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Molecular marker assisted genetic analysis of head shattering in six-rowed barley

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Abstract Head shattering in barley (*Hordeum vulgare L.*) has two forms; brittle rachis and weak rachis. Brittle rachis is not observed in cultivated barley since all cultivars carry non-brittle alleles at one of the two complementary brittle rachis loci (*Btr1*;*Btr2*). Weak rachis causes head shattering in barley cultivars and may be confused with brittle rachis. Brittle rachis has been mapped to the chromosome 3 (3H) short arm while map position(s) of the weak rachis is unknown. Two major and a putative minor QTL for head shattering were mapped using the Steptoe × Morex doubled haploid line population. The largest QTL, designated Hst-3, located on the chromosome 3 (3H) centromeric region, is associated with a major yield QTL. The Steptoe Hst-3 region, when transferred into Morex, resulted in a substantial decrease in head shattering. High-resolution mapping of Hst-3 was achieved using isogenic lines. Brittle rachis was mapped with molecular markers and shown to be located in a different position from that of Hst-3. The second major QTL, designated Hst-2 S, is located on chromosome 2 S. This locus is associated with an environmentally sensitive yield QTL.

Key words Brittle rachis · Weak rachis · QTL · Spike density · Peduncle curvature

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Introduction

Head shattering causes serious yield losses in barley throughout the world. Two mechanisms of head shattering are known. In the first type, joints in the rachis nodes disarticulate easily, resulting in segmentation of the rachis and loss of seed along with a piece of the rachis (Franckowiak and Konishi 1996). This type of head shattering, termed "brittle rachis", is found in *Hordeum vulgare* ssp. *spontaneum* and is considered an adaptive feature of non-cultivated species for seed dispersal (Takahashi 1955). In the second type, the rachis breaks, resulting in the loss of a spike segment (Kaufman and Shebeski 1954). However, the breaking point of the rachis, node or internode, is not known. Descriptions in literature indicate the possibility of a third kind of shattering where the kernel itself detaches from the rachis (Platt and Wells 1949; Chapman and Hockett 1976).

The genetics of brittle rachis has been extensively studied. Brittleness is due to two complementary genes (Takahashi 1955), now designated *Btr1* and *Btr2* (Franckowiak and Konishi 1996), with brittle dominant to non-brittle. *H. vulgare* ssp. *spontaneum* has brittle rachis as a consequence of possessing the dominant forms of both genes (Takahashi 1964). In a study to determine the distribution of *Btr1;Btr2* genes in cultivated barley, 1267 cultivars were crossed to "tester genotypes" with known genetic composition for the *Btr* genes (Takahashi 1964). Results indicated that every barley cultivar tested had either the *Btr1* or *Btr2* gene in a homozygous recessive form resulting in a non-brittle rachis. Referring to two reports by Takahashi and Hayashi, Franckowiak and Konishi (1996) noted that the two brittle rachis genes were located on the chromosome 3 (3H) short arm and closely linked. There have also been reports of independent segregation of the two brittle rachis loci (Johnson and Aberg 1943; Turcotte 1957), although the close linkage conclusion is now generally accepted.

Brittle rachis has been reported to be stable and easy to distinguish from non-brittle rachis, indicating little environmental effect, but the degree of toughness in the

non-brittle group can be variable (Aberg and Wiebe 1948). Segregation ratios that do not fit the two complementary gene models have been reported, suggesting interactions with additional loci (Smith 1951).

Head shattering as defined by rachis breaking has not been as well described as brittle rachis. Kaufman and Shebeski (1954), working with crosses between "weak" and "tough" rachis cultivars, observed that the F_1 plants had a weak rachis that shattered. In the F_2 population, plants with tough, weak, and intermediate rachis were observed. Because of the difficulty of differentiating weak rachis from intermediate ones, these researchers combined both classes and obtained a 3 (weak): 1 (tough) rachis segregation ratio. These data suggested that the weak rachis may be mediated by a major dominant gene interacting with minor genes. Kaufman and Shebeski (1954) concluded that their observed weak rachis phenotype was different from the brittle rachis phenotype. Platt and Wells (1949) and Clarke (1981) did not clearly distinguish rachis breaking from brittle rachis. Thus, the weak rachis phenotype has not been adequately differentiated from brittle rachis to date.

Platt and Wells (1949) reported that head shattering was promoted by irrigation. Planting time was another factor affecting head shattering where earlier planting resulted in more head shattering losses (Rutger et al. 1966). Reduced head shattering was observed in lodged plants (Kaufman and Shebeski 1954). Turcotte (1957) cited Ubisch's findings that "dense spikes have less tendency to be brittle". Kaufman and Shebeski (1954) speculated that varieties with curved (nodded) heads would have less head shattering because of less weathering damage on the rachis due to rain. Platt and Wells (1949) reported that heads with larger seeds would shatter more than those with smaller seeds. These reports indicate that head shattering due to rachis breaking is both influenced by the environment and associated with some morphological traits.

