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ORIGINAL PAPER

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Analysis of QTLs for yield, yield components, and malting quality in a BC_3-DH population of spring barley

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Abstract Advanced backcross (AB)-quantitative trait locus (QTL) analysis has been successfully applied for detecting and transferring QTLs from unadapted germplasm into elite breeding lines in various plant species. Here, we describe the application of a modified AB breeding scheme to spring barley. A BC_3-doubled haploid (DH) population consisting of 181 lines derived from the German spring barley cultivar 'Brenda' (*Hordeum vulgare* subsp. *vulgare*) as the recurrent parent and the wild species line 'HS213' (*H. vulgare* subsp. *spontaneum*) as the donor line was evaluated for yield and its components as well as malting quality traits. A set of 60 microsatellite markers was used to genotype the population, and phenotypic data were collected at two locations in Germany in continuous years. Altogether, 25 significant QTLs were detected by single-marker regression analysis and interval mapping. Most positive QTLs originated from the recurrent parent 'Brenda'. A QTL, *Qhd2.1*, on chromosome 2HS from 'Brenda' explained 18.3% and 20.7% of the phenotypic variation for yield and heading date, respectively. Due to the small percentage of donor-parent genome of 6.25%, the BC_3-DH lines could be directly used for the extraction of near-isogenic lines (NILs) for *Qhd2.1*. Consequently, it was possible to determine the precise location of the locus *hd2.1* within a region of 6.5 cM, using an F_2 population consisting of 234 individuals developed from a cross between an NIL containing a defined donor segment at this locus and 'Brenda'. The location of this QTL was consistent with the presence of a major photoperiod response gene, *Ppd-H1*, previously reported in this region, which is associated with pleiotropic effects on yield components. In summary, the analysis of a BC_3-DH population in barley provides a compromise between the analysis of QTLs by means of an AB scheme and the generation of defined substitution lines. Several lines carrying defined different donor segments for only one single chromosome or trait in the genetic background of 'Brenda' could be selected for further genetic studies.

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Introduction

Many agronomically and economically important traits are controlled by quantitative trait loci (QTLs). QTL analysis is a useful approach to discover and dissect complex traits and to identify favorable alleles in diverse germplasm (Paterson et al. 1988). While QTL analysis was performed in segregating populations with varying success, the extraction of identified favorable QTLs into isogenic lines for further breeding was in many cases impossible. Furthermore, due to the narrow genetic basis of elite germplasm in modern plant breeding, efficient use of the genetic variation from unadapted germplasm or wild relatives of modern cultivars is essential to the continued improvement of plant varieties (Tanksley and McCouch 1997). The advanced backcross (AB)-QTL mapping strategy (Tanksley and Nelson 1996) was introduced and utilized as an alternative in order to evaluate mapped donor introgressions in the genetic background of an elite recurrent parent. By this approach, favorable alleles and potentially valuable QTLs derived from either wild or unadapted sources of germplasm could be tagged with molecular markers and associated with the

performance of segregating offspring. In parallel, these QTL alleles were transferred into near-isogenic lines (NILs) by means of marker-associated selection breeding. Since the first report in tomato (Tanksley et al. 1996), AB-QTL analysis has been successfully applied in many plant species (Bernacchi et al. 1998; Xiao et al. 1998; Yamamoto et al. 1998; Moncada et al. 2001) and finally led to the cloning of the first plant QTLs for various traits (Fridman et al. 2000; Frary et al. 2000; Yano et al. 2000; Takahashi et al. 2001). A variation of the backcross method (Wehrhan and Allard 1965) uses genetic map information to generate lines with individual introgressions of desired chromosomal regions for the entire genome (Eshed and Zamir 1995), followed by a thorough evaluation of the resulting introgression lines for agronomic traits. The advantage of this QTL analysis method is that identified QTLs can be directly used in plant breeding or for fine mapping and cloning of the corresponding QTL. The disadvantage of this procedure is that the generation of selected introgression lines requires considerable efforts and many backcrosses in combination with marker-assisted selection in order to generate such lines.

