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Research Paper

A new method for evaluation of the resistance to rice kernel cracking based on moisture absorption in brown rice under controlled conditions

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We developed and evaluated the effectiveness of a new method to detect differences among rice cultivars in their resistance to kernel cracking. The method induces kernel cracking under laboratory controlled condition by moisture absorption to brown rice. The optimal moisture absorption conditions were determined using two *japonica* cultivars, 'Nipponbare' as a cracking-resistant cultivar and 'Yamahikari' as a cracking-susceptible cultivar: 12% initial moisture content of the brown rice, a temperature of 25°C, a duration of 5 h, and only a single absorption treatment. We then evaluated the effectiveness of these conditions using 12 *japonica* cultivars. The proportion of cracked kernels was significantly correlated with the mean 10-day maximum temperature after heading. In addition, the correlation between the proportions of cracked kernels in the 2 years of the study was higher than that for values obtained using the traditional late harvest method. The new moisture absorption method could stably evaluate the resistance to kernel cracking, and will help breeders to develop future cultivars with less cracking of the kernels.

Key Words: rice, kernel cracking, moisture absorption, late harvest method, varietal differences.

Introduction

Cracked kernels reduce the market value of rice (*Oryza sativa* L.), posing a nationwide problem in Japan (Arisaka 2002, Nakamura *et al.* 2003, Nitto *et al.* 2001, Sato *et al.* 1987, Takagi *et al.* 1980, Takahashi *et al.* 2002, Watanabe and Kodama 1991). In Fukui Prefecture, several cultivation techniques to prevent the occurrence of kernel cracking have been recommended. However, cracked kernels still accounted for 22.1% of the reported decreases in the value of rice produced in Fukui Prefecture in 2012 and 21.4% in 2013 (MAFF 2014).

Several rice cultivars with high resistance to kernel cracking have been reported: the *indica* cultivar 'Ensen 203' (Takita 1992) and the *japonica* cultivars 'Koganebare' (Watanabe and Kodama 1991), 'Ozora' and 'Nipponbare' (Takita 2002), and 'Sasanishiki', 'Hitomebore', and 'Haenuki' (Nagata *et al.* 2004, Takita 2002). However, higher temperatures during the early stages of the ripening period made kernels more sensitive to cracking (Nagata *et al.* 2004). Nagata *et al.* (2013) noted that the proportions of cracked kernels in these *japonica* cultivars tested in Takita (2002) and Nagata *et al.* (2004) increased with increasing temperature during the ripening period. This is an important finding, because air temperatures are predicted to increase during the 21st century (IPCC 2014). If temperatures during the ripening period increase, then the occurrence of kernel cracking in existing *japonica* cultivars might also increase. Cultivars with higher resistance to kernel cracking under the predicted higher temperatures are therefore needed. Clarification of the hereditary factors that create resistance to kernel cracking and the establishment of a stable evaluation method are both needed to support efficient breeding of resistant cultivars by means of methods such as marker-assisted selection.

The late-harvest method (LH method) is a traditional method for evaluating resistance to kernel cracking (Horisue 1996). In this method, mature kernels are left in the field and exposed to rain and the morning dew after maturation. The repeated absorption and release of moisture induces kernel cracking. The LH method is simple because it requires no additional system to induce kernel cracking. Unfortunately, it sometimes fails: kernel cracking may be infrequent (Takita 2002) or the measured differences in resistance between cultivars may be unstable (Nakagomi *et al.* 2012), most likely because environmental conditions in the field varied. Thus, a more stable evaluation method is needed.

