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Varietal Differences in Endosperm Cell Morphology of the Non-glutinous Rice (*Oryza sativa* L.) Released over the Past 100 Years in Hokkaido, Japan

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Abstract: Consumer demand for good eating quality of cooked rice has been growing widely not only in Japan but also in other countries. Eating quality of the non-glutinous rice varieties bred in Hokkaido, northern Japan, during the past 20 years has improved drastically due to the breeding efforts focused mainly on chemical properties, such as amylose and protein concentrations. However, the effect of physical properties, such as cell morphology, on eating quality has been less studied. To clarify the relationship between physical properties and eating quality of rice, we investigated endosperm cell morphology (ECM) of grain by using a new simple method with a stereomicroscope for 18 non-glutinous varieties released over the past 100 years in Hokkaido. There were significant varietal differences in most of the ECM characteristics measured. Especially, cell density along the central line of endosperm (CLE) correlated negatively with the released year of varieties ($r = -0.827$, $p < 0.001$), and decreased with the decrease in amylose and protein concentrations. The results indicated that the rice breeding in Hokkaido has changed not only the chemical but also physical properties. In addition, some varieties with similar amylose and protein concentrations showed large differences in CLE cell density. This suggests that the varietal difference in eating quality, which can not be explained by the difference in amylose and protein concentrations only, may be explained by the difference in CLE cell density. These findings will contribute to the further improvement in eating quality of non-glutinous rice.

Key words: Cell density, Central line of endosperm, Eating quality, Endosperm morphology, Physical properties, Rice.

Rice (*Oryza sativa* L.) is one of the three major crops in the world and the most important staple food in many countries including Japan. The breeding of the non-glutinous rice in Japan is unique in that it focuses mainly on the improvement of eating quality of cooked rice rather than the enrichment of protein and micronutrients contents in grain like in golden rice (Potrykus, 2001). Recently, consumer demand for good eating quality of cooked rice has been growing widely and japonica type rice became popular not only in Japan but also in other countries, such as China, South Korea and USA (Song and Carter, 1996; Hansen et al., 2002).

The eating quality of rice is strongly affected by the texture, such as hardness and stickiness. The variety with preferable eating quality for Japanese has generally low hardness and high stickiness (Iwasaki, 1993). This texture

of rice is affected by chemical properties (amylose, protein, lipid and mineral concentrations etc.) and physical properties (starch granule size, cell morphology etc.). In Japan, rice breeders have focused mainly on the chemical properties of grain, especially its amylose and protein concentrations.

Hokkaido, northern part of Japan, is the largest rice production area in Japan. Before the 1980s, the eating quality of the rice varieties bred in Hokkaido was markedly lower compared with the varieties bred in other prefectures, because the major breeding target of rice in Hokkaido was the improvement of cold weather resistance. In the 1980s, the breeding efforts in Hokkaido were mainly focused on the improvement of eating quality and, since then, eating quality has improved drastically. This improvement of the eating quality of rice in Hokkaido was

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Abbreviations: CLE, central line of endosperm; ECM, endosperm cell morphology.

caused mainly by the decrease of amylose and protein concentrations in grain (Inatsu, 1988). New rice varieties in Hokkaido, such as 'Oborozuki' and 'Yumepirika' have an extremely high eating quality with low amylose and protein concentrations (Ando et al., 2006; Sato et al., 2007).

However, some reports revealed that the decrease of amylose concentration does not necessarily improve the eating quality (Yoshimura and Aikawa, 1998) and that some indica type rice varieties with low amylose and protein concentrations showed low eating quality (Ohtsubo, 1995). These reports indicate that the differences in the eating quality between rice genotypes can not be explained by amylose and protein concentrations.

Physical properties are considered as another factor affecting the differences in the eating quality of rice. 'Endosperm cell morphology (ECM)' is one characteristic among the physical properties. Nagato and Kobayashi (1959) observed the ECM of rice with 50-100 μm thick cross-sections, and revealed that the array direction of endosperm cell differed between indica and japonica types. Hoshikawa (1967) also measured the endosperm cell number of 32 rice varieties by using SUMP (Suzuki's universal micro-printing) method and reported that there were large varietal differences (from 120 to 170 cells) in the endosperm cell number along the longitudinal line. However, the relationship between the varietal differences in the ECM and the eating quality of rice was obscure because of the time-consuming procedures to make microtome sections (Nagato and Kobayashi, 1959) and to prepare molding specimens (Hoshikawa, 1967).

Recently, observation techniques for the ECM have been developed markedly enabling us to get high quality images. For example, Ogawa et al. (2003) developed a new method to observe the ECM by using a microtome and an optical microscope with a mercury arc lamp and obtained good quality images of the rice grain sections. Morita et al. (2005) obtained the microscope images of rice ECM by a microtome section with 20 μm thickness and traced cell contours of the endosperm images for investigating the ECM characteristics. Aramaki et al. (2004) also investigated the ECM of brewers' rice by a scanning electron microscopy and proposed that the ECM could be divided into two types based on the array direction of endosperm cell. In these studies, however, the observation techniques for the ECM still require much time to pre-treat the sample for microscope observation, and the effect of physical properties on the eating quality has not been clarified yet.

In this study, we analyzed the varietal differences in the ECM of 18 non-glutinous rice varieties bred over the past 100 years in Hokkaido by using a quick new method, and determined the effect of breeding on this varietal difference and the relationship between ECM and the chemical properties of grain.

