Validation of QTLs for Eating Quality of *Japonica* Rice ‘Koshihikari’ Using Backcross Inbred Lines

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Abstract: Using backcross inbred lines (BILs) derived from a cross between temperate *japonica* rice cultivars, Moritawase and Koshihikari, we validated the major quantitative trait loci (QTL) for eating quality and textural characteristics on chromosomes (Chr) 1, 2, 3, 6, 7, 10, and 12. Significant genetic differences in eating quality among BILs were detected at the QTL on Chr 3 and corresponded to the differences between the parents. Although differences in eating quality on the other chromosomes were not significant by t-test, cluster analysis and principal component analysis clearly showed that the genetic effects of the QTLs on Chr 6, 7, and 10 were similar to that on Chr 3, but the genetic effects of QTLs on Chr 1, 2, and 12 were entirely different from that on Chr 3. We previously identified that textural characteristics were highly correlated with eating quality. In this study, genetic differences in textural characteristics were similar to the genetic differences in eating quality among BILs. These results reveal major QTLs for eating quality of Koshihikari on Chr 3, 6, 7, and 10. The QTL on Chr 3 contributed most to the improvement of eating quality and textural characteristics.

Eating quality of rice is one of the most important quality traits related to the consumer’s taste and influences the market share of varieties. The temperate *japonica* rice cultivar Koshihikari, which is less resistant to lodging and more susceptible to diseases, is the most widely grown cultivar in Japan because its eating quality is high and appeals to the Japanese consumers. Therefore, it has been used as a genetic resource to improve eating quality in rice breeding throughout Japan. Since much effort and time is required to evaluate eating quality accurately, it is not easy to evaluate the many lines that have to be handled in a breeding program. Moreover, because the evaluation of eating quality needs many samples with an identical genotype, much labor in polishing and cooking, and highly trained panelists, it is difficult to evaluate eating quality in early generations based on phenotypic variations. Thus, the development of a marker-assisted selection (MAS) system may accelerate the breeding for the improvement of eating quality.

Quantitative trait locus (QTL) analysis has been performed to identify the chromosome regions controlling eating quality (Tanaka et al., 2006; Takeuchi et al., 2007, 2008; Kobayashi et al., 2008; Wada et al., 2008). Although QTLs for stickiness of cooked rice were detected on chromosome (Chr) 2 (Tanaka et al., 2006; Kobayashi et al., 2008), many reports suggest that the short arm of Chr 3 has the highest effect on the eating quality of cooked rice. Takeuchi et al. (2007) detected common QTLs on Chr 3 and Chr 6 using backcross inbred lines (BILs) and chromosome segment substitution lines (CSSLs) derived from crosses between ‘Koshihikari’ and indica cultivar ‘Kasalath’. Takeuchi et al. (2008) also detected QTLs on Chr 3 (two regions) and Chr 6 using two kinds of BILs (K-BILs; derived from a cross Nipponbare/Koshihikari//Koshihikari, and N-BILs; Nipponbare/Koshihikari//Nipponbare), and showed QTLs on Chr 3 were commonly detected regions, whose nearest marker was RM4108. Kobayashi and Tomita (2008) identified QTLs for stickiness on Chr 1, 3, 6, 7, and 8 using recombinant inbred lines (RILs) derived from a cross Sakihikari, which is genetically closely related to ‘Koshihikari’, and Nipponbare. They indicated that the
nearest marker of the QTL on Chr 3 was also RM4108. Furthermore, we identified QTLs on Chr 1, 2, 3, 6, 7, 10, and 12 in RILs derived from a cross between Moritawase (low eating quality) and Koshihikari (high eating quality) (Wada et al., 2008). The QTL on Chr 3 was mapped between RM4108 and RM4853. These studies demonstrated that Koshihikari alleles of QTLs on Chr 3 increased eating quality, and suggested that the major QTL of 'Koshihikari' was located on the region of the short arm of Chr 3, which was close to RM4108.

Although Takeuchi et al. (2007) validated the effect of the QTLs for eating quality using CSSLs, the information of genetic regions for improvement of eating quality is still limited, especially, the genetic regions controlling the difference of eating quality between japonica cultivars should be unveiled. The aim of this study is to validate the effects of the QTLs we previously detected by using RILs derived from the cross between japonica-japonica cultivars (Wada et al., 2008). Here, we developed BILs from a cross between Moritawase and Koshihikari and assessed their eating quality and textural characteristics to reveal the genetic effect of each QTL.

