Effects of Variations in Starch Synthase on Starch Properties and Eating Quality of Rice

Takayuki Umemoto 1,2, Tetsuya Horibata 3, Noriaki Aoki 4, Mayu Hiratsuka 2, Masahiro Yano 1 and Naoyoshi Inouchi 1

1National Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan;
2Graduate School of Life and Environmental Sciences, University of Tsukuba, Tennodai, Tsukuba, 305-8577 Ibaraki, Japan;
3Faculty of Life Science and Biotechnology, Fukuyama University, 1 Sanzo, Gakuen-cho, Fukuyama, Hiroshima 729-0292, Japan;
4National Institute of Agrobiological Sciences, 2-1-1 Kannondai, Tsukuba, Ibaraki 305-8602, Japan

Abstract: We evaluated the effects of functional variation in three starch synthases in rice (Oryza sativa L.)—granule-bound starch synthase I (GBSSI, wx), starch synthase I (SSI, SSI), and starch synthase IIa (SSIIa, alk)—between indica cultivar Kasalath and japonica cultivar Nipponbare on starch properties and eating quality. We used three near-isogenic lines—NIL(Wx), NIL(SSI), and NIL(Alk)—containing chromosomal segments of Kasalath on a Nipponbare genetic background. The Wx allele explained most of the difference in amylose content between the two cultivars, and decreased the peak viscosity and breakdown to less than half of those of Nipponbare. These changes reduced the quality of cooked rice both just after cooking and after storage at 5°C. The variation in SSI also affected the eating quality after storage of cooked rice at 5°C: NIL(Alk) became harder and less sticky than Nipponbare, although the rices were comparable just after cooking. Differential scanning calorimetry revealed faster retrogradation of the once-gelatinized starch in NIL(Alk). The variation in SSI alleles hardly affected these properties.

Key words: Amylopectin, Amylose, Cooked rice quality, Near-isogenic line, Rice (Oryza sativa L.), Starch properties, Starch synthase.

Eating quality is one of the most important characters of rice, but preference differs greatly with the country, region, and food culture. For example, sticky glutinous rice is preferred in some regions in Asia, whereas medium- to high-amylose rice with a firm sticky glutinous rice is preferred in some regions in the country, region, and food culture. For example, characters of rice, but preference differs greatly with

synthesized through concerted reactions of starch

syntase, starch branching enzyme, and debranching enzyme (Nakamura, 2002). Each enzyme has multiple isoforms, and some of their expression patterns and organ specificities have been reported (Hirose and Terao, 2004).

Natural variations in starch synthases have been characterized. There are two major alleles of GBSSI (wx) in rice, Wx and Wx. Wx differs from Wx in having a single-nucleotide polymorphism at the splice donor site of the first intron (Wang et al., 1995). This guanine-to-thymine substitution leads to aberrant splicing and reduces the production of mRNA, and consequently reduces the Wx protein and amylose contents, which affect the eating and processing qualities of rice.

Umemoto et al. (2002) reported that varietal differences in amylopectin chain-length distribution are regulated by functional variations in starch synthase IIa (SSIa). The gene is identical to the alk gene, which is responsible for alkali disintegration of rice grains (Gao et al., 2003). However, cultivars possessing the Wx allele tend to have a functional SSIa allele, making it difficult to evaluate the effect of functional variations in wx and SSIa alleles on rice quality.
separately. Detailed analysis can be performed only by dissecting the \textit{wx} and SSIIa loci, both of which are located on the short arm of chromosome 6. Moreover, the \textit{starch synthase I} (SSI) locus, positioned between \textit{wx} and \textit{alk}, also could affect the amylopectin chain-length distribution and the gelatinization properties of starch (Takemoto-Kuno et al., 2006).

In this study, we investigated the effects of variations in these three starch synthases on rice quality by using near-isogenic lines (NILs) containing the Kasalath chromosomal segments on a Nipponbare background.

**Materials and Methods**

1. **Plant materials and cultivation**
   
   We grew three NILs−NIL(\textit{Wx}^a), NIL(\textit{SSI}^k), and NIL(\textit{Alk}), containing the Kasalath chromosomal segment including \textit{wx} (\textit{GBSSI}), \textit{SSI}, and \textit{alk} (\textit{SSIIa}) loci, respectively, on the Nipponbare background (Fig. 1)−with their parental cultivars. NIL(\textit{Wx}^a) and NIL(\textit{SSI}^k) were originally developed for research on heading date as NIL(\textit{Hd3b}) and NIL(\textit{Hd3a}), respectively (Monna et al., 2002). NIL(\textit{Alk}) was one of four NILs developed to evaluate \textit{SSIIa} variation (Umemoto et al., 2004).