Using BC_1 derived homozygous Steptoe × Morex lines, Larson et al. (1996) mapped two head shattering quantitative trait loci (QTLs) to barley chromosome 3 (3H) using DNA markers ABG396 and ABG057 to identify them. These markers are separated by about 44 cM (Kleinhofs et al. 1993). The two QTLs explained 13.9% and 13.7% of the variation, respectively. This work also showed an association between head density and head shattering with dense heads shattering less.

In another study, using an Igri × Danillo cross, three head shattering QTLs were mapped to the intervals of MWG557-MWG769 (50 cM), MWG611-MWG921 (2 cM), and MWG820C-MWG820B (13 cM) on chromosomes 2 (2H), 4 (4H), and 6 (6H), respectively. These QTLs explained 44% of the genetic variance. Ear breaking, lodging, stalk breaking, and "physical state of the plants before harvest" traits had QTLs at the same regions. It was suggested that the stability of the stem tissue was the determining factor for all of the above traits (Backes et al. 1995).

Genetic control of rachis breakage is complex, possibly due to multiple-locus inheritance and to environmental effects. Availability of a genetic map with satisfactory genome coverage facilitates simultaneous detection of multiple loci affecting complex traits. Tagging of those loci with simply inherited molecular markers is useful in developing isogenic lines, which have uniform genetic backgrounds and segregate only for the QTL of interest. The analysis of complex traits is easier in uniform genetic backgrounds such as near isogenic lines (NILs) since the interactions with modifying loci are standardized (Tanksley 1993). The development of a series of NILs where each NIL has a fragment from the target region also facilitates high-resolution mapping of the QTL. In the study rported here,, we analyzed head shattering and related traits via both QTL mapping and NIL analysis. A high-resolution map of a major head shattering QTL on barley chromosome 3 (3H) was developed and shown to be different from the brittle rachis loci.

Materials and methods

Head shattering was studied in a doubled haploid population derived from a Steptoe × Morex (SM) cross. Both Steptoe and Morex are six-rowed, spring-type cultivars. Morex heads shatter in some environments, resulting in serious yield losses (Nedel *et al.* 1993); Steptoe heads generally do not shatter. Steptoe and Morex are the parents of a 150 doubled haploid line (DHL) mapping population of the North America Barley Genome Mapping Project (http://www.css.orst.edu/barley/nabgmp.htm) which has been extensively mapped with restriction fragment length polymorphism (RFLP) markers (Kleinhofs *et al.* 1993; http://barleygenomics. wsu.edu).

QTL mapping was performed with the 150 SM DHLs grown in 3.5-m-long single rows in Pullman, Washington, in 1996 and 1997. The 1997 trial was arranged in randomized complete blocks with two replicates; the 1996 trial was not replicated. Based on the map position and completeness of marker data, 149 markers were selected to obtain a skeleton map consisting of markers spaced approximately 6–8 cM apart. Marker distances were confirmed using MAPMAKER EXP (Lander et al. 1987). QTL analysis was conducted using MQTL software that can handle data repeated over environments (Tinker and Mather 1995a, b) with the 1996 and 1997 trials constituting the two environments, respectively. Linear regression based simple interval mapping (SIM, Haley and Knott 1992) of MQTL was used first. Test statistics for QTL main effects and QTL x environment effects were calculated. Threshold values for test statistics to check type-1 error rate were calculated for both QTL main effects and QTL × environment interaction effects using permutations replicated 5000 times for the 5% significance level and 10,000 times for the 1% significance level, respectively (Churchill and Doerge 1994). Using the results of simple interval mapping, we then applied simplified composite interval mapping (sCIM) based on partial regression analysis (Zeng 1993, 1994). For sCIM, markers closest to the peaks of QTLs detected by simple interval mapping were used as background markers for each trait. This approach was advocated by QTL CARTOGRAPHER software (Basten et al. 1997), which employs linear regression based interval mapping just like MQTL. Threshold values were not calculated for sCIM since the calculation of threshold values using permutations was not advised using multiple environment data (Tinker and Mather 1995b). A strong test statistic value was accepted as an indication of a QTL. The term "secondary QTL" is used for the QTL detected only by sCIM. Additive effects calculated by MQTL are for two alleles at each locus and, therefore, are twice that obtained with other QTL software. Multiple regression analysis was used to calculate the interaction between QTLs based on principles explained by Allen (1997) using SAS software (SAS Institute 1991). The *F*-test was used to detect significance level of QTL × QTL interaction.