Three laboratory methods have been used to induce kernel cracking and to provide a stable evaluation of varietal

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differences in resistance: (1) In the drying method, grains are dried drastically in a greenhouse or in a circulation dryer (Nagata *et al.* 2004, 2005, Horisue 1996). This method simulates the kernel cracking that is caused by drastic drying due to winds (e.g., föhn, typhoon) or postharvest drying. (2) In the immersion method, panicles are dried slowly until the brown rice has a moisture content between 15% and 17% and are then submerged in water (Takita 1999); the proportion of cracked kernels in this method was correlated with the proportion using the LH method. (3) In the moisture absorption (MA) method, seeds that kernels with hull are exposed to 46°C and 100% relative humidity (RH) under laboratory conditions (Pinson *et al.* 2012). These three laboratory evaluation methods can stably clarify varietal differences in resistance to kernel cracking because the environmental conditions that induce kernel cracking are the same for all samples.

These evaluation methods use panicle or kernel samples that contain both rice kernels and the hull of the kernel or the branches of the panicles. Takagi *et al.* (1980) reported that kernel cracking increased with increasing age of the panicle branches. Waggoner *et al.* (2003) reported that factors that rapid diffusion of moisture into the endosperm and low permeability of hull were important sources of resistance to kernel cracking. In Pinson's MA method, evaluated resistance to cracking contains effects of kernel and hull. Therefore, we developed a modification of the MA method in which brown rice is directly exposed to high RH under laboratory conditions to focus on resistance to cracking of the kernel alone, unaffected by the hull or the panicle branches. The optimal treatment conditions for the use of MA method to detect varietal differences have not yet been established. Thus, in the present study, we also examined the conditions required to use the MA method with two *japonica* cultivars. On the basis of the results of this preliminary assessment, we then tested the ability of the MA method to stably distinguish among kernel cracking rates using 12 *japonica* cultivars.

Materials and Methods

Plant materials and cultivation conditions

We used two *japonica* rice cultivars, 'Nipponbare' and 'Yamahikari', to determine the optimal treatment conditions when using the MA method. 'Nipponbare' is a cracking-resistant cultivar and 'Yamahikari' is a cracking-susceptible cultivar (**Fig. 1**). Both were grown in a paddy field at the Fukui Agricultural Experiment Station (Fukui, Japan, 35.6°N) in 2011, with sowing on 19 April and transplanting on 17 May at two plants per hill. We planted a total of 48 hills per cultivar at a density of 24.2 hills m^{-2}, with three replicates. Nitrogen was applied at 0.60 kg a^{-1} as base fertilizer. The heading date was defined as the time when 50% of panicles headed. The harvest time was defined as the time when 90% of the kernels in a panicle turned yellow-ripe stage. Five moderate panicles from each of 20 hills per cultivar, for a total of 100 panicles, were harvested during the maturation period. The harvested panicles were dried in the shade to avoid drastic decrease of moisture content of kernels and packed in a plastic bag to provide a uniform moisture content for the kernels.

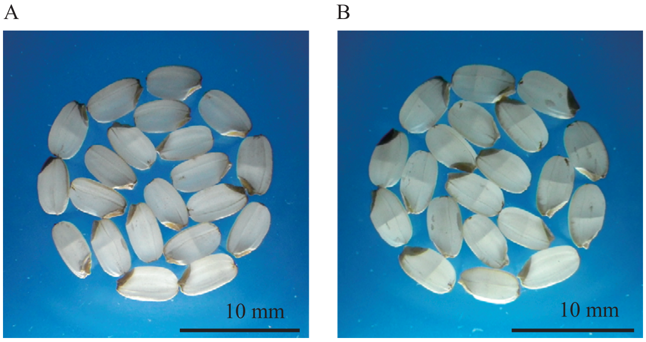

Fig. 1. Brown rice kernels of (A) 'Nipponbare' and (B) 'Yamahikari'. Images were obtained using transmitted light with a grainscope TX-200.