QTL analysis has been performed in barley after the development of molecular markers and linkage maps (Kleinhofs et al. 1993; Backes et al. 1995; Thomas et al. 1995; Bezant et al. 1997; Kicherer et al. 2000; Marquez-Cedillo et al. 2000; Marquez-Cedillo et al 2001; Teulat et al. 2001; Ayoub et al. 2002; Baum et al. 2003). Recently, the first AB-QTL studies for wheat and barley have been published by Huang et al. (2003) and Pillen et al. (2003, 2004), and the first set of introgression lines has been published for barley as well (Matus et al. 2003). In the current study, we have developed a population that combined many features of an AB population with the benefits of chromosomal substitution lines by the reduction of the proportion of donor genome and the simultaneous development of homozygous lines. Thus, a set of 181 BC_3-doubled haploid (DH) backcross lines derived from the German spring barley cultivar 'Brenda' as the recurrent parent and the wild species line 'HS213' (*Hordeum vulgare* subsp. *spontaneum*) as the donor line was developed, genotyped with microsatellite markers, and evaluated for phenotypic characters in the field. The objective of this study was to identify and map the *H. vulgare* subsp. *spontaneum* chromosome segments contributing to important agronomical characters, grain yield, and malting quality.

Materials and methods

Population development and field trials

One hundred eighty-one DH lines were produced from the BC_3 generation of the cross 'Brenda' (recurrent parent) × 'HS213' (donor parent). *H. vulgare* subsp. *spontaneum* 'HS213' was received from Bundesforschungsanstalt für Züchtungsforschung (Aschersleben, Germany) and registered as "Sp.213" in the genebank of the IPK and used in two previous studies (Ramsay et al. 2000; Li et al. 2003). It is available under the Gatersleben accession number HOR12530. 'HS213' was crossed as a male parent to 'Brenda'. F_1 plants were grown in greenhouse, and the seven F_1 plants were backcrossed to 'Brenda' (as the female). One hundred one BC_1F_1 plants were obtained and backcrossed a second time to the 'Brenda' (as the male) to produce BC_2F_1 seeds. A total of 68 BC_2F_1 plants were selected randomly for a third backcross to 'Brenda' to generate BC_3F_1 seeds. Two hundred seventy BC_3-DH plants were gained from 39 BC_3F_1 lines by anther culture performed by the Saaten-Union Resistenzlabor in 1999. Based on their performances in the field, 181 DH lines were selected for evaluation of agronomic traits.

The progeny and parents were planted in Gatersleben in the springs of 2000 and 2001, with four rows and 15 plants in each row. This population was also grown in plots (5.9×1.25 m) at Hadmersleben in 2001 (81 lines) and 2002 (110 lines). Each BC_3-DH line and the parents were evaluated for seven important quantitative traits, including grain yield (Qyld) and its components like heading date (Qhd), plant height (Qph), lodging (Qlg), grain number per spike (Qgps), spikelet number per spike (Qsps), and thousand-grain weight (Qtgw). Seed samples of the plots harvested in Hadmersleben were micromalted and used to measure three malting quality traits, which were percentage of protein content (Qpc), percentage of malt extract (Qme), and friability (Qfr). The micromalting analysis was performed at the Versuchs- und Lehranstalt für Brauerei (VLB) in Berlin.

The NIL G98/65-3/1, containing an introgressed fragment of 'HS213' on chromosome 2HS, was selected and crossed to 'Brenda'. A total of 234 F_2 plants and the two parents were planted in the greenhouse to investigate the heading date and flowering time. Phenotypic data combined with genotypic data based on four molecular markers located on chromosome 2HS were used for mapping this heading date QTL as a single Mendelian gene.

Genotyping and linkage analysis

Approximately 400 microsatellite markers from various sources (Liu et al. 1996; Ramsay et al. 2000; Li et al. 2003) were used to analyze the parents of the population. Polymorphic markers were selected for genotyping all BC_3-DH plants. The order and distance of the markers was based on the molecular consensus map of barley in the two mapping populations 'Steptoe' × 'Morex' and 'Igri' × 'Franka' (Li et al. 2003), using Mapmaker, version 3.0b (Lander et al. 1987), and CARTHAGENE (Schiex and Gaspin 1997). Microsatellite marker analysis was carried out as previously described (Li et al. 2003).

