Note

Screening and characterization of cultivar with M-type amylopectin in Japanese upland rice

Kazuyuki Okamoto*1), Hideo Hirasawa1) and Takayuki Umemoto2)

1) Ibaraki Agricultural Center Plant-Biotechnology Institute, 3402 Kamikunii, Mito, Ibaraki 311-4203, Japan
2) National Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan

Asian rice (Oryza sativa L.) cultivars were recently classified into L-type, S-type, and intermediate M-type (rich, poor and middle intermediate-chain) on the basis of the fine structure of amylopectin in their grains. We selected 4 cultivars with M-type amylopectin from 174 local Japanese nonwaxy upland rice cultivars by scoring the disintegration of starch granules in alkaline solution and measuring the pasting temperature (PT) of their rice flours. Analyzed amylopectin fine structure, these cultivars exhibited intermediate characteristics between those of S-type Koshihikari and L-type IRAT109. Moreover, compared hardness of dumpling cakes after cooled for 3 h and 24 h. The hardness was in the order L-type > M-type > S-type, each other. On the other hand, the amyllopectin chain ratio (ACR) was negatively correlated with the hardness of dumpling cakes both after cooled for 3 h and 24 h. Therefore, we concluded that the 4 cultivars—Chikanari1, Hokkaiakage, Kairyo13, and Mogamichikanari1—had M-type amylopectin, and Japanese local upland rice has wide genetic diversity about starch mutation.

Key Words: ACR, amylopectin, M-type, pasting temperature, RVA, upland rice.

In Japan, cultivation of upland rice is limited to 4100 ha in 2006, 0.24% of the total area of rice cultivation where the majority is lowland rice cultivation. Japanese local upland rice has wide genetic diversity, possess the disease tolerance and environmental stress tolerance that were lacked in Japanese lowland rice. Additionally, it is possible not only for tolerance against biotic and abiotic stresses but also possesses wide variation in starch properties.

Starch is the major component of rice endosperm, and composed of linear glucan, amylose and highly branched glucan, amylopectin. Amylose content in rice endosperm influences cooked rice firmness, and lower amylose content results in more stickiness (Juliano and Pascual 1980). There are a lot of studies on amylose content, using natural or artificial mutations (Takahashi 1993, Takahashi et al. 1998). On the other hand, a few studies on artificial mutations of amylopectin, such as amylose extender (ae), sugary-1 (sug-1), starch branching enzyme-1 (sbe1) were reported (Satoh and Omura 1981, Nishi et al. 2001, Satoh et al. 2003, Wong et al. 2003). However, the natural genetic variations that affect amylopectin structure other than alk (starch synthase IIa) have not been reported (Umemoto et al. 2002, Gao et al. 2003). Based on genetic difference in chain-length distribution, rice amylopectin was classified into S- and L-types, the former is rich in short chains compared to the later (Nakamura et al. 2002, Umemoto et al. 2002). Rice cakes made from L-type amylopectin cultivar Kantomochi172 became very hard quickly after processed—a property that we defined as hyperhardness—when compared with those made from commercial waxy cultivars with S-type amylopectin (Okamoto and Nemoto 1998, Okamoto et al. 2002). It is meaningful to screen and characterize the cultivars with a variety of amylopectin types in addition to S- or L-type amylopectin to improve eating and processing suitability not only for Japanese upland rice production but also for rice production worldwide.

In this report, we focused on screening and characterizing rice with M-type amylopectin that has short chain ratio of amylopectin and pasting temperature (PT) between those of S- and L-type, and might be useful to breed rice with novel starch properties by crossing with rice in different amylose content, for example. Only two cultivars so far have been judged to have M-type amylopectin (Nakamura et al. 2002, Nakamura et al. 2006). On the other hand, Aung et al. (2002) reported, a Myanmar cultivar shown intermediate characteristics similar to M-type cultivar. However, the judge was done without the effect of temperature during grain-filling on amylopectin chain-length into account (Umemoto et al. 1999). We analyzed rice samples harvested at least in two years and take account of the temperature after heading. It is also still unknown whether the rice with M-type amylopectin is widely distributed including in Japan or...
not. The screening was performed with Japanese local non-waxy upland rice cultivars whose starch properties have not been extensively characterized so far.

