ORIGINAL PAPER

Identification and verification of QTLs for agronomic traits using wild barley introgression lines

Inga Schmalenbach · Jens Léon · Klaus Pillen

Abstract A set of 39 wild barley introgression lines (hereafter abbreviated with S42ILs) was subjected to a QTL study to verify genetic effects for agronomic traits, previously detected in the BC$_2$DH population S42 (von Korff et al. 2006 in Theor Appl Genet 112:1221–1231) and, in addition, to identify new QTLs and favorable wild barley alleles. Each line within the S42IL set contains a single marker-defined chromosomal introgression from wild barley (*Hordeum vulgare* ssp. *spontaneum*), whereas the remaining part of the genome is exclusively derived from elite spring barley (*H. vulgare* ssp. *vulgare*). Agronomic field data of the S42ILs were collected for seven traits from three different environments during the 2007 growing season. For detection of putative QTLs, a two-factorial mixed model ANOVA and, subsequently, a Dunnett test with the recurrent parent as a control were conducted. The presence of a QTL effect on a wild barley introgression was accepted, if the trait value of a particular S42IL was significantly ($P < 0.05$) different from the control, either across all environments and/or in a particular environment. A total of 47 QTLs were localized in the S42IL set, among which 39 QTLs were significant across all tested environments. For 19 QTLs (40.4%), the wild barley introgression was associated with a favorable effect on trait performance. Von Korff et al. (2006 in Theor Appl Genet 112:1221–1231) mapped altogether 44 QTLs for six agronomic traits to genomic regions, which are represented by wild barley introgressions of the S42IL set. Here, 18 QTLs (40.9%) revealed a favorable wild barley effect on the trait performance. By means of the S42ILs, 20 out of the 44 QTLs (45.5%) and ten out of the 18 favorable effects (55.6%) were verified. Most QTL effects were confirmed for the traits days until heading and plant height. For the six corresponding traits, a total of 17 new QTLs were identified, where at six QTLs (35.3%) the exotic introgression caused an improved trait performance. In addition, eight QTLs for the newly studied trait grains per ear were detected. Here, no QTL from wild barley exhibited a favorable effect. The introgression line S42IL-107, which carries an introgression on chromosome 2H, 17–42 cM is an example for S42ILs carrying several QTL effects simultaneously. This line exhibited improved performance across all tested environments for the traits days until heading, plant height and thousand grain weight. The line can be directly used to transfer valuable *Hsp* alleles into modern elite cultivars, and, thus, for breeding of improved varieties.

I. Schmalenbach · K. Pillen
Max-Planck-Institute for Plant Breeding Research,
Barley Genetics Research Group, Carl-von-Linné-Weg 10,
50829 Cologne, Germany

J. Léon
Chair of Crop Science and Plant Breeding,
Institute of Crop Science and Resource Conservation,
University of Bonn, Katzenburgweg 5, 53115 Bonn, Germany

Present Address:
K. Pillen (✉)
Chair of Plant Breeding, Institute of Agricultural
and Nutritional Sciences, University of Halle-Wittenberg,
Ludwig-Wucherer-Str. 2, 06108 Halle, Germany
e-mail: klaus.pillen@landw.uni-halle.de

Introduction

Most agronomically important traits, such as yield and quality parameters, are quantitatively inherited. They are not conditioned by single genes, but polygenes or so called

"quantitative trait loci" (QTLs, Collard et al. 2005). Based on molecular linkage maps, many QTL studies were conducted since the late 1980s to identify and map the loci, which affect complex traits. In barley, such QTL studies focused on yield associated traits, as well as malting quality, abiotic stress tolerance, and disease resistance (e.g. Tinker et al. 1996; Thomas et al. 1996; Jefferies et al. 1999; Kicherer et al. 2000).

Tanksley and Nelson (1996) proposed the advanced backcross (AB)-QTL analysis, which has several advantages over classical QTL analyses. These are: (1) the detection of QTLs and the development of improved varieties are conducted simultaneously. (2) Unadapted germplasm from wild species or land races is used to detect exotic alleles superior to the elite alleles which might ultimately broaden the genetic variation of modern crop cultivars (Tanksley and McCouch 1997). (3) Since the AB-QTL strategy is based on BC_2 or BC_3 generations, the portion of the exotic donor genome, and thus linkage drag, is considerably reduced. The fact that wild species contain favorable alleles, which could be useful for the enhancement of quantitative traits, was proven by many AB-QTL studies for several crop species, such as tomato (e.g. deVicente and Tanksley 1993; Fulton et al. 2002; Frary et al. 2004) and rice (Xiao et al. 1996; Li et al. 2002; Thomson et al. 2003; McCouch et al. 2007). In barley, several AB-QTL studies were conducted with regard to a multitude of characters, like agronomic traits, morphological traits, yield components, disease resistances, tolerance to abiotic stress and malting quality (Pillen et al. 2003, 2004; Talame et al. 2004; Li et al. 2005, 2006; Yun et al. 2006; Gyenis et al. 2007). Comprehensive studies were also performed by von Korff et al. (2005, 2006, 2008), who generated a BC_2DH population consisting of 301 lines based on an initial cross between the malting barley cultivar 'Scarlett' (*Hordeum vulgare* ssp. *vulgare*, hereafter abbreviated with *Hv*) and the Israeli wild barley accession 'ISR 42-8' (*H. vulgare* ssp. *spontaneum,* hereafter abbreviated with *Hsp*, von Korff et al. 2004). The lines were genotyped with 98 SSR markers, and subsequently evaluated under field conditions in up to eight environments. By a three-factorial mixed model analysis of variance (ANOVA), altogether 86 putative QTLs were identified for nine agronomic traits, e.g. days until heading, plant height, thousand grain weight and grain yield, where 31 QTLs (36.0%) revealed a favorable effect of the exotic allele (von Korff et al. 2006). In addition, 18 QTLs conferring resistance to diseases (von Korff et al. 2005), and 48 QTLs for seven malting parameters (von Korff et al. 2008), were detected. For these trait complexes, the performance was improved by the *Hsp* allele at 11 QTLs (61.1%) and 18 QTLs (37.5%), respectively.

As recommended by Zamir (2001), one step further towards the nearly simultaneous identification and transfer of exotic QTL alleles associated with an improved trait performance, is the development and evaluation of introgression lines (ILs). An IL set represents the complete genome of a wild species in the uniform background of an elite cultivar, where each single line solely contains a single marker-defined segment of the exotic parent. Due to the homozygosity of the ILs, they are a stable, and, thus, immortal, genetic resource which can be used with versatility. Furthermore, the low proportion of exotic germplasm, present in each IL, is a great advantage compared to commonly used QTL mapping populations, especially F_2, BC_1 and RI, but also AB populations. It allows the statistical verification of small phenotypic effects, which are otherwise more difficult to detect (Zamir 2001). As an advancement compared to the AB-QTL analysis, the detection of QTLs and their simultaneous transfer into elite cultivars is carried out with ILs in one step. It does not require further steps of backcrossing or selfing. The usefulness of ILs for the detection of QTL effects and the identification of favorable exotic QTL alleles was demonstrated by numerous QTL studies for several different crops, first of all for tomato. The *Solanum pennellii* IL library, which was initiated by Eshed and Zamir (1994) and currently consists of 76 ILs (Lippman et al. 2007), was used to identify nearly 3,000 putative QTLs for yield associated traits (Eshed and Zamir 1995; Hanson et al. 2007), morphological traits (Holtan and Hake 2003; Semel et al. 2006) and metabolites (Baxter et al. 2005; Overy et al. 2005; Schauer et al. 2006). With regard to quantitative traits, QTL studies using ILs were also conducted for rice (e.g. Mei et al. 2006; Zheng et al. 2007; Siangliw et al. 2007), wheat (Liu et al. 2006, 2007; Pestsova et al. 2006), maize (Szalma et al. 2007), melon (Eduardo et al. 2007; Fernandez-Trujillo et al. 2007; Moreno et al. 2008; Obando et al. 2008) and *Arabidopsis* (Keurentjes et al. 2007). In spring barley, Matus et al. (2003) conducted an association analysis to identify recombinant chromosome substitution lines (RCSLs) showing positive transgressive segregation with respect to the elite parent for agronomic traits, domestication-related traits, and malting quality traits. Here, altogether 140 RCSLs, with a similar genetic structure as ILs, were evaluated in the field. A superior phenotype, compared to the elite parent, was exhibited by RCSLs for the traits number of grains per ear, ear length and thousand grain weight. A further barley QTL study using RCSLs was conducted by Hori et al. (2005). They developed a total of 134 RCSLs, each line carrying one or several *Hsp* introgressions, and containing on average 12.9% of the *Hsp* genome. By means of a core-set of 19 RCSLs and, in addition, 93 double-haploid lines (DHLs) originating from the same cross, altogether 18 and 24 QTLs could be identified, respectively. Several QTLs revealed a coincident or very close localization in both populations (Hori et al. 2005).