Statistics and QTL-analysis

Correlations among traits were analyzed with the Qgene, version 3.0, program (Nelson 1997). Single-point regression was used to determine the effect of each molecular maker on each trait. Interval mapping was preformed to identify the location of each QTL. According to Fulton et al. (1997, 2000) and Tanksley et al. (1996), regions of the genome were identified as putatively containing a QTL if the results met one or more of the following criteria: (1) a significant effect was observed for that single marker/trait combination with $P < 0.001$ in one investigation; (2) significant effects in the same direction were observed for a single marker/trait combination with $P < 0.01$ in two or more investigations; and/or (3) the significant effects in the same direction were observed for a single maker/trait combination with $P < 0.1$ in three or more investigations. The percentage of phenotypic change (%A) of each significant QTL at a given maker locus, was calculated as 100(BB-AA)/AA, where AA is the phenotypic mean for individuals homozygous for the recurrent variety 'Brenda' alleles at specified markers, and BB is the phenotypic mean for individuals homozygous for *H. vulgare* subsp. *spontaneum* (Fulton et al. 2000). The percentage of phenotypic variance (%PV) associated with each significant QTL was calculated from the regression of each maker/phenotype combination.

While originally the QTL analysis was performed with all available plants for each location/year, it turned out that the strong effect of the QTLs located in the region of GBMS229 and GBMS2 on chromosome 2H led to the detection of false-positive QTLs in other chromosomal regions. This effect was caused by few plants that had introgressions in the respective region on 2H and also on other chromosomal regions. For these plants, the extremely strong effects for chromosome 2H artificially biased the effects calculated for the other chromosomal regions with introgressions. Therefore, after determining the QTLs for the region on chromosome 2HS, plants with introgressions at marker loci GBMS229 and GBMS2 were omitted from the further analysis (one to six plants, depending on location/year). Furthermore, it turned out that one line G98/6-6/11 was tetraploid; this line was also excluded from all QTL analyses.

Results

Microsatellite polymorphism and marker segregation

Approximately 400 microsatellite makers were used to survey polymorphism between the recurrent parent 'Brenda' and the donor parent 'HS213', and 15% of the markers detected polymorphism. This degree of polymorphism is slightly lower than the 33–38% reported by Pillen et al. (2003, 2004). Three chromosomes, 1H, 3H, and 6H, were extensively monomorphic. The lack of polymorphic microsatellites resulted in large gaps on the long arm of chromosomes 2H and 5H, respectively (Fig. 1). The observed average allele frequency for 'HS213' alleles was 4.07%, close to the expected value for a BC_3-DH population of 6.25%. Of the 60 markers surveyed, 11 (18.3%) deviated significantly ($P < 0.05$) from the expected 93.75:6.25 allele frequency. Two markers showed no transmission of donor alleles for chromosome 2H and 4H, indicating the loss of the respective donor segment during the backcrossing. Eight loci were skewed toward 'Brenda', whereas three were skewed toward 'HS213'. Skewing toward the adapted, elite parent can be explained by the phenotypic selection imposed in the BC_1 and BC_2 generations during population development. Of the 181 lines scored, 110 (61%) were characterized by the presence of the overlapping introgressions from *H. vulgare* subsp. *spontaneum*, while the remaining lines did not show detectable introgressions, indicating either conversion to the recurrent parent or introgressions at chromosomal regions that were not covered by the microsatellite markers. A total of 34 plants containing a single-donor segment were found, which can be regarded as defined introgression lines. A minimum of 42 selected lines containing one or a few introgressions provided a complete coverage of the genome with introgressed donor fragments.

Correlations among traits

For grain yield and malting traits measured in Hadmersleben, correlation coefficients among traits were analyzed separately for each year. As summarized in Table 1, grain yield revealed significant positive correlations with spikelet number and grain number per spike, respectively. A significant negative relationship between grain yield and thousand-grain weight was found in one year, while a decreased protein content was associated with increased grain yield in both years. The two strongest correlations were observed between spikelet number and grain number per spike in both years ($r = 0.873$ and $r = 0.922$, $P < 0.001$). A late heading date was positively associated with grain yield, spikelet number, and grain number per ear, but had a negative correlation with plant height and protein content. For the malting traits, protein content showed a negative correlation with friability in 2001, but a strongly positive correlation in 2002.