Materials and Methods

1. Plant materials

We examined 18 non-glutinous rice varieties including three non-glutinous breeding lines (Kuiku-131, Kuiku-171, Hokkai-302) (Table 1). In addition, two brewers' rice varieties (Hatsushizuku, Ginpu) and one glutinous variety (Hakuchomochi) were also examined for the purpose of reference. These varieties are the main cultivars cultivated in the past or at present in Hokkaido, and are referred to hereafter as 'Hokkaido rice varieties'. These varieties were cultivated in the paddy field of Hokkaido Central Agricultural Experiment Station Iwamizawa Branch (Hokkaido, Japan, 43° 10' N, 141° 42' E) in 2008.

Four pre-germinated seeds were sown in each cell of a nursery box on 24 April 2008 and raised to seedlings under a vinyl house, and thereafter the seedlings with 3.5-5.0 leaf stage were transplanted to the paddy field at a planting density of 30 cm between rows and 13.3 cm between plants on 26 May 2008. The study was laid out as a randomized complete block design with three replications. Chemical fertilizer with 80, 97 and 69 kg ha⁻¹ of N, P₂O₅ and K₂O, respectively, was applied as a basal fertilizer. Days to heading was determined as the days from sowing to 50% heading. Thirty hills of plants were harvested at 6-7 weeks after heading, out of which 21 hills were used for yield survey and chemical analysis and the rest 9 hills were used for the ECM measurement.

After harvesting, the rice plants were air dried, threshed, hulled and sieved through a 1.9 mm sieve, and the weight of brown rice was measured. The grain moisture content (%) was determined with Kett's Grain Moisture Tester (model PB-1D, Kett Electric Laboratory, Japan). The weight of 500 grains was recorded three times and then the weight of 1000 grains was calculated after the moisture content of grains adjusted to 15%.

In addition to the Hokkaido rice varieties, we used four commercial rice varieties, 'Koshihikari' (released year: 1956)', 'Akitakomachi (1984)', 'Hino Hikari (1989)' and 'Hitomebore (1991)'. These four varieties, hereafter referred to as 'non-Hokkaido rice varieties', have high eating quality. Among them, 'Koshihikari' has been the leading variety in terms of cultivated area, production and eating quality for the last 30 years in Japan. Since these non-Hokkaido rice varieties can not ripen in Hokkaido because of the cold climate, the grains were obtained from central and southern parts of Japan. The data of these varieties were used for reference purposes only.

2. Chemical analysis

Polished rice grains at milling yield of 90.5% were powdered and then used for chemical analysis. Amylose and protein concentrations were measured twice for each sample by an auto analyzer (Auto Analyzer II,

Table 1. Released year, days to heading, yield, 1000 grain weight, chemical properties and endosperm cell morphology of Hokkaido and non-Hokkaido rice varieties.

