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Fine mapping of a malting-quality QTL complex near the chromosome 4H S telomere in barley

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Abstract Malting quality has long been an active objective in barley (*Hordeum vulgare* L.) breeding programs. However, it is difficult for breeders to manipulate malting-quality traits because of inheritance complexity and difficulty in evaluation of these quantitative traits. Quantitative trait locus (QTL) mapping provides breeders a promising basis with which to manipulate quantitative trait genes. A malting-quality QTL complex, QTL2, was mapped previously to a 30-cM interval in the short-arm telomere region of barley chromosome 4H in a 'Steptoe'/'Morex' doubled haploid population by the North American Barley Genome Project, using an interval mapping method with a relatively low-resolution genetic map. The QTL2 complex has moderate effects on several malting-quality traits, including malt extract percentage (ME), α-amylase activity (AA), diastatic power (DP), malt β-glucan content (BG), and seed dormancy, which makes it a promising candidate gene source in malting barley-cultivar development. Fine mapping QTL2 is desirable for precisely studying barley malting-quality trait inheritance and for efficiently manipulating QTL2 in breeding. A reciprocal-substitution mapping method was employed to fine map QTL2. Molecular marker-assisted backcrossing was used to facilitate the generation of isolines. Fourteen different types of 'Steptoe' isolines, including regenerated 'Steptoe' and 13 different types of 'Morex' isolines, including regenerated 'Morex', were made within a 41.5-cM interval between MWG634 and BCD265B on chromosome 4H. Duplicates were identified for 12 'Steptoe' and 12 'Morex' isoline types. The isolines together with 'Steptoe' and 'Morex' were grown variously at three locations in 2 years for a total of five field environments. Four malting-quality traits were measured: ME, DP, AA, and BG. Few significant differences were found between duplicate isolines for these traits. A total of 15 putative QTLs were mapped; three for ME, four for DP, six for AA, and two for BG. Background genotype seemed to make a difference in expression/detection of QTLs. Of the 15 QTLs identified, ten were from the 'Morex' and only five from the 'Steptoe' background. By combining the results from different years, field environments, and genetic backgrounds and taking into account overlapping QTL segments, six QTLs can be conservatively estimated: two each for ME and AA and one each for DP and BG with chromosome segments ranging from 0.7 cM to 27.9 cM. A segment of 15.8 cM from the telomere (MWG634–CDO669) includes all or a portion of all QTLs identified. Further study and marker-assisted breeding should focus on this 15.8-cM chromosome region.

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Introduction

Malting quality of barley grain involves a collection of complex quantitative traits (Ullrich et al. 1997). Phenotyping malting-quality parameters by micromalting and

micromashing is labor intensive, time consuming, and often has limitations on seed availability. Mapping of quantitative trait loci (QTLs) provides breeders a promising way to manipulate genes that affect malting. At least 156 distinct malting-quality QTLs for 19 traits have been reported in nine barley mapping populations (summarized by Zale et al. 2000). Among the most important chemical parameters affecting malting are malt extract percentage (ME), diastatic power (DP), α-amylase activity (AA), and malt β-glucan content (BG). From the 'Steptoe' (feed type)/'Morex' (malting type) (S/M) cross, one of the major barley mapping crosses in the North American Barley Genome Project (NABGP) (Kleinhofs et al. 1993), several malting-quality QTLs were detected using an interval mapping method (Hayes et al. 1993, 1994; Han and Ullrich 1994; Han et al. 1995). One QTL complex, QTL2, was approximately mapped in the WG622–BCD402B interval in the short-arm telomere region of barley chromosome 4H, with a relatively low-resolution genetic map (Hayes et al.1993, 1994; Han and Ullrich 1994). The QTL2 complex affected several malting-quality parameters, including ME, DP, AA, BG and seed dormancy. 'Morex' contributed all high-malting-quality alleles for ME, DP, AA, BG in the QTL2 complex.

Fine mapping can locate QTLs accurately and enhance the understanding of quantitative trait inheritance. Fine mapping can also facilitate cultivar development by molecular marker-assisted selection for quantitative traits. Gross QTL location may lead to low efficiency in quantitative trait breeding, as undesirable linkage drag may occur during molecular marker-assisted QTL manipulation in breeding lines. This is especially true because the original QTL analyses in S/M (Hayes et al. 1993) and the other primary NABGP crosses (Mather et al. 1997; Marquez-Cedillo et al. 2000) were performed on mapping populations of doubled haploid lines (DHLs) derived from F_1s. This means that only one recombinational event took place prior to mapping, and each QTL identified represents a relatively large chromosomal region. QTL2 is an interesting malting-quality QTL complex for barley breeding because it has effects on several malting-quality parameters. Molecular marker-assisted selection of QTL2 gave low selection responses and implied location inaccuracy (Han et al. 1997a). The substitution mapping method has been successfully employed to fine map several QTLs in the barley genome from the S/M cross, including malting-quality QTL1 on chromosome 7H (Han et al. 1997b) and dormancy QTLs SD1 and SD2 on chromosome 5H (Han et al.1999; Gao et al. 2003). Here, we present our study on malting-quality QTL2 fine mapping, using a reciprocal-substitution mapping approach.

Materials and methods

High-resolution linkage map

The initial S/M map developed from 150 DHLs (Kleinhofs et al. 1993) has been enhanced and is available at http://barleygenomics.wsu.edu. Han and Ullrich (1994) and Hayes et al. (1993,1994) initially reported the malting-quality QTL2.

Construction of isolines

In our previous QTL fine-mapping studies (Han et al. 1997b, 1999; Gao et al. 2003), substitution isolines were generated in one parental genetic background only. Here, the reciprocal-substitution mapping method generating substitution lines in the genetic backgrounds of both parents was used. 'Morex' carries favorable alleles for high malting quality for all phenotypes determined in this study. By reciprocal substitution, the chromosome regions with 'Morex' alleles for high malting quality replaced the corresponding 'Steptoe' alleles in the 'Steptoe' genetic background and vice versa. To generate the 'Steptoe' and 'Morex' isolines, two DHLs, DH64 and DH171, were selected from the original mapping population (Kleinhofs et al. 1993). DH64 carries 'Morex' alleles in the QTL2 region and has a high percentage (70%) of 'Steptoe' alleles in other chromosome regions. DH171 carries 'Steptoe' alleles in the QTL2 region and has a high percentage (60%) of 'Morex' alleles in other chromosome regions. DH64 and DH171 were backcrossed three times to 'Steptoe' and 'Morex', respectively. Two sets of isolines were developed: (1) lines isogenic to 'Steptoe', carrying different size 'Morex' segments in the QTL2 region and (2) lines isogenic to 'Morex', carrying different size 'Steptoe' segments in the QTL2 region. To facilitate and accelerate the process of constructing isolines, a molecular marker-assisted backcrossing strategy was employed. Molecular markers mapped around the QTL2 region (Fig. 1) were used to dissect the QTL2 region and to facilitate the selection for desirable genotypes during the backcrossing process. Because malting-quality QTLs were approximately mapped around the 30-cM WG622–BCD402B interval on chromosome 4H (Han and Ullrich 1994; Hayes et al. 1994), the actual dissected region in this research was the 41.5-cM MWG634–BCD265B interval, which spans beyond the WG622–BCD402B interval (Fig. 1). The MWG634 marker is the closest marker to the short-arm telomere (http://barleygenomics.wsu.edu). In order to obtain isolines with a clean genetic background, at least one RFLP marker was checked per 10–30-cM in map regions where DH64 carried 'Morex' segments and where DH171 carried 'Steptoe' segments after each backcross. All available markers in the MWG634–BCD265B intervals (Fig. 1) were checked. As a control, regenerated 'Steptoe' (RGS) and regenerated 'Morex' (RGM), showing the parental genotype at all markers, were also developed through the same backcrossing processes. To facilitate backcrossing, the BC_2F_1 and BC_3F_1 plants were grown in a growthroom with both temperature and photoperiod controlled to delay flowering so as to facilitate selection of plants with desirable genotypes for selfing and for potential further backcrossing. Before genotyping, the photoperiod was 12 h, and the day and night temperatures were 25°C and 15°C, respectively. After genotyping, growing conditions were adjusted to 16 h and 30°C and 25°C to accelerate flowering. Homozygous isolines were generated from the BC_3F_2. Duplicates were identified if available for each type of isoline. BC_3F_3 seed from selected BC_3F_2 plants were grown in the greenhouse to increase seed (BC_3F_4) for field tests.