QTLs detection

Grain yield

Total grain yield was investigated in the field plots at Hadmersleben in 2 years. Grain yield was affected by three significant QTLs, which explained from 4.84% to 18.24% of the phenotypic variance. In all these cases, the 'Brenda' alleles increased total grain yield. For

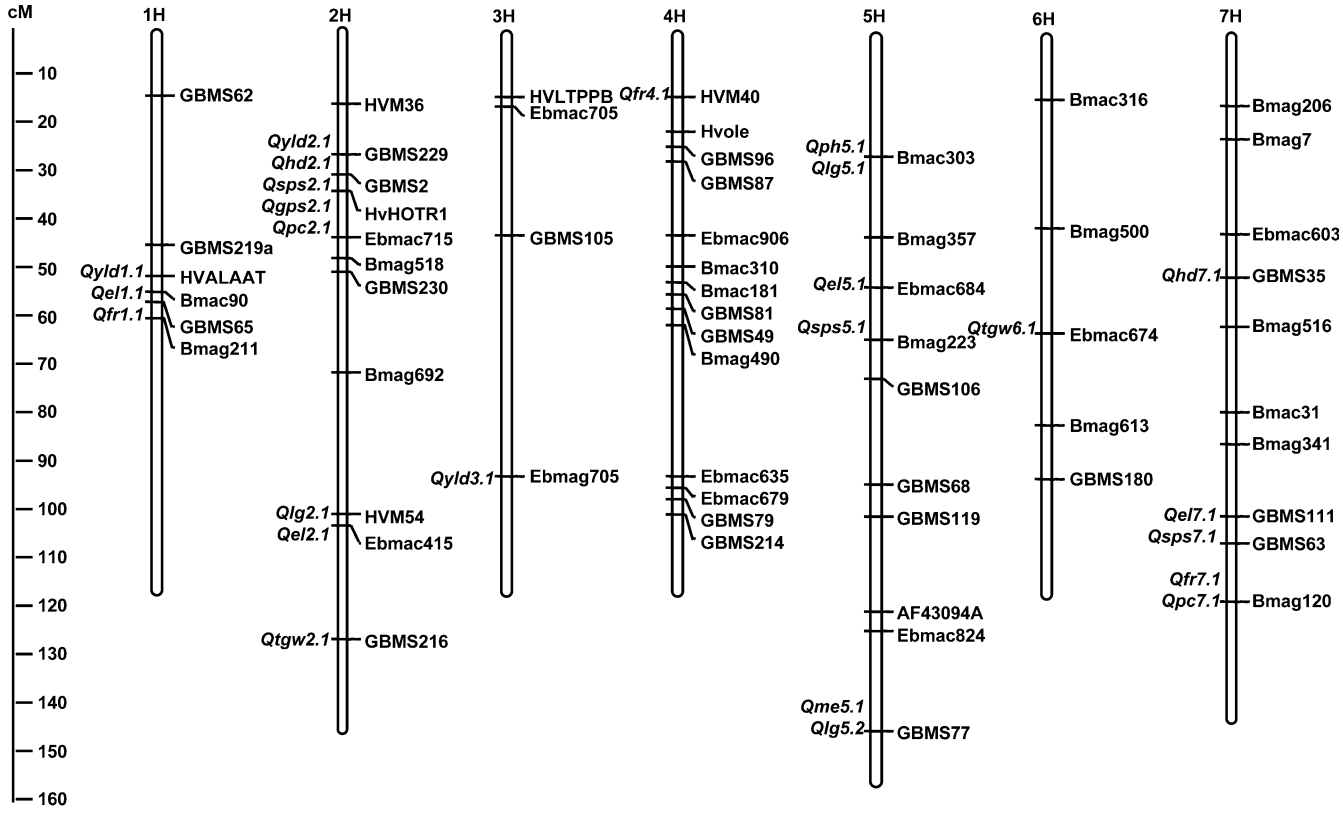

Fig. 1 Linkage map of microsatellite markers used for BC_3 doubled haploid quantitative trait locus (QTL) analysis. The order of markers and the distances in centiMorgans [(*cM*) Kosambi mapping units] are based on the barley molecular consensus map (Li et al. 2003; Ramsay et al. 2000; Liu et al. 1996). The centiMorgan scale is given on the *left*. Locus names were indicated on the *right* side of the chromosomes. QTLs are indicated to the *left* of the chromosomes

