Plant Biotechnology 24, 227–229 (2007)

Development Note

Ultrastructure of low-glutelin rice endosperm

Sachiko Furukawa[1], Mitsuaki Itou[2], Takehiro Masumura[2,3,*], Kunisuke Tanaka[2,4], Yoshifumi Kiyokawa[1], Yoshinori Wakai[1]

[1] Kizakura Co. Ltd., 223 Shioyamachi, Fushimi-ku, Kyoto 612-8046, Japan; [2] Laboratory of Genetic Engineering, Graduate School of Agriculture, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Kyoto 606-8522, Japan; [3] Kyoto Prefectural Institute of Agricultural Biotechnology, Kitaina-Yazuma, Seika-cho, Kyoto 619-0244, Japan; [4] Phytoculture Co. Ltd., 5-18-14 Sekime, Jyoutou-ku, Osaka 536-0008, Japan

* E-mail: masumura@kpu.ac.jp Tel & Fax: +81-75-703-5675

Received December 4, 2006; accepted December 26, 2006 (Edited by M. Iwano)

Abstract Low-glutelin rice has low content of digestible protein glutelin, and is expected to use as raw material for sake brewing. To characterize endosperm cell structure of low-glutelin rice, the storage proteins of endosperm cells in polished rice (degree of milling, 70%; used as raw material for sake brewing) of two low-glutelin rice varieties, LGC1 and Tashu-kei 1001, and two general rice cultivars (brewer's rice), Hyogokitanishiki and Yamadanishiki, were observed using a transmission electron microscope. Low-glutelin rice differed from general rice cultivars in the composition of major storage proteins and the transgranular distribution of storage proteins as well as in the distribution of protein bodies in endosperm cells. Furthermore, low-glutelin rice had a specific endosperm cell structure containing many type I protein bodies even in the outer layer of polished rice grains.

Key words: Low-glutelin rice, protein body, sake brewing, transmission electron microscopy.

The storage proteins of rice seed are accumulated in two types of particles called PBs. The type I PB (PB-I) stores indigestible protein prolamin, whereas the type II PB (PB-II) mainly contains digestible protein glutelin (Becthel and Juliano 1980; Tanaka et al. 1980). There are morphological differences between PB-I and PB-II. PB-I is a small spherical particle with a lamellar structure of 1–2 μm in diameter, and PB-II is an amorphous and highly electron-dense structure of 2–3 μm in diameter (Tanaka et al. 1980; Yamagata and Tanaka 1986).

In the low-glutelin rice LGC1, the content of prolamin increased, while the content of glutelin decreased (Iida et al. 1993, 1998). In sake brewing, proteins in rice seed are an important factor imparting the sake flavor, but too much protein produces an undesirable flavor. Furthermore, a morphological characteristic of brewer's rice is its white core structure, referred to as '*shinpaku*' in Japanese. Previously, the hybrid rice Tashu-kei 1001, which has a low content of digestible protein glutelin and a white core structure, was crossed between the low-glutelin rice LGC1 and brewer's rice Hyogokitanishiki by WeNARC, and its use as a raw material for sake brewing was investigated (Mizuma et al. 2002).

Furthermore, using immunofluorescent-labeling with anti-glutelin and anti-prolamin antibodies with a confocal laser scanning microscope, we previously visualized the distribution of rice storage proteins and showed that low-glutelin rice differs from general cultivar rice not only in the composition of major storage proteins but also in the transgranular distribution of storage proteins (Furukawa et al. 2003). However, there have been no reports related to the differences in the morphology and/or distribution of storage proteins (protein bodies) between the endosperm cells of low-glutelin rice and general rice cultivars. The objective of this study was to obtain further knowledge about low-glutelin rice with the purpose of increasing its use in sake brewing. Therefore, we observed the morphological characteristics of the storage proteins in endosperm cells of polished rice (degree of milling, 70%; used as raw material for sake brewing) of two low-glutelin rice varieties, LGC1 and Tashu-kei 1001, and two general rice cultivars (brewer's rice), Hyogokitanishiki and Yamadanishiki, using a TEM.