In the present study, we demonstrate the usefulness of wild barley introgression lines (S42ILs) for the detection of QTLs for important agronomic traits. The performance of a set of 39 S42ILs, each containing a single marker-defined *Hsp* introgression in the uniform genetic background of *Hv*, was evaluated with regard to seven traits in three different field environments during 2007 growing season. Subsequently, a line × phenotype association study was conducted to verify QTL effects, which were previously identified in the BC₂DH population S42 (von Korff et al. 2006), and, in addition, to detect new QTLs. A further aim was to identify S42ILs, which exhibited an enhanced trait performance compared to the recurrent parent. These lines are a potentially valuable resource for the development of new elite cultivars.

Materials and methods

Plant material

A set of 39 S42ILs (S42IL-101 to S42IL-139) were evaluated with regard to seven agronomic traits. The lines differ among each other in only one single marker-defined chromosomal segment, introgressed from the Israeli wild barley accession 'ISR 42-8' (*Hsp*), whereas the remaining genome is consistently derived from the German spring barley cultivar 'Scarlett' (*Hv*). Based on 40 BC₂DH lines, previously selected from spring barley population S42 (von Korff et al. 2004), the S42ILs were developed by a further round of backcrossing with the recurrent parent 'Scarlett', several rounds of selfing and in parallel SSR marker-assisted selection. Finally, the 39 S42ILs were selected in BC₃S₂ or BC₃S₄ generation. The approach and the genetic characterization of the 39 S42ILs, as well as the verification of QTLs for powdery mildew and leaf rust, are described in detail in Schmalenbach et al. (2008).

Phenotypic evaluation of agronomic traits

In order to evaluate the agronomic performance of the 39 S42ILs with regard to seven traits, field tests at three different locations in Germany were conducted in the 2007 growing season. The locations were the experimental field station Dikopshof (D07, University of Bonn, West Germany), and the breeders' experimental field stations in Gudow (G07, Nordsaat Saatzucht, North Germany) and Herzogenaurach (H07, Saatzucht Josef Breun, South East Germany). The field tests were designed in three randomized complete blocks (i.e. replications). The recurrent parent 'Scarlett' was tested as a control in four replications per block. In addition, the spring barley malting cultivar 'Barke' was tested in three replications per block to compare the performance of the S42ILs with a current cultivar.

Net plot sizes (4.5–6.0 m²), seed density (300–390 kernels/m²), nitrogen fertilization (30–80 kg N/ha) taking into account the N$_{min}$ content of the soil, and field management were in accordance with the local practice. The grain was harvested with a plot harvester at total maturity (EC 92). The investigated traits and methods of measurement are listed in Table 1.

Statistical analyses

Statistical analyses were carried out with SAS Enterprise Guide 4.1 (SAS Institute 2006). Genetic correlations between trait values were calculated with the least squares means (LSMEANS) for each of the 39 S42ILs averaged across all replications and environments. The LSMEANS were computed with the GLM procedure and used for calculating Pearson's correlation coefficient (*r*) with the CORR procedure.

For the detection of QTLs and identification of favorable *Hsp* introgressions, a two-factorial mixed model ANOVA was carried out. The GLM model includes the line as a fixed factor, and the environment and line × environment interaction as random factors: $Y_{ijk} = \mu + L_i + E_j + LxE_{ij} + \varepsilon_{k(ij)}$, where μ is the general mean, L_i the fixed effect of the *i*th line, E_j the random effect of the *j*th environment, $L \times E_{ij}$ is the random interaction effect of the *i*th line and the *j*th environment, $\varepsilon_{k(ij)}$ is the error of Y_{ijk}. When the analysis revealed significant differences between lines or line × environment interactions, a Dunnett multiple comparison of LSMEANS differences between the ILs with the recurrent parent 'Scarlett' as the control was conducted (Dunnett 1955). The presence of a QTL in an *Hsp* introgression was accepted, when the trait value of a particular IL was significantly ($P < 0.05$) different from 'Scarlett' across all environments (line main effect) and/or in a particular environment (line × environment interaction effect). If several lines, which contained overlapping or flanking introgressions, showed a significant effect of the same direction (increase or reduction of trait performance), it was assumed that these ILs carried the same QTL. Introgressions do overlap, if they possess at least one common *Hsp* allele. They flank each other, if they have adjacent *Hsp* alleles. The relative performance of an introgression line [RP (IL)] was calculated as follows: RP (IL) = [LSMEANS (IL) − LSMEANS ('Scarlett')] × 100/LSMEANS ('Scarlett'), where for each trait the LSMEANS were calculated across all replications and environments.

Results

Trait performances of 'Scarlett' compared to the S42IL set

In Table 2, the trait performances of the recurrent parent 'Scarlett' and the S42ILs are described per environment

Table 1 List of seven agronomic traits evaluated for 39 S42ILs in up to three environments

Abbr.	Trait	Units	Method of measurement	Breeding goal[a]	Environment tested[b]
EAR	Ears per square meter	No. of ears/m^2	Number of ears counted from a row of 50 cm (D07) or 100 cm (H07)	+	D07, H07
GEA	Grains per ear	No. of grains/ear	Number of grains per ear calculated from a row of 50 cm (D07) or 20 average ears (G07 and H07)	+	D07, G07, H07
HEA	Days until heading	d	Number of days from sowing until emergence of 50% of ears on main tillers	–	D07, G07
HEI	Plant height	cm	Average plant height (in cm) measured from soil surface to tip of spike (including awns) 2 weeks after flowering	–	D07, G07, H07
LAH	Lodging at harvest	Scores 1–9	Visual rating of the severity of lodging at harvest, where one represents no lodging and nine represents total lodging of plot	–	D07, G07, H07
TGW	Thousand grain weight	g/1,000 grains	Average weight (in g) of 1,000 grains calculated from two samples of 250 grains	+	D07, G07, H07
YLD	Grain yield	dt/ha	Weight of barley grain (in dt/ha), harvested from 6.0 m^2 (D07), 4.5 m^2 (G07), 5.0/5.5 m^2 (H07), after drying for 1–2 days	+	D07, G07, H07

[a] The breeding goals for the evaluated traits were defined according to breeding programs for spring malting barley, where (−) indicates that a reduction, and (+) that an increase of the trait values is desirable

[b] The environment names are combinations of the location [Dikopshof (D), Gudow (G), Herzogenaurach (H)] and the year 2007 (07)

and across all tested environments by the parameters mean, minimum and maximum, and coefficient of variation. Across all three environments, both 'Scarlett' and the S42IL set revealed the highest coefficient of variation for the traits lodging at harvest (73.7 and 82.3%, respectively) and grain yield (34.8 and 35.0%, respectively). In contrast, a low variation of trait performance was measured for the traits grains per ear, days until heading, and plant height. As expected, 'Scarlett' exhibited a lower coefficient of variation than the S42ILs for all traits and environments. Averaged across all environments and in G07, 'Scarlett' showed a slightly higher performance in grain yield than the S42IL set (50.8 and 60.0 dt/ha, compared to 50.3 and 57.7 dt/ha, respectively). In D07 and H07, an opposite effect was measured (Table 2).

Trait correlations

A total of 17 significant correlations were detected between the seven investigated traits (Table 3). The strongest positive correlations were found between days until heading and the traits grains per ear ($r = 0.74$) and thousand grain weight ($r = 0.73$), as well as between plant height and lodging at harvest ($r = 0.73$). In addition, days until heading exhibited strong negative correlations with lodging at harvest ($r = -0.84$) and plant height ($r = -0.61$). Grain yield revealed significant correlations with all traits. Whereas negative correlations were found with days until heading and grains per ear ($r = -0.52$ and $r = -0.27$, respectively), the other four traits showed positive correlations with grain yield ranging from 0.37 for plant height to 0.68 for thousand grain weight.

QTL detection

In order to verify QTL effects, which were previously detected within the BC$_2$DH population S42, and to identify new QTLs, the 39 S42ILs were subjected to a line × phenotype association study. The initial two-factorial mixed model ANOVA exhibited significant line effects ($P < 0.05$) for all seven evaluated traits except ears per square meter. Significant line × environment interaction effects ($P < 0.05$) were detected for all traits (data not shown).