   All plant materials were sown on 7 May 2002 and transplanted into an experimental paddy field of the National Institute of Crop Science, Japan, on 30 May at a hill spacing of 30 cm×30 cm, one seedling per hill. We applied 50 kg ha$^{-1}$ each of N, P and K as basal fertilizer, but no topdressing. Heading date was defined as the day when 50% of panicles headed. Each line was harvested upon maturity. Bulked samples consisted of 40 plants.

2. **Chemical components and chain-length distributions of debranched starch**

   We determined the starch content of polished grains with a Total Starch Assay Kit (Megazyme, Wicklow, Ireland) according to the manufacturer’s instructions, taking four individual measurements. We determined the total nitrogen content of the polished rice from three measurements by the Dumas combustion method with a Rapid N system (Elementar Americas, Inc., Mt. Laurel, NJ, USA). Multiplying the N content by 5.95 gave the protein content. After debranching of starch by \textit{Pseudomonas} isoamylase and fractionation by gel permeation chromatography (GPC) through Toyopearl HW55S and HW50S columns (Ikawa et al., 1981; Inouchi et al., 1999), we determined amylose content from the elution profiles. The first peak was composed of amylose and very long B chains of amylopectin (Takeda and Hizukuri, 1987; Inouchi et al., 2005). The second and third continuous peaks came from long B and short B plus A chains of amylopectin, respectively. We regard the ratio of this first peak area to the total peak area as the amylose content. Chain-length distributions of debranched starch were determined by high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) as described by Horibata et al. (2004) and references therein.

3. **Gelatinization and thermal properties**

   The gelatinization properties of rice flour were measured with a Rapid Visco Analyzer (RVA; RVA-4, Newport Scientific, Sydney, NSW, Australia) according to Umemoto et al. (2004). We analyzed the thermal properties of polished rice by differential scanning calorimetry (DSC; Micro DSCIII, Setaram, Caluire, France): 10 polished rice grains and 1.3 times their weight in water were added to a DSC pan, and the DSC pan was allowed to equilibrate for 1 h at 25°C before measurement. The DSC pan was heated at 1°C min$^{-1}$ from 25 to 110°C, and then cooled at the same rate to 25°C. The gelatinized samples (cooked rice) were kept at 5°C for 1 or 7 days, and then the thermogram...
was recorded from 5 to 110°C at 1°C min⁻¹. The gelatinization temperatures (Tₒ; onset temperature, Tₚ; peak temperature, Tᵣ; conclusion temperature) and gelatinization enthalpies (ΔH) of cooked rice were computed automatically from the DSC thermograms (Table 3, Fig. 4).

4. SDS-PAGE, immunoblotting, and zymogram

Starch-granule–bound proteins were solubilized, and SDS-PAGE was conducted as described by Umemoto and TeraShima (2002), except that we used 7.5% (w/v) acrylamide gels, and boiled 50 mg of purified starch in 1.5 mL of sodium dodecylsulfate (SDS) sample buffer for 10 min. Each lane contained protein from 1 mg of starch. Immunoblotting for SSI and SSIIa was conducted as reported by Umemoto et al. (2004) and Umemoto and Aoki (2005) with antisera raised against synthetic polypeptides specific to each. Starch synthases in the soluble fraction of developing endosperm at the milk-ripe stage were separated by electrophoresis in glycogen-containing native gels, the gels were incubated with ADP-glucose, and synthase activities were detected, as described by Okamoto et al. (2002). Each lane contained protein equivalent to 3.6 mg of fresh material.

5. Sensory evaluation of cooked rice

We soaked polished rice samples (450 g) with a 14.5% moisture content in 1.4 times their weight of tap water for 30 min, then cooked them in a conventional rice cooker (National, SR-ULH10, Kadoma, Osaka, Japan). Nineteen trained testers evaluated each sample about 0.5 hr after cooking and again the sample returned to room temperature after storage at 5°C for 16 hr. Evaluators recorded overall preference, gloss, stickiness, and softness. Differences in evaluated values between the recurrent parent Nipponbare and each NIL were statistically analyzed by the sign test.

Results

1. Starch synthase variations

We investigated allelic differences in each starch synthase by SDS-PAGE for GBSSI (Fig. 2A), immunoblotting for SSI and SSIIa (Figs. 2B, C), and a zymogram for SSI (Fig. 2D). NIL(Wxᵃ) and Kasalath had more Wx protein in grains than Nipponbare and the other two NILs (Fig. 2A). The molecular mass of SSI in NIL(SSIᵏ) and Kasalath was slightly smaller than that in the other lines (Fig. 2B). SSIIa in Kasalath and NIL(Alk) had a higher association with starch granules than that in the other lines (Fig. 2C). The SSI isoforms of NIL(SSIᵏ) and Kasalath had slightly higher mobility (Fig. 2D). These observations confirm that the NILs show natural variation in each starch synthase, which could affect starch properties and cooked rice qualities.