The four cultivars of *Oryza sativa* L. used in this study were harvested in 2000. Brown rice was polished to 70% of its original weight using a milling machine (THE GRAIN-TESTING MILL; Satake, Higashihiroshima). The content of crude protein in brown rice and polished rice (degree of milling, 70%) was analyzed using a near-

Abbreviations: PB(s), protein body (ies); PB-I(s), type I protein body (ies); PB-II(s), type II protein body(ies); SDS-PAGE, sodium dodecyl sulfate poly-acrylamide gel electrophoresis; SG, starch granule; TEM, transmission electron microscope; WeNARC, the National Agricultural Research Center for the Western Region

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Table 1. List of rice cultivars

Cultivar	Locality	Class	Content of crude protein (g/100 g dry seed)	
			Brown rice	Polished rice
Tashu-kei 1001	WeNARC	Low-glutelin rice for sake brewing	7.63	5.00
LGC1	WeNARC	Low-glutelin rice	7.15	5.05
Hyogokitanishiki	WeNARC	Brewer's rice	8.26	5.23
Yamadanishiki	Hyogo	Brewer's rice	7.12	4.48

infrared reflectance analyzer (InfraAlyzer 500; Bran+Lubbe, Tokyo). As shown in Table 1, the content of crude protein in the brown rice of the low-glutelin rice LGC1 was as low as that of the brewer's rice cv. Yamadanishiki. The content of crude protein in the brown rice of Tashu-kei 1001 (a hybrid between LGC1 and Hyogokitanishiki) was close to the median value of LGC1 and Hyogokitanishiki. The content of crude protein in polished rice was the lowest in Yamadanishiki and about 5% in the other cultivars.

Polished rice grains were crushed with a roller mill (Retsch; Nihonseiki Kaisha Ltd., Tokyo), and rice storage proteins were extracted with 500 μl of an SDS-urea solution (8 M urea, 4% SDS, 5% 2-mercaptoethanol, 20% glycerol, and a 50 mM Tris-HCl buffer (pH 6.8)) at 60°C for 2 h. SDS-PAGE was carried out according to the Laemmli method using 16% acrylamide gels (Laemmli 1970). The gels were stained with Coomassie Brilliant Blue R-250. The content of PBs was determined using a densitometer (Densitograph AE-6920M; ATTO, Tokyo). The content of PBs in polished rice was shown in Figure 1. The content of PB-II (glutelin and globulin) in two varieties of low-glutelin rice, Tashu-kei 1001 and LGC1, was approximately reduced to two-thirds that of Hyogokitanishiki and Yamadanishiki. The content of PB-I (prolamin) was approximately twice that of Hyogokitanishiki and Yamadanishiki, in accordance with a previous report (Iida et al.1993).

For TEM, the outer layer of polished rice grains (degree of milling, 70%) was sliced with a razor blade and fixed in 3% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4) at room temperature for 3 h. The fixed samples were rinsed several times with a 0.1 M phosphate buffer (pH 7.4) and dehydrated in a graded series of ethanol concentrations. The samples were then embedded in acrylic resin (LR white Resin; Okenshoji Co., Ltd., Tokyo). Thin sections were prepared with an ultramicrotome (ULTRA CUT; LEICA, Wetzlar, DE.) equipped with a glass knife and stained with 2% uranyl acetate and lead citrate. The stained sections corresponding to the surface layers of the grains were examined using a TEM (JEM-1220; JEOL, Tokyo).

Figure 2 shows photographs of the TEM observations and a schematic of the observation area. In the outer layer of polished rice of Hyogokitanishiki (Figure 2C)

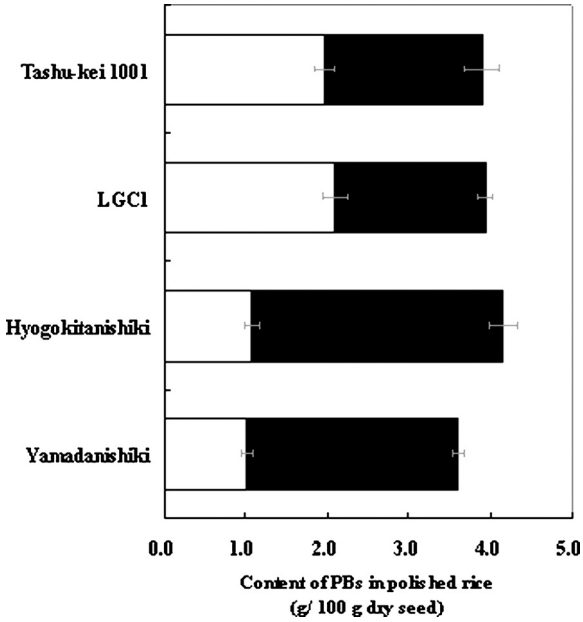

Figure 1. Content of protein bodies in polished rice (degree of milling, 70%). White bars, content of PB-I (summation of 10kDa, 13 kDa, 16 kDa prolamin polypeptides); black bars, content of PB-II (summation of 22–23 kDa, 37–39 kDa glutelin polypeptides and 26 kDa globulin polypeptide). Error bars indicate SD with five times measurements.

and Yamadanishiki (Figure 2D), PB-IIs could be mainly observed in endosperm cells. In contrast, there were few PB-IIs in the low-glutelin rice LGC1, whereas there were many PB-Is (Figure 2B), in this observation. PB-I in LGC1, seen as small spherical particles of less than 2 μm in diameter, was slightly smaller than those in other cultivars. The ultrastructure of Tashu-kei 1001 of low-glutelin rice for sake brewing (Figure 2A) differed from that of Hyogokitanishiki as a paternal line. In Tashu-kei 1001, PB-IIs were barely evident; however, PB-Is were quite evident as small spherical particles, as in the case of the LGC1 maternal line, in this observation. PBs in LGC1 and Tashu-kei 1001 had different electron density, however we had no known its cause. Further studies are needed to clarify it.