The subsequent Dunnett test revealed altogether 65 significant line × phenotype associations with four line main effects and 18 line × environment interaction effects. For 43 associations, the line main effect and the line × environment interaction effect, were significant, simultaneously (Table 4). For 29 associations (44.6%), the respective IL showed an improved trait performance in comparison to the recurrent parent 'Scarlett'. Taking into account that several ILs do overlap or are flanked, a

Table 2 Parameters describing the trait performance of the recurrent parent 'Scarlett' and the S42ILs per environment and across all tested environments

Trait and environment[a]	'Scarlett'					S42ILs				
	N[b]	Mean[c]	Min[d]	Max[d]	CV (in %)[e]	N[b]	Mean[c]	Min[d]	Max[d]	CV (in %)[e]
EAR										
D07	12	968.0	673.1	1250.0	18.0	117	1033.7	576.9	1634.6	22.2
H07	12	946.7	840.0	1088.0	10.2	117	903.9	616.0	1216.0	14.3
Across all env.	24	957.3	673.1	1250.0	14.4	234	968.8	576.9	1634.6	20.3
GEA										
D07	12	22.3	19.0	24.0	6.4	117	19.9	13.0	23.0	11.4
G07	4	23.0	22.0	24.0	3.5	39	22.5	17.0	26.0	7.8
H07	12	23.5	20.0	27.0	8.4	117	22.2	13.0	26.0	10.0
Across all env.	28	2.9	19.0	27.0	7.4	273	21.2	13.0	26.0	11.7
HEA										
D07	12	60.3	60.0	61.0	0.8	117	59.1	47.0	64.0	5.4
G07	12	72.3	70.0	75.0	2.0	117	71.0	62.0	76.0	4.7
Across all env.	24	66.3	60.0	75.0	9.4	234	65.1	47.0	76.0	10.4
HEI										
D07	12	90.9	83.0	101.0	5.7	117	89.3	70.0	100.0	6.3
G07	12	75.4	72.0	80.0	3.6	117	78.6	63.0	103.0	12.3
H07	8	76.0	70.0	82.0	5.3	78	77.5	64.0	98.0	8.6
Across all env.	32	81.4	70.0	101.0	10.4	312	82.3	63.0	103.0	11.3
LAH										
D07	12	7.4	5.0	8.0	12.1	117	7.3	3.0	9.0	16.5
G07	12	3.2	1.0	5.0	50.1	117	2.6	1.0	9.0	86.4
H07	12	1.1	1.0	2.0	26.6	117	1.3	1.0	8.0	92.4
Across all env.	36	3.9	1.0	8.0	73.7	351	3.7	1.0	9.0	82.3
TGW										
D07	12	39.7	37.1	43.0	4.8	117	39.3	34.6	47.0	5.8
G07	12	44.2	38.6	47.8	5.8	117	44.9	36.4	52.0	6.9
H07	12	33.7	32.2	36.2	4.2	117	33.5	26.9	41.8	8.7
Across all env.	36	39.2	32.2	47.8	12.3	351	39.2	26.9	52.0	13.8
YLD										
D07	12	65.7	61.8	72.4	5.0	117	66.2	52.4	78.7	6.9
G07	12	60.0	54.7	64.9	5.0	117	57.7	38.0	67.3	9.4
H07	12	26.8	20.4	30.4	11.9	117	26.9	13.9	35.9	16.6
Across all env.	36	50.8	20.4	72.4	34.8	351	50.3	13.9	78.7	35.0

[a] The trait and environment abbreviations are given in Table 1

[b] Number of observations

[c] Average trait performance

[d] Minimum and maximum trait performance

[e] Coefficient of variation (in %) = SD/mean

total of 47 putative QTLs were identified. Here, one QTL (QTgw.S42IL-2H.b) was detected solely as line main effect and eight QTLs solely as line × environment interaction effect. For the remaining 38 QTLs both the line main effect and the line × environment interaction effect, were significant. Nineteen QTLs (40.4%) showed a favorable effect of the *Hsp* introgression. In the following paragraphs, the QTL effects are described for each trait separately.

Ears per square meter (EAR)

For EAR, altogether four S42ILs revealed a significant line × phenotype association. Taking into account that

Table 3 Pearsons correlation coefficient (*r*) between seven agronomic traits in 39 S42ILs

Traits	GEA	HEA	HEI	LAH	TGW	YLD
EAR	−0.51***	−0.36*	0.03	0.39***	0.25*	0.47***
GEA		0.74***	−0.19*	−0.46***	−0.02	−0.27**
HEA			−0.61***	−0.84***	0.73***	−0.52***
HEI				0.73***	0.03	0.37***
LAH					0.13	0.67***
TGW						0.68***

The agronomic traits are defined in Table 1. For calculating correlation coefficients, the least squares means of the trait performance of each IL were averaged across replications and environments. The *r* values are significant with * $P < 0.05$, ** $P < 0.01$ or *** $P < 0.001$

several lines contain overlapping or flanking introgressions, these associations were summarized to two QTLs on chromosome 2H and 6H, respectively. At QEar.S42IL-2H.a, the line main effect and the line × environment interaction effect were significant, whereas for QEar.S42IL-6H.a a significant effect was only found in environment D07. For both QTLs, the *Hsp* introgression was associated with an increased number of ears per square meter relative to 'Scarlett'. The strongest effect was exhibited by S42IL-107, containing an *Hsp* segment in the region 2H, 17-42 cM. This line showed a significantly higher trait performance than the recurrent parent only in the environment D07. Here, the number of ears per square meter was increased by 397.4 (41.1%) relative to 'Scarlett'.

Grains per ear (GEA)

A total of 14 significant line × phenotype associations were identified for the trait GEA on all chromosomes except 5H and 6H. These associations were summarized to eight QTLs, where five QTLs showed both a significant line main effect and a line × environment interaction effect. Two QTLs each were localized on chromosomes 2H, 4H and 7H. At all eight QTLs, the *Hsp* introgressions were associated with a reduced number of GEA by up to −6.1 (−26.8%) for S42IL-107 at QGea.S42IL-2H.a.

Days until heading (HEA)

Altogether 19 significant line × phenotype associations, summarized to 14 putative QTLs, were identified for HEA on all seven barley chromosomes. For 12 QTLs both the line main effect and the line × environment interaction effect, were significant, whereas two QTLs on chromosome 6H revealed an interaction effect solely for D07. For 13 line × phenotype associations, and according to this, for nine QTLs, the exotic introgression was associated with a reduced number of days until heading relative to 'Scarlett'. The strongest favorable effect was mapped to the region 2H, 17–42 cM by S42ILs −107 and −108. Here, the number of days until heading was reduced by 10.4 (15.7%) and

10.3 (15.5%), respectively. Strong reduction effects of −6.6 days (−9.9%) and −6.1 days (−9.2%) caused by *Hsp* introgressions were mapped to the regions 4H, 80–95 cM and 7H, 146–166 cM by the S42ILs −121 and −137, respectively.

Plant height (HEI)

For HEI, eight significant line × phenotype associations were localized on all chromosomes except 6H. Due to the flanking introgressions of S42ILs −107 and −109, a total of seven QTLs were detected. At all loci, the line main effect and the line × environment interaction effect were significant. At QHei.S42IL-2H.a, the *Hsp* segment, located within the region 2H, 17–42 cM, caused a plant height reduced by 11.8 cm (14.6%) relative to the control 'Scarlett'. At the remaining six QTLs, the *Hsp* introgression increased the plant height by up to 15.9 cm (19.7%) at QHei.S42IL-7H.b.

Lodging at harvest (LAH)

Seven significant line × phenotype associations were localized for LAH on chromosomes 1H, 2H, 4H and 7H. Taking into account that two S42ILs on chromosome 2H do overlap, altogether six QTLs were detected. For five QTLs both the line main effect and the line × environment interaction effect, were significant, whereas for one QTL solely an interaction effect was significant in environment G07. At QLah.S42IL-2H.a, the exotic introgression caused a maximum reduction of lodging severity at harvest by 1.6 scores (40.0%) relative to 'Scarlett' within the region 2H, 67–92 cM. In addition, S42IL-138 containing an exotic introgression on chromosome 7H, 166–181 cM, revealed a LAH reduced by 1.4 scores (37.1%) at QLah.S42IL-7H.b.

Thousand grain weight (TGW)

For TGW, eight significant line × phenotype associations were localized on chromosomes 2H, 4H and 6H. These effects were summarized to six QTLs. For one QTL the line

Table 4 List of 65 significant line × phenotype associations for seven agronomic traits detected with 39 S42ILs

