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Research Paper

Finding a novel QTL responsible for kernel cracking resistance from CSSLs of 'Itadaki' (*O. sativa* L.) × donor *O. rufipogon*

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To find new QTLs responsible for kernel cracking resistance, we screened 50 CSSLs derived from the moderately resistant cultivar 'Itadaki' (*O. sativa* L.) and the donor *O. rufipogon*. Two lines, IRSL 30 and IRSL 37, were selected as resistant. QTL analyses of the percentage of cracked kernels (PCK) in F_4 individuals derived from "Itadaki/IRSL 30" and "Itadaki/IRSL 37" identified a major QTL, *qCR (Cracking Resistance) 8-2*, at the same position on chromosome 8 in both populations. 'IRSL 30' and 'IRSL 37' had a reduced PCK. These results show that *qCR8-2* is likely to be an important QTL for kernel cracking resistance. Both lines had long awns, linked to *qCR8-2*, but the awnless line 'Chukei 19301' was also derived from "Itadaki/IRSL 37", so *qCR8-2* is distinct from the gene for awn development. We consider that *qCR8-2* will help in the breeding of new rice cultivars with high cracking resistance and in elucidating the physiological mechanism of kernel cracking.

Key Words: chromosome segment substitution lines (CSSLs), cracking, Itadaki, *Oryza sativa*, *Oryza rufipogon*, quantitative trait locus (QTL), rice.

Introduction

Cracked rice kernels are regarded as damaged grain (MAFF 2001). They reduce the market value, increase the ratio of broken rice during milling and cooking, and diminish flavor.

Kernel cracking is caused by distortion in the endosperm caused by uneven moisture distribution due to repeated drying and wetting during the late grain filling period, when the kernel has hardened (Nagato *et al.* 1964). Cracking often follows harvest delays due to unstable weather, heavy rain, or high air temperatures caused by föhn winds after maturity (Arisaka 2002, Nitto *et al.* 2001). Without adequate topdressing at the panicle formation stage of 'Koshihikari', the percentage of cracked kernels (PCK) increased because rachis and rachis-branch withered rapidly, and the daily range of moisture content of panicle became wide (Kawaguchi and Houjou 2010).

Kernel cracking is related also to air temperature during the early grain filling stage: PCK increased when the average air temperature during the 19 days after heading increased (Takahashi *et al.* 2002), and there was high positive correlation between PCK and average daily maximum air temperature during the 10 days after heading (Nagata *et al.* 2004).

Since 2000, many cases of kernel cracking caused by high air temperature during grain filling have been reported (Arisaka 2002, Nitto *et al.* 2001, Sakai *et al.* 2012, Sakaiya *et al.* 2012). As air temperature in summer is expected to continue to rise (JMA 2017), breeding rice cultivars with high resistance to kernel cracking is urgently required.

Cultivars differ in resistance to kernel cracking (Hayashi *et al.* 2015, Nagata *et al.* 2004, Nakagomi *et al.* 2019, 2020, Takita 2002). Resistance is typically evaluated by late harvest after maturity (Horisue 1996). Two alternative methods are available: the panicle soaking method, which induces kernel cracking by soaking dried panicles or unhulled rice harvested at maturity in water (Nakagomi *et al.* 2019, Takita 1999), and the moisture absorption method, which induces kernel cracking of brown rice under controlled conditions in the laboratory by moisture absorption (Hayashi *et al.* 2015). Most standard rice cultivars used for evaluating resistance selected by the late harvest method can be evaluated also by these alternative methods (Nakagomi *et al.* 2019, 2020).

However, genetic information about kernel cracking resistance is limited. The availability of DNA markers

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linked to associated genes or QTLs would enable efficient selection without the influence of cultivation conditions. Hayashi *et al.* (2017) reported a QTL associated with resistance on chromosome (Chr.) 2 in progeny of 'Nipponbare' × 'Yamahikari'. Nakagomi *et al.* (2014) reported a QTL derived from *indica* cultivar 'Yanxuan 203' on Chr. 8 in progeny of 'Ukei 1205' × 'Ouu 390'. Nagaoka *et al.* (2018) reported two QTLs on Chr. 3 in progeny of 'Eminokizuna' × 'Tomohonami'. Pinson *et al.* (2013) reported three QTLs—*qFIS1-1* and *qFIS1-2* on Chr. 1 and *qFIS*8 on Chr. 8—by using two tropical *japonica* cultivars.

Here, to find new QTLs, we selected lines with high resistance to kernel cracking from chromosome segment substitution lines (CSSLs) derived from 'Itadaki' (*O. sativa* L.) × donor *O. rufipogon* that may have unknown and unused traits which is useful for rice breeding and conducted QTL analysis using two lines with high resistance.