In order to precisely map the major head shattering QTL, we developed a series of NILs based on preliminary information

Table 1 Summary of QTL effects for head shattering and related traits (*TS* test statistics, *ME* main effect)

Marker interval	Chr.	Interval size (cM)	SIM				sCIM		
			Additive effect[a]	ME TS	Percentage variation	Q × E effect TS	Additive effect	ME TS	Q × E effect TS
Head shattering									
(MWG858)	2	–	–1.0	48.5**	14.9	4.4	–0.9	56.9	5.9
(Dfr)	3	–	–1.4	101.2**	28.6	1.9	–1.3	111.3	1.9
CDO057B-mSrh	7	5.4	–0.4	6.3	2.1	0.4	–0.5	17.0	0.7
CDO504	7	–	–0.3	3.3	1.1	0.0	–0.4	10.1	0.1
Spike density									
ABC158	1	–	0.1	9.7	3.2	0.0	0.1	22.7	0.0
RZ242-ABC310B	1	9.2	–0.1	5.8	1.9	0.1	–0.1	22.5	0.4
ABC167B-bBE54D	2	6.0	0.0	0.5	0.0	0.7	–0.1	23.7	2.1
(Dfr)	3	–	0.7	368.9**	70.8	4.3	0.7	368.9	4.3
BCD265B-ABG003A	4	3.8	–0.1	6.8	2.2	0.4	–0.1	22.7	1.4
Number of rachis nodes									
(MWG858)	2	–	–2.1	91.7**	26.3	1.3	–2.2	108.9	1.7
(Dfr)	3	–	1.0	19.5*	6.3	1.0	1.2	36.2	1.7
(MWG798A)	6	–	–1.1	21.4*	6.9	1.7	–0.8	15.7	1.8
Peduncle curve									
ABC156A- (MWG858)	2	6.0	–0.5	33.4**	10.5	9.5**	–0.5	40.5	12.8
ABG398-Dfr	3	1.3	0.3	14.1	4.6	26.9**	0.3	18.3	43.5
MWG571B-ABG377	3	11.7	0.3	10.4	3.4	20.6**	0.4	23.9	31.6
MWG514- (CDO504)	7	4.4	–0.8	82.9**	24.1	4.1	–0.8	93.0	4.4

*,** $P < 0.05$ and 0.01, respectively

[a] Negative additive effects indicate that the Morex allele has a higher value. Markers in parenthesis were used as background markers for sCIM analysis

about the presence of a major head shattering QTL on barley chromosome 3 (3H) (T. Blake, personal communication). DHL SM23 carries the entire Steptoe chromosome 3 (3H) while DHL SM84 has a small piece from Morex at the distal part of the long arm of chromosome 3 (3H). The SM23 and SM84 lines were backcrossed to Morex four times and then selfed to produce the BC_4F_2 lines. Molecular markers were used to select the Steptoe allele of chromosome 3 (3H) in every generation starting with BC_2F_1. The genetic background of the isogenic lines was checked using markers every 20–25 cM on the SM map in the BC_4F_1 generation. Lines with recombination in the target region were grown to BC_4F_3, and homozygous individuals were selected by genotyping. The result was a series of isogenic cv. Morex lines each with a fragment of Steptoe chromosome 3 (3H) in the region presumed to contain the head shattering QTL. Only RFLP markers were used in this study except for two morphological markers – short rachilla hair and pubescent leaf. DNA isolation, Southern transfer and hybridization methods, and morphological marker phenotyping were as described by Kleinhofs et al. (1993). In order to accelerate generation turnover, we cultured 20-day-old embryos on a growth medium to start a new generation without waiting for seed maturation. The MS basal medium (Murashige and Skoog 1962) supplemented with vitamins and 30 g/l maltose but without hormones was used. Seedlings were directly transferred to pots when they reached a height of 5–10 cm.

Head shattering was observed in isogenic lines grown in single rows under field conditions in Pullman, Washington in 1997. Evaluation of head shattering phenotypes and marker genotypes of each isogenic line were used to obtain a high-resolution map of the largest head shattering QTL.

The barley genetic stock GSHO1937, which carries the brittle rachis trait from *H. vulgare* ssp. *spontaneum* introduced into cv. Bowman through BC_9F_4 generations with selection for brittle rachis (Wolfe and Franckowiak 1990), was used to map the trait. The GSHO1937 line was analyzed with chromosome 3 (3H) RFLP markers to determine the location and size of the introgressed *H. vulgare* ssp. *spontaneum* DNA fragment. The genomic DNA of Bowman was used as a control. Unfortunately, the *H. vulgare* ssp. *spontaneum* parent of GSHO1937 was not available (J.D. Franckowiak, personal communication). The high polymorphism rate between cultivated barley and *H. vulgare* ssp. *spontaneum* (Peterson *et al.* 1994) and the use of multiple markers provided us with a reasonable confidence regarding the results.

