are currently producing NILs. By means of marker-assisted backcrossing and selfing, we generate, for instance, NILs from selected BC₂F₂-lines which harbor the yield increasing *Hsp* alleles around the SSR loci HVM40_[4H] and GMS89_[4H]. Due to recombination events, the resulting NILs should possess only small *Hsp* segments in the vicinity of the selected SSR locus. The NILs will be exploited for the validation of the original favorable *Hsp* effect and, as pure introgression lines, can be utilized for further breeding cycles. Simultaneously, the NILs can be utilized for high-resolution mapping of the region of interest, ultimately leading to a map-based cloning of the QTL factor. Both strategies have already been carried out

in tomato. For example, Bernacchi et al. (1998b), Monforte and Tanksley (2000) and Monforte et al. (2001) produced detailed high-resolution maps of introgressed exotic tomato segments based on older AB-QTL analyses and validated the detected exotic effects in refined QTL-NILs. Furthermore, the production of a high-resolution map has already led to the first cloning of a QTL factor (Alpert and Tanksley 1996; Frary et al. 2000). In this case, it was shown that the QTL *fw2.2*, which controls the fruit weight of tomato, presumably acts as a negative regulator of cell division in the tomato carpel tissue.

Acknowledgements We wish to thank Dr. Eberhard Laubach and Dr. Volker Lein and their teams for conducting field experiments and collecting trait data at the breeding companies Nordsaat, Gudow, and Ackermann, Irlbach. We also thank Dr. Lizzy Kuntze and her team for measuring quality traits at the laboratory of Nordsaat, Böhnhausen. In addition, the excellent technical assistance of Karola Müller and the technical team of our experimental station Dikopshof at Wesseling is appreciated. We are indebted to Prof. G. Fischbeck, Technical University, München, for providing the wild barley accession ISR101-23. We thank Mrs. Maria von Korff for critical reading of the manuscript. This work was supported by grants Pi339/1-1 and Pi339/1-2 from the Deutsche Forschungsgemeinschaft (DFG).

References

- Alpert KB, Tanksley SD (1996) High-resolution mapping and isolation of a yeast artificial chromosome contig containing *fw2.2*: a major fruit weight quantitative trait locus in tomato. *Proc Natl Acad Sci USA* 93:15,503–15,507
- Backes G, Graner A, Foroughi Wehr B, Fischbeck G, Wenzel G, Jahoor A (1995) Localization of quantitative trait loci (QTL) for agronomic important characters by the use of a RFLP map in barley (*Hordeum vulgare* L.). *Theor Appl Genet* 90:294–302
- Basten CJ, Weir BS, Zeng ZB (1994) Zmap – as QTL CARTOGRAPHER. In: Smith C, Gavora JS, Benkel B, Chesnais J, Fairfull W, Gibson JP, Kennedy BW, Burnside EB (eds) *Proc 5th World Congr Genet Appl Livestock Production Computing Strategies Software* 22:65–66
- Becker J, Heun M (1995) Barley microsatellites: allele variation and mapping. *Plant Mol Biol* 27:835–845
- Bernacchi D, Beck Bunn T, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley S (1998a) Advanced backcross QTL analysis in tomato. I. Identification of QTLs for traits of agronomic importance from *Lycopersicon hirsutum*. *Theor Appl Genet* 97:381–397
- Bernacchi D, Beck Bunn T, Emmatty D, Eshed Y, Inai S, Lopez J, Petiard V, Sayama H, Uhlig J, Zamir D, Tanksley S (1998b) Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L. pimpinellifolium*. *Theor Appl Genet* 97:170–180
- Bezant J, Laurie D, Pratchett N, Chojecki J, Kearsey MJ (1997a) Mapping QTL controlling yield and yield components in a spring barley (*Hordeum vulgare* L.) cross using marker regression. *Mol Breed* 3:29–38
- Bezant JH, Laurie DA, Pratchett N, Chojecki J, Kearsey MJ (1997b) Mapping of QTL controlling NIR predicted hot water extract and grain nitrogen content in a spring barley cross using marker-regression. *Plant Breed* 116:141–145
- Ellis RP, Forster BP, Gordon DC, Handley LL, Keith RP, Lawrence P, Meyer R, Powell W, Robinson D, Scrimgeour CM, Young G, Thomas WTB (2002) Phenotype/genotype associations for

