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# Fine structure mapping of the barley chromosome-1 centromere region containing malting-quality QTLs

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**Abstract** Current techniques for quantitative trait locus (QTLs) analyses provide only approximate locations of QTLs on chromosomes. Further resolution of identified QTL regions is often required for detailed characterization. An important region containing malting-quality QTLs on barley (*Hordeum vulgare* L.) chromosome 1 was identified by previous QTL analyses in a Steptoe × Morex cross. This region contains two putative adjacent overlapping QTLs, each of which has effects on malt-extract percentage, α-amylase activity, diastatic power, and malt β-glucan content. All favorable alleles for these traits are attributed to Morex. The objective of the present study was fine structure mapping of this complex QTL region to elucidate whether these two putative overlapping QTLs are really one QTL. Another question was whether the apparently overlapping QTLs are due to the pleiotropic effects of a single gene, or the independent effects of several genes. A high-resolution map in the target region was developed which spans approximately 27 cM. Molecular-marker-assisted backcrossing was employed to create isogenic lines with a Steptoe background differing only in the region containing the QTLs of interest. A total of 32 different recombinants were identified, of which eight most-informative isogenic lines plus one reconstructed Steptoe control were selected for field testing. The additive effects on malt-extract percentage, α-amylase activity, diastatic power, and malt β-glucan content from eight isogenic lines were calculated based on malting data from three locations. By comparing the significant additive effects among isogenic lines carrying different Morex fragments, two QTLs each for malt extract and for α-amylase, and two to three for diastatic power were identified in certain environments and resolved into 1–8-cM genome fragments. There was a significant QTL × environment interaction for diastatic power, and there are indications that epistatic interactions for malt β-glucan content occur between the QTLs on chromosome 1 and QTLs on other chromosomes.

**Key words** Fine mapping · Additive effects · Marker assisted backcrossing · Isogenic lines

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## Introduction

Quantitative trait locus (QTL) analysis provides a powerful tool to locate the genes controlling quantitative traits on chromosomes. QTL analysis has been undertaken in virtually all plant species with molecular-marker linkage maps available. A comprehensive map of the barley (*Hordeum vulgare* L.) genome from a Steptoe × Morex cross consisting of 295 loci was constructed by the North American Barley Genome Mapping Project (Kleinhofs et al. 1993), and has been extended to include over 500 markers. This map was used to map QTLs affecting agronomic and quality traits by the interval mapping method (Hayes et al. 1993; Han et al. 1995). An important region of chromosome 1 near the centromere containing two putative adjacent overlapping QTLs for malt-extract content, α-amylase activity, diastatic power, malt β-glucan content, malt β-glucanase activity and/or dormancy was

initially identified and resolved in 29.0-cM and 16.8-cM intervals (Hayes et al. 1993; Ullrich et al. 1993; Han et al. 1995; Oberthur et al. 1995). These two intervals are flanked by markers *Brz* and ABG011, and ABG011 and *Amy2*, respectively (see Fig. 1). This complex QTL region also showed the largest and the most consistent effects for these traits over multiple locations and years (Hayes et al. 1993; Han and Ullrich 1994; Han et al. 1995), which makes this region a good candidate for further study. All favorable alleles (high malt-extract content, high amylase activity, low malt $\beta$-glucan content, and low dormancy) are attributed to Morex.

The complex malting-quality QTL region on chromosome 1 leads to three important questions: (1) whether the two overlapping QTLs on chromosome 1 are really one QTL since they are adjacent, and current QTL analysis techniques may not resolve them adequately; (2) whether the overlapping QTLs are due to pleiotropic effects of a single gene or independent effects of several loci; and (3) since this region is flanked by the *Brz* (the locus for UDP glucose flavanol 3-D-glucosyl transferase) and *Amy2* (the structural gene coding for low pI $\alpha$-amylase) loci, is the *Amy2* locus responsible for the $\alpha$-amylase part of this complex QTL region? The answers to these questions can not be gained by current technologies of QTL analysis, since most QTLs were localized in more than 10-cM regions. The total number of genes in rice has been estimated to be 20–40 000 (Song et al. 1996). If there are a similar number of genes in barley, a 10-cM region may contain up to 160–320 genes based on the comprehensive barley genetic maps averaging about 1300 cM over seven chromosomes (Kasha et al. 1995). Therefore, further resolution of the identified QTL regions is required for detailed study and precise genetic manipulation. The objective of the present study was to fine map this complex QTL region by breaking down the large complex region into small segments. More accurate localization can be obtained by making backcrosses to produce isogenic lines differing only in the region containing a QTL of interest. This will restrict the genetic variance to very specific regions and make it possible to dissect the interval by examining various recombinants between flanking markers (Paterson et al. 1990).

