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REVIEW

Utilization and Molecular Characterization of Seed Protein Composition Mutants in Rice Plants

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Abstract

Through the use of mutation breeding, we developed the cultivar LGC1, which has a low glutelin and high prolamine content, from Nihonmasari. Because of the indigestibility of prolamine, LGC1 can be used as a low-protein rice in practice. It is thus a prospective food for people such as patients with kidney disease who must restrict their protein intake. Furthermore, to reduce the content of easy-to-digest protein in LGC1, we developed two new rice cultivars, 'LGC-Katsu' and 'LGC-Jun', from a cross between LGC1 and a mutant line of Koshihikari (induced by gamma-irradiation) that was deficient in 26-kDa globulin (another easy-to-digest protein). The glutelin content of these two new cultivars is reduced to about one-third that of regular cultivars, and 26-kDa globulin is completely absent. Consequently, the total amount of easy-to-digest protein in the new cultivars is about half that of regular cultivars, and about 15% less than in LGC1. These cultivars should therefore greatly help in the dietary management of patients with chronic renal failure. We also discussed the genome structures and mechanisms of the seed protein composition mutation.

Discipline: Plant breeding
Additional key words: chronic renal failure, deletion, easy-to-digest, glutelin

Introduction

In Japan, the steep rise in medical expenses has become an object of public concern, mainly because the population is aging. In particular, the cost of blood dialysis is a very important issue for patients with kidney disease. About 15,000 new patients a year are enrolled for blood dialysis therapy, and the total patient population is more than about 200,000[5]. About 500,000 people manage chronic renal failure without blood dialysis through therapy based on a low-protein diet, and they require specially processed low-protein foods. The use of low protein rice processed by lactic acid bacterium or starchy rice is the main method of restricting their intake of protein, but these products are very expensive and not of good eating quality when compared with ordinary rice. Eating quality is a very important characteristic for maintaining diet therapy of patients with chronic renal failure. The development of rice cultivars with a low protein content would offer these people an inexpensive and delicious dietary alternative.

Breeding of 'LGC1' and molecular characterization of *Lgc1*

Usually, rice seed contains about 7% protein, and rice supplies about 15% of the average dietary intake of protein in Japan[13]. Rice seed contains two major proteins: glutelin and prolamine. Glutelin accumulates in protein body type II (PB-II) and prolamine in protein body type I (PB-I) (Fig. 1)[12]. PB-II is easily digested, but PB-I is indigestible[9]. Through the use of mutation breeding, we developed the cultivar LGC1, which has a low glutelin and high prolamine content, from Nihonmasari[1]. Glutelin is synthesized as a 57-kDa precursor and then cleaved into a 37- to 39-kDa acidic subunit and a 22- to 23-kDa basic subunit (Fig. 2). Whereas the glutelin content of LGC1 is reduced, the contents of other storage proteins, including prolamine, are increased compared with those in regular rice cultivars[1]. Because of the indigestibility of prolamine,

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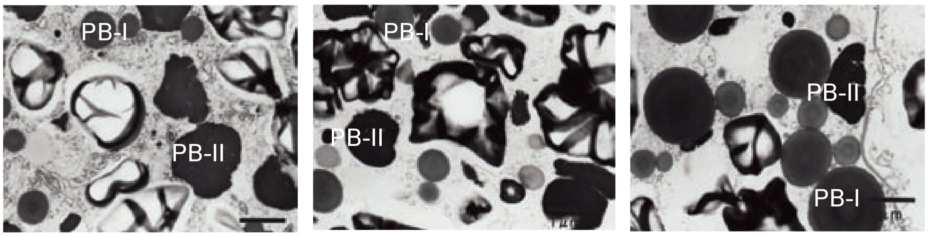

Fig. 1. Electron micrograms of protein body type-I (PB-I) and type-II (PB-II)
From left, Koshihikari, LGC-1 and LGC-Jun.