Field tests

The experiments were conducted in two consecutive years. A randomized complete block design was used. Together with 'Steptoe' and 'Morex', all isolines were grown in the field at

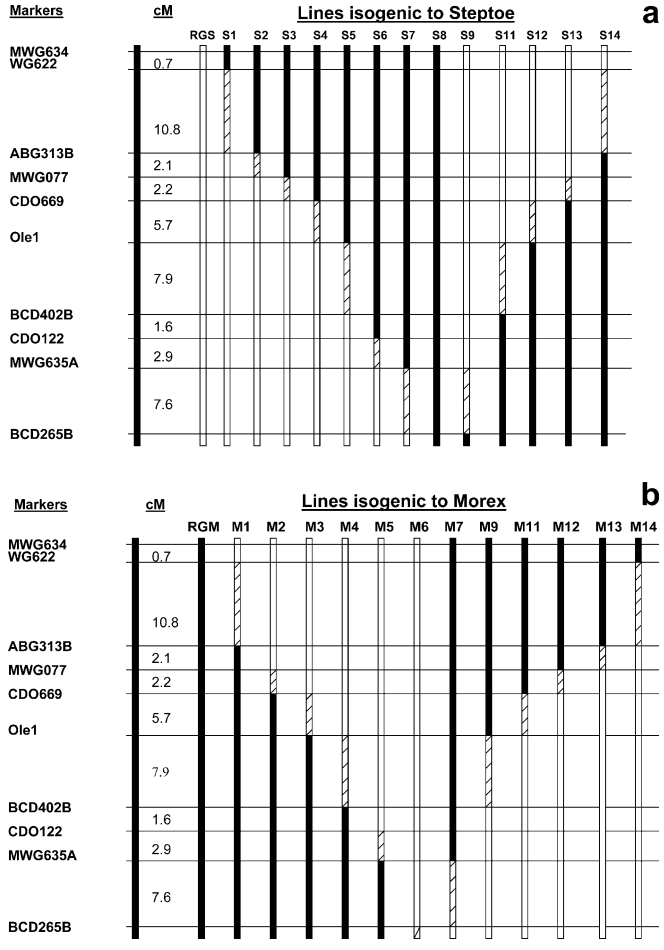

Fig. 1a, b Isoline genotypes in the MWG634–BCD265B interval of chromosome 4H. *Solid bar* 'Morex' segment, *open bar* 'Steptoe' segment, *hatched bar* crossover region with uncertain chromosomal origin. **a** 'Steptoe' isolines: *RGS* regenerated 'Steptoe', *S1 through S14* and *RGS* are 14 'Steptoe' isolines. **b** 'Morex' isolines: *RGM* regenerated 'Morex', *M1 through M14* and *RGM* are 13 'Morex' isolines

Aberdeen and Tetonia, Idaho and Pullman, Wash., USA in the summer of 2000 and at Aberdeen and Pullman in the summer of 2001. Due to seed availability limitations, duplicates were only planted in the field tests at Pullman in each year. For isolines with duplicates, only the first lines of each type listed in Tables 1 and 2 were grown at Aberdeen and Tetonia.

Phenotyping

Grain samples were malted, and grain or malt were analyzed at the USDA–ARS Barley and Malt Laboratory of the Cereal Crops Research Unit, Madison, Wisc., USA for total grain protein (PR), ME, DP, AA, and BG, using standard American Society of Brewing Chemists (1976) methods. Total grain protein content was measured to monitor its influence on the other quality traits.

Analysis of QTL effects

The principle for substitution QTL fine mapping has been described (Han et al. 1997b, 1999; Gao et al. 2003). Briefly, the difference among isolines with the same genetic background should theoretically result from their genotypic difference within the MWG634–BCD265B QTL interval (Fig. 1). By comparing the phenotypic malting-quality values from different isolines, the malting-quality QTLs should be precisely located. If similar malting-quality effects are found for several isolines, a malting-quality QTL is located in the overlapping segment among those lines. If similar malting-quality effects are found for several isolines with no overlapping segment among those lines, a malting-quality QTL is located to any unique chromosome segment(s) that each line carries. Typically, the substitution mapping method employs substitution isolines in only one parental genetic background, usually by dissecting and putting a favorable chromosomal segment from the donor parent into the genetic background of the other parent. With the reciprocal-substitution method, substitution isolines are developed and used in both parents. With the reciprocal-substitution mapping method, we could study QTL locations and effects by putting high-quality QTL segments from the malting parent 'Morex' into the feed parent 'Steptoe', as well as putting low-quality QTL segments from the feed parent 'Steptoe' into malting parent 'Morex'. By comparing results from both genetic backgrounds, a more confident conclusion on QTL locations and effects should be drawn.

Analyses of variance (ANOVA) of malting-quality trait values and mean separation tests were performed using the SAS program, version 6.12 (SAS Institute 1996). If there were no significant difference among experiments, the combined effects of each segment are presented. If a significant genotype × experiment interaction was found, ANOVA was performed separately for each experiment at first. Then, results from all experiments were compared. Comparisons of duplicates were carried out with Duncan's new multiple range test (NMRT). Similarly, comparisons among isolines were also carried out with NMRT. 'Steptoe' isolines were compared to 'Steptoe' or RGS only, while 'Morex' isolines were compared to 'Morex' or RGM only. Then, the results from both genetic backgrounds were compared. For isoline comparisons, to better control genetic background effects, an average malting-quality value for each type of isoline (Fig. 1) was calculated by combining duplicate malting-quality values for experiments at Pullman, where duplicates were employed. If there was no significant difference for a given trait between the parent and the regenerated parent, the isolines were compared to the regenerated parent by the NMRT. If there was a significant difference, they were compared to the respective original parent. By comparing fine-mapped QTLs, using reciprocal-substitution lines in both 'Steptoe' and 'Morex' genetic backgrounds, and using different experiments at different locations in different years, the location and effects of a QTL could be cross-examined.