Variety	Released year	Days to heading	Yield ¹ (kg ha ⁻¹)	1000 ² grain weight (g)	Chemical properties ³			Endosperm cell morphology								
					Amylose conc. (%)	Protein conc. (%)	Crude fiber conc. (%)	Dorsiventral line			Longitudinal line			Central line		
								Length (mm)	Cell number (cell)	Cell ⁴ density (cell mm ⁻¹)	Length (mm)	Cell number (cell)	Cell density (cell mm ⁻¹)	Length (mm)	Cell number (cell)	Cell density (cell mm ⁻¹)
Hokkaido rice varieties																
Shinriki	1877	96	5481	23.5	23.5	9.1	3.0	3.16	33.6	10.6	5.27	123	23.3	4.18	110	26.4
Akage	1905	106	4813	22.2	24.6	8.1	2.5	2.92	32.6	11.2	5.24	132	25.1	4.23	118	28.0
Bouzu-1	1919	95	3655	18.2	23.4	8.5	2.8	2.69	39.0	14.5	5.27	133	25.2	4.15	121	29.0
Mantaroumai	1928	112	5396	21.9	24.2	8.9	3.0	2.36	31.6	10.7	4.92	117	23.8	3.80	100	26.2
Fukoku	1935	106	5495	21.6	23.5	8.5	2.9	2.95	31.3	10.6	5.14	123	23.9	4.06	109	26.8
Yukara	1962	109	5075	21.5	23.2	8.0	2.9	2.85	33.0	11.6	5.46	125	22.9	4.33	110	25.3
Ishikari	1971	102	6006	21.9	24.8	7.5	2.7	2.83	35.0	12.4	5.56	127	22.9	4.45	108	24.3
Kuiku-131	1986	106	6097	19.7	21.8	7.2	2.5	2.90	33.9	11.6	5.04	114	22.7	3.96	99	24.9
Kirara397	1988	105	5839	21.9	21.7	7.6	2.4	2.96	31.3	10.6	5.29	122	23.1	4.23	107	25.3
Hakuchomochi	1989	98	4924	19.3	nd	7.3	2.4	2.99	37.0	12.4	5.52	121	22.0	4.19	105	25.0
Aya	1992	110	5812	22.1	16.7	7.5	2.6	2.94	26.5	9.0	5.35	110	20.5	4.39	96	21.9
Hoshinoyume	1996	103	5171	20.8	22.5	7.7	2.5	2.80	31.4	11.2	5.13	119	21.6	4.43	105	23.8
Hatsushizuku	1998	104	6490	22.9	24.5	7.4	2.7	3.02	31.8	10.5	5.41	122	22.5	4.28	105	24.5
Ginpu	2000	108	6597	24.2	23.6	8.0	2.6	3.09	39.3	12.7	5.14	125	24.5	4.06	109	26.7
Nanatsuboshi	2001	101	5734	21.0	20.9	7.1	2.2	2.81	30.1	10.7	5.40	119	22.0	4.36	105	24.0
Ayahime	2001	103	5752	19.8	14.4	7.1	2.1	2.88	28.5	9.9	4.95	110	22.2	4.08	96	23.4
Daichinohoshi	2003	97	6344	24.5	22.2	7.0	2.2	2.95	33.3	11.3	5.58	121	21.7	4.48	107	23.8
Oborozuki	2003	104	5489	21.4	18.1	7.8	2.3	2.86	29.8	10.4	5.40	113	21.0	4.35	101	23.2
Kuiku-171	2005	102	6242	22.4	19.1	7.5	2.2	2.87	30.8	10.8	5.38	121	22.5	4.34	106	24.6
Hokkai-302	2005	102	5647	22.2	19.2	6.8	2.0	2.91	31.9	11.0	5.56	120	21.6	4.53	107	23.7
Yumepirika	2008	103	5369	21.5	19.1	7.5	1.9	2.86	25.9	9.1	5.52	114	20.7	4.46	99	22.2
mean		103	5592	21.7	21.5	7.7	2.5	2.89	32.3	11.1	5.31	120	22.6	4.26	106	24.9
LSD (0.05) ⁵		1	406	0.4	0.4	0.5	0.3	NS	3.5	1.2	0.29	7	1.4	0.15	7	1.6
Non-Hokkaido rice varieties																
Koshihikari	1956	—	—	—	—	—	—	3.09	25.4	8.2	5.27	114	21.5	4.11	97	23.6
Akitakomachi	1984	—	—	—	—	—	—	2.94	27.8	9.4	5.41	124	23.0	4.03	103	25.5
Hinohikari	1989	—	—	—	—	—	—	3.05	27.2	8.9	5.26	121	22.9	4.09	102	25.0
Hitomebore	1991	—	—	—	—	—	—	3.11	27.8	9.0	5.32	121	22.7	4.16	102	24.5
mean		—	—	—	—	—	—	3.05	27.1	8.9	5.31	120	22.5	4.10	101	24.6

Abbreviations: conc., concentration; nd, not detectable; NS, not significant.

¹ Weight of hulled brown rice per unit area. Values are shown equivalent to 15% grain moisture content.² Values are shown equivalent to 15% grain moisture content.³ Values are shown equivalent to 0% grain moisture content.⁴ Cell density = Cell number / Length.⁵ Least significant difference (LSD) for comparison between Hokkaido rice varieties.

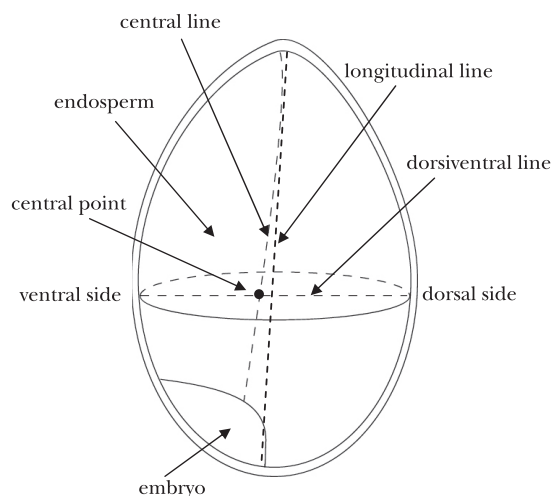


Fig. 1. Schematic diagram of rice grain morphology.

Bran+Luebbe, Germany) and a near infrared analyzer (Infra Analyzer 2000, Bran+Luebbe, Germany), respectively.

For measuring crude fiber concentration, approximately 2 g of the powdered sample (weight was recorded precisely) was put in a polypropylene tube with 30 ml of distilled water, and autoclaved for one hour at 123°C for starch gelatinization. 1 ml of solution with 5% of glucoamylase (Gluczyme AF6, Amano Enzyme Inc., Japan) and 0.5% of calcium chloride was added to the tube, and the tube was stored overnight at 40°C. Thereafter, 1 ml of acetate buffer (pH 4.8) with 1% of acid protease (Protease M “Amano” G, Amano Enzyme Inc., Japan) was added, and stored overnight at 40°C. The dry weight of filtrated substance of this solution (i.e., residue of enzymatic decomposition) was determined as crude fiber weight, and the crude fiber concentration was calculated by dividing the crude fiber dry weight by the initial powdered sample dry weight. Crude fiber concentration was measured twice for each sample.