- yield and salt tolerance in a barley mapping population segregating for two dwarfing genes. *J Exp Bot* 53:1163–1176
- Frary A, Nesbitt TC, Frary A, Grandillo S, van der Knaap E, Cong B, Liu J, Meller J, Elber R, Alpert KB, Tanksley SD (2000) *fw2.2*: a quantitative trait locus key to the evolution of tomato fruit size. *Science* 289:85–88
- Fulton TM, Beck Bunn T, Emmatty D, Eshed Y, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (1997) QTL analysis of an advanced backcross of *Lycopersicon peruvianum* to the cultivated tomato and comparisons with QTLs found in other wild species. *Theor Appl Genet* 95:881–894
- Fulton TM, Grandillo S, Beck Bunn T, Fridman E, Frampton A, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (2000) Advanced backcross QTL analysis of a *Lycopersicon esculentum* × *Lycopersicon parviflorum* cross. *Theor Appl Genet* 100:1025–1042
- Hackett CA, Ellis RP, Forster BP, McNicol JW, Macaulay M (1992) Statistical analysis of a linkage experiment in barley involving quantitative trait loci for height and ear-emergence time and two genetic markers on chromosome 4. *Theor Appl Genet* 85:120–126
- Haley CS, Knott SA (1992) A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. *Heredity* 69:315–324
- Han F, Ullrich SE, Chirat S, Menteur S, Jestin L, Sarrafi A, Hayes PM, Jones BL, Blake TK, Wesenberg DM, Kleinhofs A, Kilian A (1995) Mapping of beta-glucan content and beta-glucanase activity loci in barley grain and malt. *Theor Appl Genet* 91:921–927
- Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, Blake T, Franckowiak J, Rasmusson D, Sorrells M, Ullrich SE, Wesenberg D, Kleinhofs A (1993) Quantitative trait locus effects and environmental interaction in a sample of North American barley germplasm. *Theor Appl Genet* 87:392–401
- Hayes P, Prehn D, Vivar H, Blake T, Comeau A, Henry I, Johnston M, Jones B, Steffenson B, St Pierre CA, Chen F (1996) Multiple disease resistance loci and their relationship to agronomic and quality loci in a spring barley population. *J Quant Trait Loci* (<http://www.ncgr.org/research/jag/papers96/#paper296/jqtl22.html>)
- Jansen R, Stam P (1994) High resolution of quantitative traits into multiple loci via interval mapping. *Genetics* 136:1447–1455
- Kircherer S, Backes G, Walther U, Jahoor A (2000) Localising QTLs for leaf rust resistance and agronomic traits in barley (*Hordeum vulgare* L.). *Theor Appl Genet* 100:881–888
- Kota R, Varshney RK, Thiel T, Dehmer KJ, Graner A (2001) Generation and comparison of EST-derived SSRs and SNPs in barley (*Hordeum vulgare* L.). *Hereditas* 135:145–151
- Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* 121:185–199
- Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln S, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1:174–181
- Laurie DA, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of five major genes and eight quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. *Genome* 38:575–585
- Liu ZW, Biyashev RM, Saghai Maroof MA (1996) Development of simple sequence repeat DNA markers and their integration into a barley linkage map. *Theor Appl Genet* 93:869–876
- Marquez-Cedillo LA, Hayes PM, Jones BL, Kleinhofs A, Legge WG, Rossnagel BG, Sato K, Ullrich SE, Wesenberg DM (2000) QTL analysis of malting quality in barley based on the doubled-haploid progeny of two elite North American varieties representing different germplasm groups. *Theor Appl Genet* 101:173–184
- Marquez-Cedillo LA, Hayes PM, Kleinhofs A, Legge WG, Rossnagel BG, Sato K, Ullrich SE, Wesenberg DM (2001) QTL analysis of agronomic traits in barley based on the doubled-haploid progeny of two elite North American varieties representing different germplasm groups. *Theor Appl Genet* 103:625–637
- Mather DE, Tinker NA, LaBerge DE, Edney M, Jones BL, Rossnagel BG, Legge WG, Briggs KG, Irvine RB, Falk DE, Kasha KJ (1997) Regions of the genome that affect grain and malt quality in a North American two-row barley cross. *Crop Sci* 37:544–554
- Moncada PP, Martínez CP, Borrero J, Chatel M, Gauch H Jr, Guimaraes E, Tohme J, McCouch SR (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC₂F₂ population evaluated in an upland environment. *Theor Appl Genet* 102:41–52
- Monforte AJ, Tanksley SD (2000) Fine mapping of a quantitative trait locus (QTL) from *Lycopersicon hirsutum* chromosome 1 affecting fruit characteristics and agronomic traits: breaking linkage among QTLs affecting different traits and dissection of heterosis for yield. *Theor Appl Genet* 100:471–479
- Monforte AJ, Friedman E, Zamir D, Tanksley SD (2001) Comparison of a set of allelic QTL-NILs for chromosome 4 of tomato: deductions about natural variation and implications for germplasm utilization. *Theor Appl Genet* 102:572–590
- Nelson CJ (1997) QGENE: Software for marker-based genomic analysis and breeding. *Mol Breed* 3:229–235
- Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD (1988) Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* 335:721–726
- Pillen K, Binder A, Kreuzkam B, Ramsay L, Waugh R, Förster J, Léon J (2000) Mapping new EMBL-derived barley microsatellites and their use to differentiate German barley cultivars. *Theor Appl Genet* 101:652–660
- Powell W, Russell JR (2000) Molecular analysis of barley diversity. In: Logue S (ed) 8th Int Barley Genet Symp, vol 1. Adelaide, South Australia, pp 29–31
- Ramsay L, Macaulay M, Ivanisovich degli S, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvevsson S, Morgante M, Massari A, Maestri E, Marmioli N, Sjakste T, Ganai M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. *Genetics* 156:1997–2005
- Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganai MW (1998) A microsatellite map of wheat. *Genetics* 149:2007–2023
- Russell J, Fuller JD, Young G, Thomas B, Taramino G, Macaulay M, Waugh R, Powell W (1997) Discriminating between barley genotypes using microsatellite markers. *Genome* 40:442–450
- SAS Institute (1999) The SAS system for Windows, release 8.00. SAS Institute, Cary, N.C.
- Struss D, Plieske J (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. *Theor Appl Genet* 97:308–315
- Stuber CW, Lincoln SE, Wolff DW, Helentjaris T, Lander ES (1992) Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics* 132:823–839
- Tanksley SD, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277:1063–1066
- Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor Appl Genet* 92:191–203
- Tanksley SD, Grandillo S, Fulton TM, Zamir D, Eshed Y, Petiard V, Lopez J, Beck-Bunn T (1996) Advanced backcross QTL analysis in a cross between an elite processing line of tomato and its wild relative *L. pimpinellifolium*. *Theor Appl Genet* 92:213–224
- Thomas WTB, Powell W, Waugh R, Chalmers KJ, Barua UM, Jack P, Lea V, Forster BP, Swanson JS, Ellis RP, Hanson PR, Lance RCM (1995) Detection of quantitative trait loci for agronomic, yield, grain and disease characters in spring barley (*Hordeum vulgare* L.). *Theor Appl Genet* 91:1037–1047