2. Heading date, and starch and protein contents

The heading date of NIL(Wxᵃ) was 6 days later and that of NIL(SSIᵏ) was 2 days earlier than that of Nipponbare (Table 1). These differences can be explained by variations in the Hd₃b and Hd₃a alleles, respectively (Monna et al., 2002). The three NILs and Nipponbare had similar starch contents (Table 1). NIL(Wxᵃ) and NIL(SSIᵏ) were around 20% higher protein contents than Nipponbare and NIL(Alk).

3. Amylose content and amylopectin chain-length distribution

The amylose content of NIL(Wxᵃ) (30.7%) was similar to that of Kasalath (31.5%) and about 10% higher than that of Nipponbare (20.2%) (Table 1). This result shows that the wx allele explains most of the difference in amylose content between Nipponbare
and Kasalath. The amylose content of NIL(Alk) was 4% lower and that of NIL(SSIk) (20.1%) was close to that of Nipponbare.

We compared the chain-length distributions of amylopectin among the lines (Fig. 3). NIL(Wxa) had more short chains with a degree of polymerization (dp) of 6–15 and fewer with dp 19–30 than Nipponbare. NIL(Alk) showed very similar profiles to those of Kasalath in having fewer short chains of dp 6–11 and more mid-length chains of dp 13–23, as reported by Umemoto et al. (2004). The chain-length distribution profile of NIL(SSIk) was the most similar to that of Nipponbare.

4. RVA viscosity of rice flour

There was no clear difference in peak viscosity among Nipponbare, NIL(SSIk), and NIL(Alk) (Table 2). Kasalath had a peak viscosity of about 100 rapid viscosity units (RVU) lower than that of Nipponbare. However, NIL(Wxa) had 70RVU lower peak viscosity than Kasalath. The hot paste viscosity at minimum viscosity of both Kasalath and NIL(Wxa) was at least 30 RVU lower than that of Nipponbare. Breakdown was in the order NIL(Alk) > Nipponbare > NIL(SSIk) > Kasalath > NIL(Wxa). Nipponbare and Kasalath showed similar final viscosity values; NIL(SSIk) had a slightly higher value and NIL(Wxa) and NIL(Alk) had slightly lower values than Nipponbare.

Fig. 3. Differences in chain-length distributions of isoamylase-debranched starches relative to Nipponbare: ○, NIL(Wxa); ◇, NIL(SSIk); ■, NIL(Alk); ▲, Kasalath.

Table 1. Heading date, temperature during grain-filling, starch, amylose, and protein contents.

| Heading date | Averaged temperature(a) | Starch content(b) | Amylose content | Protein content(b) |
|--------------|-------------------------|------------------|----------------|------------------|
| Nipponbare   | 15th, Aug. 0°C          | 24.8% ± 0.5%     | 74.4% ± 20.2%  | 5.35% ± 0.02%   |
| Kasalath     | 10th, Aug. –5°C         | 25.4% ± 0.2%     | 71.4% ± 31.5%  | 8.21% ± 0.03%   |
| NIL(Wxa)     | 21st, Aug. +6°C         | 23.6% ± 0.8%     | 74.9% ± 30.7%  | 6.42% ± 0.03%   |
| NIL(SSIk)    | 13rd, Aug. –2°C         | 25.0% ± 0.2%     | 74.9% ± 20.1%  | 6.34% ± 0.01%   |
| NIL(Alk)     | 14th, Aug. –1°C         | 25.0% ± 0.2%     | 74.8% ± 16.2%  | 5.35% ± 0.19%   |

1) Differences in heading date relative to Nipponbare.
2) Average temperature over 30 days from heading.
3) Starch and protein contents on a 14.5% moisture basis.

Table 2. Viscosity properties of rice flour analyzed by RVA.

| Peak time (min) | Temperature at initial viscosity increase (°C) | Peak viscosity (RVU) | Minimum viscosity (RVU) | Breakdown (RVU) | Final viscosity (RVU) | Setback (RVU) |
|----------------|-----------------------------------------------|----------------------|------------------------|----------------|-----------------------|--------------|
| Nipponbare     | 6.43                                          | 66.3                 | 347.0                  | 137.0          | 210.0                 | 258.8        | 121.7        |
| Kasalath       | 6.20                                          | 69.3                 | 246.9                  | 105.4          | 141.5                 | 256.2        | 150.8        |
| NIL(Wxa)       | 6.27                                          | 66.5                 | 172.4                  | 92.2           | 80.2                  | 206.4        | 114.2        |
| NIL(SSIk)      | 6.53                                          | 66.4                 | 350.5                  | 148.3          | 202.2                 | 273.1        | 124.8        |
| NIL(Alk)       | 6.07                                          | 72.4                 | 368.8                  | 126.8          | 242.0                 | 233.6        | 106.8        |

1) Rapid visco units.
Fig. 4. Thermograms of polished rice grains [A] and retrograded cooked rice [B, C] measured by micro DSC III. After heating of polished rice grains, the retrograded rice were prepared by keeping the gelatinized polished rice in DSC pan at 5ºC for 1 day [B] and 7 days [C]. The thermogram of retrograded rice was measured again by micro DSC III. N; Nipponbare, K; Kasalath, Wx; NIL (Wx), SSI; NIL (SSI), Alk; NIL (Alk).