3. Endosperm cell morphology

Immediately after harvesting, specific unhulled grains (the third, fourth and fifth grain of the three uppermost primary rachis-branches of panicle) were sampled from the main culm without drying and soaked in a fixative solution (3.7% formaldehyde and 0.1% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2)), because excessively-dried grains crack easily by rapid water absorption. The soaked samples were deaerated overnight and then stored at 4°C for more than 2 weeks. Thereafter, the stored grain samples were rinsed by distilled water and cut with a razor with 0.1 mm blade thickness (Feather double-edge razor blade, Feather Safety Razor Co., Japan). The cross-sections were smoothed with super fine sandpaper (average particle diameter: 7.1–8.9 μm) followed by frost surface and glass surface of frosted glass slide. The grain samples

were cut along the longitudinal section (the section where dorsiventral and longitudinal lengths can be observed), i.e., unhulled grains were cut along main veins of lemma and palea of hull.

Smoothed grain samples were soaked and stained in 0.05% toluidine blue solution for one minute. Then, after rinsing with distilled water, the ECM was examined under a stereomicroscope. For each sample, five grains without chalky parts in the grain were observed, except for the variety Ginpu, which genetically has white core grains. The stereomicroscopic image was taken with a USB camera (model TS-CA-130M, Sugitoh Co., Ltd., Japan) connected to a personal computer, and each characteristic of the ECM was examined on the monitor. Length, cell number (exclusive of aleurone layer) and cell density (cell number / length) along dorsiventral, longitudinal and central lines of endosperm (Fig. 1) were examined as the ECM characteristics. The central line of endosperm (CLE) is the last part where the embryo sac cavity is filled with endosperm cells, which divide and multiply centripetally from periphery (Hoshikawa, 1993). This CLE is observed as the central point in the transverse section (Fig. 1).

The above procedure for the ECM observation required 5–6 minutes for each sample from cross-sectioning to capture of the stereomicroscope image, and another 2–3 minutes to determine the length and cell number of endosperm on the monitor. Compared with other methods (Nagato and Kobayashi, 1959; Hoshikawa, 1967; Aramaki et al., 2004), the ECM observation by our method required less time and effort. In addition, the quality of the captured images by our method (Fig. 2) was comparable to those obtained by a novel method of Ogawa et al. (2003).

4. Meteorological data and statistical analysis

Meteorological data of daily mean air temperature were obtained from the Automated Meteorological Data Acquisition System (AMeDAS), Iwamizawa station (43°21' N, 141°78' E).

Statistical analysis was done using the SPSS software (version 14.0J, SPSS Japan, Tokyo, Japan). The least significant difference test ($p=0.05$) was used to examine differences between varieties. Correlation coefficients between variables were calculated by Pearson product-moment analysis. The correlation analysis was conducted taking into account the non-glutinous varieties only.

Results and Discussion

1. Climatic conditions

Air temperature during the grain-filling period is an important factor affecting grain morphology (Morita et al., 2005) and chemical properties (Sano et al., 1985). In the present study, the daily mean air temperature was lower in 2008 than in the past 10 years from heading stage to two weeks after heading, but was higher thereafter (Fig. 3).

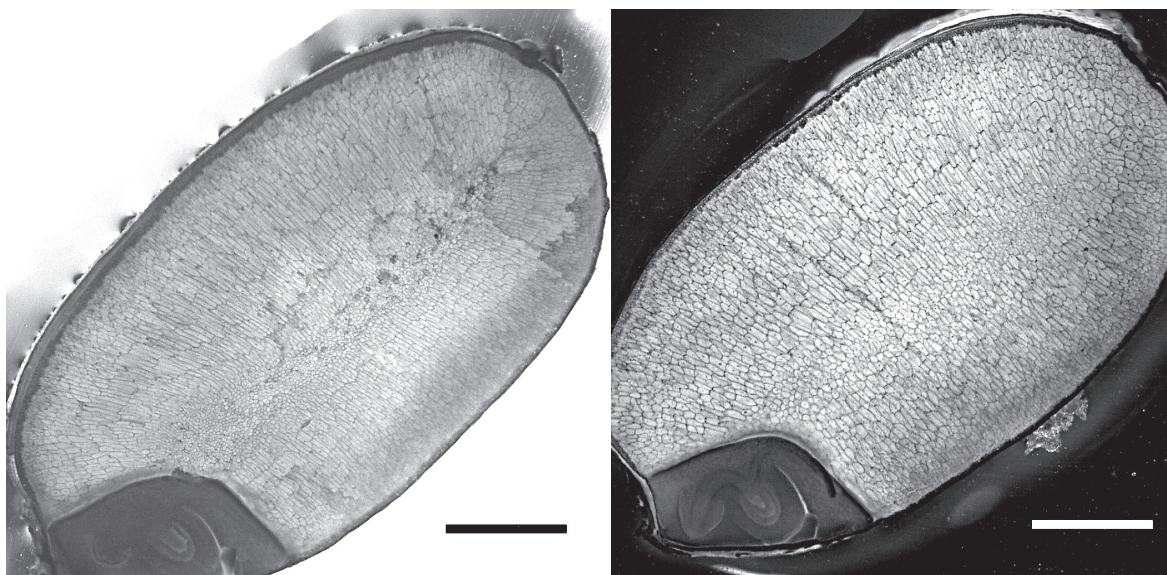


Fig. 2. Photographs of longitudinal section in old variety 'Bouzu-1' (left: higher cell density) and new variety 'Yumepirika' (right: lower cell density) under same magnification ($\times 20$). Scale bar = 1 mm.

Consequently, the mean air temperature from heading to maturity stages was lower in 2008 than in the past 10 years for all the varieties, but the difference between 2008 and the past 10 years was less than 0.5°C (from 0.05 to 0.48°C). The results suggested that all the varieties grew under normal climatic conditions, and neither cool weather nor heat damages occurred during grain filling in 2008.