Results and discussion

Development and comparison of isolines

Two sets of isolines for fine mapping QTL2 were generated in the genetic backgrounds of 'Steptoe' and 'Morex', respectively. Fourteen different types of homozygous 'Steptoe' isolines were constructed in the 'Steptoe' genetic background and designated S1 through S9, S11 through S14, and RGS. Their genotypes in the MWG634–BCD265B interval are depicted in Fig. 1a. For experimental control, one duplicate of each 'Steptoe' isoline type was identified, except for isoline types S4 and S14 (Table 1). Thirteen types of homozygous 'Morex' isolines were constructed in the 'Morex' genetic background and designated M1 through M7, M9, M11 through M14, and RGM. Their genotypes in the MWG634–BCD265B interval are depicted in Fig. 1b. Duplicates were also identified for 'Morex' isolines except for isoline type M3 (Table 2). Isoline duplicates were only grown at Pullman

Table 1 'Steptoe' isoline types, duplicates, and duplicate comparisons for malt quality traits. Isolines were grown at Pullman, Wash. *ME* Malt extract (%), *DP* diastatic power (°ASBC), *AA* α-amylase activity (20°DU), *BG* β-glucan (ppm), *PR* barley protein (%)

Types	Duplicates	Year 2000					Year 2001				
		ME	DP	AA	BG	PR	ME	DP	AA	BG	PR
RGS	S0107	74.7	52.5	35.3	772.5	10.9	77.4	52.0	40.9	471.5	11.8
	S0901	74.3	47.5	34.3	690.5	11.2	77.2	53.5	37.5	538.5	12.2
S1	S0112	75.0	53.5	37.6	760.0	11.0	77.1	53.0	40.4	533.5	12.2
	S0103	74.0	53.0	37.3	685.0	11.9	77.3	60.0	43.3	467.0	12.4
S2	S0205	74.8	46.0	35.6	665.0	11.2	77.2	45.5	37.5	445.5	11.3
	S0219	74.7	38.5	34.0	704.5	11.4	76.7	39.0	36.9	494.5	11.3
S3	S0312	74.7	45.0	34.4	789.5	10.6	77.0	47.5	35.4	566.0	11.6
	S0317	73.7	49.5	34.2	693.0	11.6	77.3	50.5	35.8	577.5	11.7
S4	S0401	74.6	43.5	33.2	683.5	10.7	77.1	45.0	35.5	576.5	11.4
S5	S0503	75.9	44.0	38.9	482.5	10.4	77.8	48.5	37.4	498.0	11.2
	S0527	74.2	46.5	32.7	606.0	11.1	77.5	50.5	40.7	431.0	11.7
S6	S0615	75.5	52.0	46.6[a]	445.0	11.0	76.8	56.0	46.2	443.0	12.3
	S0634	73.7	59.0	38.2[a]	630.5	11.7	77.7	59.0	44.1	401.5	12.0
S7	S0731	75.1	45.5	39.5	685.5	11.4	77.6	45.0	41.7	553.0	12.1
	S0707	75.1	45.0	37.5	568.5	11.4	77.3	54.0	46.7	423.5	12.3
S8	S0833	75.4	76.0[a]	43.2[a]	600.0	11.3	78.1	51.0[a]	40.9	393.0	11.8
	S0875	74.5	45.5[a]	33.8[a]	574.0	10.9	78.5	75.0[a]	43.1	350.5	11.6
S9	S0911	74.6	53.5	37.5	692.0	12.1	77.8	52.5	44.0	407.0	12.0
	S0904	74.5	51.5	35.8	609.5	11.1	76.8	56.5	41.8	486.5	12.7
S11	S1101	73.8	47.0	33.1	785.0	11.5	76.5	44.0	35.5	570.5	11.4
	S1111	74.3	39.5	32.8	608.0	10.7	76.4	45.0	34.1	615.5	11.7
S12	S1235	74.3	47.5	35.9	783.5	11.2	77.7	49.0	38.7	446.5	11.2
	S1229	74.3	48.0	32.3	748.0	11.2	77.6	50.0	39.1	459.0	11.0
S13	S1345	74.6	47.0	37.8	649.5	11.1	77.4	46.5	39.3	459.5	11.7
	S1343	74.6	42.0	36.7	543.0	11.0	77.1	43.5	39.2	512.5	11.9
S14	S1445	74.3	44.5	37.8	651.5	11.0	77.1	49.0	40.3	545.0	12.4

[a]Significant difference (α=0.05) by Duncan's new multiple range test

in 2000 and 2001 because of limited seed availability. Highly significant differences were found between the 2 years for all four traits evaluated, while significant year × genotype interaction was detected only for ME. Thus, duplicate comparison was carried out within each experimental year.

Duplicates of 'Steptoe" isolines (Table 1) For ME, BG, and PR, no significant differences (α=0.05) were found between duplicates of any isoline type in either year. For DP, significant differences were not found for any isoline except for S8, where differences were found between duplicates in both years. For AA, differences were found only for duplicate isolines of S6 and S8 in 2000.

Duplicates of 'Morex" isolines (Table 2) For ME, significant differences were not found between duplicates in either year except for M1 in 2001. No differences were found between DP values of duplicates for any isoline type in either year with the following exceptions: M1 had differences between duplicates both years, M2 had a difference between duplicates in 2001, M6 and M7 had differences between duplicates in 2000 and 2001, and M11 had a difference between duplicates in 2001. For AA, differences were not found for duplicates of any isoline except for M2, in 2001. For BG and PR, no significant differences were found between duplicates for any isoline in either year.

Using duplicate isolines enhanced control of experimental error. However, duplicate isolines also complicated genetic analysis. In this research, significant differences were found between duplicates of two (out of 12) 'Steptoe' isoline types and five (out of 12) 'Morex' isoline types for one or two traits. Overall, there were only 14 significant differences between isoline duplicates out of a total of 240 (24 isolines × 5 traits × 2 years) comparisons. The only trends observed in the data involve isoline types and traits. Multiple measurements were affected for five of the seven isoline types that showed differences, and 10 of the 14 measurements affected were of DP (Tables 1, 2). It could be that the DP measurements displayed lower accuracy or precision compared with the other three traits measured (coefficients of variance were generally over 10%). Although the genetic background of duplicate isolines appeared to be the same, based on molecular marker-assisted genotyping data, there might still have been genetic differences because it was impractical to check the whole genetic background completely. Also, even within the dissected target region, genotypic differences between duplicate isolines could have been possible, based on imperfect detection of crossover points because of limitation in molecular marker

Table 2 'Morex' isoline types, duplicates, and duplicate comparisons for malt quality traits. Isolines were grown at Pullman