Table 3. Thermal characteristics of polished rice grains determined by micro DSC III.

| Name          | T_{o1} | T_{p1} | T_{c1} | ΔH_{1} | T_{o2} | T_{p2} | T_{c2} | ΔH_{2} |
|---------------|--------|--------|--------|--------|--------|--------|--------|--------|
| Nipponbare    | 72.5   | 81.3   | 92.5   | 9.2    | 27.3   | 47.9   | 60.8   | 4.6    |
| Kasalath      | 78.4   | 91.4   | 107.5  | 13.0   | 27.8   | 48.4   | 64.2   | 5.3    |
| NIL (Wx)      | 68.0   | 81.3   | 95.2   | 7.2    | 27.6   | 40.2   | 59.7   | 3.4    |
| NIL (SSI)     | 69.7   | 83.0   | 99.2   | 10.0   | 28.1   | 47.9   | 60.2   | 4.3    |
| NIL (Alk)     | 77.8   | 89.7   | 109.2  | 14.9   | 28.5   | 51.5   | 65.5   | 6.7    |

1) T_{o1}, T_{p1}, and T_{c1} are onset, peak, and conclusion temperatures of gelatinization, and ΔH_{1} is the gelatinization enthalpy at the initial gelatinization.
2) T_{o2}, T_{p2}, and T_{c2} are onset, peak, and conclusion temperatures of gelatinization, and ΔH_{2} is the gelatinization enthalpy at re-gelatinization of the retrograded rice after 7 days of storage.

5. DSC thermograms of rice at initial gelatinization and re-gelatinization after storage

Fig. 4 shows the thermograms of polished rice obtained at initial gelatinization and re-gelatinization of gelatinized sample after 1 or 7 days of storage, and Table 3 shows the gelatinization temperatures and enthalpies of rice at initial gelatinization and re-gelatinization after 7 days of storage. Thermograms of Nipponbare, NIL (Wx), and NIL (SSI) showed a small endothermic peak around 105ºC, but those of Kasalath and NIL (Alk) did not (Fig. 4A). These peaks are thought to indicate the heat of fusion of an amylose–lipid complex (Biliaderis et al., 1985). Kasalath and NIL (Alk), which were rich in mid-length chains (dp 13–23) of amylpectin (Fig. 3), showed a higher peak temperature (T_{p1}) than the other three lines (Table 3). Thus, the peak of their amylose–lipid heat fusion overlapped with their main peak, making them hard to distinguish (Fig. 4A). The gelatinization enthalpies (ΔH_{1}) of Kasalath and NIL (Alk), with more mid-length amylpectin chains, were 13.0 and 14.9 J g\(^{-1}\), respectively, higher than those of the other three lines.

After 1 day of storage (Fig. 4B), the endothermic onset temperature of each line was unclear, but NIL (Alk) and Kasalath had higher conclusion temperatures than Nipponbare. All lines showed endothermic peaks at 50 to 60ºC. The gelatinization enthalpies of NIL (Alk) and Kasalath appeared to be higher than those of the other lines. Since cooked rice is generally eaten within 1 day in Japan as a rice ball or a box lunch, it is important to investigate retrogradation within 1 day (Ikawa and Kouno (Kikuchi), 2005). However, we also measured the
cooked rice after 7 days of storage (Fig. 4C) to investigate the retrogradation process over a longer period.

All rice samples showed larger and more distinct endothermic curves after 7 days of storage than after 1 day (Fig. 4). The thermogram of NIL(Alk), which had more mid-length amyllopectin chains than Nipponbare, showed a peak at around 52ºC, with the highest ΔH₂ (6.7 J g⁻¹) among the five lines. After 1 day of storage, only one peak at a higher temperature (50–55ºC) was apparent, but a second peak (or shoulder) at a lower temperature (around 40ºC) became apparent after 7 days of storage. In particular, after 7 days of storage, NIL(Wxa), which had a higher amylose content than Nipponbare, had an endothermic peak at around 40ºC, with the smallest ΔH₂ (3.4 J g⁻¹). Kasalath, which had a higher amylose content and more mid-length amyllopectin chains than Nipponbare, showed an endothermic peak at 48.4ºC and a ΔH₂ of 5.3 J g⁻¹, both higher than those of Nipponbare. The values in NIL(SSk) were similar to those in Nipponbare.