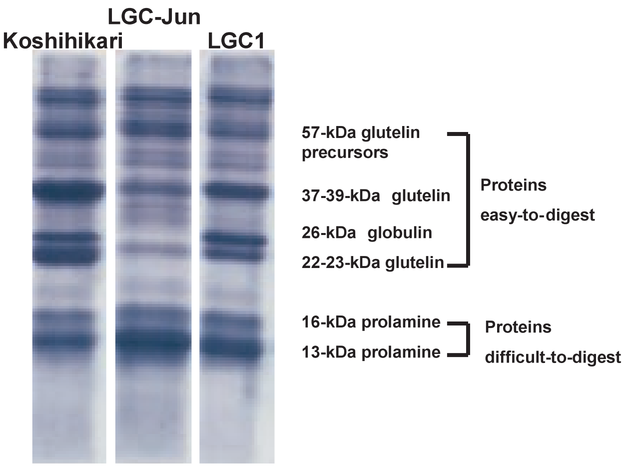

Fig. 2. SDS-PAGE analysis of total proteins in brown rice
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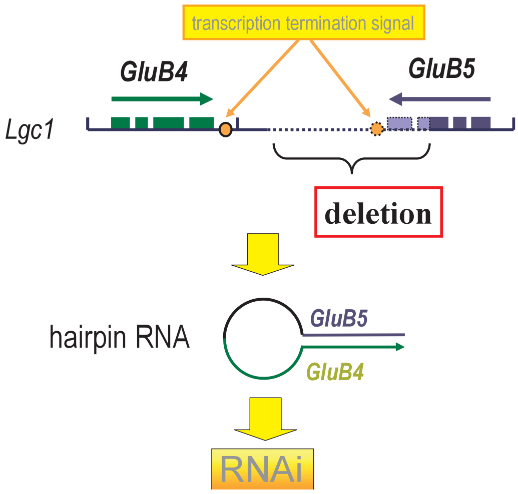

Fig. 3. Molecular characterization of *Lgc1*

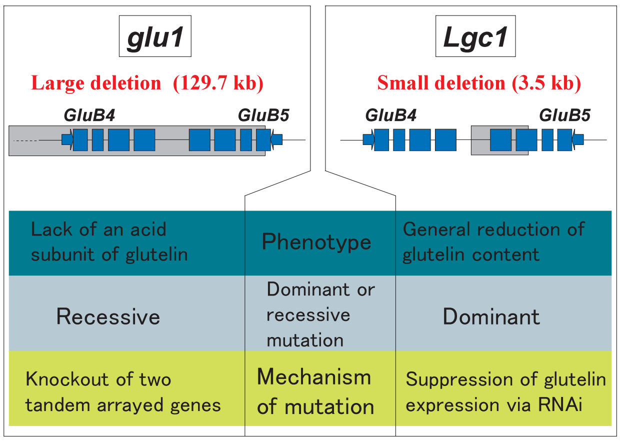

Fig. 4. Both *glu1* and *Lgc1* are the mutations involved in the *GluB4* and *GluB5* genes

LGC1 can be used as a low-protein rice in practice. We conducted a clinical test for people such as patients with kidney disease who must restrict their protein intake[6]. Patients with chronic renal failure not under blood dialysis therapy in 2 hospitals ingested 90%-milled LGC1 rice for 6 months. In those patients who had more opportunity to consume LGC1, the protein contents in the blood and the total intake of protein were decreased significantly; furthermore, their scores of 1/creatinine (an indicator of kidney function) were improved. On the other hand, in those patients who had less opportunity to consume LGC1, these scores were not improved.

This low glutelin trait is conferred by a dominant mutation *Low glutelin content 1* (*Lgc1*). The results of molecular characterization of the gene showed that *Lgc1* suppresses the expression of the glutelin multigene family. *Lgc1* associates with a 3.5-kb deletion between two highly similar and tandem repeated glutelin genes, *GluB5* and *GluB4*, with a tail-to-tail inverted arrangement (Fig. 3). As *GluB5* lacks a transcription termination signal, transcription from the *GluB5* promoter generates a read-though product containing a sense-truncated *GluB5* and an antisense *GluB4*, which can form a hairpin RNA with the *GluB4/5* dsRNA regions. Because dsRNA suppresses the expression of homologous genes, which is referred to as RNAi, this deletion is thought to be a trigger for the suppression of glutelin expression in LGC1[4].