Head shattering of DHLs and NILs was observed 2–3 weeks after the crop reached maturity (Platt and Wells 1949). Observations were made based on a visual assessment of the percentage of shattered heads in a plant row. The percentage was converted to a 1–5 scale (1 = 0%, 2 = 1–15%, 3 = 16–30%, 4 = 31–45% 5 > 46%). A spike with a missing piece was considered to be shattered.

In addition to head shattering, related traits such as the number of rachis nodes (NRN), spike density (nodes/cm) and peduncle curve were also analyzed. The NRN × 3 (three seeds per node in six-rowed barley) is equal to the number of seeds on the spike. Head density was calculated as NRN/spike length (cm). Peduncle curve was scored on a scale of 1 to 3, with 1 being the most curved, 3 being erect, and 2 intermediate. Data for these traits were collected from DHLs grown in single rows in 1996 and 1997. In addition, spike density and head shattering data were collected from NILs grown in single rows at Pullman, Washington in 1997.

Results

Head shattering was scored on 150 SM DHLs grown in the field at Pullman, Washington during 1996 and 1997. The head shattering trait showed a skewed distribution pattern in both years (Fig. 1) where half of the DHLs had

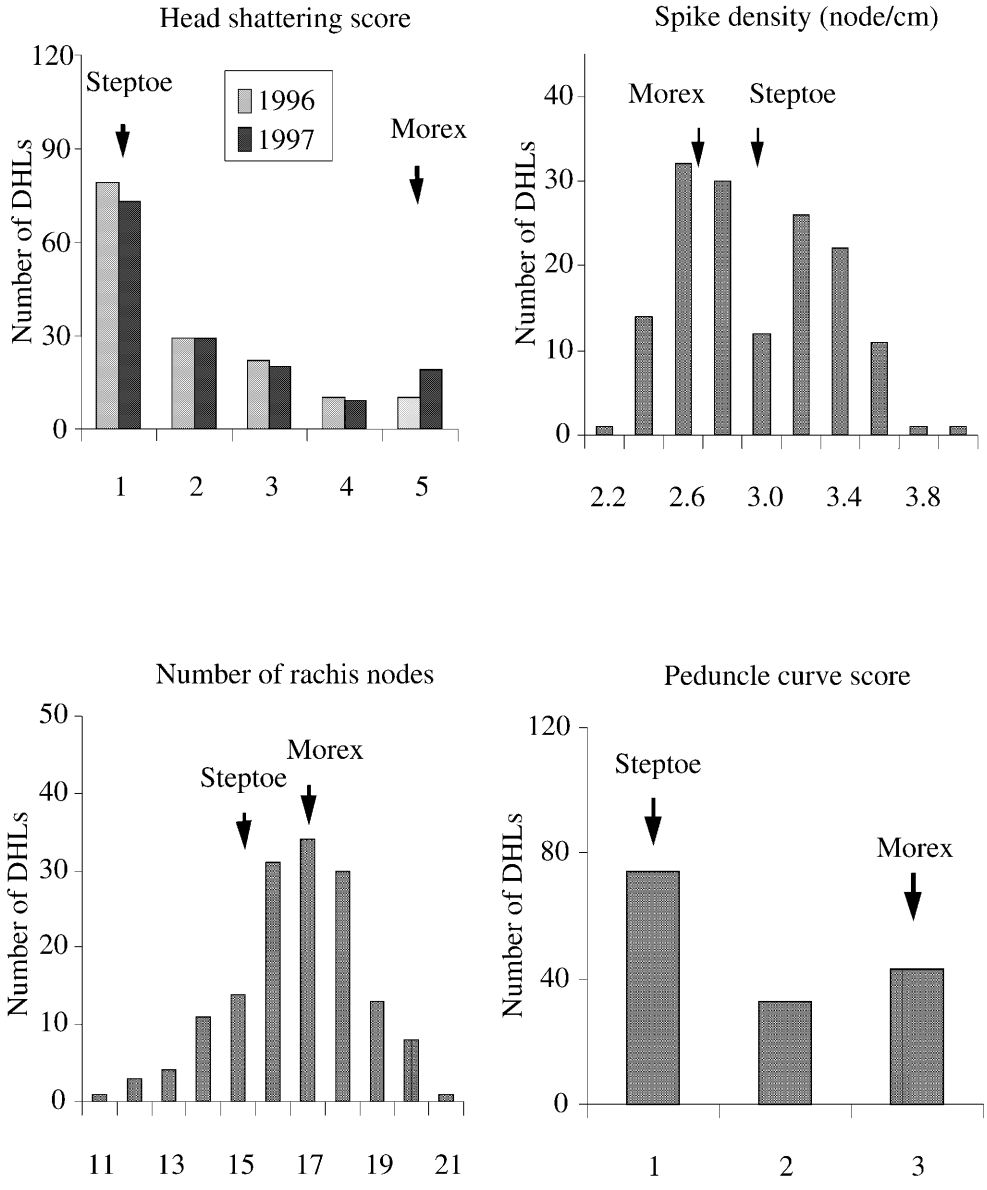

Fig. 1 Frequency distributions of SM DHLs for head shattering, spike density, number of rachis nodes, and peduncle curvature