Table 1 Correlation matrix of the traits analyzed in Hadmersleben

Trait	Yield	Heading date	Plant height	Ear length	Spikelet no.	Grain no.	Thousand-grain weight	Lodging	Friability	Protein content
Heading date	0.136									
	0.478***									
Plant height	0.138	−0.547***								
	−0.218*	−0.243*								
Ear length	–	–	–							
	0.051	0.269**	0.138							
Spikelet no.	0.354***	0.500***	0.021	–						
per spike	0.162	0.468***	0.081	0.649***						
Grain no.	0.339**	0.518***	0.049	–	0.922***					
per spike	0.342***	0.573***	−0.060	0.501***	0.873***					
Thousand-grain	−0.416***	−0.261*	0.106	–	−0.242*	−0.247*				
weight	0.084	−0.171	0.120	0.096	−0.037	−0.004				
Lodging	−0.242*	−0.643***	0.762***	–	−0.285*	−0.280*	0.292**			
	0.124	0.351***	−0.053	0.013	0.196*	0.251**	0.187			
Friability	0.163	0.308**	−0.385***	–	0.165	0.188	0.031	−0.381***		
	−0.478***	−0.295**	0.280	−0.062	−0.231*	−0.314***	0.106	−0.065		
Protein content	−0.460***	−0.134	0.034	–	−0.219	−0.235*	0.208	0.214	−0.561***	
	−0.516***	−0.410***	0.44***	−0.133	−0.299**	−0.377***	−0.088	−0.294**	0.683***	
Malt extract	0.003	−0.035	0.083	–	−0.111	−0.030	−0.009	0.069	0.215	−0.482***
	0.437***	0.283***	−0.417***	0.139	0.209*	0.242*	0.174	0.082	−0.564***	−0.804***

The *upper value* of each column is the correlation coefficient gained in 2001 and the *lower* is gained in 2002
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

Qyld2.1 and *Qyld3.1* on chromosome 2H and 3H, significant effects on grain yield were observed in continuous years. No alleles for grain yield increase from the wild parent 'HS213' were found.

Heading date

Two putative QTLs were detected on chromosomes 2H and 7H. For *Qhd7*.1, the 'HS213' allele increased the number of days to heading. A significant QTL associated with heading date on chromosome 2HS was *Qhd2.1*, which explained 20.7% of the phenotypic variance.

Plant height and lodging

Only one putative QTL on chromosome 5H was detected that significantly affected plant height. Three QTLs associated with lodging on different chromosomes were found in different years. QTL *Qlg5.1* mapped to the same region of chromosome 5H as *Qph5.1*, which was the QTL from 'HS213' that increased plant height.

Ear length

Four QTLs were found for ear length. All QTLs explained approximately 10% of the phenotypic variance. For two of the QTLs, the 'HS213' allele caused a decrease of ear length. For *Qel7.1* on chromosome 7HL, a significant increase in ear length was detected.

Yield components

Three QTLs were found for spikelet number. The QTLs explained 7.5% to 36.8% of the phenotypic variance, with LODs of 1.24–8.08. The two QTLs *Qsps2.1* and *Qgps2.1* on chromosome 2HS had the greatest and most consistent effects on spikelet number and grain number per spike.

In three investigations, two putative QTL were detected to be associated with thousand-grain weight. The most consistent *Qtgw2.1* was located on the long arm of chromosome 2H, and the donor parent 'HS213' allele decreased thousand-grain weight by 10.6%. For QTL *Qtgw6.1* on chromosome 6H, a 9.8% decrease of thousand-grain weight was observed for the 'HS213' allele in 1 year.

Malting quality traits

Three malting quality traits were evaluated in 2 years from the harvest of the field plots in Hadmersleben. However, most QTLs were detected only in the second year. Two QTLs for increased protein content for the 'HS213' allele were located on chromosome 2HS and 7HL, accounting for 11.8% and 14.6% of the phenotypic variance, respectively. Only one putative QTL was significantly associated with the percentage of malt extract. The 'HS213' alleles for *Qme5.1* caused a reduction in percentage of malt extract and explained 11% of the phenotypic variance. Three QTLs were detected for seed friability. For two of them, the 'HS213 allele' caused an increase in friability, accounting for 10.7% and 19.2% of the phenotypic variance, respectively. The donor allele mapped to chromosome 7H and caused a 20.3% diminution in the seed friability. Of six QTLs detected associated with malting quality, four alleles from donor parent severely reduced the quality of 'Brenda'.

