Major QTLs for eating quality of an elite Japanese rice cultivar, Koshihikari, on the short arm of chromosome 3

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To identify the chromosomal regions controlling the eating quality of Koshihikari rice, we performed a quantitative trait locus (QTL) analysis using two backcross inbred lines (BILs): N-BILs (79 lines derived from a cross of Nipponbare/Koshihikari/Nipponbare) and K-BILs (89 lines derived from a cross of Nipponbare/Koshihikari/Koshihikari). We evaluated several components of the eating quality of cooked rice, namely glossiness, taste, stickiness, hardness, and overall evaluation, based on sensory tests by a trained panel, and amylose and protein contents. Ten QTLs for these components were detected in N-BILs (two regions of chromosome chr. 3 and one of chr. 11) and six in K-BILs (chr. 3 and chr. 6). Each QTL explained 11.6% to 32.0% of the total phenotypic variance. QTLs at the distal end of the short arm of chr. 3 were commonly identified in both BILs. The Koshihikari alleles at these QTLs increased eating quality. The genetic effect of the Koshihikari alleles was confirmed by analysis of a chromosome segment substitution line containing a Koshihikari segment of the short arm of chr. 3 in the Nipponbare background.

**Key Words:** *Oryza sativa* L., eating quality, quantitative trait locus, backcross inbred lines, chromosome segment substitution line.

**Introduction**

In 2006, the area of production of the *japonica* rice cultivar Koshihikari was about 627,800 ha, accounting for 37.4% of the total rice cultivation area in Japan (The Ministry of Agriculture Forestry and Fisheries of Japan 2007). For the last 30 years, Koshihikari has been grown over a wide area in Japan and is favored by most Japanese consumers because of its good eating quality (Yamamoto and Ogawa 1992). In addition, Koshihikari exhibits good agronomic characteristics, such as a high tolerance to cool temperature (Takeuchi et al. 2001) and resistance to preharvest sprouting. Therefore, Koshihikari is used as a parental line to develop new rice cultivars aiming to the introduction its excellent good characteristics, such as eating quality and cool temperature tolerance at booting stage.

Eating quality is an important trait in rice breeding. In general, it is evaluated through sensory testing by trained panelists. Although its reliability depends on the experience of the panelists, the sensory test is recognized as the most effective method to determine eating quality, and it has been used extensively for selection in rice breeding. The eating quality of cooked rice is evaluated from several components, notably glossiness (GL), hardness (HA), stickiness (ST), taste (TA), and overall evaluation (OE) (Yamamoto and Ogawa 1992, Bett-Garber et al. 2001). As this evaluation must be done on advanced generations of breeding materials because of the requirement for a large amount of grain, it is very time consuming and labor intensive. Studies designed to develop a more efficient system for screening eating quality have revealed that amylose content (AC) and protein content (PC) of the endosperm are strong determinants of eating quality (Juliano et al. 1965, Ishima et al. 1974, Juliano 1985), but do not fully explain it (Bett-Garber et al. 2001). Thus, other genetic factors remain to be uncovered.

Recently, marker-assisted selection (MAS) has been used to develop new rice cultivars with particular traits (Yamamoto and Yano 2008). It has been used to introduce bacterial blight resistance, blast resistance, lodging resistance,
high yield potential, high adaptability, and submergence tolerance (Singh et al. 2001, Hayashi et al. 2004, Sugiuira et al. 2004, Ashikari et al. 2005, Wang et al. 2005, Takeuchi et al. 2006, Neeraja et al. 2007). Its effectiveness depends on the reliability of markers linked to the target gene loci. For example, high-resolution mapping and the use of tightly linked DNA markers have allowed us to develop isogenic lines of Koshihikari with either early or late heading controlled by a very tiny chromosome segment (150–625 kb) (Takeuchi et al. 2006). In terms of MAS for eating quality, only the Waxy (Wx) gene can be manipulated so far (Sato et al. 2002, Suzuki et al. 2003).