**First screening of cultivars with M-type amylopectin by PTs with RVA measurement**

Upland nonwaxy rice cultivars were grown in a paddy field at the Ibaraki Agricultural Center in Mito (36°N, 140E), Ibaraki, Japan in 1996. These included 174 local cultivars, 25 improved cultivars, and a single foreign cultivar, IRAT109, which has L-type amylopectin (Okamoto et al., 2002). All plants were grown according to the standard method for the cultivation of lowland rice in Ibaraki Prefecture, except that top dressing was omitted. The seeds were sown in a nursery box on May 9, and young seedlings were transplanted to the paddy field on June 6. Each cultivar was harvested in mid September to mid October, upon maturity.

The PTs of the rice flours were measured with an RVA-3D (Newport Scientific Pty. Ltd., Sydney, NSW, Australia) as described by Toyoshima et al. (1997). Brown rice was polished to 90.9% of its initial weight, and the polished rice flour (3.5 g) was dispersed in 25 mL of distilled water in an RVA cup. The samples were held at 50°C for 3 min, linearly heated to 93°C over 4 min, held at 93°C for 7 min, linearly cooled to 50°C over 4 min, and finally held at 50°C for 3 min. The PT was defined as the temperature at which the viscosity reached to a 5% of the difference between peak and initial viscosity. This measurement was repeated twice and PT was averaged.

The PTs measured by RVA was used for the screening of M-type cultivars since high correlation between short chain ratio of amylopectin and PT was reported (Inouchi et al., 2005, Suzuki et al., 2006). We plotted the 200 rice cultivars including local and improved cultivars with their PT and the air temperature after heading (Fig. 1). Grains of the improved cultivars were developed at the air temperatures between 23.2°C and 25.4°C, and those of the local cultivars were developed between 21.1°C and 25.7°C. The PTs of the 25 improved cultivars were ranged from 67.3°C to 79.4°C, and those of the local cultivars ranged from 67.0°C to 80.8°C. We classified the improved cultivars into two groups based on their PTs: a higher PT group (PT ≥ 78°C) consisted from 4 cultivars and a lower PT group (PT ≤ 74°C) consisted from 21 cultivars. The higher PT group was supposed to have L-type amylopectin since the typical L-type cultivar IRAT109 also showed similar PT (77.8°C). The lower PT group was supposed to have S-type amylopectin because upland Norin glutinous 20 in this group was shown to have S-type amylopectin, previously (Umemoto et al., 2004).

The first screening of M-type cultivar from the local upland nonwaxy rice cultivars was conducted based on the PT, as the higher PT and the lower PT groups used for the standards. The entries matured under the air temperature below 23.8°C were excluded because the temperature during grain-filling affect the PT, and the reference PT data with the air temperature lower than 23.8°C of the higher and lower PT groups were not available. We classified the local cultivars into 3 groups: those with PTs close to the higher PT group (n = 5), those with PTs similar to the lower PT group (n = 125), and 11 cultivars distributed between the two groups which were selected as the candidates to have M-type amylopectin (Fig. 1 plots inside the dotted line).

**Second screening by alkali-spreading score and PT**

Eleven local cultivars, Chikanariyun1, Hokkaiakage, Hoyama, Isozuki, Kairyo13, Mogamichikanari1, Owari17, Sangoku, Sankanka, Sekiyama, and Seridashi that were judged to have M-type in the first screening, 25 improved cultivars, an upland waxy cultivar ‘Naebahatamochi’, and a lowland waxy cultivar ‘Mangetsumochi’ were cultivated in 2000 and 2002. The seeds were sown on May 18 and May 15, and young seedlings were transplanted on June 8 and on June 5 in 2000 and 2002, respectively. Each cultivar was harvested in mid September to mid October.

