Identification of a novel gene (*Apq1*) from the *indica* rice cultivar ‘Habataki’ that improves the quality of grains produced under high temperature stress

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The appearance of brown rice grown under high temperature conditions is an important characteristic for improvement in Japanese rice breeding programs. We performed a QTL analysis of the appearance quality of brown rice using chromosome segment substitution lines of the *indica* cultivar ‘Habataki’ in the ‘Koshihikari’ genetic background. A line carrying a ‘Habataki’ segment on chromosome 7 showed a high percentage of perfect grains produced under high temperature conditions during the ripening period. To verify the role of this segment, and to narrow down the region containing the useful allele, substitution mapping was performed using multiple paired lines. As a result, the chromosomal location of a gene that we named *Appearance quality of brown rice 1* (*Apq1*) was delimited to a 48-kb region. In addition, we developed an *Apq1*-near isogenic line (NIL) to evaluate the effect of *Apq1* on various agronomic traits. Under high temperature conditions during the ripening period, the *Apq1*-NIL produced significantly higher percentages of perfect grains than ‘Koshihikari’. Other agronomic traits, including yield and palatability, were similar between the *Apq1*-NIL and ‘Koshihikari’. Therefore, the ‘Habataki’ allele of *Apq1* will be useful in breeding programs aimed at improving the quality of grains ripened under high temperature conditions.

Key Words: *Apq1*, appearance quality, grain, ‘Habataki’, ‘Koshihikari’, mapping, rice (*Oryza sativa* L.).

Introduction

Recently in Japan, high temperatures during the ripening period have resulted in deterioration in the quality of rice grains. This deterioration is due to increases in the abundance of chalky grains, which decrease milling quality. The price of brown rice is fixed by grain appearance, particularly the ratio of chalky grains. Therefore, the development of novel cultivars with tolerance to high temperature stress during the ripening period is a major objective in breeding programs. In particular, improvements of this trait in ‘Koshihikari’, which is a leading cultivar in Japan, are strongly needed (http://www.maff.go.jp/j/seisan/kankyo/ondanka/pdf/h22_tekiou_gijyutu_gaiyo.pdf).

The popularity of ‘Koshihikari’ is due to its good palatability. The development of near isogenic lines (NILs) with desirable traits in the ‘Koshihikari’ genetic background is a practical approach to developing new cultivars with good eating quality. Indeed, NILs for several traits, including blast resistance, early and late heading, and short culms, have already been developed using ‘Koshihikari’ as the ge-

netic background (Ebitani *et al.* 2011, Fukuoka *et al.* 2009, Ishizaki *et al.* 2005, Kojima *et al.* 2004, Takeuchi *et al.* 2006, Wan *et al.* 2005a).

The use of DNA marker-assisted selection (MAS) is highly effective in developing NILs (Ebitani *et al.* 2011, Fukuoka *et al.* 2009, Takeuchi *et al.* 2006, Wan *et al.* 2005a). To facilitate the development of lines with tolerance to high temperatures, it is required to identify quantitative trait loci (QTLs) or genes that improve the appearance quality of brown rice under high temperature stress. Previously, Ebitani *et al.* (2008) detected four QTLs in the *indica* cultivar ‘Kasalath’ that increased the percentage of perfect grains (PPG) in plants with a ‘Koshihikari’ genetic background. The ‘Kasalath’ allele of the locus on chromosome 12 that had the highest effect among those QTLs was effective at approximately 27°C, the average air temperature during the 20 days after heading (hereafter referred to as the ‘average temperature’), but not at 28°C or higher (Ebitani *et al.* 2013). Kobayashi *et al.* (2013) reported that the *qWB6* gene derived from the tolerant cultivar ‘Hana-echizen’ (HE) could decrease the abundance of white-back kernels at approximately 28°C in the ‘Niigata-wase’ genetic background, and could reduce heat-induced quality decline to the same level as in HE. However, it remains unclear whether the HE allele at *qWB6* would be effective in the ‘Koshihikari’ genetic background. Tabata *et al.* (2007) detected three QTLs

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in the heat-tolerant cultivar 'Koshiji-wase' that decreased the incidence of white-back kernels at daily mean temperatures ranging from 25.0°C to 30.7°C. These QTLs have not been mapped precisely yet.