Materials and Methods

Experiment 1: Selection of lines with high kernel cracking resistance from CSSLs

Plant materials

To select highly resistant lines, we used CSSLs derived from a cross between 'Itadaki' (*O. sativa* ssp. *japonica*) and *O. rufipogon* (IRGC-Acc103814) as a donor (Hirabayashi *et al.* unpublished data). Plants of the CSSLs and their parent lines were grown in paddy fields at the Western Region Agricultural Research Center (WRARC), NARO (Fukuyama city, Hiroshima prefecture: 34°30′7″ N, 133°23′10″ E). Fifty CSSLs were planted in 2013 for rough screening. Five selected CSSLs were planted in 2014 and 2015, and two were planted in 2016 and 2017 with standard cultivars shown in **Table 1** for check precise kernel cracking resistance.

The rice was sown on 30 May 2013, 27 May 2014, 28 May 2015, 6 May 2016, and 2 May 2017 and transplanted on 18 June 2013 to 2015, 8 June 2016, and 1 June 2017 at 22.2 hills/m^2. Ten plants of each line were transplanted with no replication in 2013 and in duplicate in other years. Each year, 5.6 g/m^2 N was applied as a basal dressing and no additional fertilizer was applied. We recorded PCK, heading date, and 1000-grain weight. We also recorded grain appearance quality by visual evaluation in 2013 and grain size in 2017. The day on which panicles had emerged on half of the plants in a line was considered to be the heading date.

Measurement and evaluation of kernel cracking

PCK was investigated by the late harvest method (LHM) in 2013 and from 2015 to 2017, and by the soaking method (SM) using unhulled kernels from 2014 to 2017. In LHM, 10 panicles were harvested from each line 10 to 30 days after maturation, after approximately the same integrated daily mean air temperature from heading to harvest time of each line. After harvest and natural drying, PCK was calculated from the number of cracked kernels per 150 or 180 kernels >1.80 mm thick counted under a Grain Scope TX-200 (Kett Electric Laboratory). In SM, 10 naturally dried panicles were harvested at maturity, and unhulled kernel (~13% moisture) were soaked in water at 15°C for 1 h. PCK was assessed as above. Resistance to kernel cracking was evaluated by comparison against standard cultivars.

Experiment 2: QTL analyses of lines with high kernel cracking resistance

Plant materials

We used 102 F_4 plants derived from 'Itadaki' × 'IRSL 30' and 73 from 'Itadaki' × 'IRSL 37'. Rice was sown on 19 December 2016 in a greenhouse and their ratoons were transplanted on 1 June 2017 in paddy fields at WRARC, NARO. We used ratoons because we could not get F_5 seeds by low temperature sterility. Plants were grown at 16.6 per m^2

Table 1. Evaluation of kernel cracking resistance of IRSLs

Line name	2014–2015				2015–2017				2017			Maturity group	Evaluation
	Heading date (mo/d)	1,000-grain weight (g)	PCK		Heading date (mo/d)	1,000-grain weight (g)	PCK		Grain size				
			LH (%)	SM (%)			LH (%)	SM (%)	Thickness (mm)	Length (mm)	Width (mm)		
CSSLs													
IRSL 4	8/14	21.6	23.7	37.7	–	–	–	–	–	–	–	Medium	Moderate
IRSL 13	8/15	24.8	27.7	25.5	–	–	–	–	–	–	–	Medium	Moderate
IRSL 30	8/14	23.4	4.0	6.0	8/9	22.4	17.1	9.4	1.92	5.79	2.72	Medium	Resistant
IRSL 37	8/12	23.6	9.0	7.6	8/4	23.6	9.6	14.1	2.06	5.29	2.91	Early	Resistant
IRSL 47	8/11	23.2	19.3	21.8	–	–	–	–	–	–	–	Early	Slightly resistant
Recipient													
Itadaki	8/11	23.1	58.6	41.3	8/4	23.5	57.3	52.0	2.01	5.26	2.93	Early	Moderate to slightly weak
Standard cultivars													
Eminokizuna	–	–	–	–	8/3	20.1	36.0	23.9	1.98	5.12	2.74	Early	Resistant
Hitomebore	8/10	22.0	53.7	37.3	8/3	22.1	52.6	53.6	2.03	5.27	2.82	Early	Moderate
Haenuki	–	–	–	–	8/1	21.2	51.6	38.8	1.99	5.27	2.76	Early	Moderate
Koshihikari	8/9	21.6	68.7	45.3	8/1	21.5	74.9	52.7	1.98	5.12	2.89	Early	Slightly weak
Yamadawara	8/18	22.5	2.3	5.9	8/11	21.8	26.7	16.6	1.91	5.50	2.83	Medium	Resistant
Nipponbare	8/18	22.3	6.0	5.0	8/13	21.6	46.1	33.3	2.00	5.14	2.79	Medium	Moderate
Akidawara	8/17	21.5	51.7	34.6	8/12	20.5	74.2	63.7	1.97	4.96	2.74	Medium	Weak

with 5.6 g/m^2 N as a basal dressing. PCK, heading date, 1000-grain weight, and grain size were recorded.