Trait[a]	QTL in S42IL	Chr.[b]	Introgression (in cM)[c]	Introgression line	Effect[d]	LSMEANS (IL)[e]	Diff.[f]	RP (IL) %[g]	QTL in BC2DH (von Korff et al. 2006)	Candidate genes[h]
EAR	QEar.S42IL-2H.a	2H	17–42	S42IL-107	I	1365.4[D07]	397.4	41.1	QEar.S42-2H.a	Ppd-H1[1]
		2H	17–92	S42IL-108	L + I	1243.4	286.1	29.9	QEar.S42-2H.a, QEar.S42-2H.b	
		2H	67–92	S42IL-109	L + I	1237.9	280.6	29.3	QEar.S42-2H.b	
	QEar.S42IL-6H.a	6H	145–155	S42IL-132	I	1359.0[D07]	391.0	40.4	QEar.S42-6H.a	
GEA	QGea.S42IL-1H.a	1H	39–70	S42IL-103	I	19.7[H07]	−3.8	−16.3		
	QGea.S42IL-2H.a	2H	17–42	S42IL-107	L + I	16.8	−6.1	−26.8		Ppd-H1[1]
		2H	17–92	S42IL-108	L + I	19.7	−3.3	−14.2		
	QGea.S42IL-2H.b	2H	67–92	S42IL-109	L + I	18.4	−4.5	−19.5		
		2H	80–86	S42IL-110	L + I	18.2	−4.7	−20.5		
	QGea.S42IL-3H.a	3H	65–70	S42IL-111	L + I	19.1	−3.8	−16.6		
	QGea.S42IL-4H.a	4H	80–95	S42IL-121	L + I	20.4	−2.5	−10.8		
		4H	125–132	S42IL-122	I	19.0[D07]	−3.3	−14.6		
		4H	125–170	S42IL-123	L + I	20.6	−2.4	−10.3		
	QGea.S42IL-4H.b	4H	170–190	S42IL-124	I	18.3[D07]	−3.3	−17.6		
	QGea.S42IL-7H.a	7H	50	S42IL-133	I	18.3[D07]	−3.9	−17.6		
		7H	62–75	S42IL-134	L + I	20.7	−2.3	−9.8		
	QGea.S42IL-7H.b	7H	146–155	S42IL-136	I	19.0[D07]	−3.3	−14.6		
		7H	146–166	S42IL-137	I	17.7[D07]	−4.6	−20.6		
HEA	QHea.S42IL-1H.a	1H	0–85	S42IL-102	L + I	68.2	1.9	2.9		
		1H	39–70	S42IL-103	L + I	68.3	2.1	3.1		
	QHea.S42IL-1H.b	1H	70–85	S42IL-105	L + I	62.0	−4.2	−6.4		
	QHea.S42IL-2H.a	2H	17–42	S42IL-107	L + I	55.8	−10.4	−15.7	QHea.S42-2H.a	Ppd-H1[1]
		2H	17–92	S42IL-108	L + I	56.0	−10.3	−15.5		
	QHea.S42IL-2H.b	2H	67–92	S42IL-109	I	59.0[D07]	−1.3	−2.1		
		2H	80–86	S42IL-110	L + I	62.3	−3.9	−5.9		
	QHea.S42IL-3H.a	3H	130–175	S42IL-114	L + I	62.8	−3.4	−5.2	QHea.S42-3H.b	sdw1(denso)[2]
		3H	155–190	S42IL-115	I	59.0[D07]	−1.3	−2.1		
	QHea.S42IL-4H.a	4H	31–57	S42IL-120	L + I	68.0	1.8	2.6		
	QHea.S42IL-4H.b	4H	80–95	S42IL-121	L + I	59.7	−6.6	−9.9		
	QHea.S42IL-4H.c	4H	170–190	S42IL-124	L + I	68.2	1.9	2.9	QHea.S42-4H.a	Vrn-H2[3]
	QHea.S42IL-5H.a	5H	43–69	S42IL-125	L + I	62.2	−4.1	−6.2		
	QHea.S42IL-6H.a	6H	96–112	S42IL-129	I	59.0[D07]	−1.3	−2.1	QHea.S42-6H.b	eps6L.1[2]
	QHea.S42IL-6H.b	6H	145–155	S42IL-132	I	61.7[D07]	1.4	2.4		
	QHea.S42IL-7H.a	7H	50	S42IL-133	L + I	69.0	2.8	4.2		Vrn-H3[4]
	QHea.S42IL-7H.b	7H	62–75	S42IL-134	L + I	63.2	−3.1	−4.7		HvCO1[5]
		7H	75–155	S42IL-135	I	59.0[D07]	−1.3	−2.1	QHea.S42-7H.b	HvCO1[5], eps7L[2]
	QHea.S42IL-7H.c	7H	146–166	S42IL-137	L + I	60.2	−6.1	−9.2		eps7L[2]
HEI	QHei.S42IL-1H.a	1H	70–85	S42IL-105	L + I	89.9	9.1	11.3		
	QHei.S42IL-2H.a	2H	17–42	S42IL-107	L + I	69.0	−11.8	−14.6	QHei.S42-2H.a	Ppd-H1[1]
		2H	67–92	S42IL-109	L + I	73.1	−7.7	−9.6	QHei.S42-2H.b	sdw3[6]
	QHei.S42IL-3H.a	3H	130–175	S42IL-114	L + I	94.9	14.1	17.5	QHei.S42-3H.b	sdw1 (denso)[2]
	QHei.S42IL-4H.a	4H	80–95	S42IL-121	L + I	95.4	14.6	18.1		
	QHei.S42IL-5H.a	5H	43–69	S42IL-125	L + I	92.9	12.2	15.1	QHei.S42-5H.a	
	QHei.S42IL-7H.a	7H	62–75	S42IL-134	L + I	90.0	9.2	11.4	QHei.S42-7H.b	HvCO1[5]
	QHei.S42IL-7H.b	7H	146–166	S42IL-137	L + I	96.7	15.9	19.7	QHei.S42-7H.c	

Table 4 continued

Trait[a]	QTL in S42IL	Chr.[b]	Introgression (in cM)[c]	Introgression line	Effect[d]	LSMEANS (IL)[e]	Diff.[f]	RP (IL) %[g]	QTL in BC2DH (von Korff et al. 2006)	Candidate genes[h]
LAH	QLah.S42IL-1H.a	1H	70–85	S42IL-105	I	6.3[G07]	3.2	100.0		
	QLah.S42IL-2H.a	2H	67–92	S42IL-109	L + I	2.3	−1.6	−40.0	QLof.S42-2H.a	*sdw3*[6]
		2H	80–86	S42IL-110	L	2.6	−1.3	−34.3		
	QLah.S42IL-4H.a	4H	80–95	S42IL-121	L + I	6.8	2.9	74.3		
	QLah.S42IL-4H.b	4H	125–170	S42IL-123	L + I	5.4	1.6	40.0		
	QLah.S42IL-7H.a	7H	146–166	S42IL-137	L + I	7.3	3.4	88.6	QLof.S42-7H.b	
	QLah.S42IL-7H.b	7H	166–181	S42IL-138	L + I	2.4	−1.4	−37.1		
TGW	QTgw.S42IL-2H.a	2H	17–42	S42IL-107	L + I	42.3	3.1	7.9	QTgw.S42-2H.a	*Ppd-H1*[1]
		2H	17–92	S42IL-108	I	38.1[H07]	4.4	13.1		
	QTgw.S42IL-2H.b	2H	80–86	S42IL-110	L	41.9	2.7	7.0	QTgw.S42-2H.a	
	QTgw.S42IL-4H.a	4H	31–95	S42IL-119	L	42.6	3.4	8.7	QTgw.S42-4H.a	
		4H	80–95	S42IL-121	I	44.6[D07]	4.9	12.3		
	QTgw.S42IL-4H.b	4H	125–132	S42IL-122	L + I	35.0	−4.2	−10.8	QTgw.S42-4H.b	
	QTgw.S42IL-4H.c	4H	170–190	S42IL-124	L + I	35.7	−3.5	−8.8		
	QTgw.S42IL-6H.a	6H	112–155	S42IL-130	L + I	42.2	3.0	7.7		
YLD	QYld.S42IL-4H.a	4H	31–57	S42IL-118	I	73.5[D07]	7.8	11.8		
	QYld.S42IL-4H.b	4H	80–95	S42IL-121	L + I	41.9	−9.0	−17.7		
	QYld.S42IL-7H.a	7H	50	S42IL-133	L	45.2	−5.6	−11.1		
		7H	62–75	S42IL-134	I	49.2[G07]	−10.8	−18.0		
	QYld.S42IL-7H.b	7H	146–166	S42IL-137	L + I	41.8	−9.1	−17.9	QYld.S42-7H.b	

[a] Abbreviations of traits see Table 1

[b] Chromosomal location of the target introgression

[c] Chromosomal extent of the target introgression in centiMorgans

[d] In the two-factorial ANOVA, significant line × phenotype associations ($P < 0.05$) were detected as line main effect (L) or line × environment interaction effect (I)

[e] If line main effects (L), or line main effects and a line × environment interaction effects (L + I) were detected, the LSMEANS[IL] across all environments are listed. If solely a line × environment interaction (I) was identified, the LSMEANS[IL] in the particular environment (D07, G07 or H07) is given

[f] Score difference = LSMEANS[IL] − LSMEANS [Scarlett]

[g] Relative performance: RP[IL] = (LSMEANS[IL] – LSMEANS [Scarlett]) × 100/LSMEANS[Scarlett]

[h] References: [1]Turner et al. (2005), [2]Laurie et al. (1995), [3]Yan et al. (2004), [4]Yan et al. (2006), [5]Griffiths et al. (2003), [6]Gottwald et al. (2004)

main effect and for three QTLs the line main effect plus the line × environment interaction effect were significant. At the remaining QTLs a line plus a line × environment interaction and a sole interaction effect was detected for the two linked ILs −107 and −108 whereas a line and a sole interaction effect was detected for the two linked ILs −119 and −121. Four QTLs revealed a favorable effect of the exotic introgression on TGW. The highest increase in trait performance was measured at QTgw.S42−2H.a in S42IL-108, which harbors a *Hsp* segment in the region 2H, 17–92 cM. Here, the thousand grain weight was increased by 4.4 g (13.1%) relative to 'Scarlett'. This effect was solely significant in environment H07. The strongest favorable *Hsp* effect, which was significant across all three environments, was mapped to 4H, 31–95 cM. Here, S42IL-119 revealed a TGW increased by 3.4 g (8.7%) relative to 'Scarlett'.