In the present study, we sought to identify a gene in the *indica* cultivar 'Habataki' that confers tolerance to high temperature stress during the ripening period. We focused on 'Habataki' because it produces very few white-back and basal-white kernels, which are the main causes of grain quality reduction under high temperature stress. This is despite the fact that the appearance quality of 'Habataki' itself is poor due to the frequent occurrence of milky-white kernels and white-berry kernels (Supplemental Fig. 1 and Kobayashi *et al.* 1990). Chromosome segment substitution lines (CSSLs) of 'Habataki' in the 'Koshihikari' genetic background were developed as experimental material for QTL analysis. We identified and mapped a QTL and named it *Apq1* (*Appearance quality of brown rice 1*). Furthermore, we developed a NIL containing the 'Habataki' *Apq1* allele in the 'Koshihikari' genetic background. The effect of this 'Habataki' allele at *Apq1* was evaluated by assessing the responses of the NIL plants to high temperature stresses in air temperature-controlled growth chambers as well as under field conditions. Various agronomic traits were also assessed in the NIL to evaluate the suitability of this line for practical use in paddy fields.

Materials and Methods

Plant materials, growth conditions, and assessment of brown rice appearance quality

Thirty-two CSSLs (hereafter referred to as 'KHSLs') were developed through a process illustrated in Supplemental Fig. 2. For QTL detection and mapping, 'Habataki', 'Koshihikari', the KHSLs, and paired line populations (fixed lines derived from recombinants in QTL candidate chromosomal regions; see Results for details) were transplanted in late April at distances of 25 cm between plants and 25 cm between rows in a paddy field at the Toyama Agricultural Research Institute (TARI) in Toyama, Japan (36.4°N, 137.1°E). Nitrogen fertilizer was applied at customary rates (basal dressing 0.8 kg/a; top dressing 0.3 kg/a). At maturity, 10 plants of each line were harvested in bulk and threshed. After de-husking, immature grains were removed. Because of differences in grain thickness among the KHSLs, a mill-grade sieve with a mesh size of 1.7 mm was used in QTL detection. For QTL mapping, a 1.9-mm sieve was used. One hundred grains were randomly selected from each line and were classified as perfect grains, chalky grains, or other grains by visual inspection. Perfect grains were defined as non-chalky with a normal shape. Grains with unusual shapes or that were stained were classified as 'other' grains. Chalky grains were sub-classified as basal-white kernels, white-back kernels, white-berry kernels, milky-white kernels, or white-core kernels, as described by Ebitani *et al.* (2008). When two of these sub-classifications

occurred simultaneously (for example, basal-white and white-berry kernels), the grains were classified based on the following list, in order of priority: milky-white, white-back, basal-white, white-berry, and white-core.

QTL detection and mapping

QTL detection using the KHSLs was performed in 2008, and QTL mapping using paired line populations was performed in 2010, 2012, and 2013. For QTL detection, the PPG of each line (including 'Habataki') was compared to that of 'Koshihikari', with three replications. The heading date and 1,000-grain weight were also evaluated since these traits are related to the appearance quality of brown rice (Hori *et al.* 2012 for heading date; Ebitani *et al.* 2008 for 1,000-grain weight). The differences between 'Habataki', the KHSLs, and 'Koshihikari' were statistically evaluated using Dunnett's multiple comparison test. For QTL mapping, *t*-tests were performed to compare PPG values between KHSL-18 and KHSL-19 or between the 'Habataki' and 'Koshihikari' homozygous lines in paired line populations, with four replications. When there were significant differences in PPGs between two lines, we hypothesized that a QTL was present in the chromosomal region that differed between the lines. A probability level of 0.05 was used as the threshold for the detection of putative QTLs.