**Materials and Methods**

1. Development of BILs

Hybrids between Moritawase and Koshihikari were backcrossed with Koshihikari or Moritawase as the recurrent parent (Fig. 1). We identified the BILs as KBILs or MBILs according to the recurrent parent. BC$_3$F$_1$ plants were selected with simple sequence repeat (SSR) markers (McCouch et al., 2002) linked to previously identified QTLs for eating quality (Wada et al., 2008; Fig. 2). KBIL-1.1 was developed with RM8083 and RM8046; KBIL-1.2 with RM8144 and RM246; KBIL-2 with RM3515 and RM5470; KBIL-3 and MBIL-3 with RM4108, RM4853 and RM4683; KBIL-6 and MBIL-6 with SSR108, RM1369 and RM8101; KBIL-7 and MBIL-7 with RM5847 and RM1330; KBIL-10 and MBIL-10 with RM2887; KBIL-12.1 with RM2529 and RM1246; KBIL-12.2 with RM1015. BC$_3$F$_2$ plants derived from the BC$_3$F$_1$ plants were classified by MAS as homozygous for the Koshihikari or Moritawase allele at each QTL, or heterozygous. We tested homozygous BC$_3$F$_3$ lines derived from the selected homozygous BC$_3$F$_2$ plants.

![Fig. 1. Scheme of BIL development.](image)

**KBIL and MBIL lines**

![Fig. 2. QTLs for eating quality detected in RILs derived from Moritawase × Koshihikari. Black bars denote 2-LOD support intervals of QTLs.](image)
KBILs with a Koshihikari segment in the targeted QTL region were designated KBILs (K); KBILs with a Moritawase segment in the targeted QTL region were designated KBILs (M). Likewise, MBILs with a Koshihikari segment in the targeted QTL region were designated MBILs (K); and MBILs with a Moritawase segment in the targeted QTL region were designated MBILs (M). The BILs were named for the QTLs of interest; for example, KBIL-1.1 comprised lines with alleles of either parent at QTL 1 of Chr 1 and Koshihikari alleles at the other QTLs (Fig. 3). KBIL-1.1 makes it possible to evaluate the effect of the 1st QTL on Chr 1 on eating quality while eliminating the effects of other QTLs. Other BILs were similarly chosen (Fig. 3). We used 8 lines of KBILs per QTL allele except for KBIL-6 (12 lines per QTL allele). Similarly, we used 8 lines of MBILs per each QTL allele except for MBIL-10 (6 lines per QTL allele). Totally, we tested 136 KBILs in 2006 and 60 MBILs in 2007.

2. DNA isolation and genotyping
Genomic DNA was extracted from mature leaves of BC$_3$F$_1$ or BC$_3$F$_2$ plants of each BIL by the cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980). PCR amplification used 40 ng DNA, 0.4 µM each primer, 100 µM each dNTP, 10 mM Tris·HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl$_2$, and 0.5 units Taq DNA polymerase (TaKaRa, Kyoto, Japan) in 20 µL of reaction mixture. The amplification profile was as follows: 5 min at 94°C; 35 cycles of 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C; and a final 5 min at 72°C. PCR was performed in a PC-808 thermal cycler (Astec, Fukuoka, Japan). Amplified products were electrophoresed in 3.0% agarose gel or 12.0% polyacrylamide non-denaturing gel. Agarose gels were stained with ethidium bromide (EtBr), and polyacrylamide gels with Vistra Green (GE Healthcare, Little Chalfont, Buckinghamshire, England).

3. Field experiments
A field experiment was performed at Fukuoka Agricultural Research Center, Chikushino (Lat: 33°31′, Lon: 130°30′), Fukuoka, Japan. KBILs were sown on 12 May 2006 and transplanted on 10 June for QTL validation. MBILs were sown on 11 May 2007 and transplanted on 11 June for validation. The date when 50% of all panicles of each line had appeared from the flag leaf sheath was recorded as heading date. Days to heading (DTH) of both BILs were calculated from transplanting. MBIL-1.1, 1.2, 12.1, and 12.2 were developed, but not provided in the field experiments because validation using KBIL-1.1, 1.2, 12.1, and 12.2 strongly demonstrated that QTLs on Chr 1 and 12 were not correlated with eating quality of rice.