## Materials and methods

### High-resolution and saturation mapping of the target region

Relatively high-resolution mapping of the target region was performed with recombinants in the *Brz–Amy2* interval identified from 355 gametes. These 355 gametes came from 150 doubled-haploid lines (DHLs), developed by the *H. bulbosum* method, which were used for the initial genetic mapping (Kleinhofs et al. 1993), 104 DHLs developed by *H. bulbosum* method (courtesy of Dr. Patrick Hayes), and 101 DHLs developed by anther culture (Devaux et al. 1995). All DHLs were derived from Steptoe × Morex crosses. Locus designations were described by Kleinhofs et al. (1993).

Saturation mapping of the target region was performed by random mapping of over 200 RFLP markers. Comparative mapping between the target region and the rice genome was also performed. The rice markers from the syntenic region were then mapped to the target barley chromosome region.

### Construction of isogenic lines

A population consisting of 150 DHLs derived from a Steptoe × Morex cross was used for the initial mapping of the malting-quality QTL. Morex carries all favorable alleles for the malting-quality QTL in this study. For the purpose of constructing isogenic lines, a DHL was selected which carries Morex alleles at the target QTL region, and a high proportion of Steptoe alleles in all other genome regions, to backcross to Steptoe (i.e., placing Morex malting-quality alleles at the target QTL region in the Steptoe background). The line, DH73, which carries Morex alleles in the QTL region on chromosome 1 and 65.1% Steptoe alleles in other genome regions was chosen and backcrossed twice to Steptoe. Two hundred and ten $BC_2$ $F_1$ plants were genotyped using all available molecular markers in the target region and some of the markers from other regions which previously had Morex alleles. The procedures for genotyping these markers are described by Kleinhofs et al. (1993). Twenty two different recombinants in the target region were identified. To facilitate the backcrossing process, plants were maintained in the vegetative growth stage (12-h day and 12-h night) during genotyping. Flowering was induced by increasing daylength to 16 h after desirable genotypes were selected for further backcrossing. In the 250 plants of the $BC_3$ $F_1$ population, a total of 32 different recombinants were identified. These recombinants have an average Morex segment size of 4 cM in the target region and essentially all Steptoe alleles in the background. Among the 32 recombinants, the eight most-informative recombinants plus one regenerated Steptoe control were selected for further testing to resolve the QTL into approximately 5–10 cM segments. Homozygosity of these nine isogenic lines was achieved by selfing, with the appropriate population size calculated based on the assumption that each heterozygous DNA segment behaves as a single gene.

### Phenotyping

The nine isogenic lines, together with Steptoe and Morex, were planted in the field at Aberdeen, Idaho, Klamath Falls, Ore. and Pullman, Wash., in the summer of 1996 in a randomized complete-block design with two replications. Seeding rate, dates and management practices were in accordance with recommended practice at each location. Multiple-environment evaluations of test genotypes were conducted to take into account genotype × environment interactions and the uncertainty of success of any given field trial. Grain samples from each replication of each location were sent to the USDA-ARS Barley and Malt Laboratory of the Cereal Crops Research Unit (CCRU) at Madison, Wis, for malting-quality analyses, mainly malt-extract percentage, $\alpha$-amylase activity, diastatic power, and malt $\beta$-glucan content, following standard ASBC (American Society of Brewing Chemists 1976) procedures.

### QTL-effect analyses

Since the recombinants only carry different Morex segments in the target QTL region in a Steptoe background, the different phenotypic effects of malting-quality traits must be conferred as a result of genes on these segments. The effect of each Morex segment on four related malting-quality parameters (malt-extract percentage, $\alpha$-amylase activity, diastatic power, and malt $\beta$-glucan content) were determined through analysis of variance (ANOVA)

using SAS/STAT (SAS Institute 1991). ANOVA was performed for each and over combined locations. If there was no genotype × location interactions, the combined phenotypic effects of each isogenic line were then presented. Since there are no dominant effects in homozygous populations, only additive effects of each segment were calculated (Falconer 1981). Then, by comparing the effects from different segments, the QTLs affecting the four related malting-quality traits can be located precisely. If like-effects for malting quality are shared by an overlap region of different segments, a QTL can be located in the overlap region; if effects for malting quality are unique to a segment region, a QTL can be located in the unique region (Paterson et al. 1990).