In order to apply the MAS to eating quality, several genetic analyses of eating quality have been conducted (Wan et al. 2004, Tanaka et al. 2006, Takeuchi et al. 2007). Tanaka et al. (2006) identified several QTLs for components of eating quality in doubled haploid lines (DHLs) derived from a cross between the japonica cultivars Akihikari and Koshihikari. The genetic effect of one of those QTLs, on chromosome (chr.) 2, has been validated by using a series of nearly isogenic lines (Kobayashi et al. 2008). The QTL reduced AC and increased ST of cooked rice. However, it could not explain all of the differences between the parental lines, so other factors must be involved in the eating quality of Koshihikari. Genetic analyses have also been performed to detect QTLs for eating and grain quality by using crosses between distantly related japonica and indica cultivars (Wan et al. 2004, Takeuchi et al. 2007). The researchers detected a major QTL in the region of the Wx gene on the short arm of chr. 6, suggesting that the difference in AC was due to the allelic difference at Wx between japonica and indica cultivars. As such studies might not be able to identify genetic factors unrelated to AC controlling eating quality, it is still necessary to develop mapping populations derived from crosses between Koshihikari and other japonica cultivars to understand the genetic basis of the eating quality of Koshihikari.

Recently, two reciprocal backcrossed inbred lines (BILs) derived from a cross between Koshihikari and Nipponbare were used for the detection of QTLs for heading date (Matsubara et al. 2008). Despite the lack of polymorphism in DNA markers (Akagi et al. 1997, Kono et al. 2000), Matsubara et al. (2008) successfully analyzed segregation of the genotypes of 102 simple sequence repeat (SSR) and nine single-nucleotide polymorphism (SNP) markers in these two BILs. Koshihikari had a glossier appearance and a stickier, soft eating quality, whereas Nipponbare had a duller appearance and a less sticky, slightly firmer eating quality. In addition, the heading date varied slightly among these two BILs. We used these BILs to conduct a QTL analysis of eating quality in rice. Here, we identified major QTLs on the short arm of chr. 3 controlling the eating quality of Koshihikari. The genetic effect of the Koshihikari alleles was confirmed by the development of a chromosome segment substitution line (CSSL) and a phenotype assay.

Materials and Methods

Plant materials

We used two populations of BILs: 79 N-BILs and 89 K-BILs (Matsubara et al. 2008, RGRC 2008a, 2008b). The 79 N-BILs and their parents were raised at the National Institute of Crop Science (Yawara, Tsukuba, Ibaraki, Japan) in 2005. Seeds were sown on 2 May and seedlings were transplanted on 27 May. Of 129 K-BILs originally developed, we randomly selected 89 on account of limited field space and limited capacity to evaluate eating quality. Seeds were sown on 2 May 2006 and seedlings were transplanted on 29 May. In both years, the planting density was 22.2 plants m−2 (one-row plots, 15 cm × 30 cm). Nitrogen, phosphorus, and potassium fertilizers were each applied at 8 g m−2 in each year. Thirty-two plants per line were raised with two replications in each year. Thirty out of the 32 plants per line were harvested at maturity. All the seeds were air-dried in a greenhouse, threshed, and hulled. Fully matured grains were used for evaluation of eating quality and chemical properties.

To verify the allelic effects of the QTLs detected, we selected plant 07-2206 from the advanced backcross progeny BC1F2 (196 plants) on the basis of the genotypes of SSR markers that showed that this plant was homozygous for the Koshihikari alleles on a segment of the short arm of chr. 3 in a homozygous Nipponbare background. Line 07-2206, Koshihikari, and Nipponbare were grown in a paddy field at the National Institute of Agrobiological Sciences (Kannonai, Tsukuba, Ibaraki, Japan) in 2007. Seeds were sown on 18 April and seedlings were transplanted at 23.1 plants m−2 on 14 May. Fertilizer was applied as before. Thirty plants with two replications were harvested at maturity. All the seeds were air-dried in a greenhouse, threshed, and hulled. Fully matured grains were used for evaluation of eating quality and chemical properties.