DNA marker analysis

RFLP marker analyses were performed according to the method of Kurata *et al.* (1994), using the ECL Direct Nucleic Acid Labeling and Detection System (Amersham Pharmacia, Uppsala, Sweden). Total plant DNA was extracted by the CTAB method from leaves (Murray and Thompson 1980). SSR marker analyses were performed as follows. Each PCR mixture contained 10 ng template DNA, 1.0 µL of 10 × PCR buffer (Hokkaido System Science, Hokkaido, Japan), 1.5 µL of 2.5 mM of each deoxynucleotide triphosphate (Hokkaido System Science), 1.5 µL of 5 × Tuning buffer (Hokkaido System Science), 0.1 µL of 2.5 units Taq DNA polymerase (Hokkaido System Science), and 0.5 µL of 20 pM of each primer in a total volume of 10.0 µL. The amplification conditions were as follows: 37 cycles of 95°C (20 s), 55°C (1 min), and 72°C (30 s), with a final extension at 72°C for 5 min. The primer sequences for the SSR markers shown in Supplemental Figs. 3, 4 and Fig. 4 (see Results) were obtained using data from McCouch *et al.* (2002) and the International Rice Genome Sequencing Project (2005), respectively. The markers EB_1, EB_2, EB_3, EB_Indel-03, EB_Indel-05, Tak6166-3, and Tak6168-8 were designed for the present study, and markers RM21975-2, RM21977-2, and RM21980-2 were modified for use in the present study (Supplemental Table 1). For analysis, the amplified DNA products were separated by electrophoresis on 3% agarose gels.

Evaluation of agronomic traits and grain appearance quality under high temperature stress of the Apq1-NIL

We used two lines, the *Apq1*-NIL and a control NIL (cont-NIL), to evaluate the usefulness of the ‘Habataki’ *Apq1* allele. These two lines were used in QTL mapping. The *Apq1*-NIL had a relatively small substituted ‘Habataki’ segment while the cont-NIL lacked this segment.

The *Apq1*-NIL, the cont-NIL, and ‘Koshihikari’ plants were transferred at the time of maximum tillering from a paddy field to 1/5000 a Wagner pots (one plant per pot), and the pots were placed in a water pool under natural conditions. When the first panicle in each pot had emerged, 10 pots per line were transferred to two growth chambers (five plants per line in each chamber). Nitrogen fertilizer was applied at 0.3 g/pot at the young panicle formation stage. The growth chambers were maintained under natural light conditions and two different temperature regimes (high and normal). The experiments were conducted in 2012 and 2013. In 2012, the high temperature conditions during the ripening period (9 A.M. to 6 P.M./6 P.M. to 9 A.M./average) were 30.5°C/25.6°C/27.4°C, respectively, and the normal temperature conditions were 28.3°C/23.6°C/25.4°C, respectively. In 2013, the high temperature conditions (31.3°C/26.3°C/28.2°C) were higher than in 2012, while the normal temperature conditions (28.6°C/23.4°C/25.3°C) were similar to those in 2012. The temperatures of the water pools in both growth chambers were kept constant at 25°C. At maturity, all kernels in each pot were harvested and the appearance quality was evaluated as described above.

In 2011 and 2012, the *Apq1*-NIL and ‘Koshihikari’ were cultivated in a paddy field at TARI. We raised 160 plants per plot, with three replications at a planting density of 22.9 hills/m². Nitrogen fertilizer was applied at customary rates (basal dressing 0.8 kg/a; top dressing 0.3 kg/a). Various agronomic traits, including heading date, culm length, panicle length, number of panicles, yield, and palatability, were evaluated using the method of Yamamoto *et al.* (1996).