## Results

### Saturated and high-resolution map of the barley chromosome-1 *Brz* to *Amy2* region

The target region was saturated with 32 markers; 18 new and 14 from the initial map of Kleinhofs et al. (1993). The refined molecular-marker linkage map in the target region from *Brz* to *Amy2* spans 27.3 cM compared with 45.8 cM in the earlier map (Fig. 1). Thus, the average distance between markers is less than 1 cM. There were four rice markers, R2736, R1010, RZ612 and RZ143, included in this map as a result of comparative mapping of this region with rice. One large gap of 7.4 cM remained in this region from R1010 to MWG911B, while the smallest interval was 0.3 cM which occurred between six pairs of markers. There were two major revisions in marker-order on the initial map of this region, which reversed the order of markers ABG011 and ABG701, and ABG476 and ABC455 (Fig. 1). The marker order differences, typing errors, and different mapping population sizes may account for the differences observed.

### Genotypes of recombinants

Thirty two recombinants were identified in the $BC_3$ $F_1$ and genotyped at selected loci within the target region (Table 1). Uniformity of the Steptoe genetic

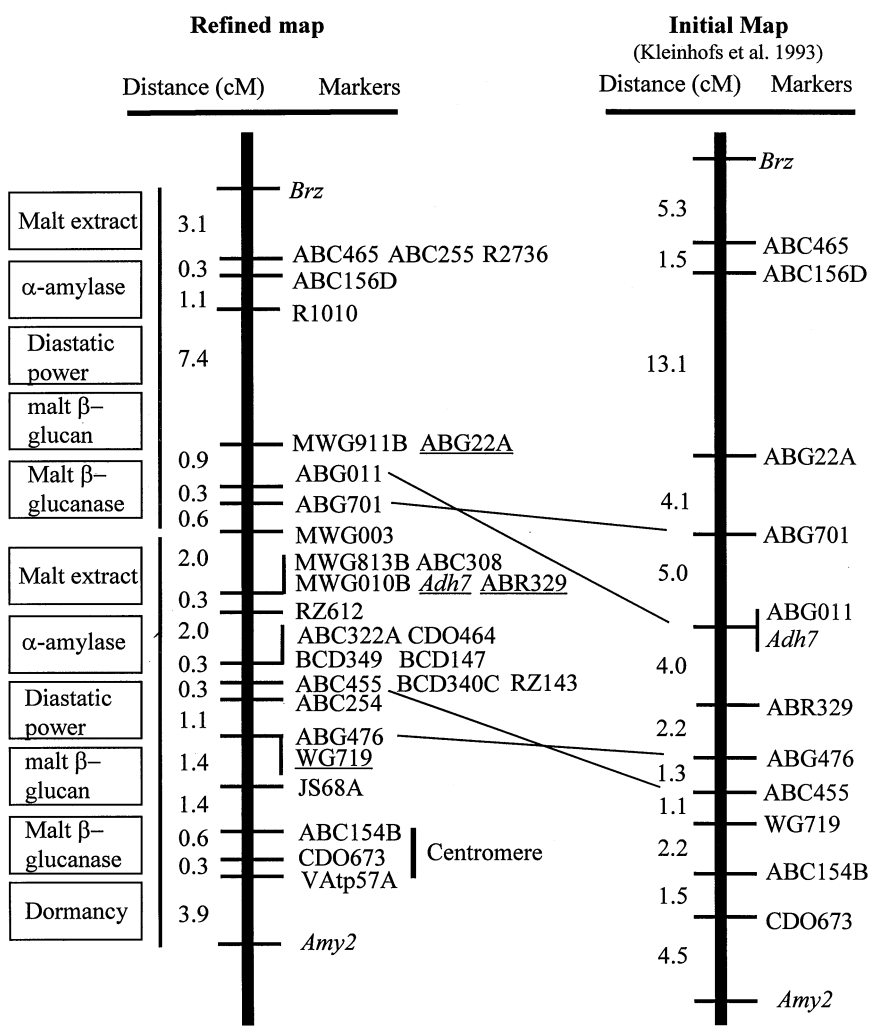



**Fig. 1** Saturated and high-resolution map of the barley chromosome-1 centromere region developed from 355 gametes with a comparison with the initial map of the target region (Kleinhofs et al. 1993). The *underlined* markers indicated that they were only re-mapped in the initial mapping population. Two putative adjacent overlapping QTLs for malt-extract percentage, α-amylase activity, diastatic power, malt β-glucan content, malt β-glucanase activity, and/or dormancy are indicated for each trait (Hayes et al. 1993; Ullrich et al. 1993; Han et al. 1995). The order of the traits indicated on the map does not imply the relative location of each QTL