We then used the 12 *japonica* rice cultivars shown in **Table 1** to evaluate the effectiveness of the MA method. These cultivars were selected on the basis of descriptions in previous reports (Nagata *et al.* 2004, Takita 2002) and our preliminary examinations of kernel cracking in a range of cultivars. The 12 cultivars were grown in a paddy field at the Fukui Agricultural Experiment Station in 2012 and 2013. Sowing and transplanting dates were 17 April and 17 May in 2012 and 19 April and 13 May in 2013. We used 16 hills (n = 1) in 2012 and eight hills (n = 2) in 2013. Other culture conditions were the same as in 2011. We harvested three moderate panicles from each of five hills per cultivar, for a total of 15 panicles, at the maturation period for the MA method, and at 21 to 24 days in 2012 and 10 to 18 days in 2013 after the maturation period for the LH method, according to the method of Horisue (1996). Harvested panicles were treated the same way as in the first experiment.

Temperature and precipitation measured at the local Fukui meteorological observatory (36.0°N) were used to describe climatic conditions during the cropping period. Temperature and RH in the room and box used for the moisture absorption and release experiments were measured using a thermo-hygrometer (CTD-TR-72wf-H, T&D Corp., Japan).

Conditions for the moisture absorption method

We examined three characteristics of the treatment conditions in the MA method using 'Nipponbare' and 'Yamahikari': (1) the initial moisture content of the brown rice before the moisture absorption treatment, (2) the temperature during and duration of the moisture absorption treatment, and (3) the number of repetitions of moisture absorption and release.

In experiment 1, we dehulled brown rice kernels of each cultivar by hand and retained only the ones that did not pass through a 1.8-mm sieve. In present study, chalky kernels

Table 1. Ripening conditions, sampling dates and proportions of cracked kernels among the 12 cultivars used to evaluate the new assessment method

Cultivars	2012							2013						
	Heading date	Sampling date			10-day max. temperature (°C)	Proportion of cracked kernels		Heading date	Sampling date			10-day max. temperature (°C)	Proportion of cracked kernels	
		MA[a]	LH[b]	LH-MA[c] (days)		MA (%)	LH (%)		MA	LH	LH-MA (days)		MA (%)	LH (%)
Koshihikari	8/2	9/4	9/28	24	33.5	53	87	7/31	9/3	9/13	10	32.3	64	60
Etsunan 222	8/5	9/13	10/5	22	33.0	41	81	8/2	9/3	9/21	18	33.0	32	79
Tenkomori	8/6	9/13	10/5	22	32.9	24	60	8/5	9/7	9/21	14	33.7	51	75
Akisakari	8/7	9/14	10/5	21	32.8	16	59	8/4	9/7	9/21	14	33.5	39	83
Eminokizuna	8/7	9/10	10/3	23	32.8	18	45	8/4	9/7	9/21	14	33.5	37	76
Yamadawara	8/9	9/21	10/15	24	33.3	15	47	8/5	9/10	9/26	16	33.7	29	40
Yamahikari	8/10	9/16	10/9	23	33.6	77	86	8/9	9/21	10/7	16	34.8	87	88
Nipponbare	8/10	9/16	10/9	23	33.6	31	38	8/10	9/21	10/7	16	34.9	47	50
Kinmaze	8/15	9/29	10/22	23	34.8	58	49	8/12	9/24	10/8	14	35.0	80	75
Koganemasari	8/15	9/29	10/22	23	34.8	55	43	8/12	9/24	10/8	14	35.0	63	40
Asominori	8/17	9/29	10/22	23	34.8	77	70	8/14	9/28	10/11	13	35.1	83	70
Hinohikari	8/19	10/3	10/27	24	34.9	67	31	8/13	9/28	10/11	13	35.2	69	42

[a] MA: moisture absorption method.
[b] LH: late harvest method.
[c] Days between the maturation period (90% of the kernels had turned yellow) and sampling for LH method.