Alkali test was performed as reported by Ebata (1968). The seeds were hulled and polished in a rice polisher (Pearlest, Kett Electric Laboratory, Tokyo, Japan) until their weight reached 90.9% of the initial weight. Eight grains for each cultivar were soaked in 8 mL of 1.7% potassium hydroxide in a polystyrene case (67 mm × 37 mm × 12 mm) at 25°C for 24 h. The degree of disintegration was scored from 1 (not dissolved) to 10 (completely dissolved) with Naebahatamochi (score 4) and Mangetsumochi (score 6) as standards. This test was repeated twice. The PT of the flours was measured by RVA as described above.

---

**Fig. 1.** Correlation of averaged air temperature with pasting temperature on upland rice. •: Local cultivar (n = 174), ○: Improved cultivar (n = 25), △: IRAT109 (L-type amylopectin). The plots in the dotted frame are candidate cultivars which had M-type amylopectin.
The alkali-spreading score (ASS) and PT of 11 M-type candidates together with 25 improved cultivars were shown in Fig. 2. ASS is widely used for the estimation of gelatinization temperature of rice flour. There is also correlation between ASS and short chain ratio of amylopectin (Inouchi et al. 2005). We classified the improved cultivars into two groups according to their ASS and PT obtained in two years: the first group estimated to have L-type amylopectin with \( \text{ASS} \leq 3.7 \) and \( \text{PT} \geq 79 \, ^\circ \text{C} \); the second group estimated to have S-type amylopectin with \( \text{ASS} \geq 5.9 \) and \( \text{PT} \leq 76 \, ^\circ \text{C} \). Among the 11 local M-type candidates, 6 cultivars were classified in the second group based on their ASS and PT of the both year. However, the rest of 5 cultivars showed ASS between 3.4 and 5.3 and PT between 76 \(^\circ\)C and 79 \(^\circ\)C, and the plots did not overlap with the both groups (Fig. 2). As the results, we selected these 5 local cultivars, Chikanarijyun1, Hokkaiakage, Kairyo13, Mogamichikanari1 and Sankanka as the candidates of M-type cultivar in the second screening.

**Polymorphisms in starch synthase activity**

Zymogram analysis of starch synthase activity to detect the polymorphisms was conducted as reported by Okamoto et al. (2002). We used 5 M-type candidates, Chikanarijyun1, Hokkaiakage, Kairyo13, Mogamichikanari1 and Sankanka. IRAT109 and Koshihikari were used as the controls. These cultivars were cultivated same condition in 2002 and 2004 as described above. Panicles were harvested at the late milky-ripening stage, frozen in liquid nitrogen, and then stored at \(-30\,^\circ\text{C}\) until the analysis.

Starch synthase 1 (SS1) activity was detected in all the cultivars used in both years, and SS1 activity in L-type was weaker than M- and S-type cultivars (Fig. 3). However, we thought this lower activity in L-type hardly affect on amylopectin fine structure. Since Fujita et al. (2006) reported, there had little influence between wild type and SS1 lacked mutant. Starch synthase 2a (SS2a) activity was detected in L-type cultivar IRAT109 and M-type candidate Sankanka in both years (Fig. 3B and 3C). SS2a activity was not detected with the other 4 M-type candidates in 2002 (Fig. 3A), however it was only slightly detected with Kairyo13 in 2004 (Fig. 3C). The SS2a activity is usually very weak in zymogram analysis compared to SS1, sometimes only faint band can be detected even with L-type lines (Umemoto et al. 2004).