Sensory test of eating quality

The sensory test was performed according to the method of Yamamoto et al. (1996). Five hundred grams of hulled grain was polished to a yield of ~90% in a rice mill (VP-31T; Yamamoto Co. Ltd., Yamagata, Japan). Then 350 g of polished rice was placed in the bowl of a rice cooker (MB-YH16; Mitsubishi Electric Co. Ltd., Tokyo, Japan) and washed five times with water. The washed rice was soaked in water for 30 min and then cooked for about 30 min at a 1:4:1 (w/w) ratio of water to polished rice. The cooked rice was then steamed in the cooker for an additional 10 min. The rice was evaluated by a panel of 20 judges (9 men and 11 women, ages 27 to 48 years), who had been trained for over 2 years in the scoring of each component of eating quality. Nine lines were evaluated at one time. Filtered water was used to cleanse the mouth between lines. The judges evaluated GL, TA, ST, HA, and OE. GL was scored by the degree of glossiness of the surface of the cooked rice. TA was scored by the degree of sweetness or bitterness. ST was scored by the degree of the force required to remove the
cooked grains from an upper tooth and a lower tooth. HA was scored by the degree of the force required to compress the cooked grains between an upper tooth and a lower tooth. OE score was determined from the total scores and balance of GL, TA, ST, and HA. The GL, TA, ST, and OE scores of Nipponbare were rated −1 (slightly low) relative to Koshihikari, and the HA score of Nipponbare was rated +1 (slightly hard). A scale between Nipponbare and Koshihikari was used to determine the score of each component of eating quality of each line. The GL, TA, ST, and OE of each line were given scores from −5 (extremely poor) to +5 (excellent) and the HA was given scores from −5 (excellent) to +5 (extremely poor), compared with that of the reference cultivar Koshihikari (score = 0). The scores from the 20 judges were averaged.

**Analysis of amylose and protein contents**

We crushed polished rice in a cyclone mill (SFC-S1; UDY Corp., CO, USA). The resulting flour was diluted with 0.5 N NaOH and left overnight at room temperature. After dilution to 0.05 N NaOH with water, AC was determined by colorimetry with iodine (Juliano 1971). The N content was determined with a protein analyzer (FT-528; LECO Corp., MI, USA). PC was calculated as 5.95 times the N content. AC and PC of each line were determined in three different samples. The average AC and PC values were used for statistical analysis.

**QTL analysis and other statistical analyses**

We performed a QTL analysis of the eating quality and chemical properties by using genotype data for 102 SSR and 9 SNP markers identified previously (Matsubara et al. 2008, RGRG 2008a, 2008b). Putative QTLs in the N-BILs and K-BILs were detected by 1-way ANOVA with SAS GLM PROC in a single-point analysis (SAS Institute, NC, USA). Owing to the nature of sensory testing, we used a stringent threshold (P = 0.001) to declare a putative QTL. We used MAPMAKER/QTL (Lander and Botstein 1989) to confirm the presence of a putative QTL and to estimate genetic parameters, namely additive effects and percentage of variance explained, in “2 backcross” mode.

**Analysis SSR markers**

We determined the genotype of 07-2206 at 192 SSR markers covering the 12 chromosomes (Temnykh et al. 2001, McCouch et al. 2002, IRGSP 2005). Total DNA was extracted from a small piece of leaf of each BC1F2 plant. The leaf was crushed in a 1.5-mL tube containing 300 μL of a solution containing 100 mM Tris-HCl, 1 M KCl, and 10 mM EDTA. The DNA in the centrifuged supernatant was precipitated with isopropanol, and the pellet was resolved in 50 μL 0.1× TE (10 mM Tris-HCl and 1 mM EDTA). The DNA extract was used as the template for PCR amplification. For the SSR analysis, the reaction mixture (6 μL total volume) consisted of 1 μL template DNA, 0.7 μL 10× PCR buffer (Promega, Madison, WI, USA), 0.4 μL 25 mM MgCl2, 0.7 μL of a solution containing 2 mM each dNTP (Boehringer Mannheim, Mannheim, Germany), 0.1 μL 5 U Taq DNA polymerase (Promega), 0.3 μL of a 20 PM solution of each primer, and 2.8 μL H2O. Amplification was performed for 30 cycles at 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min; followed by a final cycle at 72°C for 7 min. The amplified DNA products were separated by electrophoresis in 3.5% agarose gel.