Interestingly, we found that the mutant *glu1*, which is missing a subunit of Glutelin B protein, had a gamma-irradiation-induced deletion of about 129.7 kb. This deletion involved both *GluB5* and *GluB4*, suggesting that both genes were knocked out[7] (Fig. 4). Thus, the difference of deletion size and position between *Lgc1* and *glu1* is thought to cause the differences of phenotypes, suggesting that the regulation of deletion size caused by radiation induced mutation will possibly play important roles in elucidating the function of the genes.

New rice cultivars with low glutelin content and lack of 26-kDa globulin

The content of easy-to-digest protein in LGC1 is about two-thirds that of ordinary cultivars. However, there is room to further decrease this content, because levels of the 26-kDa globulin (another easy-to-digest protein) in LGC1 are slightly greater than in ordinary cultivars. Fortunately, a mutant that is deficient in 26-kDa globulin is available[3]. We therefore aimed to combine the traits of LGC1 and the mutant. We developed two new rice cultivars from a cross between LGC1 and a mutant line of Koshihikari (89WPKG30-433, induced by gamma-irradiation) that was deficient in 26-kDa globulin. The progeny were selected by a pedigree breeding method (Fig. 5), and we evaluated their agronomic characteristics. In 2001, two promising breeding lines were designated as Hoiku 2 and Hoiku 3. Both lines have a low content of easy-to-digest protein; Hoiku 2 is early-maturing and Hoiku 3 is intermediate-maturing. Both are nonglutinous. After testing for local adaptability, specific characters, yield, and dietary performance (Tables 1 & 2), they were officially registered by the Japanese Ministry of Agriculture, Forestry and Fisheries as ‘LGC-Katsu’ and ‘LGC-Jun’. *Katsu* means “vigorous” and *Jun* means “affluent” primarily, but it means “warm-hearted” secondarily in Japanese, respectively.

Because LGC-Katsu matures early it can be grown in northern Japan, from the Kanto region to the south of the Tohoku region. However, it is not cool-tolerant at the booting stage, so cultivation should be limited to regions with low risk of cool weather damage. Because LGC-Jun has much better eating quality than LGC1, patients should find it easier to eat this cultivar every day (Table 2). Judg-

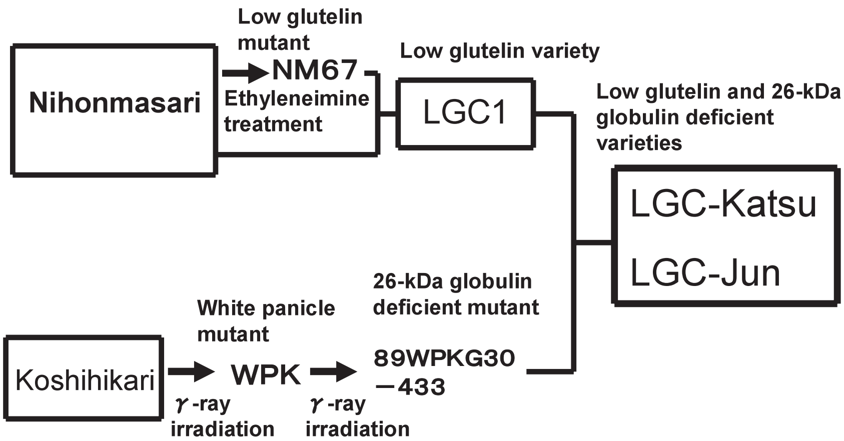

Fig. 5. Genealogy of the new cultivars
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Table 1. Agronomic characteristics of LGC1, LGC-Katsu, LGC-Jun, and Koshihikari

Cultivar name	Heading date	Culm length (cm)	Panicle length (cm)	Yield of brown rice (kg/a)	Lodging degree[1)]	Grain appearance quality[2)]
LGC1	7 Aug.	78	17.9	45.1	2	4.7
LGC-Katsu	30 July	78	17.4	46.6	2	5.5
LGC-Jun	7 Aug.	88	18.0	46.0	3	5.2
Koshihikari	5 Aug.	100	18.5	52.2	5	3.8

1): Lodging was classified into six degrees (0 = standing to 5 = lodged).
2): Grain appearance was classified into nine degrees of quality (1 = excellent to 9 = very bad).
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Table 2. Nutritional quality of milled rice[1)] from LGC1, LGC-Katsu, LGC-Jun, and Koshihikari