Types	Duplicates	Year 2000					Year 2001				
		ME	DP	AA	BG	PR	ME	DP	AA	BG	PR
RGM	M0107	78.7	144.0	53.8	168.5	13.4	79.6	155.0	57.4	197.5	15.2
	M0109	78.7	153.0	54.5	198.0	13.8	80.7	162.5	60.4	151.0	14.2
M1	M0108	77.9	118.0[b]	52.8	364.0	13.4	81.5[a]	138.0[b]	55.9	178.0	13.5
	M0105	78.6	179.5[b]	54.3	299.5	13.7	79.2[a]	174.5[b]	54.8	118.0	14.3
M2	M0208	79.9	169.0	57.0	181.5	12.8	81.0	170.5[a]	59.6[a]	131.5	14.1
	M0223	79.6	153.5	54.8	177.5	13.2	79.4	147.5[a]	50.0[a]	198.0	13.6
M3	M0307	79.1	145.0	54.0	314.5	13.5	81.7	112.5	54.6	195.5	12.8
M4	M0402	79.1	137.0	53.2	313.0	12.3	80.4	156.5	57.0	153.5	13.6
	M0403	79.0	122.0	48.4	288.5	11.4	79.8	160.5	52.0	145.5	13.8
M5	M0549	79.6	127.5	52.5	259.0	12.4	81.2	140.0	55.3	172.0	13.9
	M0542	80.0	117.5	49.3	313.0	12.6	79.7	143.5	49.7	166.5	14.0
M6	M0641	77.9	188.5[a]	50.8	324.0	13.7	80.0	190.0[b]	54.5	207.5	13.9
	M0621	78.3	156.0[a]	51.8	270.5	13.6	79.1	162.5[b]	50.2	217.0	14.4
M7	M0703	78.6	155.0[a]	56.8	253.5	13.9	80.0	153.5[b]	57.6	167.0	13.9
	M0710	78.3	124.0[a]	55.3	228.0	13.1	79.9	116.5[b]	49.7	255.0	13.8
M9	M0923	80.3	135.0	58.8	290.5	13.2	81.9	136.5	61.8	168.5	13.3
	M0902	79.7	139.0	60.1	193.0	13.3	81.0	149.0	55.5	168.5	14.3
M11	M1115	79.0	138.0	53.0	311.5	12.9	81.1	127.0[b]	53.8	181.0	12.7
	M1128	79.1	162.0	51.1	240.5	12.8	80.4	167.0[b]	53.4	214.5	14.0
M12	M1219	78.5	130.0	53.9	318.5	13.3	80.2	122.5	54.2	221.5	13.8
	M1228	78.9	116.5	55.3	202.0	12.4	79.1	131.0	52.2	265.0	13.9
M13	M1303	76.7	128.0	50.1	385.0	14.1	78.5	118.5	49.0	319.0	14.2
	M1324	77.1	116.5	52.3	333.0	13.7	78.3	128.5	50.5	249.0	14.8
M14	M1415	79.1	135.5	54.8	287.5	12.1	80.0	151.5	55.8	162.0	13.1
	M1444	78.5	136.5	51.6	322.5	12.9	79.0	157.0	50.4	192.0	14.8

[a]Significant difference (α=0.05)
[b]Highly significant difference (α=0.01) by Duncan's new multiple range test

availability in the crossover intervals. Overall, in both genetic backgrounds, most duplicates of most isoline types showed good agreement for the five traits evaluated.

QTL fine mapping

Because significant differences were generally observed among experiments, including isoline × location interactions, results from the five field trials at three locations in 2 years were analyzed separately to fine map QTL2. However, for comparison purposes, results of combined analyses over all five locations are included in the discussion. For the experiments at Pullman where duplicates were evaluated, an average malting-quality value for each isoline type (Fig. 1) was calculated by combining duplicate malting-quality values (Tables 1, 2) for each trait. The rationale included: (1) no significant differences were found between duplicates for most traits of most isoline types, (2) it was not possible to determine exact purity of the genetic backgrounds, (3) better control over genetic background should be expected when using an average value than using duplicates separately, and (4) comparison of isoline types over different environments could be carried out in a straightforward manner if all analyses were based on single values for each isoline type.

The results of the comparisons of isolines with their respective parent or regenerated parent are listed in Tables 3 and 4. For each experiment, RGS and RGM were first compared to 'Steptoe' and 'Morex', respectively, to determine the standard lines to which all other isolines would be compared. In Table 3, if no significant difference was found between RGS and 'Steptoe', the values from RGS are indicated in boldface, and the 'Steptoe' isolines were compared to RGS to fine map QTL2. Otherwise, the values from 'Steptoe' are indicated in boldface, and all 'Steptoe' isolines were compared to the original 'Steptoe' parent. Similarly, in Table 4, the values from RGM or 'Morex' are indicated in boldface, depending on the 'Morex'–RGM comparisons, and all 'Morex' isolines were compared to either RGM or 'Morex'. In general, few differences were found between the regenerated parents compared to the original parents; 17 of 20 specific comparisons were made with each of the regenerated parents.

Malt extract All QTL mapping results are depicted in Tables 3 and 4 and Fig. 2. Three QTLs for ME were mapped to two different locations from the individual environment analyses. From Pullman 2000 and 2001, Aberdeen 2000, and Tetonia 2000, no significant differences in ME were found between any of the 'Steptoe' isolines and RGS (Table 3), so no ME QTL could be