**Confirmation of M-type cultivars based on chain-length distribution and the proportion of fractions of amylopectin**

The 5 M-type candidates—Chikanarijyun1, Hokkaiakage, Kairyo13, Mogamichikanari1, and Sankanka—were grown together with L-type cultivar IRAT109 and S-type cultivar Koshihikari, a leading upland rice cultivar Toyohatamochi in 2002 and 2003. The sowing and transplanting dates in 2003 were the same as those in 2002. The rice seeds were harvested late September, in both years. Only the caryopses that flowered on the same day were marked and used in the experiments since the temperature

---

**Fig. 2.** Correlation of pasting temperature with alkali spreading score on upland rice. Alkali spreading value was scored by the method of Ebata (1968). Pasting temperature was measured by RVA. ●: Local cultivar, ○: Improved cultivar (2000), △: Improved cultivar (2002).

during grain-filling affected the chain-length distribution of amylopectin (Umemoto et al. 1999).

The seeds were hulled and polished in a rice polisher (Pearlest) for 45 seconds, and each grain crushed by a hammer to obtain fine flour. After the flour (5 mg) was gelatinized, amylopectin side chains were debranched by isoamylase, and then chain-length distribution was analyzed by a high performance anion-exchange chromatography with pulsed amperometric detection according to the methods by Umemoto et al. (1999). The area of each clear peak of linear chains up to DP54 was calculated by using PeakNet software (Dionex Corp., Sunnyvale, CA, USA). The ratios of fractions based on DPn were calculated by the method of Hanashiro et al. (1996): fa, DP6-12; fb1, DP13-24; fb2, DP25-36; and fb3, DP>37. The amylopectin chain ratio (ACR: \( \Sigma \leq \text{DP10} / \Sigma \leq \text{DP24} \)) in relative peak area basis was calculated by the method of Nakamura et al. (2002).

Chain-length distributions of amylopectin of the 5 M-type candidate cultivars were analyzed and compared with L-type IRAT109 and S-type Koshihikari. Compared to the L-type IRAT109, S-type Koshihikari was rich in short chains of DP from 6 to 12, and depleted in middle-length chains of DP from 13 to 20 (Fig. 4A and 4C). This difference between S-type and L-type was in accordance with a former report (Nakamura et al. 2002). Figure 4E and 4F show subtraction of relative peak area of 5 M-type candidates and Koshihikari with those of IRAT109. Among the 4 M-type candidates, Chikanarijyun1, Hokkaikage, Kairyo13, and

![Fig. 4. Chain-length distribution of amylopectin. Chain-length distribution S-type Koshihikari with L-type IRAT109 (A, C), and M-type Chikanarijyun1 with Koshihikari (B). Chain-length distribution of Koshihikari subtract IRAT109 (C), Chikanarijyun1 subtract IRAT109 (D). Differences among 5 local M-type cultivars in 2002 (E) and 2003 (F). X-axis showed control cultivar IRAT109. The markers shown the chain-length distributions of each cultivar subtract from IRAT109.](image-url)
M-type amylopectin in Japanese upland rice

Mogamichikanari1 had more short chains of DP from 7 to 10 compared to IRAT109, however, the short chains were clearly less than those of Koshihikari in both years. These result agreed with previous reports (Nakamura et al. 2002, Aung et al. 2002). Another candidate Sankanka showed very similar chain-length distribution to L-type IRAT109.

S- and L-type cultivars were clearly divided by the ACR of the two years (Fig. 5). The S-type cultivars, Koshihikari and Toyohatamochi have ACR of 0.198–0.209 and 0.245–0.257, and L-type cultivar IRAT109 has that of 0.130 and 0.146, in 2002 and 2003, respectively. Candidate 5 M-type cultivars separated into Sankanka and others based on the ACR. The former has the ratio of 0.135 and 0.150, and the later has that of 0.149–0.164 and 0.184–0.210 in 2002 and 2003, respectively. As the results, the eight cultivars were categorized into three groups; S-type (Koshihikari and Toyohatamochi), M-type (Chikanarijun1, Hokkaiakage, Kairyo13, and Mogamichikanari1) and L-type (IRAT109 and Sankanka) with a significance (Scheffe’s test, P = 0.05).