Each BIL, comprising 44 plants, was grown in a sandy paddy field in a randomized block design with two replications. The spacing was 30 cm between rows and 14 cm between plants. Nitrogen was applied at 2.5 g m$^{-2}$.

Weeds, insects, and diseases were controlled as necessary. Thirty-six plants from each BIL were harvested at maturity and assayed by a sensory test.

4. Evaluation of eating quality
The sensory test was performed as described by Yamamoto et al. (1996) and Matsue (1992). Each test compared one standard cultivar, one reference cultivar, and eight BILs or their parents. Four BILs with Koshihikari allele and 4 BILs with Moritawase allele on each QTL were evaluated in one sensory test simultaneously. As panelists are regularly trained using Koshihikari or Nipponbare as the standard variety for sensory test, Koshihikari was used as the standard against KBILs, as the reference against MBIL. Nipponbare, not Moritawase was used as the standard cultivar against MBILs, as the reference against KBILs to evaluate the eating quality of each BIL. Brown rice was polished to a yield of up to 90%. The polished rice was washed several times and soaked for 40 min before cooking in an electric rice cooker (RZYM15; Hitachi, Tokyo, Japan) at a ratio of 1 kg of white rice to 1.35 L of water. The cooked rice was eaten by 13 to 20 adult panelists who were selected for their ability to discriminate eating quality differences among cultivars (Oosato et al., 1998). Glossiness (GL) was estimated by the shininess of the cooked rice, and taste (TA), stickiness (ST), and hardness (HA) by chewing. Overall eating quality (OE) was determined by taking into account the total scores of GL, TA, ST, and HA. Each property was scored between −3 and +3, where 0 meant the same as the standard. OE, GL, and TA were scored higher when the evaluation of the cooked rice was better. ST was scored higher when the cooked rice was stickier. HA was scored higher when the cooked rice was harder. Mean scores of all panelists were used as trait values in the following QTL validation.

5. Evaluation of textural characteristics
Among several physicochemical properties, textural characteristics were correlated most closely with eating quality, and eating quality tended to become higher with the decrease in textural characteristics (Wada et al., 2006). We tried to validate the effects of QTLs for eating quality not only by the sensory test, but also by evaluating textural characteristics. These were measured using the polished rice of each BIL with a texturometer (GTX-2; Taketomo Electric, Tokyo, Japan) according to the method of Endo et al. (1980). The raw data were converted into a hardness-to-adhesion ratio (H / A3) and a hardness-to-adhesiveness ratio (H /-H) according to Endo et al. (1980).

6. Statistical analyses
BILs (M) and BILs (K) comprised 6 to 12 BC$_3$F$_3$ lines. Average scores of evaluation of eating quality and textural characteristics (ASE) among tested BILs with the same
Fig. 3. Graphical genotypes of QTL regions in BILs. We arbitrarily determined the recombination point to be the mid-point between markers of different genotypes: white, homozygous for Koshihikari segment; black, homozygous for Moritawase segment; shaded regions were not investigated. Pairs of black and white bars indicate two kinds of BILs: BILs (K), with the Koshihikari segment in the relevant QTL region, and BILs (M), with the Moritawase segment. Number above each bar indicates the chromosomes number.
QTL allele were calculated and were used to compare BILs (K) and BILs (M) by t-test. For this purpose, the genetic effect (GE) of each QTL was calculated as:

\[ GE = (ASE \text{ of BILs (K)}) - (ASE \text{ of BILs (M)}) \]  

The t-test showed a statistical difference between two populations in only one trait (e.g. stickiness, glossiness) at one locus and could not indicate the total difference of eating quality among QTL regions. Then cluster analysis (furthest neighbor method) and principal component analysis (PCA) were performed on the variance-covariance matrix using GE values of OE, GL, TA, ST, and HA scores as explanatory variables to evaluate the total genetic difference and similarity among BILs. Before performing cluster analysis, raw GE values were converted into the values based on Koshihikari standard as following equation:

\[ \text{Converted GE} = \text{Raw GE} \times \frac{(\text{Scores of Koshihikari} - \text{Scores of Moritawase})}{(\text{Scores of Nipponbare} - \text{Scores of Moritawase})} \]  

All statistical analyses were carried out using Microsoft Excel 2000 with the Stat Partner v. 4.5 add-in software (O-ha, Tokyo, Japan).