were excluded, although the proportion of chalky kernels in sieved samples was under 5%. This is because it was difficult to judge visually such kernels cracked or not. We then placed 100 of these kernels into a Petri dish (ϕ 35 mm) and measured their moisture contents in bulk with a moisture meter (Riceter f, Kett Electric Laboratory, Japan). The brown rice kernels in open Petri dishes were dried to seven levels of moisture content, ranging from 10% to 16% in a room with the temperature set at about 23°C and a RH of 38%. The decrease in the moisture content of the brown rice in each open Petri dish was calculated from the change in weight of the kernels during drying. The open Petri dishes were then put in a plastic box containing wetted paper at the bottom. The box was sealed and the brown rice was left to absorb moisture at 25°C for 5 h in a constant-temperature unit (BNL-110, ESPEC, Japan). After moisture absorption, we counted the number of cracked kernels by using transmitted light (grainscope TX-200, Kett Electric Laboratory; **Fig. 1**).

Before conducting experiments 2 and 3, we adjusted the initial moisture content of the brown rice to 12% on the basis of the results of experiment 1. The brown rice was prepared as in experiment 1. In experiment 2, it was allowed to absorb moisture at 20, 25, or 30°C for 3, 5, or 7 h in a constant-temperature unit. In experiment 3, it was allowed to absorb moisture at 25°C for 5 h based on the results of experiment 2 and then dried at 15°C and a RH 58% in the constant-temperature unit for about 24 h until the moisture content decreased to the initial moisture content, 12%. The moisture absorption and release (drying) were each repeated three times with the same samples, and the number of cracked kernels was counted after each treatment.

Effectiveness evaluation of the moisture absorption method

Differences between cultivars and years in the resistance to kernel cracking calculated using the MA method were compared with those calculated using the LH method. Kernel samples were dehulled in a laboratory huller (ST-50, Yanmar, Japan). The interval between the huller's rollers was set so that 60% to 70% of the kernels were dehulled in a single pass to decrease stress on the kernels. We then added 100 brown rice kernels that did not pass through a 1.8-mm sieve to a Petri dish. Seeds with an initial moisture content of 12% obtained as described above were allowed to absorb moisture one time at 25°C for 5 h. In the LH method, cracked kernels were detected immediately after sieving. To evaluate the MA method, we examined the relationship between the proportion of cracked kernels and the ripening temperature in the field, and the relationship between the cracking values in the 2 years of the study.

Proportions of cracked kernels data and correlation coefficients were analyzed using statistical software R 3.0.2 (http://www.R-project.org.)

Results

Moisture content of brown rice before absorption

After drying at 22.9°C and an RH of 38%, the brown rice moisture contents ranged from 10.2% to 16.2% in 'Nipponbare' and from 10.2% to 15.7% in 'Yamahikari' (**Fig. 2**). Following absorption of moisture at 25°C for 5 h, the proportion of cracked kernels increased with decreasing initial moisture content. The proportion of cracked kernels began to increase at about 12% in 'Nipponbare' and 14% in 'Yamahikari'. At an initial moisture content between 11% and 14%, the proportion of cracked kernels of 'Yamahikari' increased more rapidly than that of 'Nipponbare' with decreasing initial moisture content. The difference between the proportions of cracked kernels in 'Nipponbare' and

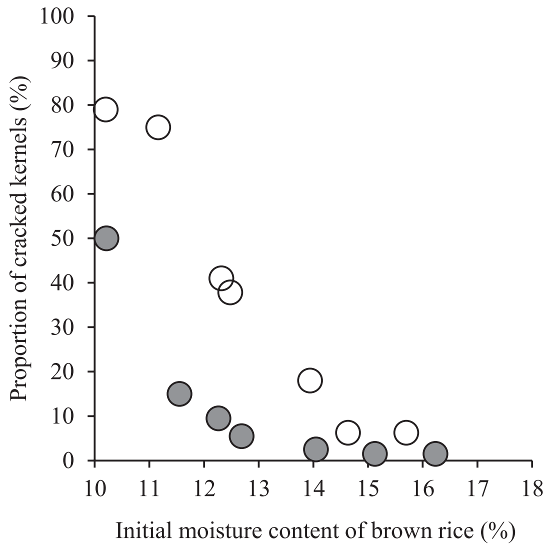

Fig. 2. Relationship between the proportion of cracked rice kernels induced by moisture absorption and the initial moisture content of the kernels. Brown rice samples absorbed moisture for 5 h at 25°C. Gray and white circles denote 'Nipponbare' and 'Yamahikari', respectively.