6. Cooked rice quality

The quality of the cooked rice was assessed by sensory tests, with Nipponbare as the standard (Fig. 5). The samples were assessed twice, 0.5 hr after cooking and after the cooked rice was stored at 5ºC for 16 hr, to test the effect of starch retrogradation on eating quality. At 0.5 hr when the rice was still warm, NIL(Wxa) and Kasalath had significantly less gloss, less stickiness, and harder texture than the preferred Nipponbare (P<0.01) (Fig. 5). NIL(SSk) had a texture and overall preference similar to that of Nipponbare, but less gloss (P<0.05). Although at 0.5 hr, NIL(Alk) was comparable to Nipponbare, after 16 hr storage, NIL(Alk) had less gloss, less stickiness and harder texture than Nipponbare (Fig. 5). The overall preference consequently was lowered. Only NIL(Alk), with the functional SSIIa, showed such dramatic differences between the first and second evaluations.

Discussion

1. Characterization of NILs in relation to starch composition and structure

NIL(Wxa) possesses the Wxa allele of GBSSI, and thus produces more Wx protein and amylose than Nipponbare (Fig. 2A, Table 1). This factor makes it suitable for evaluating the major allelic difference in the wx gene affecting starch and cooked rice qualities. The heading date of NIL(Wxa) was 6 days later and the temperature during grain filling was 1.2ºC lower than that of Nipponbare (Asaoka et al., 1984; Hirano and Sano, 1998; Umemoto and Terashima, 2002). Rice grains that mature at a lower temperature have amyllopectin rich in short chains (Umemoto et al., 1999; Inouchi et al., 2000), presumably through changes in branching enzyme activities (Jiang et al., 2003). In addition to the higher amylose content due to the Wxa allele, the higher proportion of amyllopectin short chains together with the higher protein content of NIL(Wxa) (6.42%) than in Nipponbare (5.35%) could affect the gelatinization
properties and cooked rice quality.

Functional variations in the *SSI*α gene between Nipponbare and Kasalath have been reported (Umemoto et al., 2004). Both *SSI*α alleles produce SSIIα protein, but that of Nipponbare has reduced activity and capacity to associate with starch granules. Only the SSIIα coded by the Kasalath allele had a high association with starch granules (Fig. 2C). It probably elongated the amylopectin short chains to mid-length chains with dp around 13–23 (Fig. 3). We previously reported that the amylose content of NIL(*Alk*) was slightly lower than that of Nipponbare as measured by a GPC with a reflectance index detector (Umemoto et al., 2004). Total carbohydrate assay after a GPC fractionation confirmed this difference (Table 1). This lower amylose content of NIL(*Alk*) could be caused by the additional starch synthase activity, SSIIα, which lacked in Nipponbare. It is widely accepted that availability of ADP-glucose, the substrate of starch synthase, is the rate limiting in starch biosynthesis in sink tissues including rice endosperm (Sakulsingharoj et al., 2006). Whether or not the small difference in grain filling temperature between the two lines (Table 1, Fig. 3). Takemoto-Kuno et al. (2006) reported that the allelic difference in *SSI* between *japonica* Koshihikari and *indica* Kasalath affected the amylopectin chain-length distribution in F₂ and F₃ progenies. When *SSI* expression was decreased to between 0 and 20% by retrotransposon *Tos1* insertion, the amount of dp 8–12 decreased and that of dp 6–7 and 16–19 increased (Fujita et al., 2006). Whether or not the small difference in chain lengths we observed (Fig. 3) was caused by the variation in *SSI* alleles (Figs. 2B, D) remains to be determined. The higher protein content of NIL(*SSIIα*) (6.42%) than in Nipponbare (5.35%) was probably due to an unidentified locus that controls protein content within the Kasalath chromosomal segment either on chromosome 1 or 6 in NIL(*SSIIα*), not to the minor difference in grain filling temperature between the two lines (Table 1).

2. Effects of starch synthase variations on gelatinization properties

Properties measured by RVA have been used to estimate the eating quality and processing suitability of rice (Champagne et al., 1999; Bergman et al., 2004). For example, setback (final viscosity–minimum viscosity after gelatinization) was correlated with roughness (r=0.88) and hardness (r=0.85), and was a good predictor of the baking expansion of Japanese rice crackers (Yamada et al., 1993). NIL(*Alk*) had the highest temperature at initial viscosity increase, probably because of its lower ratio of amylopectin short chains (Fig. 5). Kasalath has the same functional *Alk* allele as in NIL(*Alk*), but the temperature at initial viscosity increase was lower than that of NIL(*Alk*). This suggests that Kasalath also has genetic factors that decrease the viscosity increase temperature.