**Results**

### 1. Validation of QTLs for eating quality on Chr 3

In KBIL-3, BILs (K) scored significantly higher in OE, GL, TA, and ST and lower in HA than KBILs (M) (Fig. 4). These differences were consistent with the differences between the parental lines, namely the OE, GL, TA, and ST scores of KBILs (K) were higher than those of KBILs (M) and HA scores KBILs (K) were lower than those of
KBILs (M) like the difference between ‘Moritawase’ and ‘Koshihikari’ (Fig. 4). All differences (GE values) were found to be significant by the $t$-test. Similarly, in the MBIL-3 test, MBILs (K) scored higher in OE, GL, TA, and ST and lower in HA than MBILs (M) (Fig. 5). All the GE values in MBIL-3 were found to be significant by the $t$-test.

2. Validation of QTLs for eating quality on Chr 6, 7, and 10

KBIL-6, 7, and 10 showed eating quality patterns similar to those of KBIL-3; KBILs (K) scored higher in OE, GL, TA, and ST and lower in HA than KBILs (M), but the $t$-test showed that most of the differences were not significant (Fig. 4). MBIL-6, 7, and 10 showed patterns similar to those of MBIL-3; MBILs (K) scored higher in OE, GL, TA, and ST and lower in HA than MBILs (M) except for HA of MBIL-7, in which HA of MBILs (K) was slightly higher than that of MBILs (M). The $t$-test showed only marginal differences in most of the GE values, which was the same result as obtained for KBILs (Fig. 5).

3. Validation of QTLs for eating quality on Chr 1 and 12

KBIL-1.1, 1.2, 12.1, and 12.2 showed patterns quite different from those of KBIL-3 and most of the GE values were not significant (Fig. 4). Therefore we did not analyze the validation using MBIL-1.1, 1.2, 12.1, and 12.2 as mentioned in the section of Materials and Methods.

4. DTH and textural characteristics in KBIL and MBIL

In the test using KBIL, DTH in KBILs (K) was not significantly different from that in KBILs (M) except for KBIL-6, in which KBILs (K) headed later than KBILs (M) (Table 1). H/H and H/A3 were significantly lower in KBILs (K) than in KBILs (M) in KBIL-3. In the KBIL-6, 7, and 10, the patterns of differences were the same as in KBIL-3, but the significance was marginal. In the test using MBIL, DTH in KBILs (K) was significantly different from that in KBILs (M) in the MBIL-3 and 6; MBILs (K) headed later than MBILs (M) (Table 1). The effect of the QTL on Chr 6 was larger than that of the QTL on Chr 3, similar to the results in the KBILs. H/H and H/A3 were significantly lower in MBILs (K) than in MBILs (M) in MBIL-3. In MBIL-6, 7, and 10, the patterns of differences were the same as in MBIL-3, except for H/H in MBIL-7, but were marginally significant only in H/A3 of MBIL-6.

5. Cluster analysis and PCA of eating quality scores of BILs

Cluster analysis classified the BILs into two groups (Fig. 6): one composed of KBILs-3, 6, 7, and 10 (Cluster 1); and the other composed of KBILs-1.1, 1.2, 2, 12.1, and 12.2 (Cluster 2). PCA revealed that principal component 1 (PC1) contributed 78.7% of the total variance and PC2 16.1% (Table 2, Fig. 7). PC1 contributed substantially to increase the scores of OE, GL, TA, and ST, and PC2 to increase those of HA (Table 2). In the scatter plot of PC scores, the plots of Cluster 1 were scattered mainly in the right-hand quadrants and those of MBIL-3, 6, 7, and 10 were mainly scattered in the 1st quadrant, while those of KBIL-3, 6, 7, and 10 were mainly scattered in the 4th quadrant.