2. Varietal differences in growth and chemical properties

Days to heading and the weight of 1000 grains varied significantly with the variety in Hokkaido rice (Table 1). Grain yield also differed significantly with the variety ranging from 3655 kg ha^{-1} (Bouzu-1) to 6597 kg ha^{-1} (Ginpu). These yields were almost the same as in other reports (4100 kg ha^{-1} to 6740 kg ha^{-1}), which surveyed the rice yields in Hokkaido previously (Anzoua et al., 2009).

The released year of non-glutinous varieties correlated significantly and positively with grain yield ($r=0.543^*$, $n=18$) but not significantly with days to heading ($r=0.083^{\text{NS}}$, $n=18$) and the weight of 1000 grains ($r=-0.029^{\text{NS}}$, $n=18$). Anzoua et al. (2009) also reported a significant positive correlation between the released year and grain yield of Hokkaido rice varieties.

The chemical properties, amylose and protein concentrations, which have been considered to be the major factors affecting the eating quality, varied significantly with the variety in Hokkaido rice (Table 1): the amylose concentration from 14.4% (Ayahime) to 24.8% (Ishikari) and the protein concentration from 6.8% (Hokkai-302) to 9.1% (Shinriki). Amylose could not be detected in Hakuchomochi, because glutinous rice varieties genetically have no amylose in their grains. Another chemical property, the crude fiber concentration, also varied

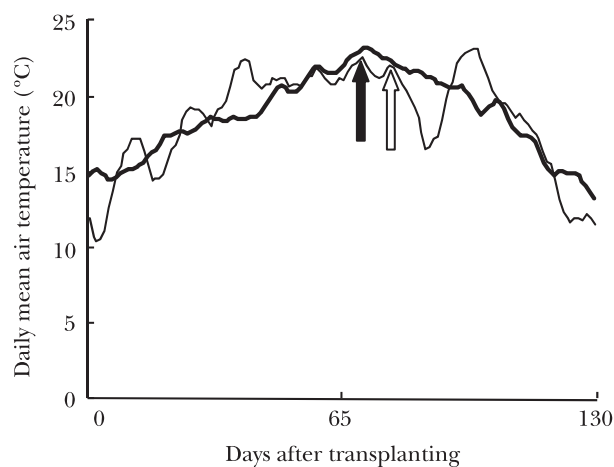


Fig. 3. Dynamics of the daily mean air temperature during the rice cropping season in 2008 (thin line) and 10-year average from 1998 to 2007 (thick line). Data were shown in 5-day moving average. Black and white arrows indicate the heading dates of the early and the late varieties, respectively.

significantly with the variety in Hokkaido rice ranging from 1.9% (Yumepirika) to 3.0% (Shinriki and Mantaroumai).

The released year of non-glutinous varieties correlated significantly and negatively with each chemical property (amylose concentration: $r=-0.656^{**}$, $n=18$; protein concentration: $r=-0.871^{***}$, $n=18$; crude fiber concentration: $r=-0.782^{***}$, $n=18$). Inatsu (1988) also reported that new rice varieties released in Hokkaido had lower amylose concentrations in grains as compared with the old varieties.

3. Varietal differences in ECM

Significant differences among the Hokkaido rice varieties were found in all the ECM characteristics except in

Table 2. Correlation coefficients of released year, days to heading, yield and 1000 grain weight with endosperm cell morphology in Hokkaido rice varieties (n=18).

Endosperm cell morphology	Released year	Days to heading	Yield	1000 grain weight
Dorsiventral line				
Length	0.014 ^{NS}	-0.368 ^{NS}	0.302 ^{NS}	0.365 ^{NS}
Cell number	-0.521 [*]	-0.446 ^{NS}	-0.411 ^{NS}	-0.206 ^{NS}
Cell density	-0.356 ^{NS}	-0.434 ^{NS}	-0.517 [*]	-0.400 ^{NS}
Longitudinal line				
Length	0.325 ^{NS}	-0.350 ^{NS}	0.165 ^{NS}	0.446 ^{NS}
Cell number	-0.606 ^{**}	-0.355 ^{NS}	-0.531 [*]	0.010 ^{NS}
Cell density	-0.795 ^{***}	-0.089 ^{NS}	-0.545 [*]	-0.236 ^{NS}
Central line				
Length	0.513 [*]	-0.330 ^{NS}	0.178 ^{NS}	0.361 ^{NS}
Cell number	-0.606 ^{**}	-0.445 ^{NS}	-0.615 ^{**}	-0.030 ^{NS}
Cell density	-0.827 ^{***}	-0.171 ^{NS}	-0.623 ^{**}	-0.220 ^{NS}

Abbreviation: NS, not significant.

***, significant at 0.1% probability level.

**, significant at 1% probability level.

*, significant at 5% probability level.

the length of dorsiventral line (Table 1). The cell densities along dorsiventral, longitudinal and central lines were the lowest for the variety 'Aya', which is characterized by its low hardness and high stickiness of cooked grain (Kunihiro et al., 1993). Brewers' rice variety, Ginpu, showed relatively high cell densities along dorsiventral, longitudinal and central lines. The cell densities along dorsiventral, longitudinal and central lines correlated significantly with each other in non-glutinous varieties (between dorsiventral and longitudinal lines: $r=0.641^{**}$, $n=18$; between dorsiventral and central lines: $r=0.667^{**}$, $n=18$; between longitudinal and central lines: $r=0.973^{***}$, $n=18$).