Results

Development of KHSLs

The procedure used to develop the 32 KHSLs is illustrated in Supplemental Fig. 2. ‘Koshihikari’ (as the female) was crossed with ‘Habataki’, and the resultant F₁ plants were backcrossed to ‘Koshihikari’ to produce 81 BC₁F₁ plants. All of these BC₁F₁ plants were again backcrossed to ‘Koshihikari’ to produce BC₂F₁ seeds. The BC₂F₁ plants (one from each backcross) were analyzed using 77 DNA markers distributed over the whole genome (Supplemental Fig. 3). Based on this whole-genome survey, we used foreground selection to select target chromosomes that were heterozygous. The selected BC₂F₁ plants were again backcrossed to ‘Koshihikari’ to produce BC₃F₁ seeds. We then used a whole-genome survey with both foreground and background selection to minimize the occurrence of heterozygotes in non-target chromosomal regions. As a result, 63

BC₃F₁ plants were selected. One plant was heterozygous only in its target region and was homozygous for ‘Koshihikari’ in all other regions; therefore, this plant was self-pollinated and its BC₃F₂ seeds were harvested. The other 62 BC₃F₁ plants were backcrossed to ‘Koshihikari’ one or two more times. Background selection was performed in the BC₄F₁ and BC₅F₁ generations. Plants that had a ‘Habataki’ homozygous segment in their target regions (candidates for KHSLs) were selected from among the BC₃F₂, BC₄F₂, and BC₅F₂ populations. If the selected plants had heterozygous segments in non-target regions, background selection was again performed in the following generations. Finally, the whole genomes of 32 plants were examined using 133 SSR markers (Supplemental Fig. 4).

The genotypes of the 32 KHSLs are represented graphically in Fig. 1. The substituted chromosome segments cover most of the 12 chromosomes. However, one region (between RM7279 and RM1359) may not be covered because the substitution segments of KHSL-8 and KHSL-9 do not overlap. In general, each line has one substituted ‘Habataki’ segment in the ‘Koshihikari’ genetic background. KHSL-12 contains a small additional ‘Habataki’ segment (at RM4584 on chromosome 7). If it is assumed that each recombination occurred midway between the two surrounding markers, and based on the physical map of the International Rice Genome Sequencing Project (2005), the average length of the major ‘Habataki’ chromosome segment in each line should be 18.6 Mbp. The percentages of substituted segments on particular chromosomes ranged from 15.9% to 99.9%, and each chromosome was covered by two to four lines (Supplemental Fig. 4).

Detection of chromosomal region affecting PPGs

The PPGs of the parental lines ‘Habataki’ and ‘Koshihikari’ and of the 32 KHSLs were measured. The PPGs of ‘Habataki’ and ‘Koshihikari’ were 18.5% and 60.0%, respectively (Fig. 2A, 2B). The inferior grain quality of ‘Habataki’ was due to increased rates of white-berry kernels (39.0%), white-core kernels (9.5%), and milky-white kernels (31.0%). Very few white-back or basal-white kernels were found in ‘Habataki’. The PPGs of KHSL-5, KHSL-6, KHSL-7, KHSL-19, KHSL-24, and KHSL-32 were significantly higher than that of ‘Koshihikari’ (Fig. 2A). KHSL-2, KHSL-8, and KHSL-20 had significantly lower PPGs than that of ‘Koshihikari’ (Fig. 2A). The heading dates of KHSL-5 (8/13) and KHSL-7 (8/16) were much later than that of ‘Koshihikari’ (7/28), and therefore the average temperatures for these lines (25.0°C for KHSL-5 and 24.5°C for KHSL-7) were much lower than that of ‘Koshihikari’ (27.4°C) (Supplemental Table 2). Moreover, many sterile spikelets developed on KHSL-5 plants (data not shown). The 1,000-grain weights of KHSL-5 (18.5 g), KHSL-6 (21.3 g), KHSL-7 (20.8 g), KHSL-24 (20.7 g), and KHSL-32 (20.4 g), were more than 1 g lighter than that of ‘Koshihikari’ (22.7 g) (Supplemental Table 2). On the other hand, the heading date (8/2) and 1,000-grain weight (23.0 g) of

