Detection of Quantitative Trait Loci for White-back and Basal-white Kernels under High Temperature Stress in *japonica* Rice Varieties

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Several quantitative trait loci (QTLs) associated with the apparent quality of brown rice were identified. QTL analysis was carried out using F_2 and F_3 populations derived from a cross between two *japonica* varieties, Hana-echizen (high quality of brown rice) and Niigatawase (low quality with numerous white-back and basal-white kernels). F_2 individuals were grown in paddy fields in 2003, and F_3 lines were grown in paddy fields and in a greenhouse to expose them to high temperature stress during the ripening period in 2004. Apparent quality of brown rice was evaluated based on the percentage of white-back and basal-white kernels. Two putative QTLs associated with white-back kernels in the F_2 population grown under low temperature conditions in paddy fields in 2003 were identified on chromosomes 3 and 6. The closest SSR markers were RM4512 and RM3034, respectively. One putative QTL associated with basal-white kernels in the F_2 population was identified on chromosome 6. The closest marker was RM3034. Two putative QTLs associated with white-back kernels in the F_3 population grown under high temperature conditions in paddy fields in 2004 were identified on chromosomes 4 and 6. The closest SSR markers were RM3288 and RM3034, respectively. One putative QTL associated with white-back plus basal-white kernels in the F_3 population grown under high temperature stress in the greenhouse was identified on chromosome 6. The closest marker was RM3034. The QTLs identified near RM3034 on the short arm of chromosome 6 contributed most to the apparent quality of brown rice. The QTLs identified near RM4512 and RM3288 which also affected the apparent quality of brown rice, were detected in either the F_2 or F_3 population. The QTLs identified in the present study should be useful for marker-assisted selection to breed varieties with a high apparent quality of brown rice, especially varieties with tolerance to kernel damage due to high temperature stress during the ripening period.

Key Words: QTL, apparent quality of brown rice, white-back kernels, basal-white kernels, high temperature stress.

Introduction

Recently serious deterioration of the apparent quality of brown rice has been reported in many areas in Japan (Arisaka 2001, Terashima *et al.* 2001, Terashima 2003, Omoteno *et al.* 2003, Tsukimori 2003).

In Fukui prefecture, from 1974 to 1998, 80 to 95% of brown rice had been ranked as first grade rice in the inspection standard for non-glutinous paddy brown rice described in the report of Hoshikawa (1989). However, from 1999 onward, the percentage of brown rice ranked as first grade sometimes fell below 70%, especially, it decreased to 69 and 62% in 1999 and 2002, respectively. The summer of these two years was very hot. The average temperature in July and August in 1999 and 2002 was 28.8°C and 28.5°C, respectively, whereas that in a normal year, 26.8°C.

Fukui prefecture is located at the southern end of the

Hokuriku region, which is characterized by high temperatures during the ripening period. This region is suitable for studies and breeding programs for tolerance to kernel damage due to high temperature during the ripening period. Nishimura *et al.* (2000) reported that many varieties derived from Koshihikari, which was bred in Fukui, displayed such tolerance. In general, early maturing varieties are susceptible to kernel damage due to high temperature stress during the ripening period. However, several early maturing varieties which are tolerant to high temperatures during the ripening period were bred in the Hokuriku region, such as Hana-echizen (Horiuchi *et al.* 1992), Koshi-ibuki (Hoshi *et al.* 2002) and Ten-takaku (Yamaguchi *et al.* 2005).

It was suggested that high temperatures during the ripening period affected the apparent quality of brown rice (Konno *et al.* 1991, Morita 2000, Terashima *et al.* 2001). Furthermore, with the progression of global warming, it is possible that temperatures during the ripening period could become higher. Therefore, tolerance to kernel damage due to high temperature stress during the ripening period has become an important objective in rice breeding in Japan.