Grain yield (YLD)

Altogether five significant line × phenotype associations were identified for YLD. Due to two flanking S42ILs on chromosome 7H, these effects were summarized to four QTLs on chromosomes 4H and 7H. Three QTLs were detected both as line main effect and line × environment interaction effect. At all three loci, the exotic segment was associated with a grain yield reduced by up to 10.8 dt/ha (18.0%) in S42IL-134 at QYld.S42IL-7H.a. The QTL QYld.S42IL-4H.a was assessed solely as line × environment interaction effect, and revealed an improved trait performance compared to the recurrent parent for S42IL-118. Here, the introgression within region 4H, 31–57 cM caused an increase in grain yield by 7.8 dt/ha (11.8%) relative to 'Scarlett' in environment D07.

Discussion

Identification and verification of QTLs

During the 2007 growing season, a set of 39 wild barley introgression lines (S42ILs) were evaluated in the field with regard to seven agronomic traits, and subsequently subjected to a line × phenotype association study. The aim of the experiment was to verify QTL effects, previously detected with 301 double haploid lines of the population S42, and to identify new QTLs. It was furthermore intended to identify *Hsp* introgressions which cause an improvement of several traits simultaneously. The S42ILs carrying these introgressions could be used directly for the development of new elite cultivars. For the five traits EAR, HEA, HEI, TGW and YLD, both the S42ILs and the population S42, were evaluated. In addition, lodging was investigated at harvest (LAH) in the S42IL set, but at flowering (LOF) in the S42 population. The QTLs for LAH and LOF are directly comparable since both traits are closely linked. Von Korff et al. (2006) identified altogether 63 putative QTLs for the six traits in the S42 population, where 23 QTLs (36.5%) showed a favorable effect of the *Hsp* allele. Forty-four QTLs, among which 18 QTLs revealed a favorable *Hsp* effect, are located in chromosomal regions which are already represented by *Hsp* introgressions in S42ILs, and are therefore comparable with the QTLs detected in the S42ILs. As illustrated in Table 4, 20 out of 44 QTLs (45.5%) and ten out of 18 QTLs with a favorable *Hsp* effect (55.6%) were verified by the evaluated S42ILs. Altogether, 18 out of 20 corresponding QTLs (90.0%) showed an effect of the same direction in both populations.

As underlined by Bernacchi et al. (1998b), the verification of a QTL can be characterized by the stability of its effect across environments. In the present study, 18 QTLs were stable across all tested environments in the S42 population as well as in the S42ILs. A further important criterion for the evaluation of QTL verification results is the magnitude of the effect measured in both populations. For all corresponding QTLs, with the exception of QHei.S42IL-7H.a and QHei.S42-7H.b, at least one S42IL exhibited a higher relative performance in comparison to 'Scarlett' than measured for the corresponding QTL in the S42 population. These differences as well as the non-confirmation of QTL effects from population S42 might be explained by the reduction of epistatic *Hsp* effects which are still active in the S42ILs. Since the S42ILs contain single exotic introgressions, whereas the BC$_2$DH lines in the S42 population carry multiple independent introgressions, potential interactions between non-linked *Hsp* alleles are expected to be reduced in the S42IL set. Furthermore, the QTL data of the S42 population were based on field tests at up to eight different environments, and thus offered a high reliability, whereas the S42ILs were only tested in three environments.

For the six traits analyzed in both populations, altogether 17 new QTLs, previously not identified in the S42 population, were detected in the S42IL set. Here, six QTLs (35.3%) revealed a favorable *Hsp* effect. Furthermore, eight QTLs for the newly identified trait GEA were mapped by S42ILs, among which no QTL showed a favorable *Hsp* effect.

A similar approach to verify QTLs was conducted in tomato by Bernacchi et al. (1998b). The authors generated a set of 23 near isogenic lines (NILs), which harbored QTLs with a favorable effect on one or several agronomic traits. Each NIL contained a single introgression of the wild species *S. hirsutum* or *S. pimpinellifolium*, whereas the remaining part of the genetic background was consistently derived from the elite parent *S. esculentum*. The aim of the study was to verify 25 QTLs for seven traits, previously mapped to 15 different genomic regions in two AB populations (Bernacchi et al. 1998a). The agronomic performance of the NILs was tested in five different environments, and a total of 22 QTLs (88%) could be confirmed by the NILs in at least one environment.

In the following paragraphs, the QTL results of the present study are discussed for each trait or trait complex separately. They are compared to the QTL effects, previously identified in population S42 (von Korff et al. 2006), and to different candidate genes which map to the same chromosomal region.

Days until heading

For HEA, von Korff et al. (2006) detected a total of ten QTLs, half of them were located in genomic regions, which are already represented by *Hsp* introgressions of S42ILs. These five QTLs were verified by QTL effects detected in the S42IL set, and exhibited an effect with the same direction in both populations (Table 4). Four QTLs were significant across all environments in the S42 population as well as in the S42ILs. In both populations, the strongest favorable *Hsp* effect, associated with a reduction of HEA, was mapped to the top of chromosome 2H (QHea.S42-2H.a and QHea.S42IL-2H.a, respectively). Here, the number of days until heading was reduced by up to 10.4 (15.7%) in S42IL-107, compared to 'Scarlett'. In the same region, *Ppd-H1*, the major photoperiod response gene of barley, whose daylength sensitive alleles promote flowering under long day conditions, is located (Turner et al. 2005). *HvCO1*, another barley photoperiod response gene of the circadian clock (Griffiths et al. 2003), coincides with the QTL QHea.S42IL-7H.b, which was detected in the S42ILs −134 and −135 on chromosome 7H. The barley vernalization response genes *Vrn-H2* and *Vrn-H3*, which regulate flower-

ing depending on low temperature (Laurie et al. 1995; Yan et al. 2004, 2006), are located on chromosomes 4H and 7H. These loci correspond to the QTLs QHea.S42IL-4H.c and QHea.S42-4H.a, and the newly identified QTL QHea.S42IL-7H.a, respectively (Table 4). All three QTLs exhibited an increased *Hsp* effect on number of days until heading. This is consistent with the fact that wild barley requires a period of low temperature as impulse for flowering (Laurie 1997). Several 'earliness per se' genes, which are relatively independent from photoperiod and vernalization conditions, were mapped by Laurie et al. (1995). The genes *eps6L.1* and *eps7L* coincide with the QTLs QHea.S42IL-6H.a and QHea.S42-6H.b, and QHea.S42IL-7H.c and QHea.S42-7H.b on chromosome 6H and 7H, respectively. Laurie et al. (1995) demonstrated a delay in flowering time in barley caused by the dwarfing gene *sdw1* (previously named *denso*). The gene location on chromosome 3H corresponds to the localization of the QTLs QHea.S42IL-3H.a and QHea.S42-3H.b, which both exhibited an enhanced *Hsp* effect on HEA. Altogether eight new QTLs, which were not detected in the S42 population, were revealed by S42ILs. Half of them showed a reducing *Hsp* effect on HEA.

Plant height and lodging at harvest

In population S42, altogether 11 QTLs were detected for HEI (von Korff et al. 2006), at which seven QTLs were localized in regions which are so far represented by S42ILs. Six out of these seven QTLs (85.7%) were confirmed by QTL effects detected in S42ILs, exhibiting effects with the same direction in both populations (Table 4). Furthermore, five verified QTLs revealed significant effects across all environments, both in S42 population and in the S42ILs. In both populations, the strongest favorable *Hsp* effect on HEI was mapped to region 2H, 17–42 cM. Here, plant height was reduced in S42IL-107 by 14.6%, relative to 'Scarlett', whereas the *Hsp* allele in the S42 population caused a reduction effect of 11.7%. As described by Laurie et al. (1994), this localization corresponds to the *Ppd-H1* locus which, in addition to flowering regulation, exerts a strong effect on plant height (Turner et al. 2005). A possible candidate gene for QHei.S42-2H.b and the effect of QHei.S42IL-2H.a in S42IL-109 is the gibberellic-acid insensitive dwarfing gene *sdw3* of barley (Gottwald et al. 2004). It might also be causal for the strong reduction of LAH in S42ILs -109 and -110 at QLah.S42IL-2H.a. As illustrated in Fig. 1, further co-localizations of QTLs for HEI and LAH are present in the chromosomal regions 1H, 70–85 cM, 4H, 80–95 cM, and 7H, 146–166 cM. For all three QTLs, the *Hsp* introgression caused an increasing effect on both traits, which coincides with their strong positive correlation of 0.73 (Table 3). The dwarfing gene *sdw1*, which reduces

plant height and lodging (Bezant et al. 1996), maps to the same genomic region as QHei.S42IL-3H.a and QHei.S42-3H.b. However, no QTL effect on LAH was detected by S42ILs in this region. The QTLs QHei.S42IL-7H.a and QHei.S42-7H.b, which both revealed an increasing *Hsp* effect on HEI, coincide with *HvCO1* (Griffiths et al. 2003). As described above, the number of days until heading is reduced by *Hsp* introgressions in the same region. These opposed effects on HEA and HEI correspond to their negative correlation ($r = -0.61$). In addition to the verified QTLs, two new unfavorable *Hsp* effects on HEI were identified in S42ILs on chromosome 1H and 4H.