Fig. 1. Graphical representation of genotypes of the 32 KHSLs. Black and white segments indicate regions homozygous for ‘Habataki’ (Ha) and ‘Koshihikari’ (Ko), respectively. The genotypes were determined using 133 SSR markers (Supplemental Fig. 4)

Fig. 2. Comparison of the PPGs of the 32 KHSLs and the parental lines ‘Habataki’ and ‘Koshihikari’. A. Black bars indicate significant differences between ‘Habataki’ or the KHSL and ‘Koshihikari’ at $P < 0.05$, according to Dunnett’s multiple comparison test. White bars indicate no significant difference. B. Black and white regions indicate regions homozygous for ‘Habataki’ and ‘Koshihikari’, respectively. ** represents significance at $P < 0.01$. The double-sided arrow indicates the candidate region of the locus affecting PPG.

KHSL-19 were similar to those of ‘Koshihikari’ (Supplemental Table 2). Therefore, we selected KHSL-19 as a promising line for carrying ‘Habataki’-derived gene(s) that may enhance the brown rice appearance quality of plants with a ‘Koshihikari’ genetic background. By comparing the locations of the substituted chromosomal segments in KHSL-18 and KHSL-19, we determined that the locus affecting appearance quality must be located between RM6767 and the end of chromosome 7 (Fig. 2B).

Mapping the locus on chromosome 7 for grain appearance quality

To precisely map the putative locus on chromosome 7, paired line populations were developed through the process shown in Fig. 3. A plant with a heterozygous segment between SSR marker RM6011 and the end of chromosome 7 was identified in the BC₅F₂ population (Supplemental Fig. 2), and its self-progeny (192 plants) were used for the selection of recombinant plants. We selected six plants in which recombination occurred between RM6432 and RM5720. We then self-pollinated each recombinant plant, and identified offspring plants that were homozygous for either the ‘Habataki’ or the ‘Koshihikari’ genomic segments in the region of interest. These plants were used as paired line populations (Pair1-1 to Pair1-6) for substitution mapping of the locus in 2010. We found significant differences in the PPGs between the lines in Pair1-2, Pair1-3, and Pair1-4, but not in Pair1-1, Pair1-5, or Pair1-6 (Fig. 4A). This indicated that the three ‘Habataki’ substitution lines, Pair1-2H, Pair1-3H, and Pair1-4H, carried the ‘Habataki’ allele at the locus. This analysis mapped the locus to the chromosomal region flanked by the markers RM1132 and RM8261 (Fig. 4A).

To further narrow down the candidate genomic region of the QTL, nine plants were selected from the progeny (576 individuals) of the same heterozygous parents of the Pair1-3 plants. The selected plants were used to produce nine paired lines (Pair2-1 to Pair2-9), which were analyzed according to the procedure mentioned above. As a result, the target locus was mapped to the chromosomal region flanked by the markers EB_indel-03 and RM21975-2 (Fig. 4B). We then selected eleven plants from the progeny (1755 individuals) of the heterozygote, which had a heterozygous segment between RM6432 and RM3555. These were used to produce eleven paired lines (Pair3-1 to Pair3-11) according to the procedure mentioned above. The analysis of the Pair3 lines clearly indicated that the target locus was located between Tak6166-3 and RM21971 (Fig. 4C). The PPGs obtained for all of the Pair1, Pair2, and Pair3 lines that carried the target locus were similar. The heading dates of all paired lines were similar to that of ‘Koshihikari’ each year. The average temperatures were 29.4°C in 2010, 28.2°C in 2012, and 27.0°C in 2013. We named the putative locus *Apq1* (*Appearance quality of brown rice 1*).