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Nagato and Ebata (1965) and Iida *et al.* (1997) reported that a large number of white-back and basal-white kernels occurred due to high temperature. White-back kernels contain a few layers of starch cells distributed along the vascular bundles on the dorsal side of a rice grain and look white and opaque due to incomplete filling. In the case of basal-white kernels, the basal portion of a grain is white and opaque (Taira 1995). Nagato and Ebata (1965) and Iida *et al.* (2002) indicated that the percentage of white-back kernels was the most appropriate index of tolerance to kernel damage due to high temperature stress during the ripening period, because they occurred only under high temperature conditions. Clear varietal differences were reported in the occurrence of white-back kernels due to high temperature (Nagato *et al.* 1961, Komaki *et al.* 2000, Nishimura *et al.* 2000).

Nishimura *et al.* (2000) and Iida and Tabata (2003) indicated that the tolerance to kernel damage due to high temperature stress during the ripening period was genetically controlled, and Tabata *et al.* (2005) performed a genetic analysis of the occurrence of white-back and basal-white kernels associated with high temperature during the ripening period.

He *et al.* (1999), Terao *et al.* (2004) and Ebitani *et al.* (2005) reported the presence of QTLs associated with the apparent quality of brown rice. These studies were conducted using a population derived from a cross between a *japonica* variety and an *indica* variety. It is preferable to use QTL information about apparent quality of brown rice using a population derived from a cross between *japonica* varieties for the breeding of *japonica* varieties, because of the differences in the shape and size of brown rice between *japonica* and *indica* varieties. Shirasawa *et al.* (2006) reported that several QTLs were associated with white-back kernels under high temperature stress using a recombinant inbred line population derived from a cross between two *japonica* varieties. However, there are very few studies in which only *japonica* varieties were used, because the construction of a genetic map is more difficult using two *japonica* varieties due to the rather low polymorphisms, compared with that of a genetic map using *indica* and *japonica* varieties.

We investigated the apparent quality of brown rice and identified several QTLs, using a population derived from a cross between two *japonica* varieties, Hana-echizen and Niigatawase (Bao *et al.* 2004, Ye *et al.* 2006). In the present study, we conducted a QTL analysis on the occurrence of white-back and basal-white kernels using F_2 and F_3 populations.

Materials and Methods

Plant materials

A cross was made between Hana-echizen and Niigatawase that are both early maturing varieties in which the apparent quality of brown rice is considerably different. The apparent quality of brown rice in Hana-echizen is high (Horiuchi *et al.* 1992, Inoue 1999), whereas, that in Niigata-

wase is low because of the presence of numerous white-back and basal-white kernels (Sasaki *et al.* 1983, Hoshi *et al.* 2002, Nagahata and Yamamoto 2005). Niigatawase showed a high percentage of white-back and basal-white kernels not only upon hot water irrigation during the ripening period but also under normal air temperature conditions in paddy fields (Kasaneyama *et al.* 1999).

A total of 180 F_2 individuals from the cross and the parents were transplanted to paddy fields in Fukui in 2003, and cultivated according to standard practices. Leaves were individually sampled from each F_2 plant to isolate genomic DNA. A total of 180 F_3 families derived from the F_2 individuals and the parents were transplanted to paddy fields and to a greenhouse in 2004, and cultivated according to standard practices. Air temperature in the greenhouse was controlled by ventilation when the temperature exceeded 35°C. Days to heading were calculated from the time of transplanting.

Seeds of 180 F_2 individuals and seeds of 10 plants from each of the 180 F_3 lines and the parents were harvested 30 days after heading, air-dried and hulled to evaluate the apparent quality of brown rice.