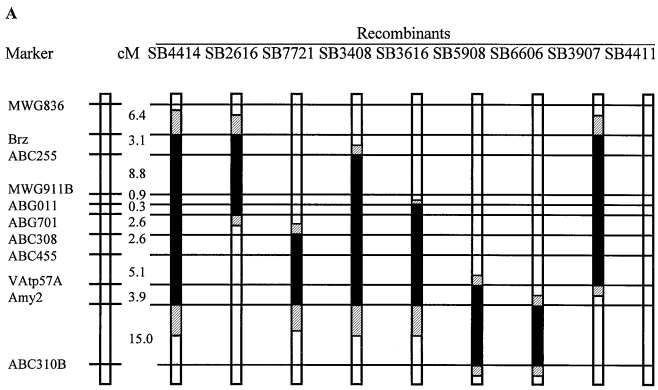
**Table 1** Selected marker genotypes of 32 recombinants in the target region at the $BC_3$ $F_1$. A – homozygous Steptoe alleles, H – heterozygous

| Markers | Recombinants | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | | | | | | | | II | | | | III | | | | | | |
| | 66 | 54 | 55 | 59 | 77 | 36 | 11 | 34 | 39 | 28 | 26 | 72 | 117 | 87 | 51 | 4 | 13 | 110 | 119 |
| *Brz* | A | A | A | A | A | A | A | A | H | H | H | H | H | H | H | H | H | H | H |
| ABC255 | A | A | A | A | A | A | A | H | H | H | H | H | A | H | H | H | H | H | A |
| MWG911B | A | A | A | A | A | A | H | H | H | H | H | A | H | A | A | H | H | A | A |
| ABG011 | A | A | A | A | A | H | H | H | H | H | H | A | H | A | A | A | H | A | A |
| ABG701 | A | A | A | A | A | H | H | H | H | H | H | A | H | A | A | A | H | A | A |
| ABC308 | A | A | A | A | H | H | H | H | H | H | A | A | H | H | A | A | A | A | A |
| ABC455 | A | A | A | H | H | H | H | H | H | H | A | A | H | H | H | A | A | A | A |
| ABG476 | A | A | H | H | H | H | H | H | H | A | A | A | H | H | H | A | A | H | H |
| VAtp57A | A | H | H | H | H | H | H | H | H | A | A | A | H | H | H | H | H | H | H |
| *Amy2* | H | H | H | H | H | H | H | H | A | A | A | A | H | H | H | H | H | H | H |

| Markers | Recombinants | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IV | | | | | | | | | | V | | VI |
| | 98 | 25 | 50 | 37 | 91 | 93 | 6 | 97 | 115 | 125 | 100 | 112 | 44 |
| *Brz* | A | A | A | A | A | A | A | A | A | A | A | A | H |
| ABC255 | A | A | A | A | A | A | A | A | A | H | H | H | H |
| MWG911B | A | A | A | A | A | A | H | H | H | H | H | H | H |
| ABG011 | A | A | A | H | H | H | H | H | H | H | H | A | H |
| ABG701 | A | H | A | H | H | H | H | H | H | H | H | A | H |
| ABC308 | A | A | H | H | H | H | H | H | H | H | A | A | H |
| ABC455 | H | A | H | A | H | H | A | H | H | H | A | A | H |
| ABG476 | A | A | A | A | A | H | A | H | H | H | A | A | H |
| VAtp57A | A | A | A | A | A | A | A | A | H | A | A | H | H |
| *Amy2* | A | A | A | A | A | A | A | A | A | A | H | H | H |