Mendelian mapping of locus *hd2.1* on chromosome arm 2HS

As shown in Table 2, a strong putative QTL for heading date *Qhd2.1* was detected on chromosome 2HS and linked to molecular marker GBMS229. This QTL was significantly associated with spikelet number per spike, grain number per spike, protein content, and grain yield. To map *Qhd2.1* precisely and to detect its effects, a population of 234 F_2 plants was derived from a cross of 'Brenda' × G98/65-3/1, an NIL, which carried a single-donor segment on chromosome 2HS covering the region of the QTL. The days to heading of line G98/65-3/1 totaled approximately 78 days under long day conditions (in light for 16 h per day) in the greenhouse, 13 to 19 days earlier than the 96 days of 'Brenda', indicating the significance of the QTL. As shown in Fig. 2, the flowering time of the F_2 progeny plants resulted in a bimodal distribution that could be described by a 3:1 segregation ratio (163:52, $\chi^2 = 0.78$, $0.25 < P < 0.50$). Four polymorphic molecular markers were used for the creation of a refined linkage map for *Qhd2.1* as a single Mendelian factor (Fig. 3). The two marker loci GBMS229 and GBMS2 were closely linked to *Qhd2.1*, with distances of 3.9 cM and 2.6 cM, respectively.

Discussion

A total of 25 putative QTLs were detected in the BC_3-DH population of the cross 'Brenda' × 'HS213' (Table 1). While most QTLs were derived from the recurrent parent 'Brenda', positive QTLs from the wild donor genome were found for increased ear length (*Qel1.1* and *Qel7.1*) and increased friability (*Qfr1.1*, *Qfr4.1*, *Qfra5.1*). One QTL from the wild donor resulted in a decrease in the days to flowering; however, early flowering severely affected grain yield, spikelet per spike, and grain number per spike.

The identified QTLs were not evenly distributed on all chromosomes, with the majority of QTLs being concentrated in three main clusters in the centromeric region of chromosome 1H, on the short arm of chromosome 2H, and the long arm of chromosome 7H. For barley chromosome 1H, the presence of the photoperiodic gene *Ppd-H2* was reported (Laurie et al. 1995), which had pleiotropic effects on grain yield and other

Table 2 Putative QTLs detected by single-marker regression analysis in the BC_3–doubled haploid population 'Brenda' × 'HS213'. Percentage of phenotypic change (*%A*) = 100(BB-AA)/AA, where AA is the phenotypic mean for individuals homozygous for 'Brenda' alleles at specified markers, and BB is the phenotypic mean for homozygotes for the 'HS213' allele. +/− indicates an increasing or decreasing effect from the 'HS213' allele. *G* Gatersleben, *H* Hadmersleben, *%PV* phenotypic variance estimated from marker regression against phenotype. *Underlined P*-values indicate locations for which %A and %PV were calculated

