QTL Mapping for Enzyme Activity and Thermostability of β-Amylase in Barley (Hordeum vulgare L.)

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We investigated the QTLs for the enzyme activity and thermostability in β-amylase in barley using two doubled haploid line (DHL) populations. QTLs for the enzyme activity were detected on chromosomes 1H and 2H in the Steptoe/Morex DHLs and on chromosome 5H in the Harrington/TR306 DHLs, respectively. Some of these QTLs were close to the QTLs for the diastatic power and grain protein which represent malt quality characteristics. We observed two QTLs for the thermostability in the Steptoe/Morex DHLs. Position of one major QTL was the same as that of the β-amylase structural gene locus on chromosome 4H, suggesting that thermostability is controlled by multiple alleles of the locus. The other QTL with a negligible effect was located on the short arm of chromosome 2H. However, no QTLs were observed in the Harrington/TR306 DHLs because the difference in the thermostability value in the DHLs was not appreciable.

Key Words: Hordeum vulgare, QTL mapping, β-amylase, thermostability, enzyme activity.

Introduction

In barley, starch-degrading enzymes in seeds such as α-, β-amylase and α-glucosidase are related to beer production. The activities of these enzymes are important for the efficiency of the mashing process. Especially, the activity of the β-amylase enzyme affected considerably the diastatic power of malt. Some QTLs for malt quality characteristics were detected, such as extract, diastatic power, nitrogen content and β-glucanase activity (Han et al. 1995, 1997, Ozziel et al. 1996). It is important to identify the chromosome location of the QTLs for each component enzyme of starch degradation, such as α-, β-amylase and α-glucosidase for effective marker-assisted selection (MAS) in diastatic power improvement.

On the other hand, the thermostability of these enzymes is also important for the efficiency of starch degradation in the mashing process. Especially the thermostability of β-amylase is a limiting factor under the high temperature conditions in a mashing process because it is relatively lower than that of other component enzymes of diastatic power in malt. Therefore, it is essential that the activity of β-amylase be maintained at high temperatures. We demonstrated that most of the β-amylases display three main thermostability types. Using Japanese malting barley progeny, we also showed that this trait is related to one characteristic of malt quality, the apparent attenuation limit (AAL) which is related to the fermentability (Kihara et al. 1998). Similar results were reported in Australian barley lines (Eglinton et al. 1998). We carried out genetic and molecular studies including the detection of the gene for the control of β-amylase thermostability and the screening of new thermostability types for utilization in practical breeding. The seed specific β-amylase gene, Bmy1 is located on the long arm of chromosome 4H. We confirmed that this β-amylase structural gene controls the thermostability by an expression analysis of different β-amylase genes in E. coli (Kaneko et al. 2000). As a result of an investigation of genetic resources, we identified the geographical distribution and detected a new mutant gene, i.e. β-amylase-less-mutant and a highly thermostable mutant (Kihara et al. 1999, Kaneko et al. In press). However, it remains to be determined whether only the β-amylase structural gene controls the thermostability and how the gene affects the trait.

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Materials and Methods

Plant materials

Steptoe/Morex and Harrington/TR306 DHLs were used for QTL analysis of the β-amylase enzyme activity and thermostability. The former was a cross between six-row feed and six-row malting varieties, and the latter was a cross within two-row malting barley. Both plant materials and genotype data of the two DHLs were supplied by the North
American Barley Genome Mapping Project (NABGMP). Seed materials from the 1994 crop in the Kurashiki field, Okayama, were used for the enzyme assay.

Assay of β-amylase enzyme activity and thermostability

The extraction and assay procedures for the enzyme were the same as those previously reported (Kihara et al. 1998). Four seeds were crushed and incubated in 1 ml of 50 mM acetate buffer (pH 5.5) containing 10 mM dithiothreitol (DTT) at 4°C for 10-15 hours with reciprocal shaking at 120 rpm. The extract was centrifuged at 18,500 x g for 10 min, and the supernatant was used as the crude enzyme solution. Each sample was diluted (100-fold) with 50 mM MOPS buffer (pH 7.1) containing 1% Bovine Albumin Fraction V, and 30 µl samples were incubated at 57°C for 30 min for the heat treatment. The β-amylase activity of the treated and untreated samples was measured at 37°C using a 2,4-dichlorophenyl β-maltopentaoside kit (DACOLOR AMY, Ono Chemical Co., Osaka, Japan). The absorbance value of an untreated sample indicates the level of the β-amylase enzyme activity. We then used the standardized value of absorbance in the untreated sample/kernel weight as an activity index for the QTL analysis of the β-amylase enzyme activity. The relative remaining activity (%) was calculated as the activity with heat treatment/activity without treatment to evaluate the β-amylase thermostability.