Measurement of kernel cracking

PCK was investigated by LHM. Ten panicles were harvested from each plant on 26 October. PCK was calculated as in Experiment 1 by checking 100 kernels of each individual.

DNA marker assays

DNA was extracted from 'Itadaki', 'IRSL 30', 'IRSL 37', and F_4 plants of each population as described by Monna *et al.* (2002). The fine-scale genotypes of 'Itadaki', 'IRSL 30', and 'IRSL 37' were investigated by the use of 768 single nucleotide polymorphism (SNP) markers positioned throughout the genome. The genotypes of F_4 individuals were investigated by 14 polymorphic SSR markers in "Itadaki/IRSL 30" and by 8 in "Itadaki/IRSL 37". Linkage maps were prepared in MAPMAKER/EXP 3.0 software (Lander *et al.* 1987) using Kosambi's mapping function (Kosambi 1944). QTL analysis used the composite interval mapping method in QTL Cartographer 2.5 software (Wang *et al.* 2012). The threshold value corresponding to $P = 5\%$ was determined by 1000 permutations. When the LOD score exceeded the threshold value, we judged that a QTL was detected.

Experiment 3: Relation between awn presence and kernel cracking resistance

We used five lines ('Chukei 19301', '2', '3' '4' and '5') selected from progeny of 'Itadaki' × 'IRSL 37' on the basis of PCK, awn presence, and genotype in 2017. The rice was sown on 1 May 2018 and 2 May 2019 and transplanted on 31 May 2018 and 28 May 2019 at 22.2 hills/m^2. Ten plants of each line were transplanted in duplicate with 5.6 g/m^2 N as a basal dressing and without additional fertilizer. PCK, heading date, 1000-grain weight, and grain size were recorded. PCK was investigated by LHM and SM. Ten panicles were harvested from each plant at maturity for SM and about 20 to 25 days after maturity on 28 September for LHM. PCK was calculated as in Experiment 1 by checking 150 kernels of each line.

Results

Experiment 1: Selection of lines with high kernel cracking resistance from CSSLs

In rough screening, the PCK of 'Itadaki' was 57% and that of most CSSLs was lower than this (**Fig. 1A**); in particular, the PCK of 18 lines was ≤30%. The grain appearance grade of 'Itadaki' was 4.5 and that of most CSSLs was equivalent or poorer (**Fig. 1B**); that of four CSSLs could not be evaluated because the grain was red. The 1000-grain weight of 'Itadaki' was 23.5 g and that of most CSSLs was lighter than this (**Fig. 1C**). The heading date of 'Itadaki' was 11 August and that of most CSSLs was within 3 days of this date (**Fig. 1D**). We selected five lines with PCK <30%, grain appearance grade <6, and 1000-grain weight >21.5 g, because kernel cracking cannot be seen clearly in grains that are red or have poor appearance, and to remove the effect of grain weight.

The PCK of 'Itadaki' (early maturity group) was 57%–59% by LHM and 41%–52% by SM, and its resistance was evaluated as "moderate to slightly weak" (**Table 1**), because the PCK of the standard cultivar 'Hitomebore' was 52%–54% by LHM and 37%–54% by SM ("moderate"), and that of 'Koshihikari' was 68%–75% by LHM and 45%–53% by SM ("slightly weak"). 'IRSL 4' and 'IRSL 13' (medium maturity) were evaluated as "moderate", because their PCKs were higher than that of 'Nipponbare' ("moderate") and lower than that of 'Akidawara' ("weak"). 'IRSL 47' (early maturity) was evaluated as "slightly resistant", because its PCK was lower than that of 'Hitomebore'. The PCK of 'IRSL 37' (early maturity) was obviously lower than that of 'Hitomebore' ("moderate") and 'Eminokizuna' ("resistant"). The PCK of 'IRSL 30' (medium maturity) was the same as that of 'Yamadawara' ("resistant") (**Table 2**). Therefore, 'IRSL 30' and 'IRSL 37', with clearly higher resistance than 'Itadaki', were evaluated as "resistant". Their 1000-grain weights were the same as that of 'Itadaki'. The grain shape of 'IRSL 30' was slightly thinner, longer, and narrower than that of 'Itadaki', and that of 'IRSL 37' was the same as that of 'Itadaki'. 'IRSL 30' and 'IRSL 37' had long many awns, but 'Itadaki' had few or none (**Fig. 2**).

Experiment 2: QTL analyses of lines with high kernel cracking resistance

Genotypes of 'IRSL 30' and 'IRSL 37'

Genetic polymorphisms between 'Itadaki' and 'IRSL 30' were detected on Chr. 1 (20.9–25.5 Mbp), Chr. 6 (4.0–4.2 Mbp), Chr. 7 (20.2–25.6 Mbp), and Chr. 8 (21.1–24.6 Mbp) by the use of 768 SNP markers (**Fig. 3A**). Genetic polymorphisms between 'Itadaki' and 'IRSL 37' were detected on Chr. 3 (33.5–34.6 Mbp), Chr. 5 (11.3 Mbp), Chr. 8 (21.0–24.6 Mbp), and Chr. 10 (20.0–21.5 Mb) (**Fig. 3B**). Both lines had an *O. rufipogon* genotype segment in the same region on Chr. 8.