Trait	QTL	Marker	Source	2000 G	2001 G	2001 H	2002 H	LOD	%A	%PV
Yield	*Qyld1.1*[a]	Bmac90	'Brenda'	ND	ND	NS	**	1.64	−18.91	7.06
	Qyld2.1	GBMS229	'Brenda'	ND	ND	****	**	3.76	−48.23	18.24
	Qyld3.1[a]	Ebmag705	'Brenda'	ND	ND	*	*	1.07	−9.04	4.84
Heading date	*Qhd2.1*	GBMS229	'Brenda'	***	*	****	****	5.43	−6.07	20.68
	Qhd7.1[a]	GBMS35	HS213	NS	NS	NS	***	2.54	3.72	10.75
Plant height	*Qph5.1*[a]	Bmac303	HS213	NS	NS	***	NS	3.17	23.38	16.86
Lodging	*Qlg2.1*	HVM54	HS213	ND	ND	NS	****	4.01	82.77	16.12
	Qlg5.1[a]	Bmac303	HS213	ND	ND	***	NS	3.42	212.50	18.09
	Qlg5.2[a]	GBMS77	HS213	ND	ND	NS	***	2.79	109.88	11.63
Ear length	*Qel1.1*[a]	Bmac90	HS213	NS	***	ND	NS	2.59	24.66	7.05
	Qel2.1[a]	HVM54	'Brenda'	**	*	ND	**	2.23	−21.74	9.31
	Qel5.1[a]	Ebmac684	'Brenda'	**	NS	ND	**	1.95	−17.68	8.86
	Qel7.1[a]	GBMS111	HS213	***	NS	ND	NS	2.81	13.70	11.38
Spikelet no. per spike	*Qsps2.1*	GBMS229	'Brenda'	***	*	****	***	8.08	−39.59	36.83
	Qsps5.1[a]	Bmag223	'Brenda'	*	NS	*	*	1.24	−19.39	7.46
	Qsps7.1[a]	GBMS63	HS213	NS	***	NS	NS	2.75	15.75	7.66
Grain no. per spike	*Qgps2.1*	GBMS229	'Brenda'	*	NS	****	**	8.77	−41.10	39.25
Thousand-grain weight	*Qtgw2.1*	GBMS216	'Brenda'	**	ND	**	*	2.20	−10.64	8.80
	Qtgw6.1[a]	Ebmac674	'Brenda'	ND	ND	NS	***	3.55	−9.79	14.67
Protein content	*Qpc2.1*	GBMS229	HS213	ND	ND	**	**	2.19	14.72	11.82
	Qpc7.1[a]	Bmag120	HS213	ND	ND	***	NS	2.50	12.02	14.60
Malt extract	*Qme5.1*[a]	GBMS77	'Brenda'	ND	ND	NS	***	2.63	−2.65	11.00
Friability	*Qfr1.1*[a]	Bmac90	HS213	ND	ND	NS	****	4.76	40.56	19.17
	Qfr4.1[a]	HVM40	HS213	ND	ND	NS	***	2.52	17.14	10.67
	Qfr7.1[a]	Bmag120	'Brenda'	ND	ND	***	NS	3.64	−19.20	20.27

ND No data, *NS* not significant
*$P < 0.1$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$
[a] QTLs were detected after the elimination of six lines with introgressions on chromosome 2HS, which had strong effects on many traits and led to artificial QTLs

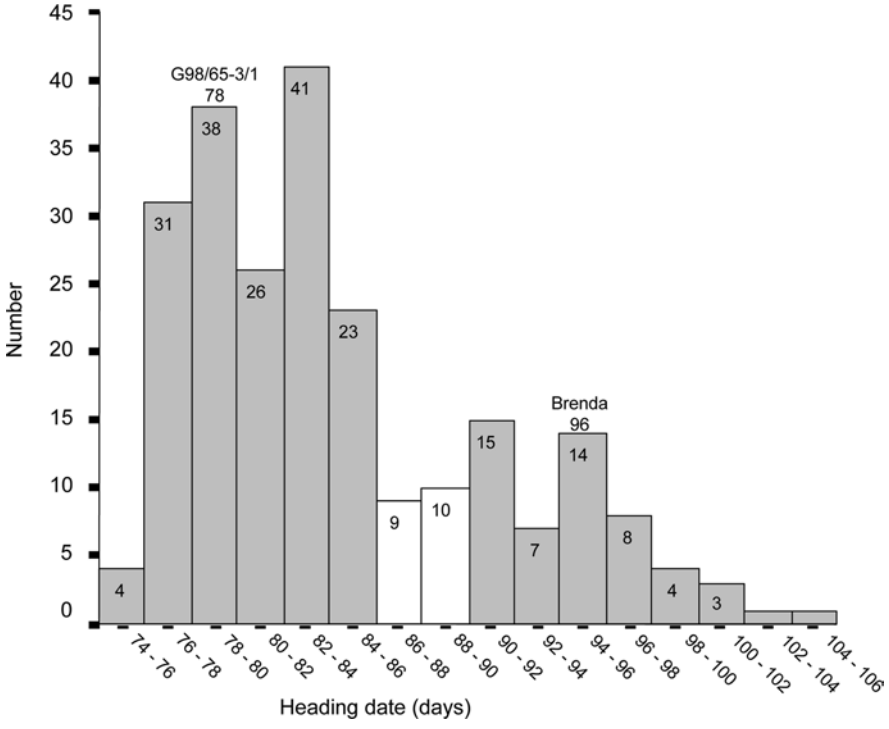

Fig. 2 Phenotypic distribution of F_2-progeny plants of the cross 'Brenda' × G98/65-3/1, investigated in the greenhouse for heading date