Discussion

Recently, studies on the genetic loci that control the eating quality of cooked rice of Koshihikari have been reported. Using doubled haploid lines derived from Akihikari × Koshihikari, Tanaka et al. (2006) found a
Table 1. Days to heading (DTH) and textural characteristics of KBILs, MBILs and parental lines.

| KBIL-3 | n = 8 | MBIL-3 | n = 8 |
|---|---|---|---|
| **Average scores** | **Average scores** | **Average scores** | **Average scores** |
| | **BILs (K)** | **BILs (M)** | **GE** | **t-test** | **BILs (K)** | **BILs (M)** | **GE** | **t-test** | **BILs (K)** | **BILs (M)** | **GE** | **t-test** |
| DTH | 48.9 | 49.0 | −0.1 | ns | 56.6 | 55.0 | 1.6 | ** | 128.9 | 338.2 | −214.3 | * |
| H / H | 18.1 | 27.2 | −9.1 | ** | 128.9 | 338.2 | −214.3 | * | 128.9 | 338.2 | −214.3 | * |
| H / A3 | 24.5 | 45.3 | −20.7 | ** | 359.0 | 2596.4 | −2237.4 | ** | 359.0 | 2596.4 | −2237.4 | ** |
| **KBIL-6** | n = 12 | **MBIL-6** | n = 8 |
| **Average scores** | **Average scores** | **Average scores** |
| | **BILs (K)** | **BILs (M)** | **GE** | **t-test** | **BILs (K)** | **BILs (M)** | **GE** | **t-test** | **BILs (K)** | **BILs (M)** | **GE** | **t-test** |
| DTH | 49.1 | 45.5 | 3.6 | ** | 60.5 | 55.1 | 5.4 | ** | 164.0 | 365.8 | −201.8 | ns |
| H / H | 22.7 | 27.3 | −4.7 | † | 532.8 | 344.0 | 188.8 | ns | 2100.6 | 3000.1 | −899.5 | ns |
| H / A3 | 31.1 | 39.2 | −8.2 | † | 79.7 | 502.5 | −422.8 | † | 79.7 | 502.5 | −422.8 | † |
| **KBIL-7** | n = 8 | **MBIL-7** | n = 8 |
| **Average scores** | **Average scores** | **Average scores** |
| | **BILs (K)** | **BILs (M)** | **GE** | **t-test** | **BILs (K)** | **BILs (M)** | **GE** | **t-test** | **BILs (K)** | **BILs (M)** | **GE** | **t-test** |
| DTH | 48.9 | 49.1 | −0.3 | ns | 54.6 | 54.8 | −0.1 | ns | 54.6 | 54.8 | −0.1 | ns |
| H / H | 22.3 | 25.3 | −1.0 | ns | 328.8 | 344.0 | −15.2 | ns | 328.8 | 344.0 | −15.2 | ns |
| H / A3 | 30.6 | 43.0 | −12.4 | † | 2100.6 | 3000.1 | −899.5 | ns | 2100.6 | 3000.1 | −899.5 | ns |
| **KBIL-10** | n = 8 | **MBIL-10** | n = 6 |
| **Average scores** | **Average scores** | **Average scores** |
| | **BILs (K)** | **BILs (M)** | **GE** | **t-test** | **BILs (K)** | **BILs (M)** | **GE** | **t-test** | **BILs (K)** | **BILs (M)** | **GE** | **t-test** |
| DTH | 49.6 | 49.1 | 0.5 | ns | 56.3 | 56.5 | −0.2 | ns | 56.3 | 56.5 | −0.2 | ns |
| H / H | 23.2 | 30.2 | −7.0 | ns | 222.0 | 270.0 | −48.0 | ns | 222.0 | 270.0 | −48.0 | ns |
| H / A3 | 35.2 | 43.3 | −8.1 | ns | 355.9 | 505.0 | −149.1 | ns | 355.9 | 505.0 | −149.1 | ns |
| **Parental lines (2006)** | n = 2 | **Parental lines (2007)** | n = 2 |
| **Average scores** | **Average scores** | **Average scores** |
| | **Koshihikari** | **Moritawase** | **GE** | | **Koshihikari** | **Moritawase** | **GE** | | **Koshihikari** | **Moritawase** | **GE** | |
| DTH | 48.0 | 46.0 | 2.0 | | 58.0 | 56.0 | 2.0 | | 58.0 | 56.0 | 2.0 | |
| H / H | 23.5 | 140.0 | −116.5 | | 30.0 | 179.0 | −149.0 | | 30.0 | 179.0 | −149.0 | |
| H / A3 | 28.2 | 918.8 | −890.5 | | 39.0 | 3158.0 | −3119.0 | | 39.0 | 3158.0 | −3119.0 | |