Distribution of PCK in F_4 individuals and correlations with factors that affect kernel cracking

The frequency distributions of PCK in F_4 individuals were continuous in both populations (**Fig. 4**). They were similar, with peaks at 0%–10% and 61%–70% in "Itadaki/IRSL 30" (**Fig. 4A**) and at 0%–10% and 31%–60% in "Itadaki/IRSL 37" (**Fig. 4B**).

There was no significant correlation of PCK with average daily maximum temperature during the 10 days after heading (30–34°C) or with integrated daily average temperature from heading to harvest in either population (**Table 2**).

There was a significant negative correlation between PCK and grain thickness in "Itadaki/IRSL 30" but not in "Itadaki/IRSL 37" (**Table 3**). There was no significant correlation of PCK with grain length, width, or weight in either population.

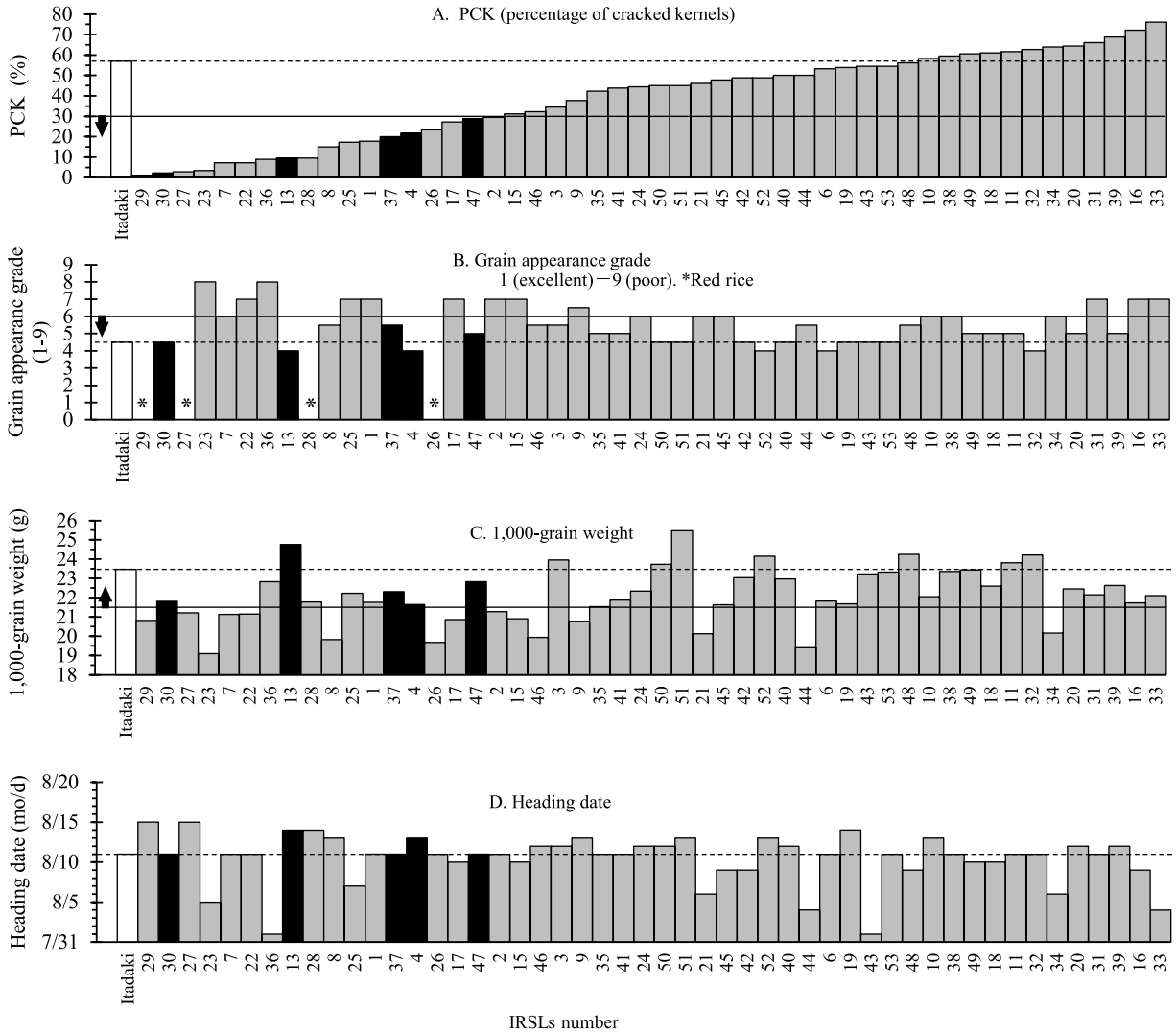

Fig. 1. PCK, grain appearance grade, grain weight, and heading date of IRSLs in 2013. - - - Scores of 'Itadaki'. — Selection standard score; ↑↓ direction.