Cultivar name	Protein content (% of dry matter)	Easy-to-digest protein[2)] (% of total protein)	Glutelin (% of total protein)	26-kDa globulin (% of total protein)	Prolamine (% of total protein)	Eating quality
LGC1	7.5	55.1	22.1	14.1	44.9	Moderate
LGC-Katsu	7.6	37.8	13.4	0.0	62.2	Moderate
LGC-Jun	7.7	40.6	16.5	0.0	59.4	Good
Koshihikari	7.5	74.6	47.5	9.4	25.4	Superior

1): Rice milled down to 90%.
2): Excluding the 13- and 16-kDa proteins (see Fig. 2).
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ing from its maturation date, LGC-Jun can be grown in the plains of south-western Japan and in the Chugoku, Kinki, Tokai, and Kanto regions. LGC-Jun has intermediate culm length and a partial panicle number type, has high yield, and is moderately resistant to lodging. Accordingly, the use of nitrogen fertilizer must be restricted in the growing of low-protein rice. LGC-Katsu and LGC-Jun are both classified as "moderately susceptible" to leaf blast, and as "susceptible" and "moderately susceptible", respectively, to panicle blast. LGC-Katsu is estimated to possess no true resistance genes[8]. LGC-Jun is estimated to possess the true resistance gene *Pia*[8]. The appearance grades of the brown rice of both are inferior to that of Koshihikari; white core, white belly and chalky rice occur frequently.

The glutelin content of these two new cultivars is reduced to about one-third that of regular cultivars, and 26-kDa globulin is completely absent (Table 2). Consequently, the total amount of easy-to-digest protein in the new cultivars is about half that of regular cultivars, and about 15% less than in LGC1. Accordingly, it is very possible that these cultivars should greatly help in the dietary management of patients with chronic renal failure. They have already been tested in clinical trials for 2 years and are proving even more suitable than LGC1 for use in low-protein diets. In the near future, Japan will be home to a huge number of elderly people, many in poor health. LGC1, LGC-Katsu and LGC-Jun have been developed as part of a movement to breed new crop cultivars with functional ingredients that can improve human health. In the future, such cultivars will also become useful in other countries.

Future prospects of rice breeding for low levels of easy-to-digest protein

What steps should we take to decrease easy-to-digest protein to levels even lower than those in LGC-Jun or LGC-Katsu? Ohyama et al.[10] reported the genetic transformation of rice plants with chimeric antisense cDNA for glutelin to achieve lower glutelin content than that of LGC1.

To avoid the issue of public acceptance of genetically modified crops, in Japan we need to use artificial mutants rather than modification through gene transfer in order to decrease easy-to-digest protein to levels even lower than those in LGC-Jun or LGC-Katsu. In these two cultivars, combining the effects of two genes, *Lgc1* and *glb1* which causes 26-kDa globulin deficiency[3], brought further decrease in the content of glutelin, lower than in the case of LGC1. Glutelin is a major rice seed storage protein and is coded by a multigene family. Depending on the homology, it is classified into two subfamilies, *GluA* and *GluB*, which show about 65% of homology in their amino-acid sequences[11]. Because *Lgc1* associates with a 3.5-kb deletion between two highly similar and tandem repeated glutelin genes, *GluB5* and *GluB4* with a tail-to-tail inverted arrangement, *Lgc1* can suppress the production of GluB protein sufficiently, but there is not necessarily adequate suppression of GluA protein production[4]. Consequently, there is still room for a further decrease in levels of easy-to-digest protein. The whole genome sequence revealed by genome projects has brought to light three genes that are responsible for the production of GluA protein. Two of them (*glu2* and *glu3*) have already been isolated by gamma-ray irradiation[2]. To isolate this unknown fifth gene (i.e. in addition to the 4 genes *Lgc1*, *glb1*, *glu2*, and *glu3*), screening by a reverse genetic approach will be important. The accumulation of these five genes will produce a line that is almost completely free of glutelin. This will be the ultimate rice low in easy-to-digest protein. To accumulate the five genes, DNA marker-assisted selection is an indispensable tool, because each of the phenotypes expressed by the 5 genes is difficult to discriminate clearly by SDS-polyacrylamide gel electrophoresis. This research will not be only marker-assisted selection breeding, but also post-genome breeding that will follow the whole genome sequence mapping.

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