Evaluation of kernel and other traits

Five-hundred grains were sampled from each plant, and the number of white-back and basal-white kernels was counted according to the criterion adopted by The Ministry of Agriculture, Forestry and Fisheries of Japan (<http://www.kanbou.maff.go.jp/www/gichou/hitemp/hannteikizyun/PAGE001.HTM>). When the opaque portion of the dorsal side was longer than 2/3 of the kernel length and wider than 1/3 of the kernel width, the kernel was classified into white-back. When the opaque portion was distributed in the basal portion and the size was larger than 1/5 of the kernel size, the kernel was classified into basal-white. In the F_3 lines grown in the greenhouse in 2004, since numerous white-back and basal-white kernels occurred simultaneously, we could not distinguish them. Therefore, the apparent quality was expressed as the percentage of the sum of white-back plus basal-white kernels. Phenotypic values of the apparent quality of brown rice were expressed as the percentage of white-back or basal-white kernels after arc sine transformation to normalize the variance.

Nagato and Ebata (1965) reported that white-back and basal-white kernels occurred due to high temperature during the ripening period, which impeded the accumulation of starch. Decrease in final grain weight at high temperatures was attributed to the early decline of the sink activity of caryopses (Kumura 1995). Since the sink size may be closely related to kernel traits, we measured the sink size, namely the panicle weight and one-thousand kernel weight in the F_2 generation. Panicle weight was measured as grain yield per plant. Panicle weight and one-thousand kernel weight of the parents (Hana-echizen and Niigatawase) were calculated based on the average value of 10 plants.

DNA isolation and QTL analysis

Genomic DNA was extracted from the leaves using 100 mM Tris-HCl (pH 8.0) buffer containing 10 mM EDTA and 1 M KCl, according to the method of Monna *et al.* (2002). PCR was performed in 10 µl volume of a reaction mixture, containing 10 ng of genomic DNA, 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 4 µM of each forward and reverse primer, 1.5 mM MgCl₂, 2 mM of dNTPs and 0.25 unit of AmpliTaq Gold DNA polymerase (Applied Biosystems). The mixture was initially heated at 95°C for 10 min, followed by 35 cycles at 94°C for 30 sec, 55°C for 1 min and 72°C for 2 min on a PCR system (iCycler Thermal Cycler System, BIO RAD). PCR products were fractionated on a 2.0% agarose TBE gel. After EtBr dyeing, the PCR products were visualized using an UV illuminator and polymorphisms were detected.

A total of 407 simple sequence repeat (SSR) markers were screened to identify polymorphisms between Hana-echizen and Niigatawase. Then a genetic map was constructed using the polymorphic SSR markers with MAPMAKER/EXP 3.0 (Lander *et al.* 1987). The Kosambi mapping function was used to estimate marker distances.

Composite interval mapping (CIM) was performed using Windows QTL Cartographer 2.5 (Wang *et al.* 2006) with the forward and backward regression model (model 6). To control the genetic background, 5 markers and 10 cM window size were used for CIM. The significance level of the experiment-wise LOD threshold was determined by computing 1000 permutations as implemented by the software. The LOD peaks at significant QTL intervals were considered to correspond to the putative QTL locations on the linkage map. The percentage of phenotypic variation explained by a QTL for traits and the additive effect were also estimated using the software. In addition, we performed single marker analysis for solitary markers which were not linked to any linkage group. This analysis fitted the data to the simple linear regression model to determine whether the marker was linked to a QTL.

Results

Days to heading and temperature during the ripening period

The average days to heading of Hana-echizen and Niigatawase in paddy fields were 56 and 58 days, respectively in 2003, and 59 and 60 days, respectively in 2004. The average days to heading for Hana-echizen and Niigatawase which were grown in the greenhouse in 2004 were both 60 days. Days to heading of the F₂ population in paddy fields ranged from 54 to 61 days, and those of the F₃ population in paddy fields and in the greenhouse ranged from 54 to 66 days and from 57 to 64 days, respectively (Table 1 and Fig. 1A, B).

Average temperatures during the ripening period, namely 30 days after heading, were 24.9°C, 27.2°C and 31.2°C, in paddy fields in 2003, 2004 and in the greenhouse in 2004, respectively. The temperature in the summer of 2003 was relatively low compared to that in a normal year in Fukui (26.7°C). On the other hand, in the paddy fields in 2004, the temperature was considered to be high. Indeed, based on the studies of Terashima (2003) on the apparent quality of brown rice, when the average temperature after heading exceeds 27°C, the apparent quality of brown rice is likely to deteriorate. The greenhouse condition was considered to correspond to strong high temperature stress.