background in the rest of the genome was tested with markers spaced 25 cM apart. Heterozygous alleles at marker loci within the target region were converted to homozygous Morex alleles after selfing. The 32 recombinants were divided into six groups. Group I contained eight isogenic lines with Morex fragments gradually increasing in size from *Amy2* to *Brz*, whereas group II contained four isogenic lines with Steptoe fragments gradually increasing in size from *Amy2* to *Brz*. These recombinants indicated that recombination occurred essentially within each marker interval along the target region. Groups III and IV had isogenic lines with Steptoe and Morex fragments gradually increasing in size outward from the middle of the target region. Some recombinants in Groups III and IV, such as 117, 87, 98, 25 and 50, most probably reflect two independent recombination events in adjacent or nearby intervals as the result of two generations of recombination. A double-crossover event in one generation is much less likely due to the small genetic interval involved. Contrasting lines could be selected from these four groups for assessing QTL effects. Group V contained two rare recombinants (recombinants 100 and 112) each with three crossovers. These presumably resulted from crossover and double-crossover events in two succeeding generations. Group VI contained a line with an intact Morex fragment in the target region.

The eight most-informative recombinants from the above 32 recombinants (Groups I, II, and VI), possessing different homozygous Morex segments in the target region plus one regenerated Steptoe control, were selected for QTL testing. As shown in Fig. 2 (panel A), recombinant SB4414 (derived from recombinant 44) has an intact Morex segment which contributed high malting quality. This line was used as a positive control. Recombinant SB4411 (reconstructed Steptoe) was the negative control. Recombinants SB2616 and SB7721 divide the target region into two subregions with each subregion containing the individual putative overlapping QTLs previously determined by Hayes et al. (1993), Ullrich et al. (1993), and Han et al. (1995) (Fig. 1). Recombinants SB3408, SB3616, SB5908, and




**B**

Additive Effect

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Malt extract (%) | | | | | | | | |
| Pullman, WA | -0.2 | 0 | 0.6 | -0.3 | 0.1 | 0.3 | 0.3 | 0.7 |
| Klamath Falls, OR | 1.0* | 1.5* | 0.5 | -0.5 | 1.9* | 0 | -0.3 | 1.4* |
| Aberdeen, ID | 0.8 | -0.5 | 0.4 | -0.3 | 0.3 | 0.6 | -0.2 | 1.6 |
| Combined Analysis | 0.6 | 0.4 | 0.5 | -0.3 | 0.8 | 0.3 | 0 | 1.3* |
| α-amylase (20 °DU) | | | | | | | | |
| Pullman, WA | 2.3* | 0.6 | 1.5* | -0.3 | 1.1* | -1.0 | -0.1 | 2.7* |
| Klamath Falls, OR | 4.3* | 0.2 | 1.9 | 1.0 | 0.8 | 0.4 | 0.9 | 2.9* |
| Aberdeen, ID | 6.8* | -2.2 | -0.9 | 2.2 | 0.3 | -1.0 | -0.4 | 1.8 |
| Combined Analysis | 3.1* | -0.6 | 0.9 | 1.3 | 0.6 | -0.1 | 0.4 | 2.5* |
| Diastatic power (°ASBC) | | | | | | | | |
| Pullman, WA | 5.5* | 4.0* | -3.0 | 0.5 | 4.0* | 2.0 | -0.5 | 7.5* |
| Klamath Falls, OR | 15.5* | 2.5 | 3.0 | 8.0* | 1.5 | 2.5 | 3.5 | 11.5* |
| Aberdeen, ID | 10.0* | -3.0 | -5.0 | 10.5* | 0 | -3.0 | -4.0 | 3.5 |
| Malt β-glucan (ppm) | | | | | | | | |
| Pullman, WA | -107 | -105 | -43 | 10 | -71 | 129 | 73 | -135* |
| Aberdeen, ID | 2 | -117 | 4 | -109 | 0 | 5 | 5 | -117 |
| Combined Analysis | -53 | -111 | -20 | -50 | -35 | 67 | 39 | -126 |

**Fig. 2** Genotypes of nine isogenic lines in the chromosome-1 centromere region (**A**) and their additive effects on malt-extract, α-amylase, diastatic power, and malt β-glucan (**B**). In panel **A**, the *open bars* represent the Steptoe segments, the *solid bars* represent the Morex segments, and the *hatched bars* represent the uncertain locations of crossovers. In panel **B**, the additive effects from eight isogenic lines were calculated based on the comparison to isogenic line SB4411 which was the regenerated Steptoe control. Significant additive effects are indicated with * at the $\alpha \leq 0.05$ level

SB6606 contain gradually smaller Morex segments. Recombinants SB6606 and SB3907 have the Morex and Steptoe alleles at the *Amy2* locus, respectively. The genotypes of the nine isogenic lines in the target region were also verified with RFLP markers on the DNA isolated from field-grown material.