no head shattering and the other half had varying degrees of shattering. Only 10 lines in 1996 and 19 lines in 1997 had a score of 5, the highest level of head shattering. Parents, Steptoe and Morex, had the extreme values of 1 and 5, respectively.

Morex and DHLs with a high head shattering score had long rachis internodes. These spikes also had a relatively high number of seeds and erect (uncurved) peduncles. The non-shattering parent Steptoe and DHLs, on the other hand, had short rachis internodes, fewer seeds per spike, and curved peduncles. Based on these observations, we analyzed spike density (nodes/cm), number of rachis nodes per spike (NRN), and peduncle curvature in the 150 DHLs (Fig. 1). NRN and the spike density traits showed transgressive segregation. Average NRN and spike density values were statistically greater ($P < 0.01$) in 1997 (18.0 and 2.9) than in 1996 (14.5 and 2.8).

QTL mapping of head shattering and related traits

Head shattering and related traits were mapped as QTLs, first using simple interval mapping (SIM) and later simplified composite interval mapping (sCIM). Test statistics calculated by both SIM and sCIM for QTL main effects and QTL × environment interaction effects are summarized in Table 1. Additive effects and percentage variance explained by each QTL are also presented in Table 1. Test statistics calculated by sCIM are presented as graphics in Fig. 2. sCIM results did not deviate with respect to the positions of QTLs detected by SIM. The magnitude of the test statistics was generally similar or higher with sCIM in QTL regions detected by SIM. However, sCIM identified additional QTLs. Hence, sCIM test statistic graphics are used to illustrate QTL positions detected by SIM.

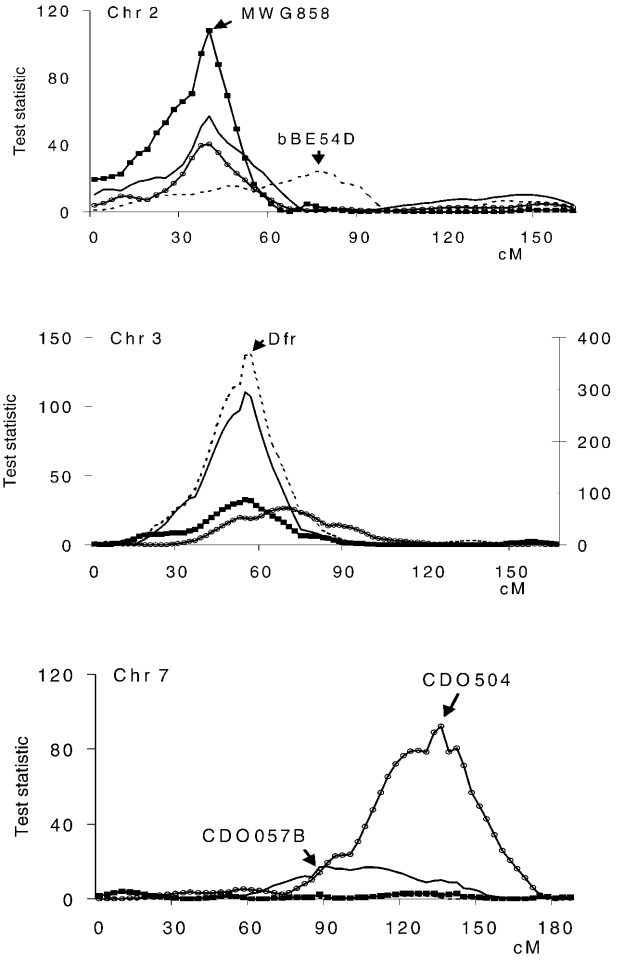

Fig. 2 Simplified composite interval QTL mapping for head shattering (——), spike density (**.....**), number of rachis nodes (–■–), and peduncle curvature (–o–).