Table 3 'Steptoe' isoline and parent comparisons for malting-quality traits

Types	Pullman 2000[a]					Pullman 2001[a]					Aberdeen 2000					Aberdeen 2001					Tetonia 2000				
	ME	DP	AA	BG	PR	ME	DP	AA	BG	PR	ME	DP	AA	BG	PR	ME	DP	AA	BG	PR	ME	DP	AA	BG	PR
'Steptoe'	74.3	48.0	32.1	821.5	11.2	76.5	44.5	35.8	637.5	12.0	73.5	**65.0**	**28.9**	**796.0**	13.7	73.9	49.5	30.5	725.0	11.3	73.4	59.5	28.4	736.5	11.1
RGS	**74.5**[b]	**50.0**	**34.8**	**731.5**	11.1	**77.3**	**52.8**	**39.2**	**505.0**	11.9	**74.3**	132.5	48.5	532.5	16.0	**74.3**	**61.0**	**35.4**	**560.5**	11.8	**73.2**	**71.5**	**31.4**	**617.0**	12.3
S1	74.5	53.3	37.4	722.5	11.5	77.2	56.5	41.8	500.3	12.3	73.7	85.5	40.4[c]	785.0	14.8	74.6	66.0	36.8	563.0	12.0	73.8	68.0	33.1	617.5	11.6
S2	74.8	42.3	34.8	684.8	11.3	76.9	42.3	37.2	470.0	11.3	74.2	71.0	33.2	726.0	13.6	72.2[c]	56.5	33.1	502.0	11.9	73.0	70.5	31.4	689.0	11.6
S3	74.2	47.3	34.3	741.3	11.1	77.1	49.0	35.6	571.8	11.7	73.7	74.5	34.0	738.0	13.3	74.2	51.5	31.1	610.0	11.7	72.0	69.0	31.7	634.0	12.6
S4	74.6	43.5	33.2	683.5	10.7	77.1	45.0	35.5	576.5	11.4	73.5	72.0	34.2	715.0	12.9	74.8	53.5	29.9	577.5	10.9	73.7	62.5	32.9	602.5	11.0
S5	75.0	45.3	35.8	544.3[c]	10.7	77.6	49.5	39.1	464.5	11.5	75.6	74.0	36.1	696.0	12.7	73.7	54.5	31.2	515.5	11.9	73.5	69.0	33.4	626.0	11.9
S6	74.6	55.5	42.4[c]	537.8[c]	11.4	77.2	57.5	45.1	422.3	12.1	76.0	76.0	41.2[c]	698.5	12.9	75.2	64.5	38.3	486.0	10.6	71.9	84.0	37.5[c]	672.0	12.8
S7	75.1	45.3	38.5	627.0	11.4	77.4	49.5	44.2	488.3	12.1	74.4	64.0	37.0	782.0	13.0	72.7	53.5	34.4	517.5	12.7	73.4	92.0	36.2[c]	575.5	12.5
S8	74.9	60.8	38.5	587.0	11.1	78.3	63.0	42.0	371.8	11.7	73.4	73.5	32.4	683.0	13.7	73.3	55.0	30.9	579.0	12.1	73.1	72.5	30.4	689.0	12.5
S9	74.5	52.5	36.6	650.8	11.6	77.3	54.5	42.9	446.8	12.3	73.8	78.5	35.8	787.5	14.0	75.0	70.0	35.4	503.5	11.6	73.4	72.5	33.1	732.5	12.2
S11	74.0	43.3	32.9	696.5	11.1	76.4	44.5	34.8	593.0	11.5	72.9	69.0	31.5	812.0	14.7	73.6	51.0	29.8	685.5	11.5	73.1	63.0	29.6	729.5	11.7
S12	74.3	47.8	34.1	765.8	11.2	77.6	49.5	38.9	452.8	11.1	74.2	70.0	32.7	832.5	13.0	73.4	57.5	31.2	598.0	11.7	73.6	68.5	29.9	744.5	11.9
S13	74.6	44.5	37.2	596.3	11.1	77.2	45.0	39.2	486.0	11.8	74.9	63.0	31.8	864.0	12.6	74.8	51.5	30.2	694.0	11.2	73.6	62.5	31.4	706.0	11.7
S14	74.3	44.5	37.8	651.5	11.0	77.1	49.0	40.3	545.0	12.4	73.7	72.5	32.7	752.5	12.9	73.8	58.0	31.2	689.0	12.1	73.1	74.5	32.7	712.5	12.7

[a]Showing mean value of duplicate when applicable
[b]Numbers in *boldface* in *RGS* (or *RGM*, *'Steptoe'*, and *'Morex'*) are the values to which values of all other isolines are compared in NMRT analyses (see text)
[c]Significant difference (α=0.05)

Table 4 'Morex' isoline and parent comparisons for malting-quality traits

Types	Pullman 2000[a]					Pullman 2001[a]					Aberdeen 2000					Aberdeen 2001					Tetonia 2000				
	ME	DP	AA	BG	PR	ME	DP	AA	BG	PR	ME	DP	AA	BG	PR	ME	DP	AA	BG	PR	ME	DP	AA	BG	PR
'Morex'	79.9	149.5	**61.0**	194.0	13.6	81.5	140.0	58.2	103.0	13.4	78.6	**165.0**	54.1	417.0	14.7	78.3	153.0	54.1	269.5	14.3	76.9	**123.0**	43.9	539.5	13.1
RGM	**78.7**	**148.5**	54.1	**183.3**	13.6	**80.1**	**158.8**	**58.9**	**174.3**	14.7	**76.5**	115.5	**46.0**	**473.5**	13.8	**77.1**	**135.5**	**48.2**	**345.5**	14.1	**75.6**	167.0	**43.6**	**415.5**	14.4
M1	78.2	148.8	53.5[b]	331.8	13.5	80.3	156.3	55.3	148.0	13.9	78.2	161.5	51.5	351.5	14.7	78.9	138.5	50.8	273.0	13.5	74.9	187.0[b]	43.8	406.5	15.5
M2	79.8	161.3	55.9	179.5	13.0	80.2	159.0	54.8	164.8	13.8	78.1	215.0[b]	54.5	475.0	15.6	78.4	170.0[b]	48.5	297.5	14.0	76.4	204.5[b]	41.4	410.0	14.2
M3	79.1	145.0	54.0[b]	314.5	13.5	81.7	112.5[b]	54.6	195.5	12.8	78.1	153.0	46.9	539.5	13.8	78.1	127.0	49.3	337.0	13.2	75.2	159.5[b]	39.2	593.0	14.3
M4	79.1	129.5	50.8[b]	300.8	11.8	80.1	158.5	54.5	149.5	13.6	77.5	183.5	53.1	545.5	14.9	77.0	160.5[b]	50.2	370.0	14.3	76.9	153.5	43.0	510.0	13.0
M5	79.8	122.5	50.9[b]	286.0	12.5	80.4	141.8	52.5	169.3	13.9	77.4	168.0	48.0	506.5	15.7	77.7	138.5	48.3	410.0	14.1	76.2	149.5	38.1[b]	493.0	14.1
M6	78.1	172.3	51.3[b]	297.3	13.6	79.5	176.3	52.3	212.3	14.1	76.4	225.0[b]	52.1	447.5	16.0	77.1	180.5[b]	49.6	362.0	14.2	75.1	223.5[b]	40.4	408.0	15.1
M7	78.4	139.5	56.1	240.8	13.5	80.0	135.0[b]	53.6	211.0	13.9	76.2	188.5	52.8	501.5	16.8	78.1	131.0	50.0	313.5	14.1	75.4	156.5[b]	39.4	434.5	14.1
M9	80.0	137.0	59.4	241.8	13.2	81.4	142.8	58.7	168.5	13.8	78.2	176.5	54.4	422.5	14.9	78.3	148.0	44.2	295.0	14.5	77.0	155.0[b]	46.0	355.5	13.6
M11	79.0	150.0	52.1[b]	276.0	12.9	80.7	147.0	53.6	197.8	13.3	76.9	177.0	48.8	461.5	15.1	77.3	148.5	49.9	295.5	13.4	75.9	165.0[b]	39.3	506.0	13.8
M12	78.7	123.3	54.6[b]	260.3	12.8	79.7	126.8[b]	53.2	243.3	13.8	75.5	163.0	49.0	471.5	15.6	77.4	135.0	51.4	317.0	13.7	73.4	176.0[b]	41.3	456.5	15.5
M13	76.9[b]	122.3	51.2[b]	359.0[b]	13.8	78.4[b]	123.5[b]	49.7[b]	284.0	14.5	74.4	176.5	49.6	495.0	16.7	75.5	120.5	42.6	323.5	14.5	74.0	146.0	38.0[b]	406.5	14.7
M14	78.8	136.0	53.2[b]	305.0	12.5	79.5	154.3	53.1	177.0	13.9	78.3	176.0	52.5	427.5	14.3	78.2	161.0[b]	51.4	354.5	13.9	75.4	182.0[b]	42.4	392.5	14.0