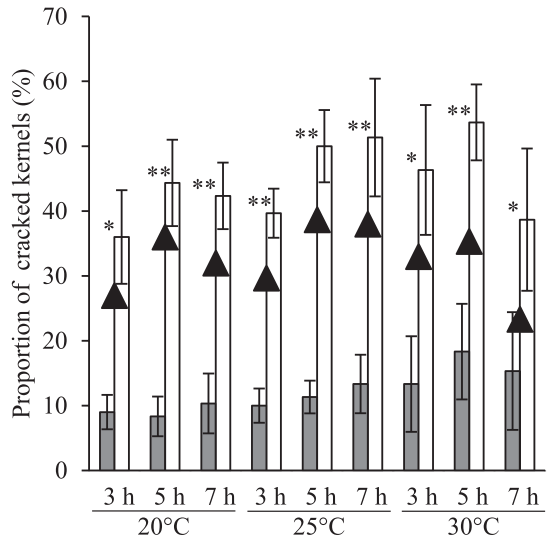

Fig. 3. Effect of the moisture absorption temperature and duration on the occurrence of kernel cracking. The initial moisture content of the kernels was set at 12%. Gray and white bars denote the proportions of cracked kernels for 'Nipponbare' and 'Yamahikari', respectively. Triangles denote the difference in this proportion between the two cultivars. Values are means and error bars denote standard deviations ($n = 3$). Significance: $^{**}P < 0.01$; $^{*}P < 0.05$.

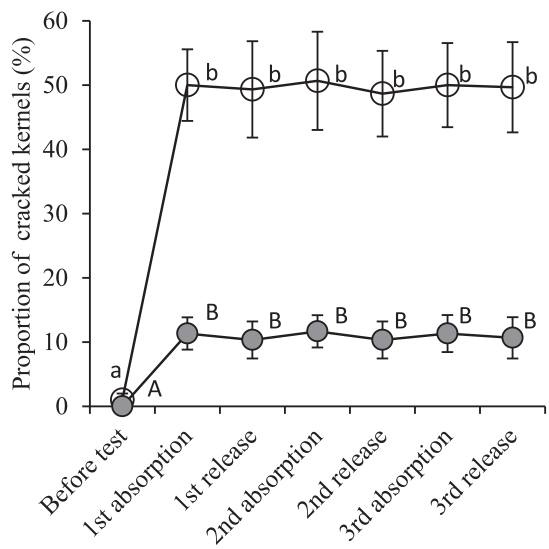

Fig. 4. Changes in the proportion of cracked kernels induced by cycles of moisture absorption and release. The moisture content of the kernels was set at 12% before treatment. The kernels then absorbed moisture for 5 h at 25°C, followed by drying for about 24 h at 15°C to a moisture content of 12%. Gray and white circles denote 'Nipponbare' and 'Yamahikari', respectively. Values are means and error bars denote standard deviations ($n = 3$). Letters show significant difference ($P < 0.05$) by Tukey's test. Capital letters and small letters denote about 'Nipponbare' and 'Yamahikari', respectively.

'Yamahikari' reached its maximum at an initial moisture content of 11% to 12%.

Moisture absorption temperature and duration

On the basis of the results of experiment 1, we set the initial moisture content of the brown rice at 12%. Average of the air temperatures in the plastic box during moisture absorption were 19.9, 24.8, or 30.2°C. And average of RH was set at 99% at all temperatures. The proportion of cracked kernels differed significantly between the cultivars at all temperatures and for all durations (**Fig. 3**). The difference was highest in the treatment at 25°C for 5 h.