Table 2. Ranges of environmental factors and coefficients of correlation between these factors and PCK

Population	Heading date	Average daily maximum temperature during 10 days after heading		Integrated daily average temperature from heading to harvest	
		°C	Correlation coefficient	°C	Correlation coefficient
Itadaki/IRSL 30, F_4	0.060 ns	29.7~34.0	–0.036 ns	1318~1661	–0.060 ns
Itadaki/IRSL 37, F_4	–0.146 ns	30.4~34.1	0.006 ns	1258~1628	0.143 ns

Not significant at $P = 5\%$.

QTL analyses

In the "Itadaki/IRSL 30" population, QTLs associated with grain thickness and width and heading date were detected on Chr. 7, and QTLs associated with PCK, grain thickness, length, weight, and heading date were detected on Chr. 8 (**Table 4**, **Fig. 5**). In the "Itadaki/IRSL 37" population, QTLs associated with PCK only were detected on Chr. 8. Those associated with PCK was detected near marker RM5485 in both populations, with a high proportion of variance explained (PVE): 69.6% in "Itadaki/IRSL 30" and 71.2% in "Itadaki/IRSL 37". We named it *qCR (Cracking Resistance) 8-2*.

In the F_4 plants of both populations, the 'IRSL 30' and 'IRSL 37' genotypes were associated with low PCK (**Fig. 6**). In the F_4 plants of "Itadaki/IRSL 37", those with the 'IRSL 37' and heterozygous genotypes had awns and those with the 'Itadaki' genotype were awnless (**Table 5**).

Fig. 2. Awns of 'IRSL 37' and 'Itadaki'.

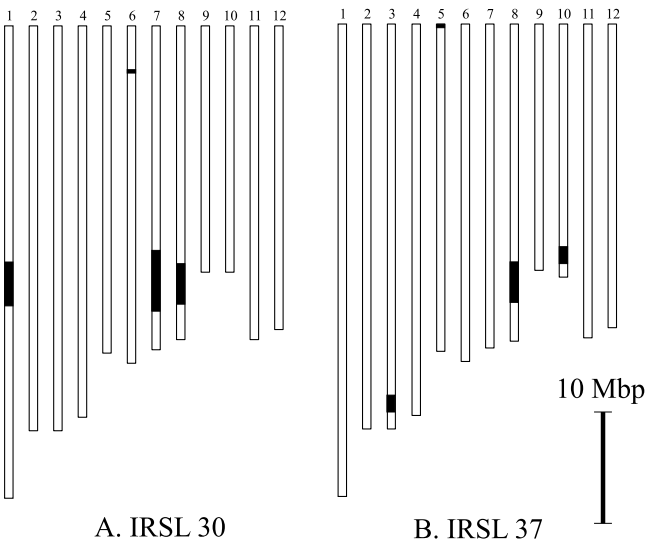

Fig. 3. Graphical genotype of IRSL 30 and IRSL 37. □ 'Itadaki' genotype (homozygous); ■ IRSL 30 or IRSL 37 genotype (homozygous for *O. rufipogon*). Genetic typing was performed on the GoldenGate BeadArray technology platform (Illumina Inc., San Diego, CA, USA), using 768 SNP markers for typing.

Table 3. Coefficients of correlation between PCK and grain size

Population	Grain thickness	Grain length	Grain width	1,000 grain weight
Itadaki/IRSL 30, F_4	–0.614**	0.189 ns	–0.035 ns	–0.022 ns
Itadaki/IRSL 37, F_4	–0.075 ns	–0.017 ns	–0.097 ns	–0.109 ns

** Significant at $P < 1\%$; ns, not significant at $P = 5\%$.

Experiment 3: Relation between awn presence and PCK

The 'Chukei 19301', '2', '3', '4' and '5' lines had an 'IRSL 37' genotype segment: 'Chukei 19301' from RM7356 (21.28 Mbp) to IDR2298 (23.01 Mbp), 'Chukei 19302' from RM7356 (21.28 Mbp) to RM5353 (24.12 Mbp), 'Chukei 19303' from RM3262 (22.38 Mbp) to RM5353 (24.12 Mbp), 'Chukei 19304' from RM5485 (24.07 Mbp) to RM3754 (26.97 Mbp) and 'Chukei 19305' from RM4487 (26.09 Mbp) to RM3754 (26.97 Mbp), on Chr. 8 (**Table 6**). 'Chukei 19301' and 'Chukei 19305' were awnless (like 'Itadaki'), and 'Chukei 19302', 'Chukei 19303' and 'Chukei 19304' were awned (like 'IRSL 37'). PCKs of 'Chukei 19301', '2', '3' and '4' were lower than that of 'Itadaki' and the same as that of 'IRSL 37'. PCK of 'Chukei 19305' was as same as that of 'Itadaki'. 1,000-grain weight of 'Chukei 19301' and 'Chukei 19305' were as same as that of 'IRSL37' and the slightly heavier than of 'Itadaki', and that of 'Chukei19304' was as same as that of 'Itadaki' (**Table 6**). Grain weight of 'Chukei19302' and 'Chukei19303' were slightly lighter than that of 'Itadaki because grain length of these lines was shorter than that of 'Itadaki'.