**Results**

**Phenotypic variations in N-BILs and K-BILs**

In 2005, the mean scores of the four components of eating quality of two parental cultivars, Nipponbare and Koshihikari, were −0.7 and 0.1 for GL, −0.9 and −0.1 for TA, −0.9 and 0.7 for ST, and 0.4 and 0.0 for HA, respectively (Fig. 1A). In 2006, these score of four components of Nipponbare and Koshihikari were −0.7 and 0.0 for GL, −0.9 and −0.1 for TA, −0.8 and 0.0 for ST, and 0.8 and 0.3 for HA, respectively (Fig. 1B). The OE scores of Nipponbare and Koshihikari were −1.1 and −0.1, respectively, in 2005 and −1.1 and 0.0, respectively, in 2006 (Fig. 1A and B). The AC values of Nipponbare and Koshihikari were 21.3% and 18.9%, respectively, in 2005 and 22.5% and 19.0%, respectively, in 2006 (Fig. 2A and B). In 2005, the PC values of Nipponbare and Koshihikari were 5.8% and 5.3%, respectively (Fig. 2A). All traits in both BILs showed continuous variations within the range of parental values (Fig. 1 and Fig. 2). No clear transgressive segregation was occurred in both populations.

**QTLs detected in N-BILs**

We detected 10 QTLs for eating quality in the N-BILs (Fig. 3A and Table 1). Five QTLs (qGL3-3, qTA3-3, qST3-3, qHA3-3, and qOE3-3) were detected at the distal end of the short arm of chr. 3 (near SSR marker RM4108). These QTLs explained 17.4% to 21.5% of the total phenotypic variance. The Koshihikari alleles at all QTLs improved the eating quality. In addition, four QTLs (qTA4-4, qST4-3, qGL3-4 and qOE3-4) were detected at the distal end of the long arm of chr. 3 (near C87C1017 and RM1038). These QTLs explained 11.6% to 18.1% of the total phenotypic variance. One additional QTL, qTA11, was detected on chr. 11 (near RM3721). It explained 17.4% of the total phenotypic variance. The Koshihikari alleles at these five QTLs decreased the eating quality. No QTL for AC and PC was detected in the N-BILs.

**QTLs detected in K-BILs**

We detected six QTLs for eating quality in the K-BILs (Fig. 3B and Table 1). Five QTLs (qGL3-3, qTA3-3, qST3-3, qHA3-3, and qOE3-3) were detected at the distal end of the short arm of chr. 3 (near RM4108 and RM8549). These five QTLs explained 12.4% to 32.0% of the total phenotypic variance. The other QTL, qST6-3, was detected on chr. 6 (near RM8101). It explained 12.6% of the total phenotypic
Fig. 1. Frequency distribution of glossiness (GL), taste (TA), stickiness (ST), hardness (HA), and overall evaluation (OE) in N-BIL (A) and K-BILs (B). Black and white arrows indicate the mean values for Koshihikari and Nipponbare, respectively. Horizontal lines indicate SD. Black, white, and shaded bars indicate the number of lines of the Koshihikari homozygous, Nipponbare homozygous, and heterozygous genotypes, respectively, for a SSR marker (RM4108 or RM5849) that is tightly linked with an eating quality QTL on the short arm of chr. 3.

Fig. 2. Frequency distribution of amylose content (AC) and protein content (PC) in N-BILs (A) and K-BILs (B). Black and white arrows indicate the mean values for Koshihikari and Nipponbare, respectively. Horizontal lines indicate SD.

variance. The Koshihikari alleles at all QTLs increased the eating quality.

One QTL for AC, qAC3-4, was detected on the long arm of chr. 3 (near C87C1017). It explained 11.2% of the total phenotypic variance. The Koshihikari allele decreased AC by 0.3%.

Eating quality in CSSL

To confirm the presence of the five common QTLs, we selected line 07-2206 from advanced backcross progeny (Fig. 4A). Analysis of 192 SSR markers confirmed that a relatively large chromosome segment of Koshihikari from the short arm of chr. 3 was substituted in the genetic background of Nipponbare. All other SSR markers were homozygous for Nipponbare, indicating that the insertion of any other Koshihikari segments was unlikely.