- Thomas WTB, Powell W, Swanston JS, Ellis RP, Chalmers KJ, Barua UM, Jack P, Lea V, Forster BP, Waugh R, Smith DB (1996) Quantitative trait loci for germination and malting quality characters in a spring barley cross. *Crop Sci* 36:265–273
- Tinker NA, Mather DE (1995) MQTL: software for simplified composite interval mapping of QTL in multiple environments. *J Quant Trait Loci* (<http://www.ncgr.org/research/jag/papers95/paper295/indexp295.html>)
- Tinker NA, Mather DE, Rossnagel BG, Kasha KJ, Kleinhofs A, Hayes PM, Falk DE, Ferguson T, Shugar LP, Legge WG, Irvine RB, Choo TM, Briggs KG, Ullrich SE, Franckowiak JD, Blake TK, Graf RJ, Dofing SM, Saghai Maroof MA, Scoles GJ, Hoffman D, Dahleen LS, Kilian A, Chen F, Biyashev RM, Kudrna DA, Steffenson BJ (1996) Regions of the genome that affect agronomic performance in two-row barley. *Crop Sci* 36:1053–1062
- Utz HF, Melchinger AE (1996) PLABQTL: a program for composite interval mapping of QTL. *J Quant Trait Loci* (<http://www.ncgr.org/research/jag/papers96/paper196/utz.html>)
- Xiao J, Grandillo S, Ahn SN, McCouch SR, Tanksley SD, Li J, Yuan L (1996) Genes from wild rice improve yield. *Nature* 384:223–224
- Xiao J, Li J, Grandillo S, Ahn S, Yuan L, Tanksley SD, McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. *Genetics* 150:899–909
- Zeng ZB (1994) Precision mapping of quantitative trait loci. *Genetics* 136:1457–1468