[a]Showing mean value of duplicate when applicable
[b]Significant difference (α=0.05)

mapped from the 'Steptoe' isolines. From Aberdeen 2001, isoline type S2 was significantly different from RGS (Table 3), so a putative ME QTL could be located in the 11.5-cM MWG634–ABG313B interval (Fig. 2). From Pullman both years, isoline type M13 was significantly different from RGM (Table 4), so that a putative ME QTL could be hypothesized in the MWG077–BCD265B interval (Fig. 2), because replacing the 'Morex' segment in that interval with the 'Steptoe' segment significantly lowered the ME value of M13. M14 also has the 'Morex' segment in the MWG077–BCD265B interval replaced by the 'Steptoe' segment, but M14 did not show a significantly lower ME value compared to RGM. In S14, the 'Steptoe' segment was replaced by the 'Morex' segment in the MWG077–BCD265B interval, but S14 did not show a significant increase in ME value from RGS. These scenarios are often observed during QTL fine mapping, using the substitution method (Han et al. 1997b). There might be several possible explanations. First, it could be due to environmental effects and/or interactions between QTL(s) and environments. Environmental effects are common for malting-quality parameters (Hayes et al. 1993). In the present research, in different experiments QTLs were mapped to different chromosomal locations or to similar chromosomal locations with different interval sizes, or even a QTL could be missing under certain environmental conditions (Fig. 2). Second, it could be due to the gene composition and nature within a QTL, which are generally not clear until the genes involved are cloned. Korstanje and Paigen (2002) summarized cloned genes from QTL mapping, and they proposed that regulatory genes or rate-limiting enzymes were more likely identified through QTL mapping than structural genes. Most known barley malting-quality structural genes were not located in mapped QTLs (Hayes et al. 1993) and even when mapped close to a QTL, were shown by high-resolution mapping to not be involved in the QTL's activity (Han et al. 1997b). It is reasonable to believe that a QTL effect would not be observed if a QTL containing a regulatory gene were transferred without the appropriate structural gene/allele it regulates or vise verse. Third, there are limitations on every experimental design and the analysis method employed. In this research, the randomized complete block design was employed. The block size was relatively big because all 'Steptoe' and 'Morex' isolines were combined. The least significant difference method gave slightly different results from that of the Duncan's NMRT method in the multiple comparisons (data not shown). In addition, even though some isolines carrying a mapped QTL segment did not show significant difference from RGS or RGM at α=0.05 level, they did show increased or decreased malting-quality values and were very close to being significant at the α=0.05 level. Fourth, there might be interaction or lack of interaction effects between genes transferred and the genetic backgrounds. This could be related to the second reason mentioned above. Increasing backcrossing generations improves the genetic background of the isolines. Our previous QTL fine-mapping studies (Han et al. 1997b, 1999; Gao et al. 2003), using the substitution mapping method indicated that three generations of backcrossing together with molecular marker-assisted selection were generally enough for generating isolines with relatively clean genetic backgrounds for QTL fine mapping. Additionally, 'Steptoe' and 'Morex' are very opposite in quality. 'Steptoe' is so poor in malting quality that its background genotype might negate a positive 'Morex' allele. Fifth, the role of grain protein content might be involved in ME as well as in other malting-quality traits, especially DP. It is well known that PR is negatively correlated with ME and positively correlated with DP (Briggs 1978; Shewry and Darlington 2002). In general, the 'Morex' isolines had higher PR levels than the 'Steptoe' isolines (Tables 1, 2, 3, 4), which appears to be a bigger factor for DP than ME (see below). Sixth, malt modification during germination might be a factor. The greater the degree of modification, the greater the development of ME and enzyme systems (DP, AA) and the lower the level of BG (greater endosperm cell wall breakdown). Modification per se was not measured in this study, but it can be seen in Tables 1, 2, 3 and 4 that modification was more complete in the 'Morex' isolines than in the 'Steptoe' isolines. Any or all of these six explanations may be reasons for the unexpected lower ME in isoline S14.

Diastatic power Four DP QTLs were mapped from four of the five environments, all with 'Morex' isolines. No isoline-parent differences from either set were detected from Pullman 2000. From Pullman 2001, M3, M7, M12, and M13 were significantly different from RGM, and the

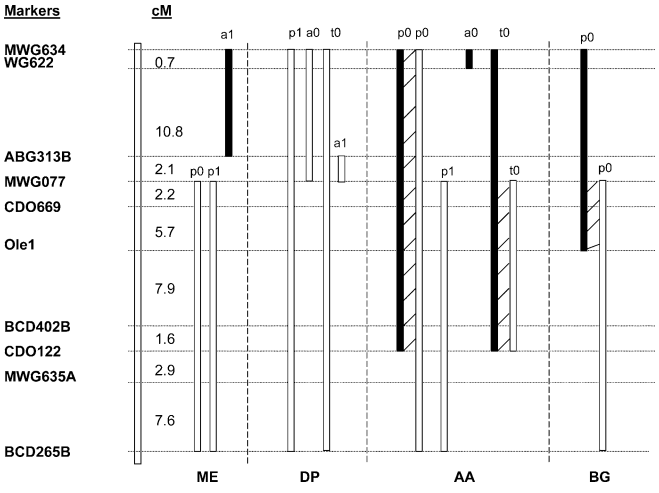

Fig. 2 Quantitative trait loci mapped in the MWG634–BCD265B interval of chromosome 4H over five field environments in two years. *ME* Malt extract (%), *DP* diastatic power (°ASBC), *AA* α-amylase activity (20°DU), *BG* malt β-glucan content (ppm), *p0* mapped from the Pullman, Wash. 2000 experiment, *p1* mapped from the Pullman 2001 experiment, *a0* mapped from the Aberdeen, Idaho 2000 experiment, *a1* mapped from the Aberdeen 2001 experiment, *t0* mapped from the Tetonia, Idaho 2000 experiment, *solid bar* mapped from 'Steptoe' isolines, *open bar* mapped from 'Morex' isolines, *bar region connected by diagonal lines* conserved chromosomal regions mapped from both 'Steptoe' and 'Morex' isolines