Discussion

The selection of lines resistant to kernel cracking is difficult because cracking is affected not only by cultivation conditions (such as fertilization, air temperature, harvest time), but also by drying and storage conditions after harvest. Resistance can be compared with that of standard cultivars (Nakagomi *et al.* 2019, 2020), but as the procedures for investigating cracking are complex, we sought genes or QTLs that could enable efficient selection without the influence of cultivation or postharvest conditions or the need for complex procedures.

First, we screened CSSLs derived from a cross between

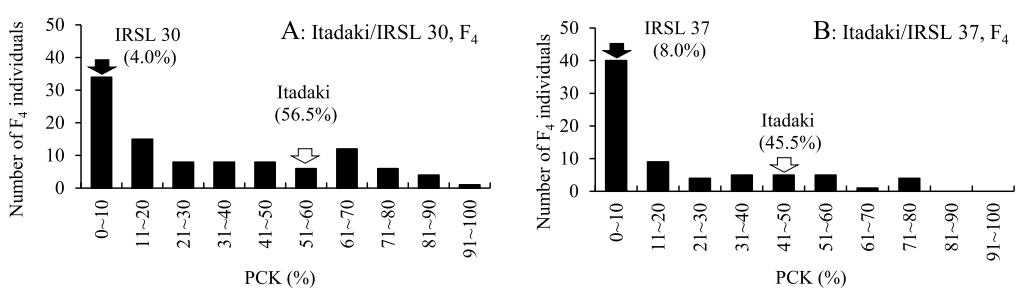

Fig. 4. Score distributions of PCK (percentage of cracked kernels) in F_4 individuals and parents (in parentheses).

Table 4. QTLs detected by composite interval mapping analyses

Population	Chr.	Trait	Nearest marker	Peak position (cM)	LOD	Additive effect	PVE	LOD threshold
Itadaki/IRSL30, F_4	7	Heading date	RM6852	15.40	12.40	2.08	47.3	2.33
		Grain thickness	RM1330-1	24.40	6.06	–0.02	32.5	2.40
		Grain width	RM1330-1	33.40	4.18	–0.02	17.9	2.36
	8	PCK	RM5485	12.49	24.67	–24.32	69.6	5.79
			RM5485	26.49	8.23	–22.63	24.6	5.79
		Grain thickness	RM5485	12.49	11.70	0.02	44.7	2.40
		Grain length	RM5485	12.49	18.43	–0.05	72.4	12.0
			RM5485	24.49	19.60	–0.06	72.3	12.0
		1,000-grain weight	RM5485	21.49	5.02	–0.97	33.8	4.75
		Heading date	RM7556	9.49	3.13	–0.79	9.3	2.33
Itadaki/IRSL37, F_4	8	PCK	RM5485	12.14	23.4	–22.35	71.2	8.54

LOD, logarithm of odds; PVE, percentage of variance explained.

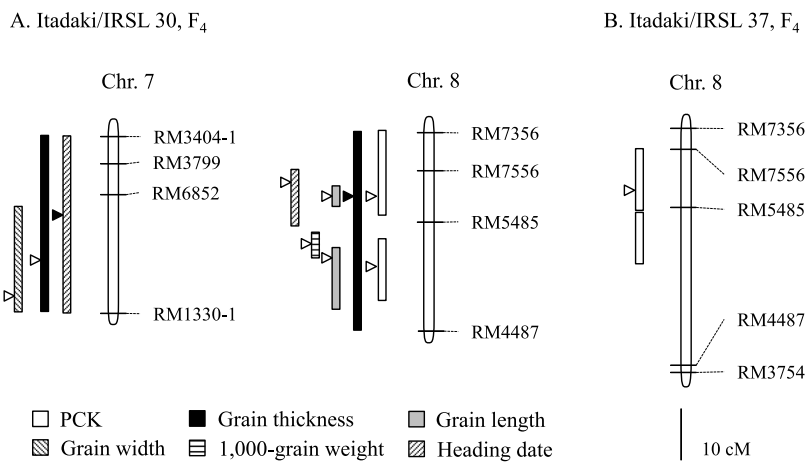

Fig. 5. QTL regions associated with PCK, grain shape, grain weight and heading date. Bars on the left side of the chromosomes indicate the intervals above the LOD threshold values. Triangles denote the position of LOD peaks. Black triangles indicate the peaks of QTLs where values increase in the IRSL 30 or IRSL 37 genotypes. Chromosome on which no QTLs were detected are not shown.