Repeated moisture absorption and release

We investigated effect of repeated moisture absorption and release on the occurrence of kernel cracking. On the basis of the results of experiments 1 and 2, moisture absorption was conducted at 25°C for 5 h using kernels with an initial moisture content of 12%. Moisture releases were conducted at 14.8°C and an RH 58% for about 24 h. The proportion of cracked kernels increased to 11.3% in 'Nipponbare' and 50.0% in 'Yamahikari' after the first moisture absorption treatment; it showed significant differences from the initial proportion of cracked kernels (**Fig. 4**). It did not change during subsequent moisture absorption and release treatments; there were no significant differences in the proportions of cracked kernels among these treatments. Thus, a single moisture absorption treatment appears to be sufficient.

Effectiveness evaluation of the MA method

On the basis of the results of the three experiments, we chose the following conditions for our comparison of multiple cultivars: an initial moisture content of 12%, a temperature of 25°C, a duration of 5 h, and a single absorption treatment. We then investigated the difference in the proportions of cracked kernels between the MA and LH methods

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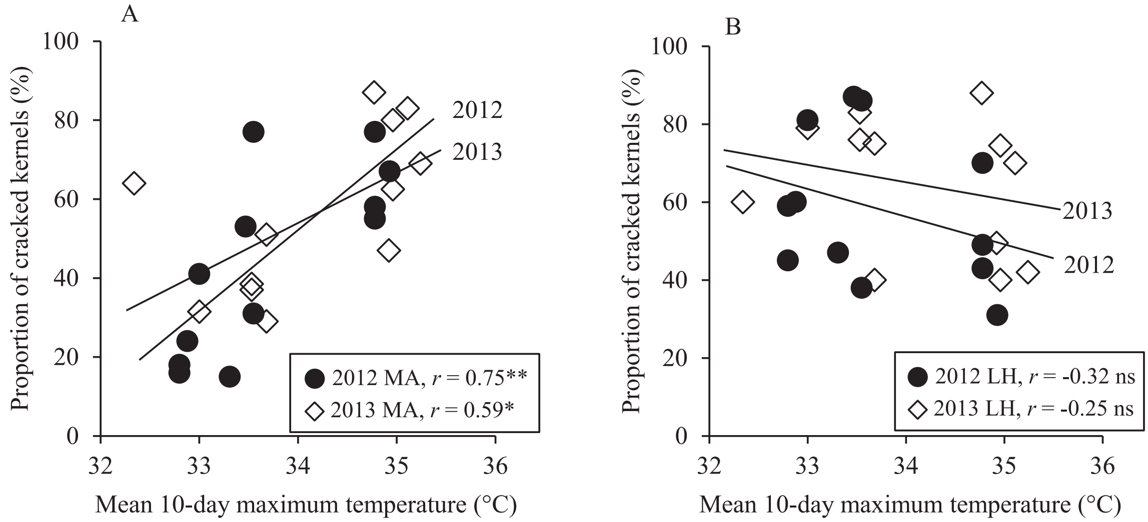

Fig. 5. Relationship between the proportion of cracked kernels and the mean 10-day maximum temperature for the 10 days after heading. Proportions were calculated using (A) the moisture absorption method and (B) the late harvest method. Circles and diamonds denote 2012 and 2013, respectively. Significance: $^{**}P < 0.01$; $^{*}P < 0.05$; ns, not significant.