The GL and TA scores of 07-2206 (−0.3 for GL and −0.4 for TA) were higher than those of Nipponbare (−1.0 for GL and −0.7 for TA) ($P<0.05$) (Fig. 4B). The ST and HA scores of 07-2206 (0.2 for ST and 0.4 for HA) ranged between those of the Nipponbare (−0.4 for ST and 0.6 for HA) and Koshihikari (Fig. 4B). The ST and HA scores of 07-2206 and Nipponbare were not significantly different ($P>0.05$).
The OE score of 07-2206 (-0.3) was higher than that of Nipponbare (-0.8) (P < 0.05) (Fig. 4B). The AC and PC values of 07-2206 (19.2% for AC and 7.2% for PC) were almost the same as those of Nipponbare (19.4% for AC and 7.0% for PC) (data not shown). These results clearly verify the effects of the Koshihikari alleles at the five QTLs (qGL3-3, qTA3-3, qST3-3, qHA3-3, and qOE3-3) on the short arm of chr. 3.

Discussion

Eating quality is an important trait in rice breeding in Japan. As many consumers prefer Koshihikari, selection for eating quality has focused on Koshihikari-like characteristics. Consequently, Koshihikari has been extensively used as a parental line in most breeding programs in Japan.

The eating quality of breeding materials is usually evaluated by sensory test. Since evaluation should be done on advanced generations, such as F₆ or later, it is very time consuming and labor intensive. Other selection methods, such as measuring chemical components and MAS, would be required to improve selection. In this regard, in order to establish MAS for eating quality we have been interested in identifying chromosomal regions affecting eating quality.

Tanaka et al. (2006) performed QTL analysis using DHLs derived from a cross between Koshihikari and Akihikari (which has inferior eating quality), and detected several QTLs for eating quality. They identified one with a major effect on chr. 2. Its effect has been confirmed in NILs (Kobayashi et al. 2008). However, an incomplete linkage map and a wide range of phenotypic variation in heading date of this population might make it difficult to identify all factors controlling eating quality. Thus, genetic dissection of eating quality among Japanese cultivars remains to be clarified.

In this study, we successfully identified one chromosomal region involved in eating quality on the short arm of chr. 3. Interestingly, several QTLs for the components of eating quality are clustered in this region. These QTLs were consistently detected in different mapping populations over three consecutive years, suggesting that they are stably expressed in different genetic backgrounds and under different environmental conditions. Their genetic effects were verified by the development of a CSSL for the short arm of chr. 3. Only one substitution resulted in significant increases in eating quality.

Fig. 3. Putative QTLs for eating quality detected in N-BILs (A) and K-BILs (B). The chromosome number is shown at the top. Vertical bars denote the linkage map (Matsubara et al. 2008, RGRC 2008a, 2008b). Putative QTLs for eating quality were detected by 1-way ANOVA and MAPMAKER/QTL. Triangle indicates the nearest marker locus revealed by 1-way ANOVA. Black and white triangles indicate that the Koshihikari alleles increase and decrease the trait score, respectively. Bold bars indicate the most likely chromosomal regions for the putative QTL within a certain confidence interval (defined by a decrease of 0.5 from the peak LOD values) according to the interval mapping analysis. Abbreviations are as follows: GL, glossiness; TA, taste; ST, stickiness; HA, hardness; OE, overall evaluation; AC, amylose content. Hd16 and Hd17 show the positions of QTLs for heading date detected in a previous study (Matsubara et al. 2008).
quality, although its QTLs do not explain all of the differences in eating quality between Nipponbare and Koshihikari.

The mapping resolution of the two BILs used in this study made it difficult to conclude whether these apparent QTLs represent pleiotropy of just one QTL, or are tightly linked but different QTLs. Other studies have also identified QTLs for multiple components of eating quality (Wan et al. 2004, Tanaka et al. 2006, Takeuchi et al. 2007). Although the individual components of eating quality (GL, TA, ST, and HA) seem to be different characteristics, they are related to each other (Takeuchi et al. 2007). In fact, each component is likely correlated with the others (Takeuchi et al. 2007). Thus, it is likely that these QTLs represent the pleiotropic effect of a single QTL. High-resolution substitution mapping should reveal this.