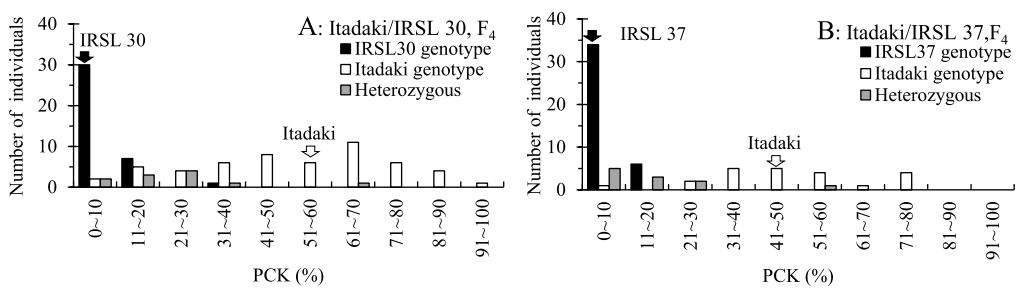

Fig. 6. Relationships between the *qCR8-2* genotype in F_4 individuals and PCK. The genotype of SSR marker RM5485 was used as the index. Arrows show scores of parents.

Table 5. Relationship between *qCR8-2* and presence of awns in F_4 individuals of "Itadaki/IRSL 37" population

	Awn	Genotype		
		Itadaki	IRSL 37	Heterozygous
Number of Individuals	Present	0	40	11
	Absent	22	0	0

Genotype of SSR marker RM5485 was used.

'Itadaki' and the donor *O. rufipogon* and selected two "resistant" lines, 'IRSL 30' and 'IRSL 37' (**Table 1**). Their PCKs were clearly lower than that of 'Itadaki', and the grain size of 'IRSL 37' was almost the same as that of 'Itadaki'. As PCK tends to increase as grain thickens (Nakagomi *et al.* 2012, Takita 2002), we consider that the cracking resistance of 'IRSL 37' is not affected by grain thickness. On the other hand, the 'IRSL 30' grain was

Table 6. Evaluation of kernel cracking resistance of "Itadaki/IRSL 37" lines and their awn presence

Line name	Marker name and distance (Mbp)								Heading date (mo/d)		PCK (%)				1,000-grain weight (g)		Grain size 2019			Awn
	RM 7356	RM 7556	RM 3262	IDR 2298	RM 5485	RM 5353	RM 4487	RM 3754			LHM		SM							
	21.28	22.21	22.38	23.01	24.07	24.12	26.09	26.97	2018	2019	2018	2019	2018	2019	2018	2019	Thick-ness	Length	Width	
Chukei 19301									8/2	8/1	4.7	25.7	18.0	64.3	22.2	24.0	2.04	5.16	2.92	Absent
Chukei 19302									8/1	8/1	2.0	11.3	13.7	60.3	21.0	22.8	2.06	5.03	2.87	Present
Chukei 19303									7/30	7/31	9.0	22.0	25.0	50.3	20.8	22.7	2.06	4.98	2.86	Present
Chukei 19304									–	7/30	–	25.0	–	57.7	–	23.6	2.05	5.14	2.88	Present
Chukei 19305									–	7/30	–	48.3	–	77.7	–	24.2	2.05	5.24	2.88	Absent
IRSL 37									7/31	7/31	1.7	10.7	8.7	47.3	22.7	24.2	2.06	5.28	2.86	Present
Itadaki									7/29	7/30	37.3	70.0	70.7	82.0	22.1	23.6	2.05	5.15	2.89	Absent

slightly thinner than the 'Itadaki' grain. Therefore, its resistance to kernel cracking may have been affected in Experiment 1.

'IRSL 30' and 'IRSL 37' had the *O. rufipogon* segment in the same region on Chr. 8, within the 'Itadaki' background (**Fig. 3**). This result indicates that the QTL associated with kernel cracking resistance derived from *O. rufipogon* is on Chr. 8.

To confirm the QTL position, we conducted QTL analyses with F_4 individuals of "Itadaki/IRSL 30" and "Itadaki/IRSL 37" populations. The distribution frequencies of PCK were continuous, from 0% to 96% (**Fig. 4**). Nagata *et al.* (2004) reported a high positive correlation between PCK and average daily maximum air temperature during the 10 days after heading. However, we found no correlation in either population within a wide range of maximum temperatures (30–34°C) (**Table 2**). Harvest time also affects kernel cracking (Arisaka 2002, Nagato *et al.* 1964, Nitto *et al.* 2001). However, there was no significant correlation between PCK and integrated daily average temperature from heading to harvest (**Table 2**). These results suggest the presence of another factor that affects PCK besides temperature after heading and harvest.