DTH, H / H and H / A3 indicate Days to heading, Hardness / Adhesion and Hardness / Adhesiveness, respectively. Genetic effect (GE) indicates difference between average scores of BILs (K) and those of BILs (M), or average scores between Koshihikari and Moritawase. **, *: significant at 1%, 5%, 10% level, respectively. ns: not significant.

major QTL for stickiness on Chr 2. Kobayashi et al. (2008) validated its effect by using near-isogenic lines with the Koshihikari segment on Chr 2 in the Akihikari genetic background. Using BILs and chromosome segment substitution lines (CSSLs) derived from Koshihikari × indica Kasalath, Takeuchi et al. (2007) found major QTLs for eating quality in the terminal region of the short arm of Chr 3. Using two reciprocal BILs derived from Nipponbare × Koshihikari, they later detected QTLs for eating quality in the same region of Chr 3 (Takeuchi et al., 2008). Using RILs derived from Sakihikari × Nipponbare, Kobayashi and Tomita (2008) identified a QTL for stickiness on the short arm of Chr 3. Sakihikari is derived from Hinohikari × Kinuhikari, both of which are
Table 2. Eigenvalues, contributions, and eigenvectors for eating quality scores in principal component analysis of KBILs and MBILs.

| Principal component | PC1   | PC2   | PC3   |
|---------------------|-------|-------|-------|
| Eigenvalue          | 3.93  | 0.80  | 0.12  |
| Percentage contribution | 0.787 | 0.161 | 0.023 |
| Cumulative contribution | 0.787 | 0.947 | 0.971 |
| Trait               |       |       |       |
| Overall eating quality | 0.977 | 0.120 | -0.075 |
| Glossiness          | 0.937 | 0.200 | 0.189 |
| Taste               | 0.953 | 0.118 | 0.114 |
| Stickiness          | 0.964 | 0.024 | -0.243 |
| Hardness            | -0.513 | 0.857 | -0.042 |

The effect of the QTL on Chr 3 was the highest, and the difference in eating quality (GE value) was significant for all measures of eating quality. The QTLs on Chr 6, 7, and 10 showed a similar tendency, although not significant (Figs. 4, 5). However, cluster analysis and PCA grouped KBILs-6, 7, and 10 and MBILs-6, 7, and 10 in the same cluster (Cluster 1) as KBIL-3 and MBIL-3 (Figs. 6, 7). These facts suggest that the QTLs on Chr 6, 7, and 10 contributed to the high eating quality of Koshihikari along with that on Chr 3. Takeuchi et al. (2008) detected QTLs for eating quality in similar regions on Chr 3 and 6, and Kobayashi and Tomita (2008) detected QTLs for stickiness in similar regions on Chr 3, 6, and 7. Both studies showed that the effect of the QTL on Chr 3 was the highest and support the present results.

In contrast, we could not validate the effects of the putative QTLs on Chr 1, 2, and 12 (Fig. 4). Cluster analysis
and PCA placed KBILs-1.1, 1.2, 2, 12.1, and 12.2 in a different cluster (Cluster 2) from KBIL-3 and MBIL-3 (Fig. 6, 7). The reason why they were detected in previous QTL analyses is difficult to explain, but could have been due to a false positive result (type I error). However, we had not validated the QTLs on Chr 1 and 12 using MBILs. Furthermore, there is a possibility that those QTLs have positive functions with the genetic background of Moritawase (MBILs) and/or interact with the QTLs on Chr 3, 6, 7, and 10. More investigations are needed to understand the effects of QTLs on Chr 1 and 12 comprehensively.

Cluster analysis and PCA also provided meaningful results; MBIL-3, 6, 7, and 10 were mainly scattered in the 1st quadrant, while KBIL-3, 6, 7, and 10 were mainly scattered in 4th quadrant. That meant there was a difference in PC2 scores between MBILs and KBILs. According to Table 2, hardness mainly contributed to the difference of PC2. These results suggested that ‘Moritawase’ harbored unknown genetic regions, which control the hardness of cooked rice.