The facts that Sankanka had similar SS2a activity to IRAT109 (Fig. 3) in addition to similar ACR, strongly support the view that this cultivar belongs to L-type.

Table 1 shows the ratios of fraction fa, fb1, fb2, and fb3 based on the DPn of side chain of amylopectin. The proportion of fraction of M-type cultivars was different from that of L- and S-type cultivars. The ratio of fa of the 4 M-type cultivars were lower than S-type and higher than L-type cultivars. On the other hand, the values of fb1, fb1/fa, and (fb1 + fb2 + fb3) were in the order L-type > M-type > S-type. The fb2 ratio of Toyohatamochi was larger than that of others, and the fb3 ratio was not so much different among the three types. Basically same trends in the proportion of fractions were observed in 2002 and 2003. Therefore, we confirmed that the 4 upland local nonwaxy cultivars, Chikanarijun1, Hokkaiakage, Kairyo13, and Mogamichikanari1 as the M-type cultivars defined by Nakamura et al. (2002).

Hardness of dumpling cake and ACR
L-type upland cultivar IRAT109 and Sankanka, 4 M-type cultivars, and S-type lowland cultivar Koshihikari were cultivated in 2003. The cultivation was the same as above described. The rice flour samples were prepared same as for RVA measurement. The rice flour (25 g) was dispersed in 35 mL of distilled water in a plastic bag, and mixed thoroughly to be a paste. The paste was transferred to a steel cup (diameter 38 mm × height 31 mm). The samples in the cup

| Table 1. The proportion of fractions, fa, fb1, fb2, and fb3 of the samples |
|-----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Year | Cultivar | Type of amylopectin | fa** | fb1 | fb2 | fb3 | fb1/fa | fb1+fb2+fb3 (%) |
| 2003 | Toyohatamochi | S | 26.87 a | 46.27 f | 14.36 a | 12.51 a | 1.72 d | 73.13 c |
| Koshihikari | S | 27.50 a | 48.81 e | 13.01 b | 10.68 d | 1.77 d | 72.50 c |
| Chikanarijun1 | M | 24.34 b | 51.99 b | 12.45 b | 11.22 cd | 2.14 b | 75.66 b |
| Kairyo13 | M | 24.92 b | 50.75 d | 12.86 b | 11.47 bc | 2.04 c | 75.08 b |
| Hokkaiakage | M | 24.61 b | 51.00 cd | 12.84 b | 11.55 bc | 2.07 bc | 75.39 b |
| Mogamichikanari1 | M | 24.31 b | 51.46 bc | 12.85 b | 11.37 c | 2.12 b | 75.69 b |
| Sankanka | L | 21.97 c | 53.98 a | 12.51 b | 11.53 bc | 2.46 a | 78.03 a |
| IRAT109 | L | 21.37 c | 53.62 a | 13.04 b | 11.98 ab | 2.51 a | 78.63 a |

| 2002 | Toyohatamochi | S | 27.97 a | 46.31 b | 13.67 a | 12.05 a | 1.66 c | 72.03 c |
| Koshihikari | S | 27.29 a | 47.69 b | 12.98 a | 12.05 a | 1.75 c | 72.71 c |
| Chikanarijun1 | M | 24.24 b | 50.56 a | 12.90 a | 12.30 a | 2.09 b | 75.76 b |
| Kairyo13 | M | 23.79 b | 50.84 a | 12.75 a | 12.62 a | 2.14 b | 76.21 b |
| Hokkaiakage | M | 23.93 b | 49.95 a | 13.34 a | 12.78 a | 2.09 b | 76.07 b |
| Mogamichikanari1 | M | 23.81 b | 51.67 a | 12.42 a | 12.09 a | 2.17 b | 76.19 b |
| Sankanka | L | 22.22 c | 52.51 a | 12.67 a | 12.60 a | 2.36 a | 77.78 a |
| IRAT109 | L | 21.90 c | 52.81 a | 12.55 a | 12.74 a | 2.41 a | 78.10 a |