Variation in the percentage of white-back and basal-white kernels in the parents, F₂ and F₃ populations

The percentage of white-back and basal-white kernels in Hana-echizen and Niigatawase is listed in Table 1. In general, the apparent quality of brown rice in Hana-echizen was much higher than that in Niigatawase. All the differences in the percentage of white-back and basal-white kernels between Hana-echizen and Niigatawase were significant. Especially, Hana-echizen did not exhibit white-back kernels under paddy field conditions either in 2003 or 2004, and very few basal-white kernels occurred under paddy fields conditions in 2003 (low temperature summer). The values of

Table 1. Kernel and other traits of Hana-echizen, Niigatawase, F₂ population cultivated in paddy fields in 2003, and F₃ population cultivated in paddy fields and greenhouse in 2004

Traits	Condition		2003						2004					
			Hana- echizen ¹⁾	Niigata- wase ¹⁾	F ₂			Hana- echizen ¹⁾	Niigata- wase ¹⁾	F ₃				
					Ave.	Max.	Min.			Ave.	Max.	Min.		
White-back kernels	Paddy fields	(%)	0.0	4.8* ²⁾	6	38	0	0.0	15.4**	11	48	1		
Basal-white kernels	Paddy fields	(%)	0.7	17.4** ²⁾	8	44	0	7.8	34.8**	20	39	0		
White-back plus basal-white kernels	Greenhouse	(%)	—	—	—	—	—	51.6	82.6**	74	100	32		
Days to heading	Paddy fields	(days)	56	58 ns ²⁾	57	61	54	59	60 ns	60	66	54		
	Greenhouse	(days)	—	—	—	—	—	60	60 ns	61	64	57		
Panicle weight	Paddy fields	(g/plant)	28.7	24.5**	28.7	39.8	17.1	—	—	—	—	—		
One-thousand kernel weight	Paddy fields	(g)	20.6	18.5**	19.9	21.8	18.2	—	—	—	—	—		

¹⁾ Value for Hana-echizen and Niigatawase is the average of 10 plants.

²⁾ **, * and ns: t-test significant at 1%, 5% levels and not significant, respectively.

Fig. 1. Frequency distribution of days to heading, percentage of white-back and basal-white kernels and one-thousand kernel weight in the F_2 and F_3 populations. Bars indicate the average and range of the parent plants.

one-thousand kernel weight and panicle weight of Hana-echizen were significantly higher than those of Niigatawase, as shown in Table 1.

Frequency distribution of the percentage of white-back and basal-white kernels in the F_2 and F_3 populations was mostly continuous (Fig. 1). As indicated in Figure 1, some F_2

individuals and some F_3 lines showed a higher percentage of white-back kernels than Niigatawase, the parent with the lowest apparent quality of brown rice (Fig. 1C and D). On the other hand, the percentage of basal-white kernels in the progenies was distributed mostly between that of their parents (Fig. 1E and F). The distribution of the percentage of

white-back plus basal-white kernels under the greenhouse conditions was not symmetrical: the skewness was -2.16 , leaning to the average value recorded in Niigatawase (Fig. 1H). The values of one-thousand kernel weight (Fig. 1G) and panicle weight (not in the figure) were distributed mostly between those of the parents.

Correlation analysis was conducted among the traits (Table 2). The percentage of white-back kernels and basal-white kernels was positively and highly correlated in both the F_2 and F_3 populations. The correlation of the occurrence of white-back and basal-white kernels between the F_2 and F_3 populations is shown in Figure 2. There was a positive correlation between them. As shown in Table 2, there was no relationship between the days to heading and the percentage of white-back or basal-white kernels in the F_2 population. However, in the F_3 population, the percentage of basal-white kernels was positively correlated with the days to heading. The values of one-thousand kernel weight and the percentage of white-back or basal-white kernels in the F_2 population were positively correlated, while the values of panicle weight and the percentage of white-back or basal-white kernels in the F_2 population were not significantly related.