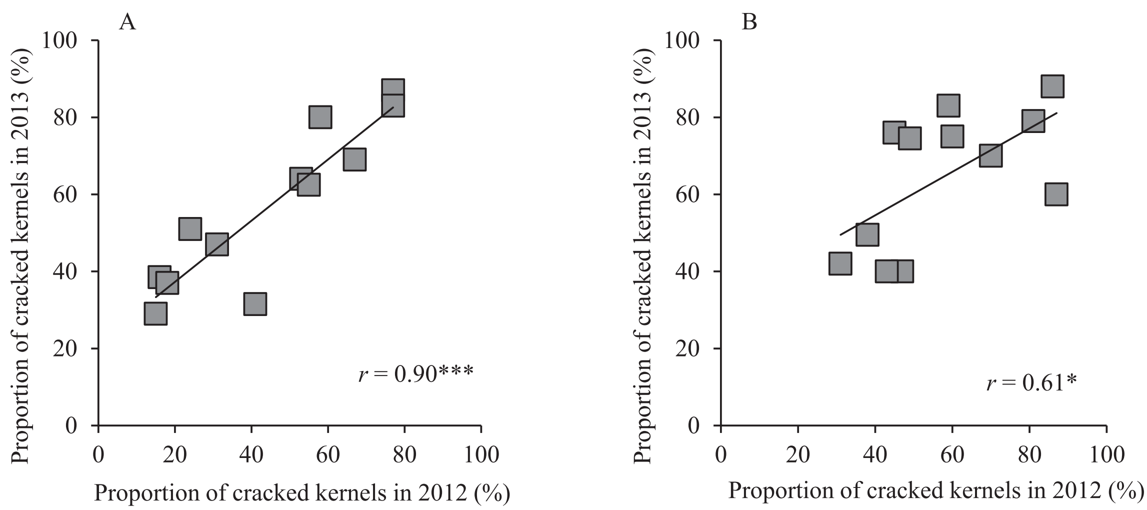

Fig. 6. Correlation between the proportion of cracked kernels in both years of the study, with the proportions calculated using (A) the moisture absorption method and (B) the late harvest method. Significance: $^{***}P < 0.001$; $^{*}P < 0.05$.

using 12 cultivars. The heading date of the 12 cultivars ranged from 2 to 19 August in 2012 and from 31 July to 14 August in 2013 (**Table 1**). The mean 10-day maximum temperature after heading ranged from 32.8 to 34.9°C in 2012 and from 32.3 to 35.2°C in 2013. Weather conditions after the heading period are shown in **Supplemental Fig. 1**. The proportion of cracked kernels calculated using the MA method ranged from 15% to 77% in 2012 and from 29% to 87% in 2013 among the 12 cultivars. The proportion calculated using the LH method ranged from 31% to 87% in 2012 and from 40% to 88% in 2013. 'Eminokizuna' and 'Yamadawara' had the lowest proportions of cracked kernels, and 'Yamahikari' and 'Asominori' had the highest proportions.

The proportion of cracked kernels calculated using the MA method was strongly and significantly correlated with the mean 10-day maximum temperature in both years (**Fig. 5**). In contrast, the proportion of cracked kernels calculated using the LH method was not significantly correlated with this temperature in either year. The correlation between the proportion of cracked kernels in the 2 years calculated using the MA method was strong and significant ($r = 0.90$, $P < 0.001$), whereas that for the LH method was significant but weaker ($r = 0.61$, $P < 0.05$) (**Fig. 6**). The correlation between the proportions of cracked kernels calculated using the MA and LH methods was not significant in either year (data not shown).

Discussion

Our three experiments revealed that the initial moisture content, temperature and duration affected the kernel cracking results, but that the number of wetting and drying cycles did not. Based on these results, we determined that the optimal conditions for the MA method were an initial moisture content of 12%, a temperature 25°C, a duration of 5 h and a single absorption treatment. These conditions produced stable results and let us distinguish among cultivars with different degrees of resistance to kernel cracking.