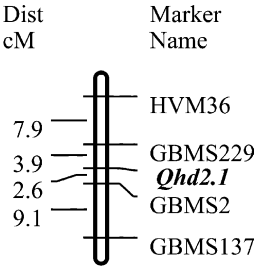

Fig. 3 Linkage map of a chromosomal region of 2HS based on 234 F_2-progeny plants of the cross 'Brenda' × G98/65-3/1. The locus *Qhd2.1* was mapped as a single Mendelian locus, based on segregation in flowering time

traits. Based on the current mapping information for this gene, it is in a more distal position on the long arm of chromosome 1H than the cluster identified here. Therefore, it is possible that the observed cluster of QTLs could be caused by a pleiotropic gene that has not been described and designated so far. Effects on heading date, plant height, and lodging at harvest for a similar position on chromosome 1H were also described by Pillen et al. (2003) in an AB population of spring barley.

Since big gaps existed on chromosome 3H, and no QTLs were detected for plant height in this region, it is difficult to decide whether the grain yield QTL *Qyld3.1* is caused by pleiotropic effects of the *denso* gene. This semi-dwarfing gene was mapped to a comparable position on chromosome 3H (Barua et al. 1993) and was associated with pleiotropic effects on heading date, thousand-grain weight, and grain yield (Thomas et al. 1991).

For *Qhd2.1*, the mapping position on 2HS was very similar to the photoperiodism gene *Ppd-H1* (Laurie et al. 1994), indicating that this QTL is controlled by this gene. From the BC_3-DH lines, it was possible to directly extract an NIL that could be used to fine map this gene. The analysis of an F_2 population derived from the cross between the introgression line carrying this segment from 'HS213' and 'Brenda' proofed the presence of a single gene affecting flowering time on chromosome 2HS. It is highly likely that this gene is identical to the photoperiodism gene *Ppd-H1*, which decreases the days to flowering under long day conditions (Laurie et al. 1994). The most likely location of *Ppd-H1* in that publication was in a 6-cM interval between RFLP loci MWG858 and PsrB9, 1 cM proximal to MWG858. In the linkage map of 'Stepote' × 'Morex', the RFLP marker MWG858 was located in a 5.1-cM interval between GBMS229 and GBMS2 (Li et al. 2003), where also *Qhd2.1* was mapped, suggesting the identity of the QTL and *Ppd-H1*. A second locus affecting flowering time, *eps2H*, was described in a more proximal position close to marker Psr571 on chromosome 2HS (Laurie et al. 1994, 1995). Effects on agronomic characters such as plant height, tiller biomass, tiller grain weight, ear grain number, and harvest index were attributed to pleiotropic effects of both flowering time genes on 2HS (Laurie et al. 1994). Therefore, it is possible that beside the *Ppd-H1* gene, the *eps2H* gene might be in part responsible for the big cluster of QTLs for various traits observed in our population on 2HS.

In summary, the results of this study show that a BC_3-DH population can be used for the detection of QTLs associated with agronomically important traits in a cross between an elite barley variety and *H. vulgare* subsp. *spontaneum*. In comparison to a typical BC_2 AB population as it has been described by Pillen et al. (2003, 2004), the number of valuable QTLs that could be extracted from the wild donor parent was, however, relatively low, and no QTLs could be identified that significantly increased grain yield in barley. This result is consistent with the analysis of recombinant chromosome substitution lines recently published by Matus et al. (2003).

This study has demonstrated that the analysis of a BC_3-DH population is a highly valuable approach toward the genetic dissection of quantitative traits in barley. The main advantage of the BC_3-DH population is that it represents on the one side a population structure, where a number of lines carry an introgression at a specific locus, producing statistically quite significant results. On the other side, the number of introgressions is so low that in many cases, it is possible to directly extract NILs for a further dissection or fine mapping of the respective QTL locus into single Mendelian factors as demonstrated for *Qhd2.1*. The easy development of an F_2 mapping population from an NIL is an important step toward the map-based cloning of genes underlying quantitative traits.

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