Genetic map construction

Among the 407 SSR markers screened, 64 (15.7%) showed polymorphisms between Hana-echizen and Niigatawase. These markers were classified in 13 linkage groups by linkage analysis, except for 8 markers which did not belong to any linkage group. The information about these 8 solitary markers is listed in Figure 3. Finally, the genetic map was constructed using 56 SSR markers on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 10 and 11 (Fig. 3).

QTL identification

The analysis by CIM revealed the presence of QTLs associated with white-back and basal-white kernels and one-thousand kernel weight (Table 3 and Fig. 3). The threshold values for $P < 0.05$ ranged from 3.64 to 4.38. For all the QTLs associated with white-back, basal-white and white-back plus basal-white kernels, the Hana-echizen alleles decreased the ratio of damaged kernels.

Table 4 shows the solitary markers which were linked to a QTL by single marker analysis.

White-back kernels

Significant threshold LOD values ($P < 0.05$) of white-back kernels under low temperature conditions in paddy

Table 2. Correlation matrix between measured traits of F_2 individuals and F_3 lines

Traits	Dh F_2	Dh F_3	Wb F_2	Bw F_2	Wb F_3	Bw F_3	Gh	Tw F_2	Pw F_2
Days to heading of F_2 (Dh F_2)	1.00								
Days to heading of F_3 (Dh F_3)	0.37** ²⁾	1.00							
White-back kernels of F_2 (Wb F_2)	0.08	-0.07	1.00						
Basal-white kernels of F_2 (Bw F_2)	0.08	-0.02	0.60**	1.00					
White-back kernels of F_3 (Wb F_3)	-0.02	-0.13	0.46**	0.38**	1.00				
Basal-white kernels of F_3 (Bw F_3)	0.17* ²⁾	0.20**	0.32**	0.32**	0.52**	1.00			
White-back plus basal-white kernels in Greenhouse ¹⁾ (Gh)	0.14*	0.06	0.24**	0.17*	0.36**	0.39**	1.00		
One-thousand kernel weight of F_2 (Tw F_2)	-0.29**	-0.09	0.27**	0.24**	0.27**	0.23**	0.22**	1.00	
Panicle weight of F_2 (Pw F_2)	-0.11	0.01	0.02	0.07	-0.01	-0.09	-0.03	0.16*	1.00

¹⁾ White-back plus basal-white kernels occurred under high temperature stress in the greenhouse in the F_3 population.

²⁾ ** and *: significant at 1% and 5% levels, respectively.

Fig. 2. Correlation of the percentage of white-back (A) and basal-white (B) kernels between F_2 individuals and average value of F_3 lines.

Fig. 3. Linkage map and positions of putative QTLs associated with white-back and basal-white kernels and one-thousand kernel weight, and solitary markers which were not linked to any linkage group. Bars on the left side of the chromosomes indicate 1-LOD likelihood intervals, and the triangles indicate the position of the peak LOD in the interval. wb: white-back kernels, bw: basal-white kernels, gh: white-back plus basal-white kernels under greenhouse conditions, tw: one-thousand kernel weight, F₂: traits measured in the F₂ population in 2003, F₃: traits measured in the F₃ population in 2004.

Table 3. QTLs of kernel and other traits detected by composite interval mapping

Year	Population	Traits	Chr.	CML ¹⁾	LOD	AE ²⁾	PVE ³⁾	Threshold ⁴⁾
2003	F ₂	White-back kernels	3	RM4512	4.60	-0.73	25.9	3.93
			6	RM3034	6.80	-0.84	31.2	
		Basal-white kernels	6	RM3034	8.08	-1.58	14.1	4.02
		One-thousand kernel weight	3	RM4512	4.98	-0.17	12.3	4.09
2004	F ₃	White-back kernels	4	RM3288	4.36	-0.53	15.2	3.96
			6	RM3034	13.39	-1.14	59.6	
		White-back plus basal-white kernels in Greenhouse	6	RM3034	7.80	-0.19	75.1	3.64

¹⁾ Closest marker locus of putative QTLs.

