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Mutants that have shorter amylopectin chains are promising materials for slow-hardening rice bread

Noriaki Aoki [a, b], Takayuki Umemoto [c], Kazuyuki Okamoto [d], Yasuhiro Suzuki [e], Junichi Tanaka [b, e, *]

[a] National Agriculture and Food Research Organization, Headquarters 3-1-1 Kannondai, Tsukuba, Ibaraki 305-8666, Japan
[b] University of Tsukuba Graduate School of Life and Environmental Sciences, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan
[c] NARO Hokkaido Agricultural Research Center, 1 Hitsujigaoka, Toyohiraku, Sapporo 062-8555, Japan
[d] Ibaraki Agricultural Center Plant-Biotechnology Institute, 3402 Kamikunii, Mito, Ibaraki 311-4203, Japan
[e] NARO Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan

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ABSTRACT

Bread staling is a serious economic issue for the baking industry. Here, we found that shorter amylopectin chains caused by mutations play a role in maintaining the softer texture of rice bread. We used three rice cultivars that have a high proportion of short amylopectin chains in endosperm starch, two of which were *starch branching enzyme I* mutants, to make gluten-free and gluten-containing bread. Compression tests showed that the hardening rates for both types of bread made from these cultivars were markedly lower than those for control rice breads (gluten-free bread: 14%–39%, gluten-containing bread: 13%–27%), although there were no clear differences in the hardness values among the breads one day after baking. Sensory tests conducted two days after baking showed that gluten-free breads made from the three cultivars were softer than the control breads. Amylose contents, flour particle sizes, and damaged starch contents were similar among the flour samples, indicating that shorter amylopectin chains led to the slow-hardening of the rice bread. This finding can be applied not only to the breeding of rice cultivars for softer bread, but also to breeding of wheat and other cereals for bread.

1. Introduction

Bread easily stales within a few days and its palatability reduces. Bread staling is not only a serious issue for bread quality to consumers but also causes huge economic losses to the baking and restaurant industries. Rice flour is used to make gluten-free bread, which is helpful for those who are allergic to wheat protein or have celiac disease (Cureton and Fasano, 2009); however, it stales faster than regular wheat bread (Ahlborn et al., 2005). Starch is thought to affect the staling of both gluten-free and regular wheat bread (Gray and Bemiller, 2003; Morgan et al., 1997). Therefore, one approach to solving the problem is to breed cultivars that produce breads that show less staling during preservation by changing the starch properties of such cultivars through mutations.

Rice is suitable for investigating the relationship between starch and bread staling for two reasons. First, rice proteins do not form a gluten matrix like wheat proteins, hence the properties of starch directly affect bread qualities. Second, rice is a good material for genetic analyses, because it is a diploid self-pollinating species, that is, it is relatively easy to correct recessive alleles. Many rice mutants have been screened and characterized, including mutants with unique starch properties (Nakamura, 2002).

Starch consists of two components, amylose and amylopectin. Amylose is essentially a linear molecule containing α-(1-4)-linked glucose units with few branches, whereas amylopectin is a branched molecule with linear chains of α-(1-4)-linked glucose units with α-(1-6)-linked branches (Hizukuri et al., 1989). Native starch is closely packed to form clusters of double helices, and starch granules comprise an amorphous region and a semi-crystalline region (Donald, 2001). Starch is gelatinized by denaturing its structure under high temperature in the presence of water and the gelatinization temperature depends on amylopectin structure (Jane et al., 1999). When the gelatinized starch is cooled,

Abbreviations: DP, degree of polymerization; *SbeI*, *starch branching enzyme I*.

* Corresponding author. NARO Institute of Crop Science, 2-1-18 Kannondai, Tsukuba, Ibaraki 305-8518, Japan. Tel.: +81 29 838 8536.
E-mail address: tanajun@affrc.go.jp (J. Tanaka).

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starch is retrograded by partial recrystallization of the linear chains. This starch retrogradation is believed to play a major role in the staling process (Bosmans et al., 2013; Hug-Iten et al., 2003). Although both amylose and amylopectin undergo retrogradation, amylose is retrograded within hours, whereas amylopectin is retrograded for days (Miles et al., 1985; Ribotta and Le-Bail, 2007). Therefore, we hypothesized that amylopectin plays a major role in bread staling during long-term storage.

Rice amylopectin is mainly classified into S-type and L-type (Nakamura, 2002) based on the absence or presence of Starch Synthase IIa activity (Umemoto et al., 2004). S-type amylopectin has a higher ratio of short chains than does L-type amylopectin. Moreover, the gelatinization temperature of starch with S-type amylopectin is lower than that of starch with L-type amylopectin. In our previous report, gluten-containing rice breads made from cultivars that have L-type amylopectin were more prone to hardening than bread made from cultivars that have S-type amylopectin (Aoki et al., 2012). Therefore, we hypothesized that softer bread (both gluten-containing and gluten-free) can be made by using cultivars that have shorter amylopectin chains than those in S-type amylopectin. Rice mutants of *starch branching enzyme I* (*SbeI*) have a high proportion of shorter amylopectin chains and a lower gelatinization temperature compared with cultivars that have regular S-type amylopectin (Okamoto et al., 2013a; Satoh et al., 2003). Here, we report that slow-hardening gluten-free and gluten-containing breads can be obtained by using mutants that have shorter amylopectin chains than those in S-type amylopectin.