*In 2003, value within a column followed by different letters are significantly according to Tukey’s multiple range test (P = 0.05)
In 2002, value within a column followed by different letters are significantly according to Scife’s multiple range test (P = 0.05)
Sample was analyzed once and three times, in 2002 and in 2003, respectively

Fig. 5. Relationship between amylopectin structure in 2002 and in 2003. ACR: The amylopectin chain ratio (Σ ≤ DP10/Σ ≤ DP24)
were boiled at 100°C for 10 min, cooled in running water for 10 min, and then transferred to a refrigerator (4°C) until the measurements. Hardness of dumpling cakes was evaluated by a fruit hardness meter (KM-5: Fujiwara Scientific Company, Tokyo), after cooled for 3 h and 24 h.

Hardness of dumpling cakes after cooled for 3 h were between 1.50 and 3.42 kg/cm², whereas those for 24 h were between 3.40 and 4.17 kg/cm² (Fig. 6). The hardness was in the order L-type > M-type > S-type. Differences in the hardness of cultivars were clear after 3 h cooling compared to 24 h cooling. The ACR was negatively correlated with the hardness of dumpling cakes both after cooled for 3 h and 24 h (r = −0.904*, −0.870*). Furthermore, the ACR negatively correlated with SS2a activity, since ACR was in the order S->M->L-type and SS2a activity was in the order L->M->S-type (Fig. 3 and Table 1).

Fig. 6. Hardness of dumpling cake of rice. Dumpling cake was after cooled (4°C) for 3 h and 24 h, and evaluated hardness by fruit hardness meter on 2003. ▲: hardness of dumpling cake after cooled 3 h, △: hardness of dumpling cake after cooled 24 h. ACR: The amylopectin chain ratio (ΣDP10/ΣDP24). C: Chikanariyujin 1, H: Hokkaikage, K: Koshihikari, K13: Kairyo 13, M: Mogami chikanari 1, S: Sankanka, 109: IRAT 109.

Significance of M-type cultivars for genetic resources and food processing

Nakamura et al. (2002, 2006) pointed out that the ACR value for amylopectin greatly influences the thermal properties of starch measured by a differential scanning calorimetry (DSC). Two M-type cultivars, ‘Khauk Yoe’ and ‘Malagkit Sungsong’ showed intermediate values both in the ACR and the thermal properties. Additionally, Myanmar cultivar ‘MMR310’ had intermediated pasting properties (Aung et al. 2002). These 4 cultivars that we classified to have M-type amylopectin, also had PT measured by RVA and the ACR between the L- and S- type (Fig. 1, 2 and 5). Fig. 4D showed subtraction of relative peak area of M-type Chikanariyujin1 with L-type IRAT 109. Chikanariyujin1 was rich in short chains and depleted in intermediate-length chains, compared to IRAT109. However, the difference in relative peak area between L-type and M-type was smaller than that of the area between L-type and S-type (Fig. 4A–4D). Those characteristics were similar to those of M-type cultivars reported by Nakamura et al. (2002). Recently, differences in chain-length distribution of amylopectin among Japanese lowland rice cultivars have been reported (Horibata et al. 2004, Suzuki et al. 2006, Igarashi et al. 2008). It is difficult to assess the presence of rice cultivar with M-type amylopectin among the cultivars they used, since they have not taken the effect of temperature during ripening on chain-length distribution into account. Nakamura et al. (2002, 2006) have reported 2 M-type cultivars. But they also have not considered the effect of temperature on amylopectin chain-length. In this report, we have screened and found 4 M-type amylopectin cultivars in Japanese local upland nonwaxy rice collection. Extra attention was paid to disintegrate the varietal differences and the differences caused by the temperature during grain-filling on the amylopectin chain-length (Fig. 1). The data was obtained from rice samples harvested in two years for the confirmation. It is noteworthy that only one M-type was found among 129 Asian lowland rice cultivars (Nakamura et al. 2002), whereas we found 4 cultivars among 174 Japanese local nonwaxy upland cultivars. This may suggest that Japanese upland rice is a precious genetic resource of natural variations in starch properties and hence rice qualities.