Previous studies reported several QTLs for eating quality on the short arm of chr. 3. Tanaka et al. (2006) identified one QTL for appearance in the center of the short arm of chr. 3 in DHLs derived from a cross between two japonica cultivars (Akihikari and Koshihikari). Takeuchi et al. (2007) identified four QTLs for eating quality on each of the distal end of the short arm of chr. 3 (qGL3-2, qST3-2, qHA3, and qOE3-2) and the center of the short arm of chr. 3 (qGL3-1, qTA3, qST3-1, and qOE3-1) in populations derived from crosses between japonica Koshihikari and indica Kasalath. The five QTLs we detected here (qGL3-3, qTA3-3, qST3-3, qHA3-3, and qOE3-3) appear to be different loci from the one identified by Tanaka et al. (2006) and four of the QTLs (qGL3-1, qTA3, qST3-1, and qOE3-1) identified by Takeuchi et al. (2007), but they appear to coincide with the other four QTLs (qGL3-2, qST3-2, qHA3, and qOE3-2) identified by Takeuchi et al. (2007). However, it is difficult to confirm the allelic relationships among these QTLs from the linkage map data. Further analysis, including fine mapping and cloning of genes at these QTLs, should be conducted to clarify the relationships between the QTLs detected in this study and other eating quality QTLs.

Five QTLs for eating quality (qGL3-3, qTA3-3, qST3-3, qHA3-3, and qOE3-3) were commonly identified in 2005 in N-BILs, in 2006 in K-BILs, and in 2007 in the CSSL. This result suggests that the effects of these QTLs are reproducible in different genetic backgrounds of Nipponbare and Koshihikari. The average temperature of the ripening period was significantly higher in 2007 than in 2005 and 2006, and the temperature was abnormally high in the middle of August 2007. This result suggests that the effects of these QTLs are reproducible in different environmental conditions too.

Five QTLs (qGL3-3, qTA3-3, qST3-3, qHA3-3, and qOE3-3) were introduced from Koshihikari into Nipponbare, in the process of the establishing CSSL, 07-2206, in this study. The CSSL verified that the alleles from Koshihikari increased the level of eating quality of Nipponbare. However, the level of eating quality of the CSSL did not meet that of Koshihikari. This difference might be explained by following two possibilities. The first is that other QTLs are involved in eating quality, although we did not find any. It will be necessary to use different materials or different evaluation criteria to identify additional QTLs. With this aim, we are developing a series of CSSLs to cover the whole genome of Koshihikari in the Nipponbare background. With these lines, it may be

### Table 1. Putative QTLs controlling the eating quality detected in the N-BILs and K-BILs

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\[a\] Overall evaluation; GL, glossiness; TA, taste; ST, stickiness; HA, hardness; AC, amylose content.

\[b\] Percentage of total phenotypic variance explained by the QTL.

\[c\] Additive effect of the Koshihikari allele.
possible to identify additional QTLs with minor effects on eating quality. Another explanation is that eating quality may be influenced by heading date; differences in heading date may affect temperature exposure during ripening and it may affect eating quality. To verify this possibility, it will be necessary to compare eating quality under the same environmental conditions during ripening between Koshihikari and 07-2206 by adjusting sowing time. It will also be necessary to develop CSSLs in which chromosomal segments of Nipponbare are inserted in the Koshihikari background and evaluate their eating quality.

In general, eating quality was affected by heading date. Tanaka et al. (2006) reported that components of eating quality such as GL and ST were positively correlated with heading date. In this regard, attention should be paid to the relationship between heading date and eating quality. Although two QTLs for heading date, $Hd16$ and $Hd17$, were identified on the long arm of chr. 3 and the short arm of chr. 6, respectively, by QTL analysis using N-BILs and K-BILs (Fig. 3) (Matsubara et al. 2008), no QTL for heading date was detected on the short arm of chr. 3. Furthermore, the heading date of a CSSL, 07-2206, with QTLs for eating quality on the short arm of chr. 3 was the same as that of Nipponbare (data not shown). These results indicate that QTLs for eating quality on the short arm of chr. 3 were not detected by the effect of the variation in heading date.