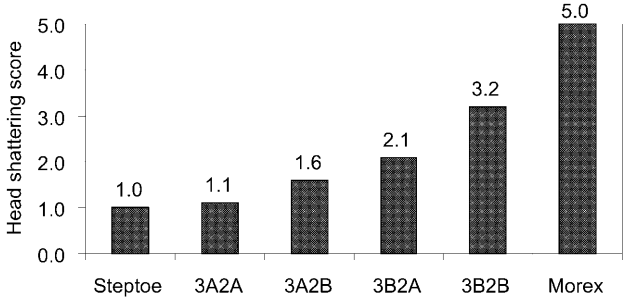

Fig. 3 Interaction between chromosome 3 (3H) and chromosome 2 (2H) head shattering QTL. Marker alleles for chromosome QTL regions are: *A* Steptoe parent, *B* Morex parent

Head shattering

Head shattering QTL analysis using SIM detected two major QTL on chromosome 2S (2HS) and the centromeric region of chromosome 3 (3H), designated Hst-2 S and Hst-3, respectively. The Hst-3 QTL peaked at the RFLP marker *Dfr* (dihydroflavonol-4-reductase, Kristiansen and Rohde 1991) on chromosome 3 (3H). This QTL had a test statistic of 101.2 with SIM (Table 1, $P < 0.01$) and explained 28.6% of the variation. DHLs with the Steptoe *Dfr* allele had a 1.3 lower head shattering score then DHLs with the Morex allele. None of the 19 DHLs with the highest head shattering score in 1996 and 1997 had the Steptoe *Dfr* allele, while about one-third of the non-shattering group had the Morex *Dfr* allele. Hst-3 x environment interaction was not significant.

The peak of the Hst-2S QTL coincided with the RFLP marker MWG858. The presence of the Steptoe MWG858 allele reduced head shattering by a score of 1.0 compared to the Morex allele (Table 1). This QTL had a test statistic of 48.5 with SIM ($P < 0.01$) and explained 14.9% of the variation for head shattering based on 2-year data. On the other hand, QTL analysis conducted for each year showed that head shattering variance due to the QTL represented by MWG858 was 23.0% of the total variance in 1997 but only 7.4% in 1996 (data not shown). This difference is possibly the reason for the significant QTL × environment interaction detected by 2-year QTL analyses (Table 1, $P < 0.05$).

Hst-3 and Hst-2S showed an additive gene effect (Fig. 3). When the Morex alleles of the two QTL peak markers were present, the average head shattering score was 3.2. The presence of Steptoe alleles resulted in a head shattering score of 1.1. Based on multiple regression analysis, the two major head shattering QTLs had a highly significant ($P < 0.01$) interaction that explained 2.1% of the variation. Together, Hst-3, Hst-2S and their interactions explained about 45% of the variation for head shattering. A major part of the variation remained unexplained and possibly was due to unidentified loci and non-genetic factors, including variability caused by the environment and experimental error.

sCIM was performed using MWG858 and *Dfr* as background markers. This yielded a secondary QTL with strong test statistics on chromosome 7L (5HL), designated Hst-7L (Fig. 2, Table 1). The QTL profile was broad without a distinct peak, perhaps due to the presence of a 31.2-cM gap between markers in this region. Two markers, CDO057B and CDO504, were used to represent this putative QTL. The CDO057B was the closest marker to the test statistic peak (17.0 with sCIM) in the interval of CDO057B to mSrh markers. The marker CDO504 on the distal side of the gap had a test statistic of 10.1. CDO057B and CDO504 explained 2.1% and 1.1%, respectively, of the variation for head shattering. The Steptoe alleles of these markers decreased the head shattering score by 0.5 and 0.4, respectively (Table 1). Interactions between Hst-7L and Hst-3 or Hst-2S were not calculated due to the uncertainty of the map location of the putative chromosome 7 QTL.

Spike density

The only spike density QTL detected by SIM was on chromosome 3 (3H) in the same position and shape as the

head shattering QTL Hst-3 (Fig. 2). The spike density QTL had a very large test statistic of 368.9 with SIM (Table 1, $P < 0.01$) and explained 70.8% of the variation for spike density. The Steptoe *Dfr* allele was associated with the dense spike trait (an average of 0.7 more nodes/cm spike length than Morex). During the backcross and selfing generations, we observed that the lax spike character is dominant to dense spike. sCIM mapping using the *Dfr* marker as background yielded four secondary QTLs for spike density, each explaining a relatively small part of the variance (Table 1). Two of these QTL were on chromosome 1 (7H), one on chromosome 2 (2H), and one on chromosome 4 (4H). None of these QTL regions showed any correspondence with head shattering.

Number of rachis nodes

The largest NRN QTL coincided with the head shattering QTL Hst-2S, located on chromosome 2 (2H) (Fig. 2). This QTL had a test statistic of 91.7 with SIM (Table 1) and explained 26.3% of the variation for NRN. Presence of the Morex MWG858 allele resulted in 2.1 more nodes (about six seeds) per spike on the average. The second largest QTL had a peak at the MWG798A marker of chromosome 6, explaining 6.9% of the variance (Table 1). The Morex allele of this marker conferred 1.1 more nodes per spike. A third NRN QTL explained 6.3% of the variance and peaked at the *Dfr* marker on chromosome 3 (3H) where Hst-3 was mapped (Table 1, Fig. 2). In this case, it was the Steptoe *Dfr* allele that resulted in increased NRN values (about 1.0 node/spike).