Fig. 3. Flowchart illustrating the development of the paired line populations. Grey, white, and black boxes indicate heterozygous, ‘Koshihikari’ homozygous, and ‘Habataki’ homozygous chromosomal regions, respectively. A. The genotype of the plant from which KHSL-19 was derived. B. Only chromosome 7 is illustrated. Heterozygous plants, recombinant plants, and paired line populations are shown. K and H indicate lines homozygous for ‘Koshihikari’ and ‘Habataki’, respectively.

Grain appearance quality and agronomic traits in the *Apq1*-NIL

The Pair2-8H line (Fig. 4B) showed substitution for the chromosome region flanked by the markers EB_2 to RM3555 (2.8 Mbp). We used Pair2-8H as the *Apq1*-NIL and Pair2-8K as the cont-NIL.

The effect of *Apq1* under high temperature stress during the ripening period was evaluated in temperature-controlled growth chambers. In 2012 under high temperature conditions, the PPG of the *Apq1*-NIL (90.0%) was much higher than those of ‘Koshihikari’ (44.7%) and the cont-NIL (47.3%) (Fig. 5A). Under normal temperature conditions, the PPGs of the *Apq1*-NIL, ‘Koshihikari’, and the cont-NIL

Fig. 4. Mapping of *Apq1* using paired line populations. Black and white regions indicate regions homozygous for ‘Habataki’ and ‘Koshihikari’, respectively. The PPG data were obtained in 2010 for Pair1 lines (A), 2012 for Pair2 lines (B), and 2013 for Pair3 lines (C). * and ** represent significance at $P < 0.05$ and $P < 0.01$, respectively, according to the Student’s *t*-test. The double-sided arrow indicates the localized region of *Apq1*.

were 96.3%, 85.3%, and 77.3%, respectively. The results in 2013 showed similar trends as in 2012, though the PPG of the *Apq1*-NIL in 2013 was lower than in 2012 due to increased proportions of milky-white, white-back, and basal-white kernels (Fig. 5B). These results indicated that the

Apq1-NIL could produce grains with higher PPG values than those of ‘Koshihikari’ and the cont-NIL under high temperature stress conditions.

We also compared various agronomic traits between the *Apq1*-NIL and ‘Koshihikari’ under field conditions (Table 1).

Fig. 5. Grain appearance qualities of *Apq1*-NIL, 'Koshihikari', and cont-NIL plants grown in growth chambers under normal and high temperature conditions in 2012 (A) and 2013 (B). Photos (left, 'Koshihikari'; center, *Apq1*-NIL; right, cont-NIL) show grains which were produced under high temperature conditions (28.2°C average temperature). Scale bar: 1cm.

The average temperature in 2011 and 2012 were 27.7°C and 28.2°C, respectively. In 2011 there were no significant differences between the two lines regarding heading date, 1,000-grain weight, or any other important trait. In 2012, the culm length and the 1,000-grain weight differed significantly between the *Apq1*-NIL and 'Koshihikari'. The PPG of the *Apq1*-NIL appeared to be superior to that of 'Koshihikari'.

Discussion

In the present study, we identified a genomic region on chromosome 7 in 'Habataki' that enhances tolerance to high temperature stress during the ripening period. This tolerance is conferred by the 'Habataki' allele of *Apq1* as indicated by clear phenotypic differences in paired lines carrying the 'Habataki' allele (Fig. 4). Several QTLs affecting brown rice quality have previously been reported: He *et al.* (1999) detected a QTL on chromosome 8; Tan *et al.* (2000) detected a QTL on chromosome 5; Wan *et al.* (2005b) identified QTLs on chromosomes 8 and 9; Tabata *et al.* (2007) found QTLs on chromosomes 1, 2, and 8; Ebitani *et al.* (2008) detected QTLs on chromosomes 2, 9, 11, and 12; and Kobayashi *et al.* (2013) identified QTLs on chromosomes 6 and 9. Based on these chromosomal locations, the locus detected in the present study is different from the previously reported QTLs.