Grain thickness also affects kernel cracking (Nakagomi *et al.* 2012, Takita 2002). 'IRSL 30' grain was slightly thinner than 'Itadaki' grain. However, there was a negative correlation ($r = -0.614$) between PCK and grain thickness in the "Itadaki/IRSL 30" population (**Table 3**), opposite to previous reports that PCK tends to increase with grain thickness. Therefore, we consider that the thinner grain of 'IRSL 30' does not affect kernel cracking resistance at the genetic level. On the other hand, there was no significant correlation between PCK and other traits in the "Itadaki/IRSL 37" population. These results indicate that other genetic factors affect kernel cracking independent of grain thickness in both populations.

QTL analysis detected a QTL, *qCR8-2*, associated with PCK, near marker RM5485 (24.07 Mbp) on Chr. 8 in both populations (**Table 4**, **Fig. 5**), with PVEs near 70%; the 'IRSL 30' and 'IRSL 37' genotypes decreased PCK (**Fig. 6**). In experiment 3, 'Chukei 19301', '2', '3' and '4' is likely to have *rufipogon* (IRSL37) segment at *qCR8-2* because PCKs of 'Chukei 19301', '2', '3' and '4' were lower than that of 'Itadaki' and the same as that of 'IRSL 37', and that of 'Chukei 19305' was as same as that of 'Itadaki' though grain thickness of these lines were almost same in experiment 3 (**Table 6**). These results indicate that *qCR8-2* is likely to lie between IDR2298 (23.01 Mbp) and RM5485 (24.07 Mbp). Pinson *et al.* (2013) detected a QTL (*qFIS8*) associated with kernel cracking on Chr. 8, but it positioned near SSR marker RM404 (15.4 Mbp) far from position of *qCR8-2*. Nakagomi *et al.* (2014) detected a QTL associated with kernel cracking on Chr. 8 derived from *indica* cultivar 'Yanxuan 23' between SSR markers RM223 (20.6 Mbp) and RM210 (22.5 Mbp), near *qCR8-2*, but *qCR8-2* is likely to be different from the QTL derived from 'Yanxuan 23', because there is no region where these two QTLs positions overlap. QTLs associated with grain thickness were also detected near *qCR8-2* in the "Itadaki/IRSL 30" population, but the 'IRSL 30' genotype increased kernel thickness, and not detected in the "Itadaki/IRSL 37" population. This is why there was negative correlation between PCK and grain thickness in the "Itadaki/IRSL 30" population (**Table 3**). These results indicate that *qCR8-2* is a novel and important QTL associated with the kernel cracking resistance of 'IRSL 30' and 'IRSL 37', and not affected by grain thickness.

QTLs associated with grain length were also detected at or near *qCR8-2* in the "Itadaki/IRSL 30" population, consistent with a report that a major gene, *GAD1*, derived from *O. rufipogon*, which regulates grain number, grain length, and awn development, is located near RM5485 on Chr. 8 (Jin *et al.* 2016). But grain length decreased in 'IRSL30' (*O. rufipogon*) genotype, opposite to Jin *et al.* (2016) report. The reason for this is considered to be that the combinations were different. On the other hand, no QTLs associated with grain length and weight was detected in the "Itadaki/IRSL 37" (**Table 4**, **Fig. 5**). One of reason for this is considered that difference in grain size between "Itadaki" and "IRSL37" was very small (**Tables 1**, **4**). But grain length and 1,000-grain weight of selected lines was slightly different in each other though PCKs of 'Chukei 19301', '2', '3' and '4' were lower than that of 'Itadaki' and the same as that of 'IRSL 37' (**Table 6**). Although grain

thickness affects kernel cracking, the effect of grain length has not been reported previously (Nakagomi *et al.* 2012, Takita 2002). Therefore, further studies including fine mapping of *qCR8-2* and the QTL for grain shape and 1000-grain weight are needed.

The presence of awns was linked to *qCR8-2* (**Table 5**), as previously reported (Jin *et al.* 2016). Transpiration from awns is about 13% to 26% of that from the whole panicle during the early grain filling stage (Tsuda 1933). Fukuoka *et al.* (2012) reported a relationship between panicle transpiration and panicle temperature, and the average daily maximum temperature during the 10 days after heading increases kernel cracking (Nagata *et al.* 2004). Therefore, the presence of awns may affect kernel cracking. However, in Experiment 3, PCK of the awnless 'Chukei 19301' was obviously lower than that of the awnless 'Itadaki' and the same as that of the awned 'IRSL 37', 'Chukei 19302', 'Chukei 19303' and 'Chukei 19304' (**Table 6**). This result indicates that *qCR8-2* is different from *GAD1*, and awns do not affect kernel cracking.

Little is known about the physiological mechanism of kernel cracking. *qCR8-2* will help to breed new rice cultivars with high cracking resistance and to elucidate the physiological mechanisms through the use of NILs for *qCR8-2* such as 'Chukei 19301' and 'Chukei 19302'.

Author Contribution Statement

KN carried out screening and genetic analyses and wrote the manuscript; HH developed CSSLs; UY carried out genotyping of plant materials; HS, AS, TA and OI helped to develop plant materials and to write the manuscript.

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