The cell density was lower in the non-Hokkaido rice varieties than in the Hokkaido rice varieties (Table 1). 'Koshihikari', which is considered to have the highest eating quality in Japan, had a considerably low cell density, and the other varieties with high eating quality (Akitakomachi, Hinohikari and Hitomebore) also had a low cell density, implying that the cell density has some effects on the eating quality. Hoshikawa (1967) reported, however, that the cell density in 'Koshihikari' was not particularly low among 32 japonica-type rice varieties. The reason for the discrepancy between our study and that of Hoshikawa (1967) is probably that the rice varieties in the present study were selected based on the eating quality unlike in the latter study.

The released year of non-glutinous varieties correlated significantly with the six ECM characteristics (cell number along the dorsiventral line, cell number and density along the longitudinal line and length, cell number and density along the CLE) (Table 2). Among these ECM characteristics, the CLE cell density correlated most strongly with the

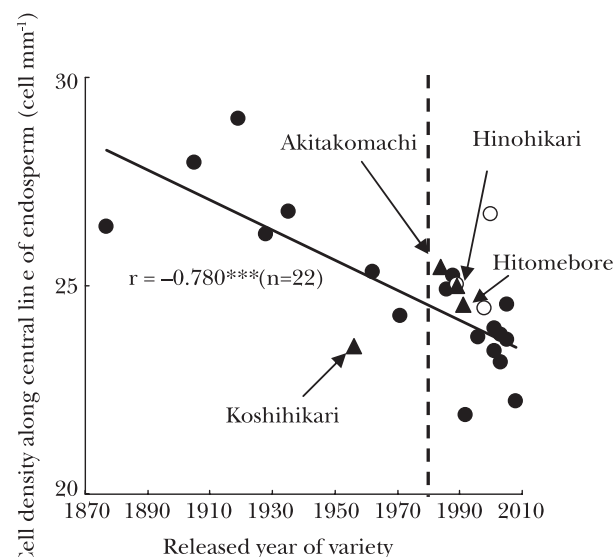


Fig. 4. Correlation between released year and cell density along central line of endosperm in non-glutinous rice varieties released over the past 100 years in Hokkaido and in areas other than Hokkaido. ●, non-glutinous variety released in Hokkaido; ▲, non-glutinous variety released in areas other than Hokkaido; ○, glutinous and brewers' rice variety released in Hokkaido. Arrows indicate the data for varieties cultivated in areas other than Hokkaido. Dashed line indicates the year 1980 when the main breeding objective in Hokkaido changed from the improvement of cold weather resistance to the improvement of eating quality. *** Significant at 0.1% probability level.

released year of varieties: the CLE cell density was lower in new varieties than in old varieties (Table 1 and Fig. 2). This correlation between released year of non-glutinous varieties and CLE cell density was significant, even if four

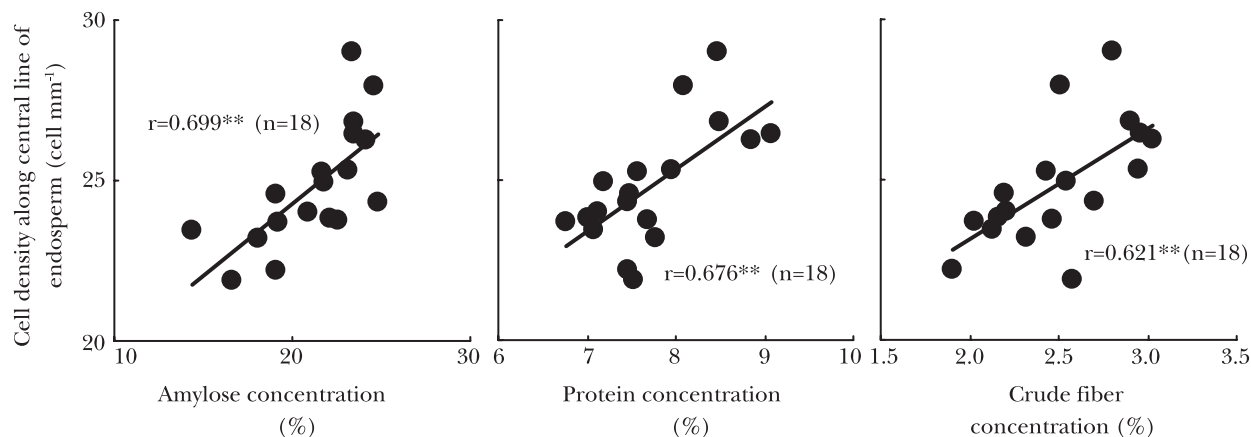


Fig. 5. Correlations between chemical properties (amylose, protein and crude fiber concentrations) and cell density along central line of endosperm (CLE) in non-glutinous rice varieties released over the past 100 years in Hokkaido. ** Significant at 1% probability level.

non-Hokkaido rice varieties were included (Fig. 4).