²⁾ Additive effects (1/2 weight) of Hana-echizen allele. Numbers expressed in italics indicate additive effects based on arc sine transformation of the percentage of white-back or basal-white kernels.

³⁾ Percentage of phenotypic variation explained by each QTL.

⁴⁾ Significant threshold LOD value ($P < 0.05$) determined by computing 1000 permutations.

fields in the F₂ population (2003) and under high temperature conditions in paddy fields condition in the F₃ population (2004) were 3.93 and 3.96, respectively. Two QTLs for

white-back kernels in the F₂ population were detected on chromosomes 3 and 6. The closest markers to the QTLs were RM4512 (chromosome 3) and RM3034 (chromosome 6),

Table 4. Markers linked to a QTL by a single marker analysis

Year	Population	Traits	Chr.	Marker	Significance ¹⁾	AE ²⁾
2003	F ₂	White-back kernels	11	RM552	*	-0.21
		Basal-white kernels	11	RM552	*	-0.19
		Days to heading	12	RM2197	*	0.35
2004	F ₃	White-back kernels	1	RM3440	*	0.26
			2	RM5654	*	-0.21
		Basal-white kernels	12	RM2197	**	0.15
		White-back plus basal-white kernels in Greenhouse	12	RM2197	*	0.01
		Days to heading	12	RM2197	**	0.40

¹⁾ Marker was linked to a QTL at significant 5% and 1% levels indicated by * and **, respectively.

²⁾ Additive effects (1/2 weight) of Hana-echizen allele. Numbers in italics indicate additive effects based on arc sine transformation of the percentage of white-back and basal-white kernels.

respectively. Two QTLs for white-back kernels in the F₃ population were detected on chromosomes 4 and 6. The closest markers were RM3288 (chromosome 4) and RM3034, respectively. The QTLs near RM3034 were detected in both populations. The values of the phenotypic variance explained by the individual QTLs were 25.9% (RM4512), 31.2% and 59.6% (RM3034), and 15.2% (RM3288).

Basal-white kernels

Significant threshold LOD values ($P < 0.05$) of basal-white kernels in the F₂ population (2003) and in the F₃ population (2004) were 4.02 and 3.61, respectively. A QTL for basal-white kernels in the F₂ population was detected on chromosome 6. The closest marker to the QTL was RM3034. The phenotypic variance explained by the QTLs was 14.1%. No QTLs for basal-white kernels were detected in the F₃ population.

White-back plus basal-white kernels in the greenhouse

Significant threshold LOD value ($P < 0.05$) of the white-back plus basal-white kernels under high temperature stress in the greenhouse in the F₃ population (2004) was 3.64. One QTL for white-back plus basal-white kernels in the greenhouse was detected on chromosome 6. The closest marker to the QTL was RM3034. The phenotypic variance explained by the QTL was 75.1%.

Other traits

Significant threshold LOD values ($P < 0.05$) of days to heading in the F₂ population (2003) and F₃ population (2004) were 3.93 and 4.24, respectively. No QTLs for days to heading were detected in either the F₂ or F₃ population. Significant threshold LOD value ($P < 0.05$) of one-thousand kernel weight in the F₂ population was 4.09. One QTL for one-thousand kernel weight was detected near RM4512 (chromosome 3). The phenotypic variance explained by the QTL was 12.3%. Significant threshold LOD value ($P < 0.05$) of panicle weight in the F₂ population was 4.38. No QTLs for panicle weight were detected.