The nine isogenic lines had essentially a clean Steptoe background. In the $BC_3F_2$, SB3408 and SB3907 were heterozygous at the locus ABG458 on chromosome 6 which had no detectable malting-quality QTLs. SB3616 was heterozygous at the locus MWG813A on the telomere of chromosome 7 which had a minor QTL detected for malt extract only at the Aberdeen, Idaho, location. SB5908 was heterozygous at the locus ABG452 on chromosome 5 which had no detectable malting-quality QTL.

## Additive effects of isogenic lines and implications for QTL locations

Data over the three test locations for malt-extract percentage, α-amylase activity, diastatic power, and the malt β-glucan content of the nine isogenic lines are presented in Table 2. The additive effects of each line were calculated for each trait by comparing the phenotypic value of each line to that of SB4411 (regenerated Steptoe). There were no significant phenotypic differences between SB4411 and Steptoe for the traits in this study. Significant positive effects at $\alpha = 0.05$ for high malt-extract, α-amylase activity, and diastatic power, and low malt β-glucan are indicated in Fig. 2 (panel B).

**Table 2** Malting-quality parameters based on three locations for nine isogenic lines and Steptoe and Morex

| Parameter | Nine isogenic lines | | Steptoe Mean | Morex Mean |
|---|---|---|---|---|
| | Range | Mean | | |
| Malt-extract (%) | 72.9–75.5 | 73.9 | 73.9 | 77.5 |
| α-amylase (20°DU) | 19.1–28.9 | 24.6 | 23.6 | 42.3 |
| Diastatic power (°ASBC) | 49.5–83.0 | 64.1 | 59.3 | 148.9 |
| Malt β-glucan (ppm) | 843–1549 | 1183 | 1466 | 719 |

For malt-extract, there were no significant additive effects detected for the eight isogenic lines at Pullman, Wash. and Aberdeen, Idaho. Significant additive effects were identified for SB3616, SB2616, SB3907, and SB4414 at Klamath Falls, Ore. A common Morex fragment among these four lines is the ABG011–ABG701 fragment with a distance of 0.3 cM. This fragment might be responsible for the QTL effect for malt-extract. However, SB3408 which has the ABG011–ABG701 Morex fragment did not have a significant additive effect. Comparing SB3408 with SB4414, SB2616, SB3907 and SB3616, we speculate that the ABC255–MWG911B Morex fragment contains a gene(s) which had negative effects on malt-extract and, conversely, the *Brz*–ABC255 fragment of 3.1 cM carries a gene(s) responsible for an increase in malt-extract. Based on this hypothesis, SB4414, SB3907 and SB2616 would possess two fragments carrying positive QTL effects for malt extract. This would explain why these three isogenic lines had similar malt-extract values compared with SB3616, which does not contain the presumed negative malt-extract QTL (ABC255–MWG911B).

Significant additive effects for α-amylase activity were detected for SB4414, SB7721, SB3616 and SB3907 at Pullman, Wash., for SB4414 and SB3907 at Klamath Falls, Ore., and for SB4414 at Aberdeen, Idaho. The common fragment shared by SB4414, SB7721, SB3616 and SB3907 is ABC308–VAtp57A, with a distance of 7.7 cM. Again SB3408 did not show a significant additive effect for α-amylase. The explanation proposed above may also apply to this situation. The larger additive effects detected for SB4414 and SB3907 at Klamath Falls, Ore., and Aberdeen, Iaho, indicate that the *Brz*–ABC255 fragment might also carry a QTL for α-amylase. Only SB4414 and SB3907 showed significant additive effects in the combined analysis of all three locations.