Isoelectric focusing (IEF) analysis

The β-amylase extract obtained by the method described above was analyzed by electrophoresis using the PhastSystem and ready-made gels (PhastGEL IEF 4-6.5; Amasiam Pharmacia Co. California, USA). The gels were incubated at 37°C in 1% starch for 30 min, followed by staining with a KI-I2 solution.

QTL analysis

The QTL analysis was performed using genotype data of RFLP markers for the 150 lines in the Steptoe/Morex DHLs and 146 lines in the Harrington/TR306 DHLs, referred to as NABGMP. The QTL analysis was performed using a computer program, Mapmaker QTL (Lincoln et al. 1993) with the backcross population analysis mode. The threshold of log-likelihood (LOD) score was set at 2.0 for detecting the QTLs.

Results

QTL analysis of β-amylase enzyme activity

The activity index value was distributed more widely in the Steptoe/Morex DHLs than in the Harrington/TR306 DHLs (Fig. 1a, b). Both parents of the Harrington/TR306 DHLs showed higher values (11.0, 10.3) than those of the progeny. Table 1 and Fig. 3 show the results of the QTL analysis of the β-amylase enzyme activity. In the Steptoe/Morex DHLs, QTLs were detected at the end of the short arm of chromosome 1H and the short arm of chromosome 2H. The LOD scores of each QTL were 7.03 and 7.02, and the values explaining the variance were 20.7% and 20.1%, respectively. In both QTLs, the alleles from Morex contributed significantly to the enzyme activity value. In the Harrington/TR306 DHLs, a QTL with a negligible effect (LOD score = 2.83) was detected on the short arm of chromosome 5H. No common QTLs were observed between the two DHLs. Fig. 1a and Fig. 1b show the distribution of the activity index values among the genotypes of the QTL loci in the Steptoe/Morex DHLs and Harrington/TR306 DHLs, respectively. Fig. 1a indicates a significant and cumulative effect of two QTLs from Morex, while Fig. 1b shows that the effect of ksuA1B as QTL on chromosome 5H in the Harrington/TR306 DHLs was very limited.

QTL analysis of β-amylase thermostability

In the Steptoe/Morex DHL population, the thermostability values of the parents were 37.3% in Steptoe and 33.4% in Morex, respectively, whereas the values for the DH lines were widely distributed between 22.7% and 44.4% (Fig. 2a, b). Two QTLs were mapped on the short arm of chromosome 2H (LOD score = 2.88, Variance ex-

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**Table 1.** Location and effect of QTLs on the β-amylase enzyme activity and thermostability

<table>
<thead>
<tr>
<th>Trait* / DHL</th>
<th>Chr.</th>
<th>Marker interval</th>
<th>LOD of peak</th>
<th>Variance explained (%)</th>
<th>Weight**</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC (S/M)</td>
<td>1H</td>
<td>MWG938-MWG036A</td>
<td>7.03</td>
<td>20.7</td>
<td>1.68 M</td>
</tr>
<tr>
<td></td>
<td>2H</td>
<td>ABC156A-MWG858</td>
<td>7.02</td>
<td>20.1</td>
<td>1.66 M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multilocus</td>
<td>16.19</td>
<td>40.9</td>
<td></td>
</tr>
<tr>
<td>AC (H/T)</td>
<td>5H</td>
<td>CDO348B-ksuA1B</td>
<td>2.83</td>
<td>14.3</td>
<td>0.82 T</td>
</tr>
<tr>
<td>RTS (S/M)</td>
<td>2H</td>
<td>ABG008-RbcS</td>
<td>2.88</td>
<td>8.8</td>
<td>2.75 M</td>
</tr>
<tr>
<td></td>
<td>4H</td>
<td>Bmy1-ksuH11</td>
<td>16.11</td>
<td>39.5</td>
<td>5.81 S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multilocus</td>
<td>18.90</td>
<td>44.8</td>
<td></td>
</tr>
</tbody>
</table>

* AC: Enzyme activity, RTS: Thermostability, (S/M): Steptoe/Morex DHLs (H/T): Harrington/TR306 DHLs
** M: Morex, S: Steptoe allele.
plained = 8.8\%) and on the long arm of chromosome 4H (LOD score = 16.11, Variance explained = 39.5\%) (Table 1, Fig. 4). The QTL on chromosome 4H was near the Bmy1 locus which corresponds to the \( \beta \)-amylase structural gene. The allele from Steptoe increased the stability, whereas the QTL on chromosome 2H exhibited a negligible phenotypic effect and the allele from Morex showed a positive effect. Fig. 2a shows the distribution of the thermostability values, in reference to the genotype, of two linked markers ABC008 and Bmy1 located on chromosome 2H and 4H, respectively. The effect of Bmy1 was clearly detected in this figure.