2. Experimental

2.1. Materials

Rice (*Oryza sativa* L.) cultivars, 'Akita Sake 44', 'Hiderishirazu D', and 'Kurnai' that are rich in shorter chains of amylopectin, were used. 'Koshihikari', which is the most popular cultivar in Japan, was used as a control (Table 1). 'Hiderishirazu D' and 'Kurnai' are natural loss-of-function mutants of *SbeI*. The mutations of 'Hiderishirazu D' and 'Kurnai' are considered to be of common origin, because no difference was detected between the DNA sequences of *SbeI* between 'Hiderishirazu D' and 'Kurnai' (Okamoto et al., 2013a). 'Akita Sake 44' is a gamma-ray-induced mutant with a low gelatinization temperature (Okamoto, unpublished result). Polished grains of rice were ground by using a jet mill (SPM-R290; Nishimura Machine Works, Osaka, Japan) after being soaked in a solution containing 0.3% trisodium citrate dihydrate and 0.05% pectinase (Pectinase G; Amano Enzyme, Nagoya, Japan) for 1 h at 40 °C.

2.1.1. Genotyping of the wx and alk locus

Total DNA was extracted by using diatomaceous earth and a spin filter (Tanaka and Ikeda, 2002). The *waxy* (*wx*) locus was genotyped by using the *wx*-allele-specific dCPAS marker (Yamanaka et al., 2004). The *alk* locus, which encodes starch synthase IIa, was genotyped by using SNP markers (Hiratsuka et al., 2010).

2.2. Flour property measurements

Flour particle sizes were measured with a laser diffraction particle size analyzer (LS 13 320; Beckman Coulter, Brea, USA). Damaged starch contents were measured according to the American Association of Cereal Chemists (AACC) method 76-31 (AACC, 2000b) with a starch damage assay kit (Megazyme International Ireland, Wicklow, Ireland). Apparent amylose contents were determined by using the iodine absorption method with an Auto Analyzer (BRAN + LUEBBE, Norderstedt, Germany). The nitrogen content of flour was determined by using a Sumigraph NC-22F (Sumika Company Ltd., Osaka, Japan) and acetanilide as a standard; protein values were calculated by multiplying the nitrogen content by 5.95. Pasting properties were determined with a Rapid Visco Analyzer (RVA Model 3D; Newport Scientific, Warriewood, Australia) as previously described (Toyoshima et al., 1997). The temperature at the onset of the rise in viscosity was determined as the pasting temperature. The thermograms of the starch granules were recorded on a differential scanning calorimeter (DSC7; PerkinElmer Inc., Waltham, USA) with distilled water as the reference. Rice flour (10 mg) and distilled water (25 μL) were sealed in a platinum pan. Samples were heated from 10 °C to 100 °C at a rate of 10 °C/min. The peak temperatures were determined as the gelatinization temperature. Water absorption of rice flour was determined as the amount of water needed to develop a standard dough of 500 Brabender units at the peak of the curve, as determined by using a Farinograph (Brabender Inc., Duisburg, Germany) according to the AACC method 54-21 (AACC, 2000a). A mixture of rice flour (40 g) and vital wheat gluten (10 g) was used (14% moisture basis). Amylopectin chain-length distribution was analyzed by using capillary electrophoresis as described by Fujita et al. (2001). Statistical evaluations were performed by using Tukey's test.

2.3. Bread making

We made two types of bread, gluten-free bread and gluten-containing bread. Gluten-free bread was made by using 480 g of rice flour, 120 g of tapioca starch, 12.0 g of hydroxypropyl methylcellulose (Metolose; Shin-Etsu Chemical, Tokyo, Japan), 48.0 g of sugar, 24.0 g of skimmed milk, 12.0 g of salt, 42.0 g of shortening, 24.0 g of dry yeast, and 630 mL of water. Tapioca starch, which is often used in gluten-free bread-making (Schober, 2009), was added in an attempt to make bread larger (Kusunose et al., 1999; Sanchez et al., 2002). The dough was mixed in a Kanto mixer (HPi-20M; Kanto Mixer, Tokyo, Japan). Portions of dough (400 g) were placed in bread pans and proofed at 27 °C and 80% humidity for 60 min. Breads were baked at 200 °C for 20 min without steam.

Gluten-containing bread was made by using 600 g of rice flour (14% moisture basis), 150 g of vital wheat gluten (14% moisture

Table 1
Properties of rice flour samples.

	Average particle size (μm)[a]	Damaged starch content (%)[b]	Amylose content (%)[b]	Protein content (%)[b]	Pasting temperature (°C)[b]	Gelatinization temperature (°C)[b]	Water absorption (%)
Koshihiakri	32.9	1.9 ± 0.1d	18.0 ± 0.2b	5.1 ± 0.1c	68.8 ± 0.8a	69.9 ± 0.3a	77.4
Akita Sake 44	30.3	2.4 ± 0.1c	20.2 ± 0.4a	5.1 ± 0.2c	66.4 ± 0.7a	66.8 ± 0.1b	77.1
Hiderishirazu D	31.3	2.7 ± 0.1b	20.7 ± 0.2a	7.0 ± 0.0a	65.7 ± 0.7b	65.2 ± 0.1c	77.1
Kurnai	34.9	3.0 ± 0.1a	17.6 ± 0.3b	5.9 ± 0.1b	65.2 ± 0.4b	65.8 ± 0.4c	79.3

[a] Means of two replicates are shown.
[b] Means and standard deviations from three replicates are shown. Means ± standard deviations with the same letter are not significantly different from each other ($p < 0.05$, Tukey's test).