The initial moisture content was the most effective factor on the proportion of cracked kernels among these characteristics of the treatment condition in tested ranges. The optimal initial moisture content of 12% to detect varietal difference was the same level with another MA method using seeds (Pinson *et al.* 2012), though their optimal condition was decided with two long grain cultivars, 'Cypress' which was resistant to kernel cracking and 'LaGrue' which was sensitive. In their conditions, humidity exposure was conducted at 46°C with duration of 8 to 16 h which was harsher than our condition, and it suggested that the hull protected the kernel from cracking by moisture absorption. In our MA method, elimination of the hull made the conditions of absorption temperature and duration milder than Pinson's conditions. In addition, all the tested conditions of absorption temperature and duration could detect varietal difference (**Fig. 3**). It indicates that making the initial moisture content at the same level is important to evaluate resistant to kernel cracking accurately by the MA method.

Heading periods of tested cultivars were 4–5 days (data not shown), and distinct differences in duration of flowering periods among tested cultivars were not observed. Ohnishi *et al.* (2013) showed the proportion of cracked kernels increased when kernels in the stage of 4–7 days after flowering were counted with a three-day high temperature condition: 32°C 11 h in light period and 27°C 13 h in dark period. In our test, harvested panicles headed within a few days around heading date, and the heat sensitive period of these panicles was within 10 days after heading date. Therefore, the duration of flowering period was thought to have little effect on the proportions of cracked kernels in present study.

During the testing of the 12 cultivars in 2012 and 2013, the mean 10-day maximum temperature ranged from 32 to 35°C (**Table 1**). Nagata *et al.* (2013) reported that cultivars that showed high resistance to kernel cracking at temperatures below 33°C showed increased cracking at higher temperatures. Therefore, the temperatures in the present study were high enough to induce kernel cracking.

The proportion of cracked kernels calculated using the MA method was strongly and significantly correlated with the mean 10-day maximum temperature (**Fig. 5**). High temperatures during the early ripening period make rice kernels more sensitive to cracking (Nagata *et al.* 2004, 2013), and wetting and drying after the maturation period induce kernel cracking (Kondo and Okamura 1932, Nagato *et al.* 1964). In the new MA method, the wetting–drying condition is unified. Therefore, the MA method was able to reveal the effect of high temperature during the early ripening period on the occurrence of kernel cracking. In addition, the strong and significant correlation between the proportions of cracked kernels in both years in the MA method and the absence of a significant correlation in the LH method (**Fig. 6**) suggest that the MA method can stably evaluate differences among cultivars in resistance to kernel cracking.

The proportion of cracked kernels by the MA method did not always coincide with that by the LH method (**Table 1**). In both years, three cultivars, 'Etsunan 222', 'Akisakari' and 'Eminokizuna' showed higher proportions of cracked kernels by the LH method than that by the MA method. These three cultivars might have characteristics in degree of decrease rate in moisture content of seed. Watanabe and Kodama (1991) reported that there was a varietal difference in the degree of decrease rate in moisture content of seed and the degree was related to the proportion of cracked kernels. On the other hand, 'Hinohikari' found a higher proportion of cracked kernels by the MA method than that by the LH method. 'Hinohikari' might have another factor related to resistance to kernel cracking than brown rice. For example, Waggoner *et al.* (2003) reported that low permeability of hull were important sources of resistance to kernel cracking. Comparison of data by the MA method and that by the LH method can give us some information about characteristics of resistance to kernel cracking.

We confirmed the effectiveness of the MA method using 12 cultivars. However, further study is needed to confirm the applicability of the method for cultivars with earlier or later heading, as their mean 10-day maximum temperature would differ from those in the present study.

Screening of cultivars using the MA method would support the discovery of cultivars with high resistance to kernel cracking and of cultivars that are not susceptible to high temperatures during the early ripening period. In the present study, such cultivars included 'Eminokizuna' and 'Yamadawara'; these may be promising materials for use in future breeding. We are currently trying to reveal the hereditary factors related to high resistance to kernel cracking, with the goal of helping breeders to select cultivars with high resistance, possibly using techniques such as marker-assisted selection.

Acknowledgements

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