In addition, the decrease in the CLE cell density in the non-glutinous Hokkaido rice varieties showed drastic reduction after the 1980s (Fig. 4). The main breeding target in non-glutinous rice varieties before the 1980s (i.e., from Shinriki to Ishikari) was the improvement of cold weather resistance. The focus of the breeding efforts have shifted towards the improvement of eating quality after the 1980s (i.e., from Kuiku-131 to Yumepirika). As a result, the eating quality improved in the new varieties compared to the old varieties, especially after the 1980s (Yoshimura and Aikawa, 1998). Our results imply that the decrease of the CLE cell density progressed concurrently with the drastic improvement of eating quality after the 1980s in Hokkaido.

4. Correlation of growth and chemical properties with ECM

Days to heading and the weight of 1000 grains did not correlate significantly with all the ECM characteristics in non-glutinous varieties (Table 2). This indicates that the earliness and yield components of the Hokkaido rice varieties had no effect on the ECM. Although the yield correlated significantly with some ECM characteristics (cell densities along dorsiventral, longitudinal and central lines and cell number of longitudinal and central lines), these correlation coefficients were not high, suggesting that the yield of the Hokkaido rice varieties had less effect on the ECM.

There were significant positive correlations of amylose and protein concentrations with the CLE cell density (Fig. 5). These results indicate that not only amylose and protein concentrations but also the CLE cell density decreased as a result of the rice breeding in Hokkaido. The correlation coefficients were, however, not very high and there were some varieties with small differences in amylose and protein concentrations but with large differences in the CLE cell density (Table 1 and Fig. 5). This implies that the varietal differences in the eating

quality of rice, which can not be explained only by the differences in amylose and protein concentrations, can be explained by the differences in the CLE cell density.

Some characteristics of the CLE in relation to water penetration have been reported. For example, Horigane et al. (2006) monitored three-dimensionally the water migration into a rice grain during soaking by using compact magnetic resonance imaging, and proposed that the CLE served as a main channel for water penetration into grain. The CLE was also considered as the route of water migration from the pericarp vascular bundle to the embryo during development of caryopses (Horigane et al., 2001). These reports imply that the CLE plays an important role in the process of water absorption during soaking, which is one of the major factors affecting the eating quality of rice (Okuno and Adachi, 1992).

The crude fiber concentration also correlated significantly and positively with the CLE cell density (Fig. 5). This suggests that the concentration of cell wall substances increased with the increase in the CLE cell density through the rice breeding in Hokkaido, because crude fiber mainly consists of the apoplastic components including cellulose, hemicellulose, pectin and lignin.

Although there are few reports suggesting the effect of cell wall substances on the texture, Tohno-Oka et al. (2004) reported that the concentrations of two principal substances of endosperm cell wall, beta-glucan and arabinoxylan, correlated significantly and positively with grain hardness in barley (*Hordeum vulgare* L.). Arabinoxylan is also known as a principal substance of endosperm cell wall in rice (Shibuya et al., 1985). In addition, it is reported that the concentration of glucomannan in the cell wall was higher in low eating quality varieties, particularly those bred in Hokkaido, than in high eating quality varieties (Shibuya, 1993). Takano (2002) also indicated a significant negative correlation between pectin degradation rate by cooking and hardness of cooked rice. However, these results were

based on experiments with a few rice varieties and, therefore, further investigations are needed to clarify the effect of the cell density and cell wall substances on the eating quality of rice. These questions could be addressed by using varieties and/or breeding lines with various characteristics such as high hardness and low stickiness even with low amylose and protein concentrations.

Conclusions

This study revealed morphological variation in endosperm cells among the non-glutinous rice varieties bred in Hokkaido by using a simple method for observation of the ECM. Among the ECM characteristics, the CLE cell density correlated most strongly with the released year of variety and decreased with the decrease in amylose and protein concentrations, indicating that the rice breeding in Hokkaido has changed not only the chemical properties of grains but also their physical properties. Though some varieties showed small differences in amylose and protein concentrations, large differences in the CLE cell density were noticed. These results suggest that the varietal difference in eating quality, which can not be explained only by the difference in amylose and protein concentrations, may be explained by the difference in the CLE cell density. This simple observation method enables us to investigate the varietal difference in ECM with less time and effort and should be useful in determining the effect of the physical properties on eating quality. Although our results were based on only a one-year experiment and the reproducibility of the results needs to be confirmed, they are expected to contribute to the further improvement in eating quality of non-glutinous rice varieties.