'Steptoe' allele(s) decreased DP as expected (Table 4). There is no common overlapping 'Steptoe' segment among these four lines, so the putative QTL could be located in unique chromosomal regions, which includes the whole target region from MWG634 to BCD265B (Fig. 2). However, one DP QTL could reside between MWG635A and BCD265B, which includes a 'Steptoe' segment overlap between M12 and M13, and M7's crossover interval. A second DP QTL could be located in the MWG077–Ole1 interval, which is a 'Steptoe' segment overlap among M3, M12, and M13, including the crossover intervals for M3 and M12. Mapping new markers into the MWG635A–BCD265B and MWG077–Ole1 intervals and using them to generate new isolines could test the above two hypotheses. A third DP QTL could be outside the MWG634–BCD265B region, where 'Steptoe' carries a gene(s) for decreased DP. Because all markers in known DP QTLs were checked and showed 'Morex' patterns, we believe that there are undetected DP QTLs in the 'Steptoe'/'Morex' mapping population. Here, we noticed that significant differences existed between two M7 duplicates for DP in 2000 and 2001 that could be explained if they had differences in their genetic backgrounds, as discussed previously. Additional backcrossing of M7 could be helpful to further explain these results. These data certainly demonstrate the complexity of the inheritance of DP. From Tetonia 2000, M1, M2, M3, M6, M7, M9, M11, M12, and M14 were significantly different from 'Morex'. Because there is no overlapping 'Steptoe' segment among all of these isolines, the DP QTL could only be located with certainty to the whole MWG634–BCD265B region or even beyond it. From Aberdeen 2000, M2 and M6 were significantly different from 'Morex', so a DP QTL could be located in the 13.6-cM MWG634–MWG077 interval. From Aberdeen 2001, M2, M4, M6, and M14 were significantly different from RGM, so a DP QTL could be located in the 2.1-cM ABG313B–MWG077 overlapping interval among these isolines. The last three QTL regions detected appear to carry 'Steptoe' alleles that increase DP, which is contrary to the original QTL analysis result in which the 'Morex' allele is positive (Han and Ullrich 1994; Hayes et al. 1993, 1994). However, the PR contents were very high in the 'Morex' isolines from the Aberdeen and Tetonia nurseries. The isolines with significantly elevated DP values also had very high PR levels (Table 4). As described above the positive relationship between PR content and DP is well known (Briggs 1978; Shewry and Darlington 2002).

α-Amylase Individual analyses revealed six QTLs for AA, three from each isoline set. QTLs were detected in four environments (Tables 3, 4; Fig. 2). From Pullman 2000, a QTL could be located in the MWG634–CDO122 interval because S6 was significantly different from RGS (Table 3). 'Morex' isolines M1, M3, M4, M5, M6, M11, M12, M13, and M14 were significantly different from 'Morex' (Table 4). No overlapped 'Steptoe' sequence exists among these lines, so AA QTLs could be located to different unique regions. In other words, no refinement of QTL location(s) is certain from these data, and (an) AA QTL(s) could only be mapped in the whole MWG634–BCD265B region. However, there could be a QTL in the common 'Steptoe' overlap segment for M1, M3, M4, M5, M6, and M14 of WG622-ABG313B, including uncertain crossover intervals. Likewise, another QTL could be in the common CDO669–Ole1 for M3, M4, M5, M6, M11, M12, M13, and M14. Another hypothesis is that a QTL is located in the MWG634–CDO122 interval, which is the segment common to the QTLs mapped individually from the two isoline sets (hatched region, Fig. 2). When the QTL2 region was detected from field tests at Pullman in 1991 and 1992 with a relatively lower resolution linkage map, it was mapped in the CDO669–BCD402B interval (Han et al. 1994; Hayes et al. 1994). This result indicates that the actual QTL2 region is larger than that originally detected (Han and Ullrich 1994; Hayes et al. 1994), which we suspected (Han et al. 1997a). From Pullman 2001, M13 was significantly different from RGM, so an AA QTL could be located in the MWG077–BCD265B interval. From Aberdeen 2000, S1 and S6 were significantly different from 'Steptoe', so a QTL could be mapped into the 0.7-cM MWG634–WG622 interval (Fig. 2; Table 3). From Tetonia 2000, S6 and S7 were significantly different from RGS, so an AA QTL could be mapped in the common MWG634–CDO122 interval. M5 and M13 were significantly different from RGM, so a QTL could be located in their common MWG077–CDO122 interval. All the significant differences between isolines and background parent for AA were in the direction expected if 'Morex' contributes the positive alleles (higher AA values in 'Steptoe' isolines and lower AA values in 'Morex' isolines). Combining results from both genetic backgrounds, an AA QTL is most likely in this MWG077–CDO122 interval (hatched area, Fig. 2). This result demonstrates the advantage of the reciprocal-substitution mapping method.

β-Glucan QTL mapping results for BG were from both sets of isolines and only from Pullman 2000. S5 and S6 had significantly lower BG values than RGS as expected (Table 3), so a QTL could be mapped in the S5 and S6 common interval of MWG634–Ole1 (Fig. 2). M13 had a significantly higher BG than RGM as predicted (Table 4), so a QTL could be located in the MWG077–BCD265B interval (Fig. 2). A comparison of these two QTLs showed an overlapping chromosomal region in the MWG077–Ole1 interval (hatched region, Fig. 2). It is likely that the MWG077–Ole1 interval is the actual BG QTL location, based on the strength of positive analyses in both the 'Morex' and 'Steptoe' genetic backgrounds.

Conservation of QTL locations All QTLs mapped from the different environments (sites and years) are depicted in Fig. 2. In this research, two ME QTLs were mapped from the Pullman experiments and the results between the 2 years were the same (MWG077–BCD265B, 27.9 cM). The combined analysis over all trial environments reinforced the Pullman results (data not shown). One

ME QTL was mapped from the Aberdeen experiment in 2001 only (MWG634–ABG313B, 11.5 cM). There were no overlapping regions between the QTLs from these two locations. However, those QTLs might overlap in the ABG313B–MWG077 interval of 2.1 cM, where the exact location of the crossover could not be identified in corresponding isolines (M13 and S2). Additional markers within this interval could test this possibility. Four DP QTLs were mapped from four of the five trial environments. The 2.1-cM ABG313B–MWG077 interval overlaps all four mapped intervals. The overall combined analysis (data not shown) singled out only this 2.1-cM interval as well. This interval is the most likely DP QTL location and could be a target DP QTL in future study, even though the QTL direction effect is different in one of the QTLs (from Pullman 2001, Table 4; Fig. 2). It just means there could be another QTL site along the target chromosome interval of this study. All DP QTLs detected were mapped through 'Morex' isolines, that is, mapped by replacing the 'Morex' segment with corresponding 'Steptoe' segments in the 'Morex' genetic background. There were a total of six AA QTLs mapped. Individually mapped AA QTLs span relatively long chromosome intervals up to the entire target region (MWG634–BCD265B) of 41.5 cM (including the combined analysis results). However, reciprocal backcross isolines and multiple location analyses allowed for the identification of two relatively short intervals that are the most likely QTL locations. These are the MWG634–WG622 interval of 0.7 cM, which is an overlapping segment of four separate analyses and the MWG077–CDO122 interval of 17.4 cM, which is the overlapping segment of five analyses. The two BG QTLs, which mapped to 21.5 cM and 27.9 cM intervals, most likely identify a single QTL in the common MWG077–Ole1 interval of 7.9 cM. The combined analysis results spanned the entire 41.5-cM target interval.

The five-environment (three locations and 2 years) set of analyses identified 15 QTLs for the four malting-quality traits. Of these 10 were mapped in the 'Morex' background and only five in the 'Steptoe' background. The reason for this disparity is not clear. However, it could be related to the structural and/or functional integrity of each given QTL. That is to say that the gene(s) within a QTL region and its genetic background act in concert to be fully functional. Whereas 15 QTLs were mapped through the individual analyses by taking into account reciprocal backcross isoline results and overlapping QTL segments, only six QTLs are conservatively estimated, two each for ME and AA and one each for DP and BG as described above. Individual conservative QTL segments range from 0.7 cM to 27.9 cM.