Altogether seven QTLs for lodging at flowering which mapped to regions covered by exotic introgressions in the S42IL set, were identified by von Korff et al. (2006). As shown in Table 4, the QTLs QLof.S42-2H.a and QLof.S42-7H.b were verified by QLah.S42IL-2H.a and QLah.S42IL-7H.a, detected by the S42ILs -109 and -110, and -137, respectively. In the S42 population as well as in the S42ILs, both QTLs were detected as significant effects of the same direction across all environments. In the present study, four new QTLs for LAH were localized on chromosomes 1H, 4H and 7H. Here, QLah.S42IL-7H.b exhibited a favorable *Hsp* effect.

Yield components

In the present study, the S42IL set was evaluated with regard to the three yield components EAR, GEA and TGW. Von Korff et al. (2006) detected altogether 11 and nine QTLs for EAR and TGW, respectively. Nine and eight QTLs are localized in chromosomal regions which are also represented by S42ILs. For both traits, three QTLs could be confirmed in the S42IL study (Table 4). For EAR as well as for TGW, one QTL revealed an unfavorable *Hsp* effect in the S42 population, but a favorable exotic effect in the S42ILs. Furthermore, one new QTL effect for TGW with an enhanced trait performance of S42IL-130 was mapped to the genomic region 6H, 112–155 cM. For EAR and GEA, the S42ILs -107, -108 and -109 revealed co-localizations of significant line × phenotype associations in the region 2H, 17–92 cM. All three S42ILs showed an increased EAR and simultaneously a reduced GEA, compared to 'Scarlett' (Fig. 1). These effects with an opposed direction coincide with a negative correlation of -0.51 between both traits. For all three yield components, QTL effects were detected by the S42ILs -107 and -109 on the top of chromosome 2H. As described by Laurie et al. (1994), the *Ppd-H1* locus, which mapped to the same region, also has an effect on yield components, probably as a direct result of the effect on flowering time. Also on chromosome 2H, but not directly at the *Ppd-H1* locus, Li et al. (2005, 2006)

Fig. 1 SSR map with 65 significant ($P < 0.05$) line × phenotype associations for 39 S42ILs and seven agronomic traits. The chromosomes are shown as *black bars* with centiMorgan values for SSR loci following the order of von Korff et al. (2004). The extent of *Hsp* introgressions are given in *grey bars* right to the chromosomes. The associations are illustrated as *symbols* below the S42ILs. They either reveal a favorable (*filled symbols*) or unfavorable (*empty symbols*) *Hsp* effect. Associations solely detected as line × environment interaction effect are marked by an *asterisk* right to the symbol
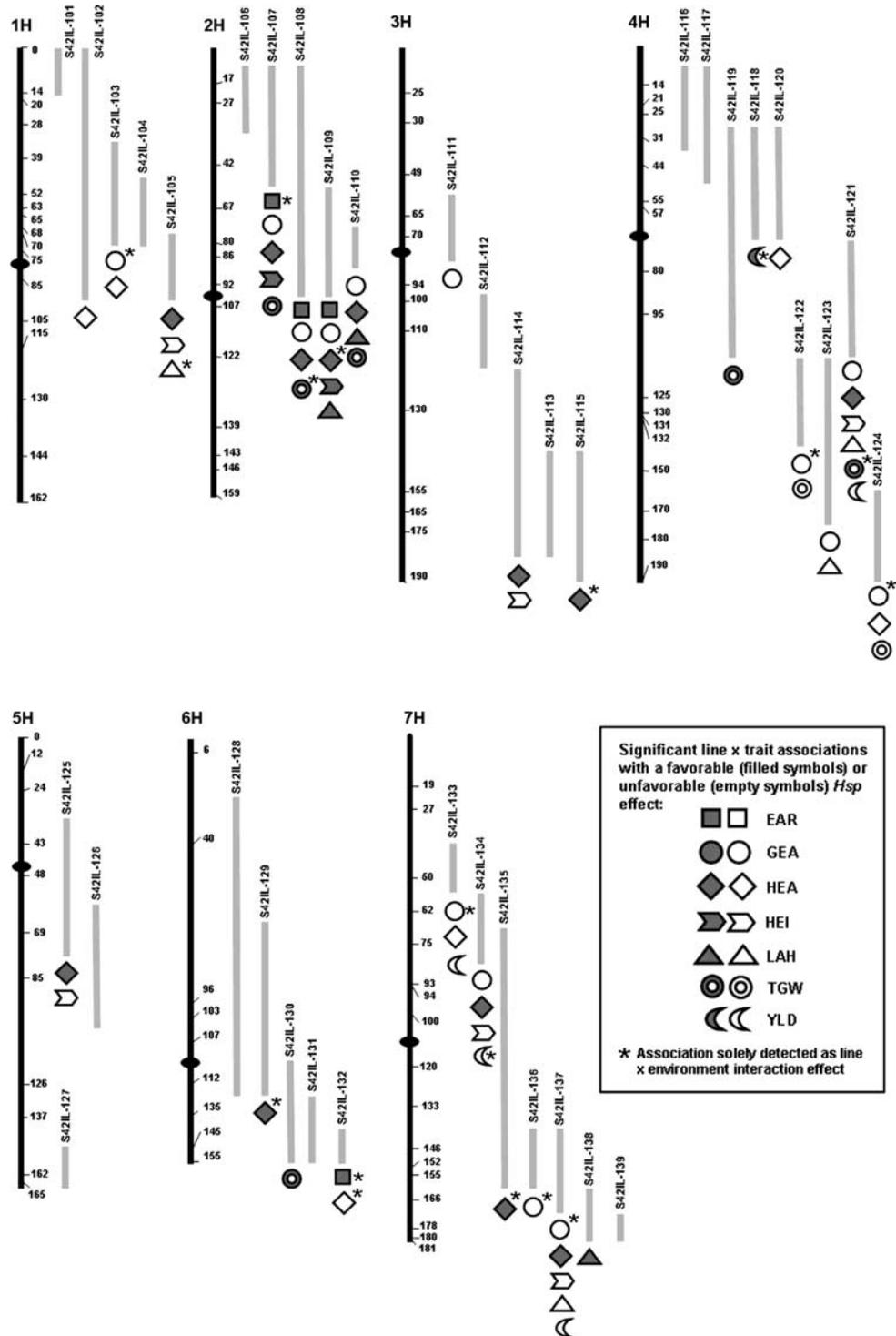
detected altogether eight QTLs for the five yield components ear length, spikelet number per spike, grain number per spike, spike number per plant, and thousand grain mass. At one QTL each for spike number per plant and thousand grain mass, the *Hsp* allele was associated with an improved trait performance.

Grain yield

For YLD, von Korff et al. (2006) detected 13 QTLs on all chromosomes. At three QTLs the *Hsp* allele caused an increased grain yield compared to the recurrent parent. Nine QTLs could theoretically be verified by the 39

S42ILs since they are located in genomic regions which are covered by *Hsp* introgressions. Solely the QTL QYld.S42-7H.b, showing an unfavorable *Hsp* effect in the S42 population, was verified by QYld.S42IL-7H.b. The latter revealed a reduced grain yield in S42IL-137, compared to 'Scarlett'. In addition, three new QTLs for YLD were mapped to chromosomes 4H and 7H present in four different S42ILs (Table 4). A potentially valuable introgression line for breeding of cultivars with enhanced grain yield might be S42IL-118 where yield was increased by 11.8% relative to 'Scarlett'. However, the stability of this favorable effect has to be verified since the YLD effect was only detected as a line × environment interaction active in D07.

Several times, co-localizations of QTLs for YLD and yield associated traits were detected which were consistent with the genetic correlations, for example, for HEA and TGW (Fig. 1). In the region 4H, 31–57 cM, a QTL effect for YLD as well as for TGW was detected. For both traits, the *Hsp* introgression caused an enhanced performance compared to the recurrent parent. This coincides with the strong positive correlation ($r = 0.68$) between YLD and TGW (Table 3). One coincidence of QTLs for YLD and HEA was identified in the genomic region 7H, 50 cM. Here, an opposite *Hsp* effect was in accordance with the negative trait correlation of -0.52. For YLD and LAH, two QTL co-localizations were detected in the regions 4H, 80–95 cM and 7H, 146–166 cM. On both QTLs the exotic introgression was associated with a reducing effect on YLD and an increasing effect on LAH. This is contrary to the positive genetic correlation between both traits. Due to the fact that a strong severity of lodging is assumed to be associated with a loss of grains, and, thus, a reduced grain yield, a negative trait correlation would be expected. For instance, von Korff et al. (2006) measured a genetic correlation of -0.55 between the traits grain yield and lodging at flowering. In the present study, the unexpected positive correlation between YLD and LAH might be due to the fact, that both traits differed substantially between the three examined environments. Whereas LAH was extremely high in D07, YLD was extremely low in H07 (Table 2). We assume that the observed positive correlation might be artificial since it disappeared when the calculation was based on single plot entries per environment (data not shown). Despite a positive genetic correlation ($r = 0.47$) between YLD and EAR, no co-localizations of QTLs could be revealed. In contrast, three co-localizations for YLD and GEA were detected on chromosomes 4H and 7H (Fig. 1). In all cases, an unfavorable *Hsp* effect on both traits was observed. Here, the negative correlation of -0.27 was contradictory.