In the Harrington/TR306 DHLs, the thermostability values of Harrington and TR306 were almost the same, 34.6\% and 33.7\%, respectively, whereas in the case of the Steptoe/Morex DHLs, the thermostability values of the DHL population showed a narrow range (25.8-35.3\%) (Fig. 2b). As a result, no significant QTLs were observed in these DHLs.

**IEF analysis of \( \beta \)-amylase isozyme**

Since the IEF pattern of \( \beta \)-amylase differed between Steptoe and Morex (Fig. 5), a segregation of the IEF pattern was observed in the Steptoe/Morex DHLs. As a result, the IEF pattern and the RFLP of Bmy1 cosegregated in the DHLs with 13 missing lines for which Bmy1 mapping data were lacking. No polymorphism of the IEF pattern was observed between Harrington and TR306 and these IEF patterns were the same as that of Morex.

**Discussion**

**QTL analysis of \( \beta \)-amylase enzyme activity**

Because the properties of various enzymes in malt are important for beer production, several QTL analyses of seed enzyme activity have been reported, such as \( \beta \)-glucanase (Han et al. 1995) and total diastatic power (Oziel et al. 1996, Han et al. 1997). However, no study had been reported on QTL analysis of the \( \beta \)-amylase enzyme activity in...
Fig. 2. Frequency distribution of β-amylase thermostability. a; the distribution of ABG008 and Bmy1 genotype groups in Steptoe/Morex DHLs. b; the distribution in Harrington/TR306 DHLs. Arrows indicate the parents' value.

In the present study, we identified the QTLs of the β-amylase enzyme activity using two sets of DHLs. We identified two QTLs on chromosomes 1H and 2H in the Steptoe/Morex DHLs and one QTL on chromosome 5H in the Harrington/TR306 DHLs (Table 1, Fig. 3). There was no common QTL between the two DHLs. In the Steptoe/Morex DHLs, the QTL position on chromosome 1H was close to that of the previously reported QTL for diastatic power (Han et al. 1997). This is understandable because β-amylase is a very important component enzyme for starch degradation in the same way as α-amylase and α-D-glucosidase. The other QTL on chromosome 2H covered a relatively broad area. QTLs for kernel weight and grain protein were present within the area (Han et al. 1997). The results obtained are in agreement with the fact that the diastic power is affected by the protein content.

The characteristics of these two DHLs were quite different. The Steptoe/Morex DHLs consisted of a combination of six-row feed and six-row malting barley. Thus, a wide genetic variation may occur for malt qualities in the DHLs. On the other hand, the Harrington/TR306 DHLs consist of a cross within two-row malting barley which was highly selected and well-balanced for malting quality characteristics, including diastatic power. Thus the β-amylase activity values of the parents were relatively high, whereas those of the progeny were lower than the parent values. This narrow diversity between the parents resulted in a small genetic variation among the offsprings of the DHLs. The QTLs observed in the Steptoe/Morex DHLs are essential for a high activity of malt barley, while the QTL in the Harrington/TR 306 DHLs is effective for β-amylase activity in elite malt barley.

Based on the QTL analysis, the β-amylase structural gene, Bmy1 did not affect on the enzyme activity. The enzyme activity was considered to be controlled by other gene regions in the two DHLs. A QTL for diastic power near,
but not at the Bmy1 locus, was reported in QTL mapping for malt qualities (Oziel et al. 1996). This fact suggests that breeding for a high fermentability by introducing the thermostable β-amylase gene could be achieved without a negative effect on the diastatic power value.

**QTL analysis of β-amylase thermostability**

In a previous study, we confirmed that the seed-specific β-amylase structural gene, Bmy1 on chromosome 4H controls the thermostability, based on the enzyme expression analysis in *E. coli* (Kaneko et al. 2000). Eglinton et al. (1998) reported that the QTL for β-amylase thermostability was located near Bmy1. In addition, we mapped the gene region for the control of the β-amylase thermostability at the Bmy1 locus by QTL analysis for the Steptoe/Morex DHLs in the present study (Table 1, Fig. 4). We also confirmed that the
Thus we concluded that the Bmy1 locus controls the protein structure and thermostability of β-amylase, based on two different analyses: gene expression (Kaneko et al. 2000) and QTL mapping.

In the present study, we identified another QTL for the thermostability, derived from Morex on the short arm of chromosome 2H. This QTL had a relatively low LOD score and value for explaining variance (Table 1, Fig. 4).

We investigated the QTLs for the β-amylase enzyme activity and thermostability using two sets of DHLs. The results showed that the QTL regions for the β-amylase enzyme activity corresponded to those of the QTLs for the diastatic power and total nitrogen content. We also observed that β-amylase thermostability is mainly controlled by the structural gene itself, Bmy1. There was a minor QTL for the trait on chromosome 2H. The results obtained in the present study may contribute to the modification or improvement of the enzyme activity and thermostability of β-amylase in barley.

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