This study demonstrates that low-glutelin rice has a different composition of major storage proteins and a different transgranular distribution of storage proteins, as well as a different distribution of PBs in the endosperm cells from those of general cultivars. The most remarkable difference is that low-glutelin rice has a

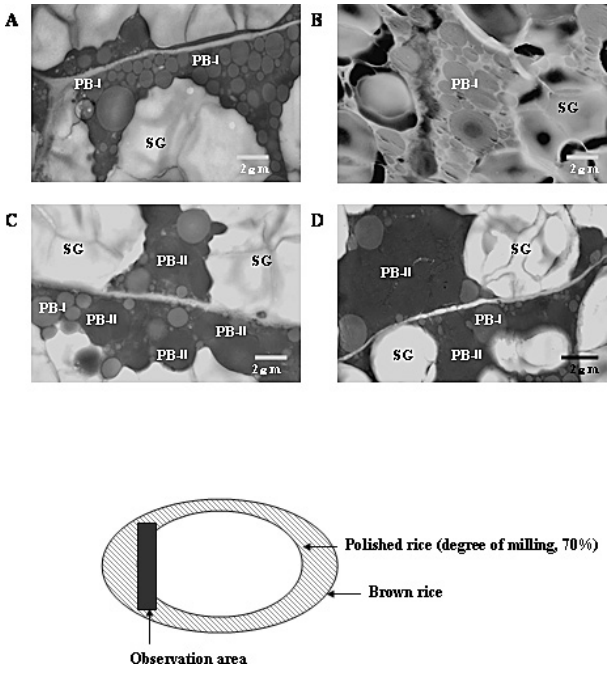

Figure 2. TEM observation of PBs of rice endosperm cells. (A) Tashukei-1001; (B) LGC1; (C) Hyogokitanishiki; (D) Yamadanishiki. The schematic indicates the observation area.

specific endosperm cell structure containing many PB-Is even in the outer layer of polished rice grains (degree of milling, 70%). Tashu-kei 1001 of low-glutelin rice for sake brewing inherits morphological traits from a paternal line, cv. Hyogokitanishiki, but it inherits the trait of the composition of major storage proteins (Mizuma et al. 2002) and the transgranular distribution of storage proteins from LGC1. From this study, it was confirmed that Tashu-kei 1001 also inherits the trait of distribution of PBs in endosperm cells from LGC1.

Acknowledgements

We are grateful to Dr. Shuichi Iida of the National Agricultural Research Center for the Western Region for providing the rice cultivars. We are deeply grateful to Prof. Hiroshi Matsui of Kyoto Prefectural University for his critical review of the manuscript.

References

Becthel DB, Juliano BO (1980) Formation of protein bodies in the starchy endosperm of rice (*Oryza sativa* L.). *A re-investigation Ann Bot* 45: 503–509

Furukawa S, Mizuma T, Kiyokawa Y, Masumura T, Tanaka K, Wakai Y (2003) Distribution of storage proteins in low-glutelin rice seed determined using a fluorescent antibody. *J Biosci Bioeng* 96: 467–473

Iida S, Amano E, Nishio T (1993) A rice (*Oryza sativa* L.) mutant having a low content of glutelin and a high content of prolamine. *Theor Appl Genet* 87: 374–378

Iida S, Miyahara K, Nishio T (1998) Rice mutant lines lacking α-globulin. *Breeding Science* 48: 45–49

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature* 227: 680–685

Mizuma T, Furukawa S, Kiyokawa Y, Wakai Y, Yamamoto Y, Tsutsui N, Matsushita K, Maeda H, Iida S, Nemoto H (2002) Characteristics of low-glutelin rice for sake brewing –Studies on rice for sake brewing–. *Jpn J Biosci Bioeng* (*Seibutsu-Kougaku Kaishi*) 80: 503–511 (in Japanese)

Tanaka K, Sugimoto T, Ogawa M, Kasai Z (1980) Isolation and characterization of two types of protein bodies in the rice endosperm. *Agric Biol Chem* 47: 1633–1639

Yamagata H, Tanaka K (1986) The site of synthesis and accumulation of rice storage proteins. *Plant Cell Physiol* 27: 135–145