Examples of S42ILs harboring QTL effects for several traits simultaneously

As illustrated in Fig. 1, several chromosomal regions revealed clusters of QTLs for different traits. Among the set of 39 S42ILs, altogether seven lines on chromosomes 2H, 4H and 7H exhibited at least four QTLs simultaneously. Four of these lines carry introgressions on chromosome 2H, among which the S42ILs −107 and −109 exhibited a favorable *Hsp* effect on four different traits. S42IL-107, carrying a *Hsp* segment in the region 2H, 17–42 cM, showed a significant improved performance relative to the recurrent parent for the traits HEA, HEI and TGW across all tested environments. In addition, one favorable *Hsp* effect on EAR, only significant in environment D07 was measured (Table 4). In S42IL-109, the exotic introgression in the region 2H, 67–92 cM was associated with an improved performance for EAR, HEI and LAH. All three effects were stable across environments, whereas an additional favorable effect on HEA was only significant in environment D07 (Fig. 1). Amongst others, S42ILs −107 and −109 represent valuable genetic resources for the development of new elite cultivars with enhanced agronomic performance. The relatively small portion of the *Hsp* genome present in most of the 39 S42ILs facilitates and accelerates the direct transfer of favorable exotic alleles into modern cultivars through backcrossing and marker-assisted selection. However, during the selection of favorable introgressions for a particular trait, it is worthwhile to assure the complete absence of unfavorable effects on other traits. ILs, exhibiting negative side effects, should not be transferred directly into new cultivars without selection of recombination events. It is, thus, advisable to select ILs with small *Hsp* introgressions, only exhibiting favorable exotic QTL effects.

Future prospects

The present study describes the application of wild barley introgression lines for the identification and verification of QTL effects. Altogether, 45.5% of the QTLs for six agronomic traits, previously identified in population S42 by von Korff et al. (2006), were verified by S42ILs. In future, promising *Hsp* effects will be subjected to map-based cloning. These effort will focus on effects chosen by the following criteria: the QTL effects are (1) verified, (2) significant across all environments and (3) strong, i.e. the elite and the exotic allele differ substantially in their phenotypic performance. Appropriate S42ILs for map-based cloning projects are the S42ILs −110 and −137 carrying an exotic segment in the region 2H, 80−86 cM and 7H, 146–166 cM, respectively. Both S42ILs exhibit significant and verified QTL

effects for multiple traits. A high-resolution mapping approach will eventually help to distinguish between gene linkage and true pleiotropy as the alternative hypotheses to explain the effects on multiple traits which are observed in these S42ILs. The required high-resolution mapping populations for chosen S42ILs are currently under construction. For this reason, all 39 S42ILs are currently backcrossed again with 'Scarlett'. In subsequent generations, recombination events and SUB-ILs with smaller introgressions will be selected. This material can serve as a starting point for both high-resolution mapping and molecular breeding.

In future, the S42IL set will also be characterized in detail through genotyping with Illumina SNPs (Rostoks et al. 2006) or Diversity Arrays Technology (DArT) markers (Wenzl et al. 2006), which will facilitate the fine-mapping of QTL effects. Currently, the S42ILs are also extensively tested with regard to malting quality and abiotic stress tolerance. All genotype and phenotype data of the S42ILs will be archived in the public IL data base 'Phenom Networks' (http://phn.huji.ac.il/RTQ/).

References

Baxter CJ, Carrari F, Bauke A, Overy S, Hill SA, Quick PW, Fernie AW, Sweetlove LJ (2005) Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. Plant Cell Physiol 46:425–437

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 back-cross 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 97:1191–1196

Bezant JH, Laurie DA, Pratchett N, Chojecki J, Kearsey MJ (1996) Marker regression mapping of QTL controlling flowering time and plant height in a spring barley (*Hordeum vulgare* L.) cross. Heredity 77:64–71

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. Euphytica 142:169–196

deVicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134:585–596

Dunnett CW (1955) A multiple comparison procedure for comparing several treatments with a control. J Am Stat Assoc 50:1096–1121

Eduardo I, Arus P, Monforte AJ, Obando J, Fernandez-Trujillo JP, Martinez JA, Alarcon AL, Alvarez JM, van der Knaap E (2007) Estimating the genetic architecture of fruit quality traits in melon using a genomic library of near isogenic lines. J Am Soc Hort Sci 132:80–89

Eshed Y, Zamir D (1994) A genomic library of *Lycopersicon pennellii* in *Lycopersicon esculentum*—a tool for fine mapping of genes. Euphytica 79:175–179

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147–1162

Fernandez-Trujillo JP, Obando J, Martinez JA, Alarcon AL, Eduardo I, Arus P, Monforte AJ (2007) Mapping fruit susceptibility to postharvest physiological disorders and decay using a collection of near-isogenic lines of melon. J Am Soc Hort Sci 132:739–748

Frary A, Fulton TM, Zamir D, Tanksley SD (2004) Advanced back-cross QTL analysis of a *Lycopersicon esculentum* × *L.* pennellii cross and identification of possible orthologs in the Solanaceae. Theor Appl Genet 108:485–496

Fulton TM, Bucheli P, Voirol E, Lopez J, Petiard V, Tanksley SD (2002) Quantitative trait loci (QTL) affecting sugars, organic acids and other biochemical properties possibly contributing to flavor, identified in four advanced backcross populations of tomato. Euphytica 127:163–177

Gottwald S, Stein N, Börner A, Sasaki T, Graner A (2004) The gibberellic-acid insensitive dwarfing gene *sdw3* of barley is located on chromosome 2HS in a region that shows high colinearity with rice chromosome 7L. Mol Gen Genomics 271:426–436

Griffiths S, Dunford RP, Coupland G, Laurie DA (2003) The evolution of *CONSTANS*-like gene families in barley, rice, and *Arabidopsis*. Plant Physiol 131:1855–1867

Gyenis L, Yun SJ, Smith KP, Steffenson BJ, Bossolini E, Sanguineti MC, Muehlbauer GJ (2007) Genetic architecture of quantitative trait loci associated with morphological and agronomic trait differences in a wild by cultivated barley cross. Genome 50:714–723

Hanson PM, Sitathani K, Sadashiva AT, Yang RY, Graham E, Ledesma D (2007) Performance of *Solanum habrochaites* LA 1777 introgression line hybrids for marketable tomato fruit yield in Asia. Euphytica 158:167–178

Holtan HE, Hake S (2003) Quantitative trait locus analysis of leaf dissection in tomato using *Lycopersicon penellii* segmental introgression lines. Genetics 165:1541–1550

Hori K, Sato K, Nankaku N, Takeda K (2005) QTL analysis in recombinant chromosome substitution lines and doubled haploid lines derived from a cross between *Hordeum vulgare* ssp. *vulgare* and *Hordeum vulgare* ssp. spontaneum. Mol Breed 16:295–311

Jefferies SP, Barr AR, Karakousis A, Kretschmer JM, Manning S, Chalmers KJ, Nelson JC, Islam AKMR, Langridge P (1999) Mapping of chromosome regions conferring boron toxicity tolerance in barley (*Hordeum vulgare* L.). Theor Appl Genet 98:1293–1303

Keurentjes JJB, Bentsink L, Alonso-Blanco C, Hanhart CJ, Vries HBD, Effgen S, Vreugdenhil D, Koornneef M (2007) Development of a near-isogenic line population of *Arabidopsis thaliana* and comparison of mapping power with a recombinant inbred line population. Genetics 175:891–905

Kicherer 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

Laurie DA (1997) Comparative genetics of flowering time. Plant Mol Biol 35:167–177

Laurie DA, Pratchett N, Bezant JH, Snape JW (1994) Genetic analysis of a photoperiod-response gene on the short arm of chromosome 2(2H) of *Hordeum vulgare* (barley). Heredity 72:619–627

Laurie DA, Pratchett N, Bezant JH, Snape JW (1995) RFLP mapping of 5 major genes and 8 quantitative trait loci controlling flowering time in a winter × spring barley (*Hordeum vulgare* L.) cross. Genome 38:575–585