Discussion

Tolerance to kernel damage due to high temperature during the ripening period is one of the major objectives in rice breeding in Japan. Genetic and QTL information is necessary for efficient breeding, using DNA marker-assisted selection. Ebitani *et al.* (2005) identified three QTLs for the apparent quality of brown rice on chromosomes 2, 9 and 12, using chromosome segment substitution lines in which Kasalath (*indica*) segments had been introgressed into the Koshihikari (*japonica*) background. Terao *et al.* (2004) identified four QTLs for the apparent quality of brown rice on chromosomes 2, 4, 9 and 10 using a population derived from a cross between Habataki (*indica*) and Sasanishiki (*japonica*). He *et al.* (1999) found two QTLs for the percentage of white core kernels on chromosomes 8 and 12 using a doubled haploid population derived from a cross between ZYQ8 (*indica*) and JX17 (*japonica*). These are all the studies using a population derived from *indica/japonica* crossings.

In the present study, QTLs for the apparent quality of brown rice were analyzed using a progeny population derived from a cross between two *japonica* varieties, Hana-echizen and Niigatawase. The apparent quality of brown rice was evaluated based on the percentage of white-back and basal-white kernels. Hana-echizen showed a very low percentage of white-back and basal-white kernels under paddy field conditions. The occurrence of white-back and basal-white kernels in Hana-echizen was significantly lower than that in Niigatawase not only under low and high temperature conditions in paddy fields but also under high temperature stress in the greenhouse. Under the high temperature stress in the greenhouse, the percentage of white-back plus basal-white kernels in Hana-echizen was about 30% lower than that in Niigatawase. On the other hand, the values of panicle weight and one-thousand kernel weight in Hana-echizen were significantly higher than those in Niigatawase. In general, a larger sink size, namely higher value of panicle weight or one-thousand kernel weight, is more unfavorable

for starch accumulation. For example, one-thousand kernel weight and the occurrence of white-back and basal-white kernels were positively correlated (Table 2). In this regard, the tolerance of Hana-echizen to kernel damage due to high temperature stress was very unique. Although the sink size of Hana-echizen was larger, the apparent quality of brown rice was much higher than that of Niigatawase.

We identified several QTLs for the apparent quality of brown rice. A putative QTL identified near the RM3034 marker on the short arm of chromosome 6 contributed most to the apparent quality of brown rice. This QTL was detected for white-back kernels, basal-white kernels and white-back plus basal-white kernels under greenhouse conditions. For the white-back kernels, QTLs were detected under both low temperature conditions in paddy fields in the F_2 population (2003) and under high temperature conditions in paddy fields in the F_3 population (2004). For the basal-white kernels, a QTL was detected only in the F_2 population (2003). These QTLs near the RM3034 marker displayed high LOD scores (6.80–13.39) and relatively high phenotypic variations (14.1%–75.1%), as shown in Table 3.

Based on these results, it appears that a gene with a major effect on the short arm of chromosome 6 controls the apparent quality of brown rice. Near the RM3034 marker, QTLs for days to heading or sink size (one-thousand kernel weight or panicle weight) were not identified in the present study. Therefore, the influence of days to heading or sink size on the apparent quality of brown rice was considered to be limited.

On the short arm of chromosome 6, several QTLs associated with chalkiness had been detected, according to other studies. Chalkiness is the visual rating of the chalky proportion in the grain. This trait is considerably affected by genes and also by the environment, especially high temperature immediately after heading. Li *et al.* (2003) reported the presence of a QTL associated with chalkiness of the endosperm on the short arm of chromosome 6 using an F_2 population derived from the cross Nipponbare (*japonica*)/Kasalath (*indica*)/Nipponbare. Xu *et al.* (2000) also reported the presence of a QTL associated with chalkiness of the endosperm and the percentage of occurrence of white-core on the short arm of chromosome 6, using F_2 and F_3 populations and an F_{10} recombinant inbred line population from a cross between Zh97 and Ming63, the parents of Shanyou63, the most widely grown *indica* hybrid rice variety in China. Shirasawa *et al.* (2006) also detected a QTL for white-back kernels under high temperature stress on the short arm of chromosome 6 using recombinant inbred lines derived from a cross between Kokoromachi (*japonica*) and Tohoku168 (*japonica*). The phenotypic variation was the largest among the QTLs they found. It is interesting to note that QTLs for the apparent quality of brown rice were detected on the short arm of chromosome 6, though mapping populations differed from each other. Due to the lack of common DNA marker shared in their and our studies, it is not possible to determine the allelism of these QTLs.