For diastatic power, isogenic lines showing significant additive effects were SB4414, SB2616, SB3616 and SB3907 at Pullman, Wash.; SB4414, SB3408 and SB3907 at Klamath Falls, Ore., and SB4414 and SB3408 at Aberdeen, Idaho. The isogenic lines having significant additive effects for diastatic power at Pullman were the same lines having significant additive effects for malt-extract at Klamath Falls, suggesting that the same common fragments ABG011–ABG701, and *Brz*–ABC255, contain the QTLs for both malt-extract and diastatic power. SB3907 and SB4414 had a larger additive effect than SB2616 and SB3616, indicating that there might be another gene(s) in the ABG701–VAtp57A fragment responsible for diastatic power, and that these fragments (genes) interacted with each other. SB3408, which had no detectable additive effect on malt-extract and α-amylase at any location, or on diastatic power at Pullman, showed a significant additive effect on diastatic power at Klamath Falls and Aberdeen, which gives rise to a very complex situation. The gene(s) in the fragment ABC255–MWG911B may not just have a negative effect, but may interact with certain environments and with genes from the fragments of *Brz*–ABC255, ABG011–ABG701, and ABG701–VAtp57A to produce larger positive effects. Since a significant genotype × environment interaction was detected, combined analysis was not performed.

There were no significant additive effects from the eight isogenic lines on malt β-glucan content at Aberdeen, nor in the combined analysis. Only SB3907 showed a significant additive effect on malt β-glucan content at Pullman. Due to a large coefficient of variation (29.9%) in the Klamath Falls β-glucan data, analysis with these data was not performed.

## Discussion

### Recovery of QTL effects

In the original mapping population studies, the additive effects of QTLs in the *Brz–Amy2* interval were 1.0% for malt-extract content, 3.7 20°DU for α-amylase activity, 13.6° ASBC for diastatic power and −408 ppm for malt β-glucan content (Hayes et al. 1993; Han et al. 1995). In the present study, significant ($\alpha \leq 0.05$) additive effects were detected in five isogenic lines (SB4414, SB2616, SB7721, SB3408, SB3616, and SB3907) for certain traits at certain locations. The additive effects detected ranged from 1.0 to 1.9% for malt-extract, 1.1 to 6.8 20°DU for α-amylase, and 4.0 to 15.5° ASBC for diastatic power, and were −135 ppm for malt β-glucan. The comparable additive effects of QTLs on chromosome 1 between the isogenic lines and the original mapping population suggest that there are no apparent epistatic interactions between these QTLs and the QTLs from the other genome regions, except for the malt β-glucan content QTL. From the plant breeding point of view, this result is very significant in that breeders should have good success in transferring favorable alleles and the effects of malting-quality QTLs from one genetic background to another. Most of the detectable additive effects came from SB4414 and SB3907 which contain almost the intact QTL region, indicating that there may be interactions among the QTLs within this region.

There appear to be at least two genes for malt-extract and two genes for α-amylase with one of each in the *Brz*–ABC255 fragment of 3.1 cM, and one each in the adjacent ABG011–ABG701 and ABC308–VAtp57A fragments of 0.3 and 7.7 cM, respectively. Depending upon where the crossovers occurred and the distance between the markers, the actual fragment size may be larger. For example, the ABG011–ABG701 Morex fragment in SB2616 and SB3616 may actually extend close to MWG911B and ABC308 with a distance of about 3.8 cM. For diastatic power, besides the two genes in the common fragments with malt-extract, there may be an additional gene in the ABG701–VAtp57A fragment. For QTLs of traits identified in a common fragment, there may be pleiotropy, and/or multigene-family situations, since these malting-quality traits are related to each other biochemically and physiologically (Fincher and Stone 1993). However, close linkage can not be excluded, since these fragments are still quite large. The different, but closely linked, genes may represent a cluster of functionally related genes, as was observed by Paterson et al. (1990) in their QTL fine mapping study. The hypothesis that the ABC255–MWG911B fragment may contain the genes responsible for malting-quality traits and strong interactions with the environment is currently being tested. Recombinants with Morex alleles in the ABC255–MWG911B fragment, with Steptoe alleles in ABC255–MWG911B fragment and Morex alleles in the *Brz*–ABC255 and MWG911B–*Amy2* fragments have been selected for field and quality testing. In actual malting-quality breeding, removing the ABC255–MWG911B Morex fragment may stabilize genotype × environment interactions, thus resulting in varieties with better adaptation for malting quality.

These initial results provide useful information for continuing fine-mapping efforts. The further fine-structure mapping and characterization of the QTL region of interest will focus on much smaller fragment(s). Since a large number of valuable recombinants have already been developed in the region, subsequent dissecting efforts in this region should be promising.