The difference between L-type and S-type amylopectin is caused by natural variations in SS2a (Umamoto et al. 2002, Gao et al. 2003, Umamoto et al. 2004, Nakamura et al. 2005, Umamoto and Aoki 2005, Waters et al. 2006, Bao et al. 2006). Two individual nucleotide polymorphisms result in amino acid substitutions in conserved regions and inactivate the enzyme in S-type cultivars. Nakamura et al. (2005) reported that when the SS2a gene from L-type cultivar IR36 was transformed to S-type cultivar Kinmaze, a series of rice plants with chain-length profile between L-type and S-type was obtained. The chain-length profile of intermediate type between L- and S-types is very similar to that of M-type in this study. To see whether the cause of the M-type amylopectin is in SS2a gene or not, we have to perform allelism tests between M-type and L-type, and M-type and S-type cultivars. On the other hand, activities of branching enzymes 1, 2a, and 2b in the 4 M-type cultivars were about the same as those in the S-type Koshihikari and the L-type IRAT109 with zymogram analysis (data not shown). Thus, the formation of M-type amylopectin does not caused by changes in branching enzyme activities.

It was possible that the amylose content other than the chain-length distribution of amylopectin also affect the PT in Fig. 1. The apparent amylose contents of 25 improved cultivars were ranged from 16.5 to 24.5%, and those of 174 local cultivars were ranged from 14.7 to 29.2%, in 1996. There wasn’t significant correlation between amylose content and PT in both improved cultivars and local cultivars (data not shown). This is in agreement with the results of
Nakamura et al. (2002) showing no relationship between amylose content and PT measured by DSC, while a very strong relationship between PT and the ACR. We supposed that the fine structure of M-type amyllopectin was influenced by amylose content weakly.

For the evaluation of processing property of M-type cultivars, dumpling cakes were prepared and the hardness was measured (Fig. 6). We previously reported that the dumpling cakes made from L-type waxy rice became hard faster than those made from S-type waxy rice (Okamoto et al. 2002). The hardness of dumpling cake in the present study with nonwaxy rice was in the order L-type > M-type > S-type. Although the amylose content could affect the hardness of dumpling cakes, we assume the ACR is the strong determinant. This is based on the observation that the amylose content of L-type IRAT109 was lower than those of M-type cultivars (data not shown), while the hardness of dumpling cake of IRAT109 was clearly harder after cooling the dumplings for 3 h at 4°C (Fig. 6). These results imply that the rheological properties of products made from M-type amyllopectin were different from S- and L-type amyllopectin, and can use as a novel food material.

In summary, we surveyed and found the cultivars that have M-type amyllopectin in the Japanese upland nonwaxy rice collection, and characterized their gelatinization properties and the chain-length distribution of amyllopectin in the grain. Allelism tests between the cultivars with L- and S-type, and within M-type have to be performed to confirm if they are under same genetic control. Nevertheless, our study strongly supports the view that there is the M-type amyllopectin group in addition to L- and S-type group in Oryza sativa. Combining the three amyllopectin types, L-, M-, and S-type with a various amylose content, we can produce wide varieties of rice that may useful to develop novel food products for the world market.

Acknowledgment

We thank all the members of the Laboratory of Crop Breeding at the Plant-Biotechnology Institute of the Ibaraki Agricultural Center. This research was partially supported by the Japanese Ministry of Agriculture, Forestry and Fisheries (Designated station for upland rice breeding).

Literature Cited