Peduncle curve

The peduncle curve trait was associated with head shattering in the extremely shattering SM DHLs, i.e., DHLs with the highest shattering (score of 5) had erect peduncles while the DHLs with the lowest shattering (score of 1) had curved peduncles. SIM showed a QTL on chromosomes 2 (2H) and 7 (5H). The larger QTL, marked by CDO504 on chromosome 7 (5H), was in the vicinity of a minor head shattering QTL, had a test statistic of 82.9 with SIM (Fig. 2 and Table 1, $P < 0.01$), and explained 24.1% of the variation. The second QTL mapped close to marker MWG858 on chromosome 2 (2H), had a test statistic of 33.4, and coincided with the second largest head shattering QTL, Hst-2S, and the largest NRN QTL (Fig. 2). MWG858 explained 10.5% of the variation for the peduncle curve trait. sCIM using CDO504 and MWG858 as background markers yielded two secondary QTLs for the peduncle curve, both on chromosome 3 (3H) and 11 cM apart. These two QTLs had test statistic peaks in the MWG571B-ABG377 and ABG398-*Dfr* intervals and explained 3.4% and 4.6% of the variance, respectively. The Steptoe alleles of the two chromosome 3 (3H) peduncle curve markers resulted in erect peduncles, while the Steptoe alleles of the chromosome 2 (2H) and 7 (5H) QTLs resulted in curved peduncles.

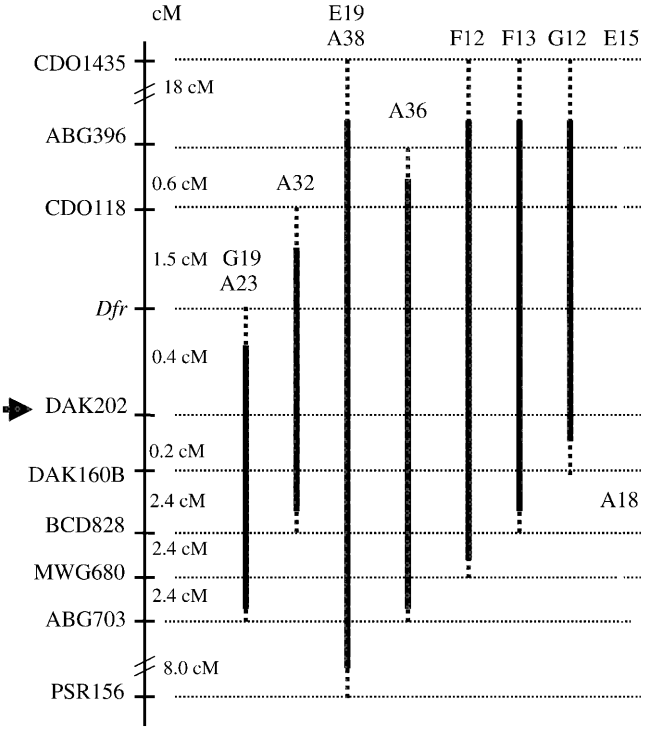

Fig. 4 Genomic composition of near-isogenic lines evaluated for head shattering. *Thick, solid lines* Steptoe fragments in a Morex genetic background, *dashed lines* putative cross-over regions, *dark* and *gray lines* non-shattering and shattering lines, respectively. *Arrow* shows the closest marker to the head shattering locus. Markers cosegregating with the indicated markers in this near isogenic line population are *Dfr* – ABG462, ksuA3C; DAK202 – *Dor4A*, RSB020; DAK160B – *Adh5*, ksuF2B, PSR626A, ABC156C

High resolution mapping of chromosome 3 (3H) head shattering QTL via NILs

It was observed that the breaking of the rachis in shattered heads always occurred at the attachment points of rachis internodes. However, this breakage was different from the segmentation or disarticulation of rachis internodes, which in the brittle rachis trait occurs at almost every attachment point. All lines with a Steptoe segment in the ABG396-ABG703 region, except E15 and A18, had a shattering score of 2 and short rachis internodes (Fig. 4). Since the shortest proximal segment terminated between *Dfr* and DAK202 and the shortest distal segment terminated between DAK202 and DAK160B, we conclude that the head shattering locus Hst-3 resides in a 0.6-cM interval between the *Dfr* and DAK160B markers. The spike density trait cosegregated with this locus in NILs.