The gelatinization temperatures and enthalpies of cooked rice were high in NIL(*Alk*) and Kasalath, which had lower ratios of amylopectin short chains. Side chains of amylopectin clusters form double helices (the minimum length of the α-1,4 portion without α-1,6 branch points for a double helix should be approximately 10 glucose units), the length of which is correlated with *Tg* (Gidley and Bulpin, 1987). Our result is consistent with reports that an increase in amylopectin chains of dp < ~10 decreased the gelatinization temperature of starch as measured by DSC (Jane et al., 1999; Umemoto et al., 2004; Nakamura et al., 2006).

The enthalpies of stored cooked rice of NIL(*Alk*) and Kasalath were higher than those of Nipponbare. This is probably due to the enrichment of amylopectin side chains of dp 13–23 in both lines (Fig. 3). More hydrogen bonds form between these chains than in other short-chain-enriched lines, giving tolerance to heat gelatinization (Inouchi et al., 1991; Liu and Thompson, 1998). After 1 day of storage, only one peak at a higher temperature was apparent, but a second peak at a lower temperature became apparent after 7 days of storage. Similar results were reported in maize starches (Inouchi et al., 1991, 1995; Liu and Thompson, 1998). At the early stage of retrogradation (1 day), the starch gel is strongly resistant to heat. This resistance may be due to early formation of nuclei of retrograded starch. The lower temperature peak (around 40°C) after 7 days corresponds to the thermal behavior of re-melting of a weaker structure. The formation of relatively longer double helices may dominate retrogradation within 1 day. A lower peak temperature of the endosperm after 7 days may indicate more numerous shorter double helices.

Rice cakes made from waxy rice with the functional *SSIIα* and rich in amylopectin mid-length chains tend to harden faster (Okamoto et al., 2002). We found by DSC measurements that rice starch rich in amylopectin mid-length chains (NIL(*Alk*) and Kasalath) retrograded further during storage at 5°C. This observation could explain the differences in hardening speed of rice cakes. Vandeputte et al. (2003a, b) also showed that a higher proportion of mid-length chains of dp 12–22 increased both the starting gel point temperature measured by RVA and the melting enthalpy of retrograded amylopectin measured by DSC. Horibata et al. (2004), Inouchi et al. (2005), and Nakamura et al. (2006) found a negative correlation between the ratio of short chains (dp 6–12) and
gelatinization temperature by DSC measurement.

3. Effects of starch synthase variations on cooked rice quality

As both NIL(Wx) and Kasalath have the Wx allele and a high amylose content (Table 1), the amylose content is the key to the differences in gloss, stickiness, and softness. Especially, the eating quality of NIL(Wx) rice was rated lower than that of Nipponbare on all scores (Fig. 5). This result indicates that amylose content is solely a strong determinant of eating quality both just after cooked and after stored 16 hr at 5ºC. Bao et al. (2000) suggested that eating quality could be improved by introducing the japonica wx allele (Wx) into an indica background. Our converse introduction of indica Wx into a japonica background supports this suggestion (Figs. 1, 5). Takeuchi et al. (2007) detected a QTL for eating quality at or very close to the wx locus, finding that the japonica Wx gave a higher eating quality. A higher protein content, in addition to a higher amylose content, gives a harder texture and thus inferior eating quality (Okadome et al., 1999). The higher protein content of NIL(Wx) than that of Nipponbare (Table 1) could have contributed to its inferior eating quality.

The functional SSIIa decreased the ratio of amyllopectin short chains (Fig. 3), and the starch showed a higher gelatinization temperature and degree of retrogradation (Fig. 4). These obvious alterations in starch properties affected the eating quality only after the rice had been cooked and stored (Fig. 5). This is probably due to the high retrogradation of NIL(Alk) starch as revealed by DSC analysis (Fig. 4, Table 3). Juliano (2003) reviewed extensive studies of the effect of combinations of amylose content type and gelatinization temperature on cooked rice hardness of freshly cooked and stored cooked rice. Among low-amylose cultivars, similar to Nipponbare, only the high-gelatinization-temperature cultivar Century Patna 231 showed more amyllopectin retrogradation as evaluated by the Instron hardness test (Perez et al., 1993). Since the high gelatinization temperature of Century Patna 231 was estimated from the alkali spreading value, it is very likely that this cultivar has the functional SSIIa allele (Alk) and similar cooked rice properties to those of NIL(Alk).

NIL(SSIIa) was less preferable to Nipponbare in the first sensory test (Fig. 5). Since its amylose content and amyllopectin chain lengths are not much different from those of Nipponbare, its higher protein content may adversely affect its eating quality (Table 1, Fig. 3).

Using NILs for each starch synthase allele we elucidated the effects of natural variation in GBSSI, SSIIa, and SSI on starch properties and eating quality of rice. Although additional work is needed for the precise evaluation of the variation in SSI, we found clear effects of GBSSI and SSIIa on starch properties and cooked rice qualities. It was confirmed that rice with the combination of Wx for GBSSI allele and alk for SSIIa allele had soft and sticky texture both just after cooked and after storage, and was suited to the preference of Japanese consumers.