Li DJ, Sun CQ, Fu YC, Li C, Zhu ZF, Chen L, Cai HW, Wang XK (2002) Identification and mapping of genes for improving yield from Chinese common wild rice (*O. rufipogon* Griff.) using advanced backcross QTL analysis. Chin Sci Bull 47:1533–1537

Li J, Huang XQ, Heinrichs F, Ganal MW, Röder MS (2005) Analysis of QTLs for yield, yield components, and malting quality in a BC$_3$-DH population of spring barley. Theor Appl Genet 110:356–363

Li JZ, Huang XQ, Heinrichs F, Ganal MW, Röder MS (2006) Analysis of QTLs for yield components, agronomic traits, and disease resistance in an advanced backcross population of spring barley. Genome 49:454–466

Lippman ZB, Semel Y, Zamir D (2007) An integrated view of quantitative trait variation using tomato interspecific introgression lines. Curr Opin Gen Dev 17:545–552

Liu SB, Zhou RG, Dong YC, Li P, Jia JZ (2006) Development, utilization of introgression lines using a synthetic wheat as donor. Theor Appl Genet 112:1360–1373

Liu SW, Zhao SY, Chen F, Xia GM (2007) Generation of novel high quality HMW-GS genes in two introgression lines of *Triticum aestivum/Agropyron elongatum*. BMC Evol Biol 7, article number 76

Matus I, Corey A, Filichkin T, Hayes PM, Vales MI, Kling J, Riera-Lizarazu O, Sato K, Powell W, Waugh R (2003) Development and characterization of recombinant chromosome substitution lines (RCSLs) using *Hordeum vulgare* subsp *spontaneum* as a source of donor alleles in a *Hordeum vulgare* subsp *vulgare* background. Genome 46:1010–1023

McCouch SR, Sweeney M, Li JM, Jiang H, Thomson M, Septiningsih E, Edwards J, Moncada P, Xiao JH, Garris A, Tai T, Martinez C, Tohme J, Sugiono M, McClung A, Yuan LP, Ahn SN (2007) Through the genetic bottleneck: *O. rufipogon* as a source of trait-enhancing alleles for *O. sativa*. Euphytica 154:317–339

Mei HW, Xu JL, Li ZK, Yu XQ, Guo LB, Wang YP, Ying CS, Luo LJ (2006) QTLs influencing panicle size detected in two reciprocal introgressive line (IL) populations in rice (*Oryza sativa* L.). Theor Appl Genet 112:648–656

Moreno E, Obando JM, Dos-Santos N, Fernandez-Trujillo JP, Monforte AJ, Garcia-Mas J (2008) Candidate genes and QTLs for fruit ripening and softening in melon. Theor Appl Genet 116:589–602

Obando J, Fernandez-Trujillo JP, Martinez JA, Alarcon AL, Eduardo I, Arus P, Monforte AJ (2008) Identification of melon fruit quality quantitative trait loci using near-isogenic lines. J Am Soc Hort Sci 133:139–151

Overy A, Walker HJ, Malone S, Howard TP, Baxter CJ, Sweetlove LJ, Hill SA, Quick WP (2005) Application of metabolite profiling to the identification of traits in a population of tomato introgression lines. J Exp Bot 56:287–296

Pestsova EG, Börner A, Röder MS (2006) Development and QTL assessment of *Triticum aestivum–Aegilops tauschii* introgression lines. Theor Appl Genet 112:634–647

Pillen K, Zacharias A, Léon J (2003) Advanced backcross QTL analysis in barley (*Hordeum vulgare* L.). Theor Appl Genet 107:340–352

Pillen K, Zacharias A, Léon J (2004) Comparative AB-QTL analysis in barley using a single exotic donor of *Hordeum vulgare* ssp. spontaneum. Theor Appl Genet 108:1591–1601

Rostoks N, Ramsay L, MacKenzie K, Cardle L, Bhat PR, Roose ML, Svensson JT, Stein N, Varshney RK, Marshall DF, Graner A,

Close TJ, Waugh R (2006) Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. Proc Natl Acad Sci 103:18656–18661

SAS Institute (2006) The SAS Enterprise guide for Windows, release 41. SAS Institute, Cary

Schauer N, Semel Y, Roessner U, Gur A, Balbo I, Carrari F, Pleban T, Perez-Melis A, Bruedigam C, Kopka J, Willmitzer L, Zamir D, Fernie AR (2006) Comprehensive metabolic profiling and phenotyping of interspecific introgression lines for tomato improvement. Nature Biotech 24:447–454

Schmalenbach I, Körber N, Pillen K (2008) Selecting a set of wild barley introgression lines and verification of QTL effects for resistance to powdery mildew and leaf rust. Theor Appl Genet 117:1093–1106

Semel Y, Nissenbaum J, Menda N, Zinder M, Krieger U, Issman N, Pleban T, Lippman Z, Gur A, Zamir D (2006) Overdominant quantitative trait loci for yield and fitness in tomato. Proc Natl Acad Sci USA 103:12981–12986

Siangliw L, Jongdee B, Pantuwan G, Toojinda T (2007) Developing KDML105 backcross introgression lines using marker-assisted selection for QTLs associated with drought tolerance in rice. Sci Asia 33:207–214

Szalma SJ, Hostert BM, LeDeaux JR, Stuber CW, Holland JB (2007) QTL mapping with near-isogenic lines in maize. Theor Appl Genet 114:1211–1228

Talame V, Sanguineti MC, Chiapparino E, Bahri H, Ben Salem M, Forster BP, Ellis RP, Rhouma S, Zoumarou W, Waugh R, Tuberosa R (2004) Identification of *Hordeum spontaneum* QTL alleles improving field performance of barley grown under rainfed conditions. Ann Appl Bot 144:309–319

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, McCouch SR (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277:1063–1066

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

Thomson MJ, Tai TH, McClung AM, Lai XH, Hinga ME, Lobos KB, Xu Y, Martinez CP, McCouch SR (2003) Mapping quantitative trait loci for yield, yield components and morphological traits in an advanced backcross population between *Oryza rufipogon* and the *Oryza sativa* cultivar Jefferson. Theor Appl Genet 107:479–493

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, Maroof MAS, 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

Turner A, Beales J, Faure S, Dunford RP, Laurie DA (2005) The pseudo-response regulator *Ppd-H1* provides adaptation to photoperiod in barley. Science 310:1031–1034

von Korff M, Wang H, Léon J, Pillen K (2004) Development of candidate introgression lines using an exotic barley accession (*Hordeum vulgare* ssp. *spontaneum*) as donor. Theor Appl Genet 109:1736–1745

von Korff M, Wang H, Léon J, Pillen K (2005) AB-QTL analysis in spring barley. I. Detection of resistance genes against powdery mildew, leaf rust and scald introgressed from wild barley. Theor Appl Genet 111:583–590

von Korff M, Wang H, Léon J, Pillen K (2006) AB-QTL analysis in spring barley: II. Detection of favourable exotic alleles for agro-

nomic traits introgressed from wild barley (*H. vulgare* ssp. *spontaneum*). Theor Appl Genet 112:1221–1231

von Korff M, Wang H, Léon J, Pillen K (2008) AB-QTL analysis in spring barley: III. Identification of exotic alleles for the improvement of malting quality in spring barley (*H. vulgare* ssp. *spontaneum*). Mol Breed 21:81–93

Wenzl P, Li HB, Carling J, Zhou MX, Raman H, Paul E, Hearnden P, Maier C, Xia L, Caig V, Ovesna J, Cakir M, Poulsen D, Wang JP, Raman R, Smith KP, Muehlbauer GJ, Chalmers KJ, Kleinhofs A, Huttner E, Kilian A (2006) A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics 7, article no. 206

Xiao JH, Grandillo S, Ahn SN, McCouch SR, Tanksley SD, Li JM, Yuan LP (1996) Genes from wild rice improve yield. Nature 384:223–224

Yan LL, Loukoianov A, Blechl A, Tranquilli G, Ramakrishna W, SanMiguel P, Bennetzen JL, Echenique V, Dubcovsky J (2004) The wheat VRN2 gene is a flowering repressor down-regulated by vernalization. Science 303:1640–1644

Yan L, Fu D, Li C, Blechl A, Tranquilli G, Bonafede M, Sanchez A, Valarik M, Yasuda S, Dubcovsky J (2006) The wheat and barley vernalization gene VRN3 is an orthologue of FT. Proc Natl Acad Sci USA 103:19581–19586

Yun SJ, Gyenis L, Bossolini E, Hayes PM, Matus I, Smith KP, Steffenson BJ, Tuberosa R, Muehlbauer GJ (2006) Validation of quantitative trait loci for multiple disease resistance in barley using advanced backcross lines developed with a wild barley. Crop Sci 46:1179–1186

Zamir D (2001) Improving plant breeding with exotic genetic libraries. Nature Rev Genet 2:983–989

Zheng TQ, Xu JL, Li ZK, Zhai HQ, Wan JM (2007) Genomic regions associated with milling quality and grain shape identified in a set of random introgression lines of rice (*Oryza sativa* L.). Plant Breed 126:158–163