Another way of looking at the results of this study is to consider the relatedness of the four traits analyzed. Diastatic power and AA are related in that DP includes both α- and β-amylase activity. Malt extract is related to the other three traits in that amylolytic and glucanolytic enzyme activity release soluble and fermentable saccharides, which defines ME. Protein content and malt modification are also related to these traits as pointed out above. It should be noted though, that no protein QTL was found in the target chromosome region of this study (Hayes et al. 1993, 1994; Han and Ullrich 1994). Modification per se was not measured. Therefore, common areas of QTL interval overlap among traits may actually represent one QTL or gene or gene family that affects more than one trait (Fig. 2). This could even involve modification directly or indirectly. Such common areas of overlap include the 0.7-cM interval of MWG634–WG622 or the 11.5-cM interval of MWG634–ABG313B and the 2.1-cM interval of ABG313B–MWG077 or the 4.3-cM interval of ABG313B–CDO699. These intervals include all or nearly all of the fine-mapped QTL intervals, especially if the uncertain crossover intervals are included (Figs. 12).

The combined results from this experiment demonstrate the complexity of malting-quality trait inheritance. First, the MWG634–BCD265B region studied affected all four malting-quality traits. This region spans beyond the previously identified QTL2 region of WG622–BCD402B (Han and Ullrich 1994; Hayes et al. 1994). The newly mapped extended QTL2 region could help explain why low molecular marker-assisted selection responses were observed using only the WG622–BCD402B region (Han et al. 1997). Second, this research would suggest that (an) additional undetected QTL(s) affecting malting quality exist(s) in the barley genome. Third, the fine-mapped QTL2 region is still a malting-quality QTL complex, which carries unique and common region(s) for several malting-quality traits—not unusual for malting quality (Zale et al. 2000). Fourth, environment effects were obvious. In a previous analysis from the S/M cross, different environments often located a QTL into different sized intervals, and a number of minor QTLs were detected in limited environmental situations (Hayes et al. 1994). In this research, no QTL was detected in all five field environments. However, relative consistency was also significant for all four traits.

By comparing and combining results from multiple environments (locations and years), the reciprocal-substitution method allowed mapping of conserved chromosomal regions for a QTL or locate a QTL to a more accurate location along a chromosome than an original QTL analysis using interval mapping. We believe that the conserved segments more accurately reflect true QTL regions and could be used for further QTL fine mapping and QTL gene cloning, as well as for molecular marker-assisted selection in breeding. This study indicated some difficulties in detecting the effect of a given QTL under certain conditions, which could result in some difficulties in molecular breeding for quantitative traits. Widely conserved QTL chromosomal regions might be targets for selection to maintain malting quality, while selection for unique QTL regions might lead to new improvements. Specifically in this study, the conserved MWG634–ABG313B (11.5 cM) and/or the ABG313B–CDO669 (4.3 cM) segments are the most logical targets for further research and marker-assisted breeding. Further studies are required to understand the gene composition and regula-

tion of malting-quality QTL in the MWG634–BCD265B interval so as to efficiently manipulate malting-quality QTLs in practical barley breeding programs.

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References

American Society of Brewing Chemists (1976) In: Kneen E (ed) Methods of analysis, 7th edn. American Society of Brewing Chemists, St Paul., Minnesota

Briggs DE (1978) Barley. Chapman and Hall, London, p 612

Gao W, Clancy JA, Han F, Prada D, Kleinhofs A, Ullrich SE, North American Barley Genome Project (2003) Molecular dissection of a dormancy QTL region near the chromosome 7 (5H) L telomere in barley. Theor Appl Genet 107:552–559

Han F, Ullrich SE (1994) Mapping of quantitative trait loci associated with malting quality in barley. Barley Genet Newsl 23:84–97

Han F, Ullrich SE, Chirat S, Menteur S, Jestin L, Sarrafi A, Hayes PM, Jones BL, Blake TK, Wesenberg DM, Kleinhofs A, Kilian A (1995) Mapping of β-glucan content and β-glucanase activity loci in barley grain and malt. Theor Appl Genet 91:921–927

Han F, Romagosa I, Ullrich SE, Jones BL, Hayes PM, Wesenberg DM (1997a) Molecular marker-assisted selection for malting-quality traits in barley. Mol Breed 3:427–437

Han F, Ullrich SE, Kleinhofs A, Jones B, Hayes PM, Wesenberg DM (1997b) Fine-structure mapping of the barley chromosome 1 centromere region containing malting-quality QTLs. Theor Appl Genet 95:903–910

Han F, Ullrich SE, Clancy JA, Romagosa I (1999) Inheritance and fine mapping of a major barley seed dormancy QTL. Plant Sci 143:113–118

Hayes PM, Liu BH, Knapp SJ, Chen F, Jones B, Blake T, Franckowiak J, Rasmusson D, Sorrells M, Ullrich SE, Wesenberg D, Kleinhofs A (1993) Quantitative trait locus effects and environmental interaction in a sample of North American barley germplasm. Theor Appl Genet 87:392–401

Hayes PM, Iyamabo O, the North American Barley Genome Mapping Project (1994) Summary of QTL effects in the 'Steptoe' × 'Morex' population. Barley Genet Newsl 23:98–143

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hayes P, Chen FQ, Lapitan N,Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffman D, Skadsen R, Steffenson BJ (1993) A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor Appl Genet 86:705–712

Korstanje R, Paigen B (2002) From QTL to gene: the harvest begins. Nat Genet 31:235–236

Marquez-Cedillo LA, Hayes PM, Jones BL, Kleinhofs A, Legge WG, Rossnagel BG, Sato K, Ullrich SE, Wesenberg DM, the NABGP (2000) QTL analysis of malting quality in barley based on the doubled haploid progeny of two elite North American varieties representing different germplasm groups. Theor Appl Genet 101:173–184

Mather DE, Tinker NA, LaBerge DE, Edney M, Jones BL, Rossnagel BG, Legge WG, Briggs KG, Irvine RB, Falk DE, Kasha KJ (1997) Regions of the genome that affect grain and malt quality in a North American two-row barley cross. Crop Sci 37:544–554

SAS Institute (1996) SAS user's guide, version 6.12. SAS Institue, Cary, N.C.

Shewry PR, Darlington H (2002) The proteins of the mature barley grain and their role in determining malting performance. In: Slafer GA, Molina-Cano JL, Savin R, Araus JL, Romagosa I (eds) Barley science recent advances from molecular biology to agronomy of yield and quality. Haworth, Oxford, pp 503–521

Ullrich SE, Han F, Jones BL (1997) Genetic complexity of the malt extract trait in barley suggested by QTL analysis. J Am Soc Brew Chem 55:1–4

Zale JM, Clancy JA, Ullrich SE, Jones BL, Hayes PM, North American Barley Genome Project (2000) Summary of barley malting-quality QTLs mapped in various populations. Barley Genet Newsl 30:44–54