Acknowledgments

We thank Dr. Hisatoshi Ohta for his aid in the evaluation tests, and members of the National Institute of Crop Science who acted as testers. We also thank Ms. Sachiko Gonokami for her technical assistance in preparing plant materials.

References

Asaoka, M., Okuno, K., Sugimoto, Y., Kawakami, J. and Fuwa, H. 1984. Effect of environmental temperature during development of rice plants on some properties of endosperm starch. Starch 36 : 189-193.
Bao, J.S., Zheng, X.W., Xia, Y.W., He, P., Shu, Q.Y., Lu, X., Chen, Y. and Zhu, L.H. 2000. QTL mapping for the paste viscosity characteristics in rice (Oryza sativa L.). Theor. Appl. Genet. 100 : 280-284.
Bergman, C.J., Bhattacharyya, K.R. and Ohsubo, K. 2004. Rice end-use quality analysis. Chapter 15 In : E. Champagne ed., Chemistry and Technology, Third Edition. American Association of Cereal Chemists, Inc., St. Paul, Minnesota, USA, 415-472.
Biliaderis, C.G., Page, C.M., Slade, L. and Sirett, R.R. 1985. Thermal behavior of amylose-lipid complexes. Carbohydr. Polym. 5 : 367-389.
Champagne, E.T., Bett, K.L., Vinyard, B.T., McClung, A.M., Barton, F.E. II, Moldenhauer, K.M., Linscombe, S. and McKenzie, K. 1999. Correlation between cooked rice texture and Rapid Visco Analyser measurement. Cereal Chem. 76 : 764-771.
Fujita, N., Yoshida, M., Asakura, N., Ohdan, T., Miyao, A., Hirochika, H. and Nakamura, Y. 2006. Function and characterization of starch synthase I using mutants in rice. Plant Physiol. 140 : 1070-1084.
Gao, Z., Zeng, D., Cui, X., Zhou, Y., Yan, M., Huang, D., Li, J. and Qian, Q. 2003. Map-based cloning of the ALK gene, which controls the gelatinization temperature of rice. Sci. China (Ser. C) 46 : 661-668.
Gidley, M. and Bulpin, P.V. 1987. Crystallization of malt-oligosaccharides as models of the crystalline forms of starch : minimum chain-length requirement for the formation of double helices. Carbohydr. Res. 161 : 291-300.
Hirano, H.Y. and Sano, Y. 1998. Enhancement of Wx gene expression and the accumulation of amylose in response to cool temperatures during seed development in rice. Plant Cell Physiol. 39 : 807-812.
Hirose, T. and Terao, T. 2004. A comprehensive expression analysis of the starch synthase gene family in rice (Oryza sativa L.). Planta 220 : 9-16.
Horibata, T., Nakamoto, M., Fuwa, H. and Inouchi, N. 2004. Structural and physicochemical characteristics of endosperm starches of rice cultivars recently bred in Japan. J. Appl. Glycosci. 51 : 303-313.
genetic background. Starch 33 : 9-13.
Ikawa, Y. and Kouno (Kikuchi), C. 2005. Multiple evaluation of cooked rice retrogradation in early stage of storage. J. Appl. Glycosci. 52 : 293-298.
Inouchi, N., Glover, D.V., Sugimoto, Y. and Fuwa, H. 1991. DSC characteristics of retrograded starches of single-, double-, and triple-mutants and their normal counterpart in the Inbred Oh43 maize (Zea mays L.) background. Starch 43 : 473-477.
Inouchi, N., Glover, D.V. and Fuwa, H. 1995. Structure and physicochemical properties of endosperm starches of a waxy allelic series and their respective normal counterparts in the Inbred Oh43 maize background. Starch 47 : 421-426.
Inouchi, N., Nishi, K., Tanaka, S., Asai, M., Kawase, Y., Hata, Y., Konishi, Y., Yue, S. and Fuwa, H. 1999. Characterization of amaranth and quinoa starches. J. Appl. Glycosci. 46 : 233-240.
Inouchi, N., Ando, H., Asaoka, M., Okuno, K. and Fuwa, H. 2000. The effect of environmental temperature on distribution of unit of rice amylopectin. Starch 52 : 8-12.
Inouchi, N., Hibi, H., Li, T., Horihata, T., Fuwa, H. and Itani, T. 2005. Structure and properties of endosperm starches from cultivated rice of Asia and other countries. J. Appl. Glycosci. 52 : 299-246.
Jane, J., Chen, Y.Y., Lee, L.F., McPherson, A.E., Wong, K.S., Radosavljevic, M. and Kasemsuwan, T. 1999. Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. Cereal Chem. 76 : 629-637.