The putative QTL associated with white-back kernels was detected near the RM4512 marker on chromosome 3 under low temperature conditions in paddy fields in the F_2 population (2003). This QTL was not identified under high temperature conditions in paddy fields in the F_3 population (2004) and the LOD score and phenotypic variation were smaller than those of the QTL near the RM3034 marker. Therefore, this QTL is likely to exert a minor effect on the apparent quality of brown rice, or it may act only under low temperature conditions (this QTL would not be effective under high temperature conditions such as those in 2004). Li *et al.* (2003) also reported the presence of a QTL associated with chalkiness of the endosperm on chromosome 3. Due to the lack of common DNA marker shared in their and our studies, it is not possible to determine the allelism of these QTLs. Near the RM4512 marker on chromosome 3, a putative QTL for one-thousand kernel weight was also detected. The percentage of white-back or basal-white kernels was positively correlated with one-thousand kernel weight (Table 2). Therefore, further studies should be carried out to determine whether this QTL for the apparent quality of brown rice actually exists, or represents a side-effect of the QTL for one-thousand kernel weight.

The putative QTL associated with white-back kernels under high temperature conditions in paddy fields in the F_3 population (2004) was detected near the RM3288 marker on chromosome 4. This QTL was not identified under low temperature conditions in paddy fields in the F_2 population (2003). Therefore, it is likely to exert a minor effect on the apparent quality of brown rice, or it may act only under high temperature conditions. On chromosome 4, we found only two polymorphic SSR markers. Further studies using a larger number of markers should be carried out.

Single marker analysis enabled to identify markers which were linked to QTLs (Table 4), namely RM3440 on chromosome 1, RM5654 on chromosome 2, RM552 on chromosome 11 and RM2197 on chromosome 12 were associated with QTLs for white-back or basal-white kernels. The QTLs for days to heading were linked to the RM2197 marker on chromosome 12 both in the F_2 and F_3 populations based on the single marker analysis. Further studies should be conducted in these regions where the genetic map is incomplete.

The QTLs detected in the present study represent the minimum number among the possible QTLs, because the genetic map is not complete and shows many gaps across the chromosomes. However the QTL information obtained in the present study should be useful for marker-assisted selection to breed varieties with a high apparent quality of brown rice, especially varieties with tolerance to kernel damage due to high temperature stress during the ripening period. The temperature during the ripening period in 2003 was lower than that in a normal year in Fukui. However, the QTL information obtained in the F_2 population grown in 2003 could be used for DNA marker selection for high apparent quality of brown rice. As the temperature during the ripening period in

paddy fields in 2004 was considered to correspond to high temperature stress, the QTL information obtained in the F_3 population grown in paddy fields in 2004 could be useful to develop DNA markers to select varieties tolerant to kernel damage due to high temperature. The QTL information obtained in the F_3 population grown in the greenhouse in 2004 could be important when global warming intensifies.

To use marker-assisted selection in a rice breeding program for tolerance to kernel damage due to high temperature stress, it will be necessary to identify DNA markers tightly linked to the genes controlling the tolerance by fine mapping. The development of near-isogenic lines differing only in the presence of a single and specific QTL for white-back or basal-white kernels should be promoted to perform fine mapping. We are currently constructing a genetic map by adding SSR and single nucleotide polymorphism markers to fill in the gap regions, and analyzing QTLs for the apparent quality of brown rice again, using recombinant inbred lines derived from the F_3 lines to confirm the existence of the QTLs detected in the present study. In addition, backcrosses to develop near-isogenic lines have been initiated.

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