Based on PPGs, the appearance quality of brown rice derived from the *Apq1*-NIL grown at high temperatures in the growth chamber was clearly superior to those of the cont-NIL and 'Koshihikari' in 2013 (28.2°C) as well as 2012 (27.4°C) (Fig. 5). Moreover, the PPGs of almost all lines harboring the 'Habataki' *Apq1* allele were consistently above 85% in each of the three years, including 2010, which had a very high average temperature of 29.4°C (Fig. 4). Kondo *et al.* (2006) and Wakamatsu *et al.* (2007) reported that the PPG decreased when the average temperature rose to more than 27°C. Moreover, in recent years, the average temperatures in areas where 'Koshihikari' is grown have often exceeded 28°C. Therefore, the effects of the *Apq1*

Table 1. Comparison of agronomic traits between the *Apq1*-NIL and 'Koshihikari'

Year	Line/Variety	Heading date (m/d)	Culm length (cm)	Panicle length (cm)	Panicle number (No./m ²)	Lodging degree ^a	Yield (kg/a)	1000-grain weight (g)	Percentage of perfect grains (%)	Eating quality ^b
2011	<i>Apq1</i> -NIL	8/3	92	18.7	440	3.3	56.5	22.7	88.7	-0.04
	Koshihikari	8/3	91	19.2	402	1.8	59.4	23.5	83.0	-0.06
		n.s. ^c	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
2012	<i>Apq1</i> -NIL	8/1	82	17.8	396	0.8	57.8	23.1	91.5	-0.02
	Koshihikari	8/2	85	18.2	397	1.0	57.3	23.4	73.5	0.03
		n.s.	*	n.s.	n.s.	n.s.	n.s.	*	**	n.s.

^a Lodging degree was classified into 6 levels (0 indicating standing to 5 indicating lodged).

^b Eating quality shows the aggregate value for overall evaluation and was classified into 9 levels (2 indicating excellent to -2 indicating especially bad).

^c n.s. denotes no significant difference between the *Apq1*-NIL and 'Koshihikari'. * and ** indicate that the differences between *Apq1*-NIL and 'Koshihikari' were significant at $P < 0.05$ and $P < 0.01$, respectively.

allele evidently are useful.

The chromosomal location of the *Apq1* locus was delimited to a region of 48 kb between Tak6166-3 and RM21971 (Fig. 4C). In the Rice Annotation Project Database (<http://rapdb.dna.affrc.go.jp/>), there are five predicted genes (*Os07g0616800*, *Os07g0616900*, *Os07g0617000*, *Os07g0617100*, and *Os07g0617500*) within this region. The Rice Expression Profile Database (<http://ricexpro.dna.affrc.go.jp/>) provides gene expression profiles from microarray analyses. Data from this database indicate that *Os07g0616800* is expressed more highly than the other four genes and that *Os07g0616800* is particularly strongly expressed in the ovary and the endosperm at the filling stage. In addition, RNA-seq data from the Rice Annotation Project Database indicate that *Os07g0616800* is highly expressed in seeds and panicles after flowering. Although linkage analysis allowed us to identify several positional candidate genes, further studies, including a more detailed expression analysis and genetic complementation analysis, will be required to clone the *Apq1* gene.

All agronomic traits of the *Apq1*-NIL were similar to those of 'Koshihikari' (Table 1). The culm length and 1,000-grain weight differed slightly between the *Apq1*-NIL and 'Koshihikari' in 2012; however, these small differences would cause no practical difficulties in a commercially grown crop. The appearance quality of brown rice is generally affected by heading date because of changes in temperature during the ripening period (Hori *et al.* 2012). Ebitani *et al.* (2008) reported that high PPG lines tend to have lighter 1,000-grain weights. On the other hand, the heading date and 1,000-grain weight of the *Apq1*-NIL were similar to those of 'Koshihikari'. In addition, the *Apq1*-NIL shows no disadvantages in yield and eating quality compared with 'Koshihikari'. Therefore, we conclude that the *Apq1* allele from 'Habataki' improves grain quality without affecting other important agronomic traits.

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