Jiang, H., Dian, W. and Wu, P. 2003. Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme. Phytochemistry 63 : 53-59.
Juliano, B.O. 2001. Asian perspective on rice sensory quality. Cereal Foods World 46 : 531-535.
Juliano, B.O. 2003. Chemical Constituents. Chapter 3 In B.O. Juliano, Rice Chemistry and Quality. Island Publishing House, Inc., Manila, Philippines. 57-129.
Liu, Q. and Thompson, D.B. 1998. Retrogradation of dw1 wx and su2 wx maize starches after different gelatinization heat treatments. Cereal Chem. 75 : 868-874.
Monna, L., Lin, X., Kojima, S., Sasaki, T. and Yano, M. 2002. Genetic dissection of a genomic region for a quantitative trait locus, Hd3i, into two loci, HdiSu and Hdi3b, controlling heading date in rice. Theor. Appl. Genet. 104 : 772-778.
Nakamura, Y. 2002. Towards a better understanding of the metabolic system for amylopectin biosynthesis in plants : rice endosperm as a model tissue. Plant Cell Physiol. 43 : 718-725.
Nakamura, Y., Sato, A. and Juliano, B.O. 2006. Short-chain-length distribution in debranched rice starches differing in gelatinization temperature or cooked rice hardness. Starch 58 : 155-160.
Okadome, H., Kurihara, M., Kusuda, O., Toyoshima, H., Kim, J.I., Shimotsubo, K., Matsuda, T. and Ohtsubo, K. 1999. Multiple measurements of physical properties of cooked rice grain with different nitrogenous fertilizers. Jpn. J. Crop Sci. 68 : 211-216.
Okamoto, K., Kobayashi, K., Hirasawa, H. and Umemoto, T. 2002. Structural differences in amylopectin affect waxy rice processing. Plant Prod. Sci. 5 : 45-50.
Perez, C.M., Villereal, C.P., Juliano, B.O. and Biliaderis, C.G. 1993. Amylopectin-staling of cooked nonwaxy milled rices and starch gels. Cereal Chem. 70 : 567-571.
Sakulsingharoj, C., Choi, S.B., Hwang, S.K., Edwards, G.E., Bork, J., Meyer, C.R., Preiss, J. and Okita, T.W. 2004. Engineering starch biosynthesis for increasing rice seed weight : the role of the cytoplasmic ADP-glucose pyrophosphorylase. Plant Sci. 167 : 1323-1333.
Sano, Y. 1984. Differential regulation of waxy gene expression in rice endosperm. Theor. Appl. Genet. 68 : 467-473.
Takeda, Y. and Hizukuri, S. 1987. Structures of rice amyllopectins with low and high affinities for iodine. Carbohydr. Res. 168 : 79-88.
Takekuchi, Y., Nomoue, Y., Ebihata, T., Suzuki, K., Aoki, N., Sato, H., Ideta, O., Hirabayashi, H., Hirayama, M., Ohta, H., Nemoto, H., Kato, H., Ando, I., Ohtsubo, K., Yano, M. and Imbe, T. 2007. QTL detection for eating quality including glossiness, stickiness, taste and hardness of cooked rice. Breed. Sci. 57 : 231-242.
Tomlinson, K. and Denyer, K. 2003. Starch synthesis in cereal grains. Adv. Bot. Res. 40 : 1-40.
Umemoto, T., Nakamura, Y., Satoh, H. and Terashima, K. 1999. Differences in amylopectin structure between indica and japonica rice varieties. J. Agric. Food Chem. 54 : 9234-9240.
Vandeputte, G.E., Vandeputte, G.E., Vermeylen, R., Geeroms, J. and Delcour, J.A. 2003a. Rice starches. I. Structural aspects provide insight into amylopectin-staling properties and gel texture. J. Cereal Sci. 38 : 43-52.
Vandeputte, G.E., Derycke, V., Geeroms, J. and Delcour, J.A. 2003b. Rice starches. III. Structural aspects provide insight into amylopectin retrogradation properties and gel texture. J. Cereal Sci. 38 : 69-76.
Wang, Z.Y., Zheng, F.Q., Shen, G.Z., Gao, J.P., Snustad, P., Li, M.G., Zhang, J.L. and Hong, M.M. 1995. The amylose content in rice endosperm is related to the effects of temperature during grain-filling. Starch 51 : 58-62.
Umemoto, T., Yano, M., Satoh, H., Shomura, A. and Nakamura, Y. 2002. Mapping of a gene responsible for the difference in amylopectin structure between japonica-type and indica-type rice varieties. Theor. Appl. Genet. 104 : 1-8.
Umemoto, T. and Terashima, K. 2002. Activity of granule-bound starch synthase is an important determinant of amylose content in rice endosperm. Funct. Plant Biol. 29 : 1121-1124.