### Structural genes and QTL effects

Morex had a much higher malt α-amylase activity than Steptoe (42.3 and 23.6 20°DU, respectively), but SB5908 and SB6606 which have Morex alleles for the α-amylase structural gene (*Amy2*) showed very low α-amylase activity (23.4 and 23.9 20°DU, respectively).

The additive effect on α-amylase activity from SB5908 was even negative compared to regenerated Steptoe (SB4411) (Fig. 2). In contrast, SB3907 which had the Steptoe allele at the α-amylase structural gene but Morex alleles at the α-amylase QTL had high α-amylase activity (26.0 20°DU). Therefore, the *Amy2* locus does not appear to be responsible for the QTL effects on α-amylase activity. Although Steptoe and Morex had quite different malt α-amylase activity, they may have similar alleles at the structural gene. Thus, α-amylase activity QTLs may come from presumed regulatory genes which encode proteins interacting with the structural genes or their products. These could be any number of transcription factors, enhancers, activators, or inhibitors acting at the transcriptional or translational levels. Continued fine mapping and ultimate cloning of QTLs will further elucidate the complex nature of these malting-quality QTLs.

## Strategies for molecular-marker-assisted backcrossing

Molecular-marker-assisted backcrossing was employed to create isogenic lines for fine-structure mapping of QTLs in this study. To facilitate the backcrossing process, plants were maintained in vegetative growth in the greenhouse during genotyping. Flowering was induced by increasing daylength after desirable genotypes were selected for further backcrossing. In this way, genotyping and backcrossing can be achieved in the same generation. For obtaining isogenic lines in the target region of this study, recombinants were identified in each backcrossing population followed by selection for the recurrent parent background. An alternate approach was to transfer the target region into the recurrent parent background followed by the generation of recombinants after the last backcrossing. The second approach was employed to create isogenic lines for fine-mapping of dormancy QTLs on chromosome 7. The second approach was preferred since it allowed work with small populations until the generation of recombinants. At that time, the number of progeny examined was based on the number of recombinants desired. However, the identification of desirable recombinants was somewhat faster with the first approach. Three generations of molecular-marker-assisted backcrossing were usually sufficient to remove the donor parent background. To obtain homozygous isogenic lines, one generation of selfing was effective with the appropriate population size calculated based on the assumption that each heterozygous DNA segment behaved as a single unit (i.e., gene). The whole process (starting with backcrossing to obtain homozygous isogenic lines) took less than 24 months. These strategies are very useful not only to create isogenic lines for fine structure mapping of QTLs, but also to transfer desirable QTLs to elite varieties in applied breeding programs.

## Approaches for fine-structure mapping

Fine-structure mapping of QTLs was first proposed by Paterson et al. (1990). They used a method, called substitution mapping, which utilizes meiotic recombinants. These recombinants were identified by all available molecular markers in the target region and subjected to QTL analysis. Precise QTL locations then were assigned by comparing QTL effects of these recombinants carrying different chromosome fragments. They performed phenotyping and QTL analyses on $BC_2$ $F_2$ plants (i.e., segregating populations) so that both additive and dominant effects could be determined. However, since they were locating several QTLs at the same time, there might be interactions among the QTLs which actually interfere with the detection of a specific QTL effect. Moreover, two backcrosses are not sufficient to remove all donor parent alleles in order to achieve a uniform genetic background. Constructing isogenic lines which differ only in the target region provides another approach to assay recombinant chromosomes (Paterson et al. 1990). We employed this second approach for the fine mapping of only one chromosome region at a time by: (1) performing three backcrosses to obtain a more uniform genetic background, and (2) selecting homozygous recombinants in the $BC_3$ $F_2$. The advantages of this approach are as follows: (1) with only one chromosome region under study and a uniform genetic background, QTL effects identified from recombinants will clearly come from the target region; (2) homozygous recombinants can be tested extensively in different environments to detect small effects; and (3) further dissection of QTL fragments can be achieved by additional mating to the recurrent parent and checking only the target region for recombinants. The disadvantages of this isogenic line approach are: (1) the effect of a target QTL may not be detected if there are epistatic interactions with QTLs on other genome regions, which may be the case for the malt $\beta$-glucan content QTL in this study; and (2) some very small donor chromosome segments may persist in isogenic lines to interfere with target QTL effects, but such segments should occur at random in large segregating populations. The use of several different lines for a segment can solve the second problem (Paterson et al. 1990). Putting unfavorable alleles into a favorable background may solve the first problem, i.e., the opposite of what was done in this study.

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