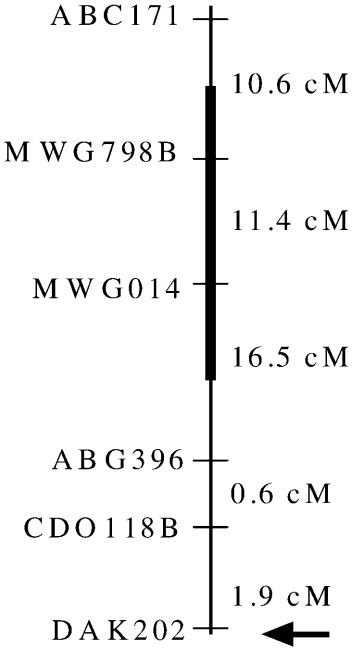

Fig. 5 *H. vulgare* ssp. *spontaneum* fragment (thick line) identified in the brittle rachis stock GSH01937. Arrow shows the location of the chromosome 3 (3H) head shattering QTL (Hst-3) mapped in the Steptoe × Morex population

Mapping of the *H. vulgare* ssp. *spontaneum Btr2* locus

RFLP analysis of the GSHO1937, *H. vulgare* ssp. *spontaneum*-derived brittle rachis line, and its backcross parent line cv. Bowman revealed an *H. vulgare* ssp. *spontaneum* segment containing chromosome 3 (3H) markers MWG798B and MWG014 but not markers ABC171 and ABG396 (Fig. 5). We conclude that the *Btr2* gene resides in this region and does not overlap with Hst-3 in the *Dfr* to DAK160B interval. Since the Bowman parent is believed to carry *Btr1* (J. Franckowiak, personal communication) it is not possible to draw any conclusions regarding the location of *Btr1*.

Discussion

It has been speculated that the head shattering QTL on chromosome 3 (3H) may represent weak alleles of the brittle rachis genes *Btr1* and *Btr2*. Our data indicate that the *Btr2* brittle rachis gene, and presumably *Btr1* due to close linkage, are distal from the weak rachis QTL Hst-3. These data are in agreement with the different characteristics of these two traits: i.e., disarticulation of rachis internodes in every attachment point in brittle rachis versus, one or two breaks in weak rachis and environmental effects. However, the mapping data do not exclude the possibility that the two traits may be due to diverged duplicated genes.

The Hst-3 QTL is probably the same as the head shattering QTL reported by Larson et al. (1996) although they observed two peaks with the main one at marker ABG396 and the other one about 44 cM away on the short arm of chromosome 3 (3H). We found a single QTL with a peak at the marker *Dfr*, 2.1 cM proximal to ABG396. The Hst-3 QTL is also probably the same as a previously reported major yield, lodging, plant height and head shattering QTL (Hayes and Iyamabo 1993; Hayes et al. 1993, Larson et al. 1996).

Hst-3 was associated with a major QTL for spike density. An association between head shattering and spike density association was reported previously (Turcotte 1957; Larson et al. 1996). The presence of clear transgressive segregation for spike density (Figs. 1 and 6) but not for head shattering suggests that head shattering is not a direct result of lax spikes. The spike density locus around the *Dfr* marker probably coincides with head shattering due to either pleiotropic effects or tightly linked genes.

Hst-3 explained nearly one-third of the head shattering variation in the SM DHL population. Skewed distribution and the presence of intermediate types in the DHL population suggested the involvement of additional loci. QTL mapping uncovered a second major head shattering locus on chromosome 2(2H) (Hst-2S) and a putative minor locus on chromosome 7(5H) (Hst-7L). Further evidence for multiple head shattering loci was provided by the Hst-3 NILs. Head shattering in these NILs showed a clear phenotypic separation with scores of either 2 or 5. The Steptoe head shattering score of 1 was not recovered.

The Hst-2S QTL is possibly associated with the NRN QTL which is also marked with MWG858. Hst-2S had a significant QTL x environment interaction and explained a much higher proportion of head shattering variance in 1997 when NRN values were higher than in 1996. Higher head shattering in spikes that carry more seeds is in accordance with higher head shattering damage under growing conditions that favor the development of large spikes – e.g., early planting dates (Rutger *et al.* 1966) and irrigated conditions (Platt and Wells 1949). It is probable that Hst-2S is associated with the yield QTL mapped previously on chromosome 2S (2HS) (Hayes and Iyamabo 1993). The only previous report indicating the presence of a head shattering QTL on chromosome 2 (2H) is from a two-rowed barley cross (Backes et al. 1995). The size of the reported QTL interval (50 cM) prevented comparison with our mapping results.

In conclusion, we demonstrated that the head shattering trait of Morex maps to a different position on chromosome 3(3H) than the brittle rachis trait of *Hordeum vulgare* ssp *spontaneum*. The head shattering QTL Hst-3 was mapped at a high resolution, and molecular markers were identified that may be useful for breeding in six-rowed barley.

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