In this study, we did not analyze several morphological grain traits, such as grain shape, weight, and size. In general, very small variations have been observed in these traits in populations developed from temperate japonica × japonica crosses. A few genetic analyses have demonstrated QTLs for such traits using japonica × japonica crosses (Kobayashi et al. 2007, Kwon et al. 2008). In these studies, two QTLs for grain weight, tw3 (Kobayashi et al. 2007) and g3 (Kwon et al. 2008), were detected on the short arm of chr. 3. On the basis of a comparison of SSR markers (IRGSP 2005), the QTLs detected on the short arm of chr. 3 in this study were not likely to be the same loci as tw3 and g3. Fine mapping of these QTLs will be required to clarify the relationships between the QTLs detected in this study and other QTLs.

It is well known that chemical properties, including AC and PC, affect eating quality (Juliano et al. 1965, Ishima et al. 1974). PC of Nipponbare was slightly higher than that of Koshihikari and a small variation was observed in PC of N-BILs. QTL analysis also revealed no QTL for PC in N-BILs. These results suggested that the variation in PC in N-BILs might not greatly affect eating quality; however, it could not be rejected that QTL for PC might be located in the chromosomal region with a lack of polymorphism markers in N-BILs. To further clarify the relationship between PC and eating quality, it is necessary to develop more high-density markers and a series of CSSLs. In rice breeding, the Wx gene, controlling AC, has been used to improve eating quality (Higashi et al. 1999, Sato et al. 2002, Suzuki et al. 2003). AC showed a small variation in the BILs, and one QTL, qAC3-4, was mapped to the long arm of chr. 3. No QTL for AC was detected on the short arm of chr. 3. AC and PC values of a CSSL, 07-2206, with QTLs for eating quality on the short arm of chr. 3 were also the same as those of Nipponbare. These results suggest that the higher eating quality due to the Koshihikari alleles on the short arm of chr. 3 was not related to the AC and PC in endosperm.
On the other hand, we detected four QTLs (qGL3-4, qTA3-4, qST3-4, and qOE3-4) on the distal end of the long arm of chr. 3, where Hdi6 for heading date has been detected. The Koshihikari allele at Hdi6 decreases days-to-heading (DTH) (Matsubara et al. 2008). This is a likely reason for the low AC. It is considered that low AC decreases the stickiness of cooked rice, and thus eating quality. However, the Koshihikari alleles at the four QTLs (qGL3-4, qTA3-4, qST3-4, and qOE3-4) in our study decreased eating quality. This discrepancy might be due to the effects of temperature on amyllopectin structure as well as AC (Asaoka et al. 1985, Umemoto et al. 2003): higher temperature decreases the proportion of short chains of amyllopectin (Umemoto et al. 2003), causing a hard texture (Juliano et al. 1987) and decreasing eating quality. Although we did not measure changes in amyllopectin structure, the QTLs on the long arm of chr. 3 seem to have responded to DTH.

In rice breeding, sensory tests of advanced lines are time consuming and labor intensive. It is difficult to establish standards for eating quality owing to the variable reliability of sensory tests, which depend on the talents of panelists. Establishing more effective and reliable methods of screening for eating quality will be a very important task in future rice breeding in Japan. MAS is one solution, using DNA markers RM4108 and RM5849, located near the QTLs for eating quality. To evaluate the potential of these markers for indirect selection, researchers should investigate the allelic frequency of these markers among elite cultivars developed recently in Japan.

In the last decade, several QTLs with relatively large phenotypic effects have been cloned by map-based strategies (Yano 2001, Yamamoto and Yano 2008). Molecular identification of such genes has brought new insights into phenotypic traits, such as stress tolerance and yield potential (Ashikari et al. 2005, Ren et al. 2005). As long as eating quality of cooked rice is evaluated by sensory testing, it will be difficult to reveal the genetic basis of components of eating quality, except in terms of chemicals such as amylose and protein. Here, we identified QTLs with relatively large phenotypic effects on eating quality independent of amylose content. This will provide an opportunity to clone the genes involved in eating quality. Molecular identification of such genes will help to create new methods of evaluation and selection in rice breeding.

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