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References

- Ando, I., Araki, H., Shimizu, H., Kuroki, M., Miura, K., Nagano, K. and Konno, K. 2006. "Oborozuki", A new rice cultivar with low amylose content and superior eating quality. *Research Bulletin of the National Agricultural Research Center for Hokkaido Region* 186: 31-46*.
- Kossohou G.A., Kashiwagi, J., Hasegawa, T., Iwama, K. and Jitsuyama Y. 2009. Genetic improvements for high yield and low soil nitrogen tolerance in rice (*Oryza Sativa* L.) under a cold environment. *Field Crops Res.* 116: 38-45.
- Aramaki, I., Kikunaga, Y., Yoshii, M., Okuda, M., Koseki, T., Ogawa, M., Kumamaru, T., Sato, H. and Hashizume, K. 2004. Observation of the endosperm-mutant rice grains having a white core (Shinpaku) and analysis of their physical and chemical properties for Sake brewing. *J. Brew. Soc. Jpn.* 99: 134-144**.
- Hansen, J., Fuller, F., Gale, F., Crook, F., Wailes, E. and Moore, M. 2002. China's japonica rice market: growth and competitiveness. *In Rice Situation and Outlook Yearbook*. Washington, D.C., U.S. Department of Agriculture, Economic Research Service, Market and Trade Economics Division. 32-37
- Horigane, A.K., Engelaar, W.M.H.G., Toyoshima, H., Maruyama, S., Yoshida, M., Okubo, A. and Nagata, T. 2001. Visualization of moisture distribution during development of rice caryopses (*Oryza sativa* L.) by nuclear magnetic resonance microimaging. *J. Cereal Sci.* 33: 105-114.
- Horigane, A.K., Takahashi, H., Maruyama, S., Ohtsubo, K. and Yoshida, M. 2006. Water penetration into rice grains during soaking observed by gradient echo magnetic resonance imaging. *J. Cereal Sci.* 44: 307-316.
- Hoshikawa, K. 1967. Studies on the development of endosperm in rice: 7. Size of endosperm and number of cell layers of endosperm of paddy rice varieties in Japan. *Jpn. J. Crop Sci.* 36: 395-402**.
- Hoshikawa, K. 1993. III Morphology and development of reproductive organs: chapter 2 Anthesis, fertilization and development of caryopsis. *In T. Matsuo and K. Hoshikawa eds., Science of the Rice Plant, vol.1: Morphology*. Tokyo, Japan: Food and Agriculture Policy Research Center. 339-376.
- Inatsu, O. 1988. Studies on improving the eating quality of Hokkaido rice. *Report of Hokkaido Prefectural Agricultural Experiment Stations* 66: 1-89**.
- Iwasaki, T. 1993. III Morphology and development of reproductive organs: chapter 3 Quality and shape of rice grain: eating quality of the cooked rice. *In T. Matsuo and K. Hoshikawa eds., Science of the Rice Plant, vol.1: Morphology*. Tokyo, Japan: Food and Agriculture Policy Research Center. 396-404.
- Kunihiro, Y., Ebe, Y., Shinbashi, N., Kikuchi, H., Tanno, H. and Sugawara, K. 1993. A new paddy rice variety "Aya" with good eating quality due to low amylose content developed by anther culture breeding. *Jpn. J. Breed.* 43: 155-163**.
- Morita, S., Yonemaru, J. and Takanashi, J. 2005. Grain growth and endosperm cell size under high night temperatures in rice (*Oryza sativa* L.). *Ann. Bot.* 95: 695-701.
- Nagato, K. and Kobayashi, Y. 1959. On varietal differences in the tissue of starch-cell of rice kernels. *Jpn. J. Crop Sci.* 27: 443-445**.
- Ogawa, Y., Orts, W.J., Glenn, G.M. and Wood, D.F. 2003. A simple method for studying whole sections of rice grain. *Biotech. Histochem.* 78: 237-242.
- Ohtsubo, K. 1995. 2.2. Chemical property of rice: chapter 2 Quality of rice. *In T. Ishitani and K. Ohtsubo eds. Science of Rice (Series 'Science of foods')*. Tokyo, Japan: Asakura Publishing. 18-48***.
- Okuno, M. and Adachi, K. 1992. Relationship between physical properties and water absorption characteristics of milled rice. *Jpn. J. Crop Sci.* 61: 244-250**.
- Potrykus, I. 2001. Golden rice and beyond. *Plant Physiol.* 125: 1157-1161.

- Sano, T., Maekawa, M. and Kikuchi, H. 1985. Temperature effects on the Wx protein level and amylose content in the endosperm of rice. *J. Hered.* 76: 221-222.
- Sato, T., Numao, Y., Yoshimura, T., Ozaki, H., Kinoshita, M., Shinada, H., Kasuya, M., Kiuchi, H., Maekawa, T., Hirayama, Y., Sasaki, T., Aikawa, M., Kikuchi, H., Tanno, H., Tanaka, K. and Shinbashi, N. 2007. A new rice variety "Jouiku No.453". *New research findings -Hokkaido Region-* 4-7***.
- Shibuya, N., Nakane, R., Yasui, A., Tanaka, K. and Iwasaki, T. 1985. Comparative study on cell wall preparations from rice bran, germ and endosperm. *Cereal Chem.* 62: 252-258.
- Shibuya, N. 1993. Varietal differences of chemical structure of rice endosperm cell wall and their genetic analysis. *J. Brew. Soc. Jpn.* 88: 910-913***.
- Song, J.H. and Carter, C.A. 1996. Rice trade liberalization and implications for US policy. *Am. J. Agr. Econ.* 78: 891-905.
- Takano, K. 2002. *Kenkyuseika 384*. Japan, Agriculture, Forestry and Fisheries Research Council Secretariat***.
- Tohno-Oka, T., Kawada, N. and Yoshioka, T. 2004. Relationship between grain hardness and endosperm cell wall polysaccharides in barley. *In Proceedings of the Ninth International Barley Genetic Symposium, Brno, Czech Republic.* 595-600.
- Yoshimura, T. and Aikawa, M. 1998. Comparison of eating qualities old and new rice cultivars in Hokkaido. 2. Comparison of Physico-chemical characteristics. *Hokuno* 65: 273-279***.

* In Japanese with English summary.

** In Japanese with English abstract.

*** In Japanese.