The average scores of textural characteristics in KBILs were quite different from those in MBILs (Table 1). Although this fact was due to the difference in genetic background of BILs, we assume that environmental conditions affected the textural characteristics, because the amylose content of ‘Koshihikari’ in 2006 was 14.7%, and that in 2007 was 15.7%. Because a lower amylose content leads to lower textural characteristics and higher evaluation of eating quality (Wada et al., 2006), this annual difference suggests that the eating quality of samples in 2006 was totally higher than those in 2007. As shown in Table 1, the evaluation of textural characteristics also supported the sensory test results.

Thus, we can conclude that QTLs for the eating quality of Koshihikari are located on Chr 3, 6, 7, and 10. Takeuchi et al. (2007) validated the QTLs on Chr 3 and 6 in a japonica × indica population. Furthermore, they also validated the genetic effect of the Koshihikari allele on the segment of Chr 3 using CSSLs with a genetic background of Nipponbare (Takeuchi et al., 2008). They validated the effects of QTLs for eating quality in a japonica × japonica population. Likewise, the present results validated the effects of the QTLs on Chr 3, and strongly suggested that QTLs on Chr 6, 7, and 10 improved eating quality like the QTL on Chr 3.

DTH had a significant genetic effect in KBIL-6, MBIL-6, and MBIL-3, and the differences were highest in KBIL-6 and MBIL-6. In the present study, we validated the QTL for DTH on Chr 6 in the same RILs detected previously (Wada et al., 2008). Kojima et al. (2002) detected a QTL for DTH, Hd3a, in a Nipponbare × Kasalath population. Whether the QTL on Chr 6 that we detected was the same as Hd3a or not needs to be investigated. Furthermore, several previous studies suggested that DTH affected the air temperature of ripening duration and the temperature during ripening influenced the amylose content of rice endosperm (Asaoka et al., 1985; Sano et al., 1984; Umemoto et al., 1995). The previous findings and the results of this study indicate that a difference in heading date due to the QTL on Chr 6 might cause the difference of amylose content and eating quality.

MBIL-3 (K) headed 1 to 2 days later than MBIL-3 (M) and the difference was statistically significant. Furthermore, in our preliminary study, the line, which carries Koshihikari alleles on Chr 3 and Chr 6 with a Moritawase genetic background, headed 10 days later than Moritawase. This fact suggested that there was an obvious interaction between QTLs on Chr 3 and Chr 6.

The terminal region of the short arm of Chr 3 harbors many QTLs for heading date and several other traits. Takeuchi et al. (2003) carried out fine mapping of QTLs for DTH and seed dormancy in this region. Hori et al. (2010) detected a QTL for preharvest sprouting that is allelic with qLTG3-1 identified by Fujino et al. (2008). Further studies are needed to clarify the relationship between eating quality and other agronomic traits.

Takeuchi et al. (2008) identified QTLs for eating quality on Chr 3 and 6 in a cross between Nipponbare and Koshihikari. Kobayashi and Tomita (2008) detected QTLs on Chr 3, 6, and 7. The marker locations show that the QTLs we detected here are similar to those detected in their studies. These results suggest that the QTLs on Chr 3, 6, and 7 contribute most to the eating quality of Koshihikari in spite of the difference in genetic background. The short arm of Chr 6 houses several starch synthase-related genes, including wx (Sano, 1984), starch synthase I (Baba et al., 1993; Tanaka et al., 1995), and starch synthase IIa (Umemoto et al., 2002). However, there have not been any reports of a QTL for eating quality on Chr 10.

Although we validated the effects of the QTLs for eating quality on Chr 3, 6, 7, and 10, we do not yet know whether pyramiding multiple QTLs will improve eating quality. After several kinds of NILs, such as NILs with 2 QTLs, those with 3 QTLs and that with 4 QTLs, were developed, and their eating quality were analyzed thoroughly, we could conclude this argument. The line that we developed with the Koshihikari QTLs on Chr 3 and 6 in the Moritawase genetic background headed 10 days later than Moritawase. As this difference in heading date might affect eating quality, we need to develop NILs in which the linkage drag of Hd3a or QTL for DTH is canceled using genome information. Furthermore, in order to confirm whether QTLs on Chr 3, 6, 7, and 10 are effective or not with different genetic background, we have to develop NILs with a different genetic background except for Moritawase, focusing on the application of these QTLs to our practical breeding program.
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* In Japanese with English abstract.
** In Japanese with English summary.
*** In Japanese.