

## New rice cultivars with low levels of easy-to-digest protein

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### Abstract

Rice seed contains 2 major proteins: glutelin and prolamine. Glutelin accumulates in protein body type II (PB-II), and prolamine in PB-I. PB-II is easily digested by humans, but PB-I is indigestible. We previously developed a new rice cultivar, LGC1, from 'Nihonmasari' by means of ethyleneimine treatment. LGC1 seed contains low levels of glutelin and high levels of prolamine. This makes LGC1 suitable for use by patients with chronic renal failure, even though the total protein content is nearly the same as in the original cultivar. In the present study, we bred 2 new cultivars from a cross between LGC1 and a mutant line of 'Koshihikari' that is deficient in 26-kDa globulin. Their glutelin content is about 1/3 of that of regular cultivars, and they completely lack 26-kDa globulin. Overall, these cultivars have about half the easy-to-digest protein content of regular cultivars. They are thus even more suitable for use in a low-protein diet than LGC1.

### Media summary

New rice cultivars with a low content of easy-to-digest protein were developed. They are suitable for patients with kidney disease.

### Key words

diet therapy, dietary food, endosperm, mutation breeding, *Oryza sativa*

### Introduction

In Japan, the steep rise of medical expenses has become an object of public concern, particularly 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 about 200,000. About 500,000 people manage chronic renal failure without blood dialysis through therapy based on a low-protein diet. They require specially processed low-protein foods. The use of hyper-polished rice or starch rice is the main method of restricting their intake of protein, but these products are very expensive compared with ordinary rice. Rice cultivars with low protein content would offer a cheaper diet.

Usually, rice seed contains about 7% protein, and rice supplies about 15% of the average dietary intake of protein in Japan. Rice seed contains 2 major proteins: glutelin and prolamine. Glutelin accumulates in protein body type II (PB-II), and prolamine in PB-I (Tanaka *et al.*, 1980). PB-II is easily digested, but PB-I is indigestible (Ogawa *et al.*, 1987). Through the use of mutation breeding, we developed a new cultivar, LGC1, from 'Nihonmasari', with a low glutelin and high prolamine content (Iida *et al.*, 1993). 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. The glutelin content of LGC1 is reduced and the contents of other storage proteins, including prolamine, are increased compared with more common rice cultivars (Iida *et al.*, 1993). Because

of the indigestibility of prolamine, LGC1 can be used as a low-protein rice. It is thus useful for people who must restrict their protein intake, such as patients with kidney disease (Mochizuki and Hara, 2000).

The molecular characterization of *Low glutelin content 1 (Lgc1)* has been recently reported. *Lgc1* is a dominant mutation that suppresses expression of the glutelin multigene family. It causes a 3.5-kb deletion between 2 *GluB* genes that form a tail-to-tail inverted molecule, which is thought to be a trigger for post-transcriptional gene silencing (PTGS) (Kusaba *et al.*, 2003). It is very rare for a single study to simultaneously reveal both the molecular characterization of a gene and its use.

The content of easy-to-digest protein in LGC1 is about 2/3 that of ordinary cultivars. But there is room to further decrease the content, because levels of the 26-kDa globulin in LGC1 are slightly increased compared with ordinary cultivars. Fortunately, a mutant that is deficient in 26-kDa globulin is available (Iida *et al.*, 1998). In the present study, we aimed to combine the traits of LGC1 and the mutant.

## Methods

We developed 2 new rice cultivars from a cross between LGC1 and a mutant line of Koshihikari (89WPKG30-433) that is deficient in 26-kDa globulin. Both have a low content of easy-to-digest protein and are nonglutinous: LGC-Katsu is early-maturing and LGC-Jun is intermediate-maturing. (*Katsu* means vigorous and *Jun* means warm-hearted in Japanese.) After testing for local adaptability, specific characters, yield, and dietary performance, they were registered with the Japanese Ministry of Agriculture, Forestry and Fisheries.

## Results and discussion

The glutelin content of these 2 new cultivars is reduced to about 1/3 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. They should thus greatly help in the dietary management of patients with chronic renal failure.

LGC-Katsu matures early, so it can be cultivated in northern Japan, from the Kanto region to the 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 a much better eating quality than LGC1, patients should find it easier to eat this cultivar every day. Judging from its maturation date, LGC-Jun can be grown in the plains of southwestern Japan, in the Chugoku, Kinki, Tokai, and Kanto regions. LGC-Jun is intermediate in 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.

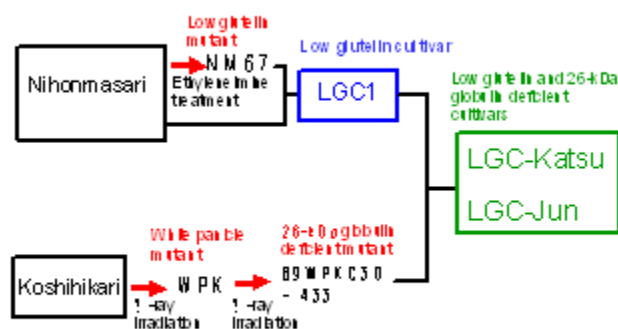


Fig. 1 Genealogy of the new cultivars

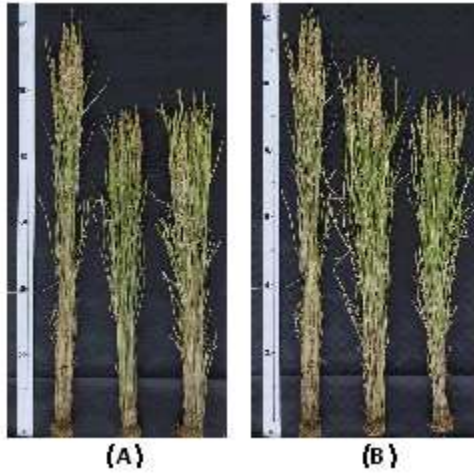


Fig. 2 Plant type. (A) From left, Koshihikari, LGC-Katsu, and Shunyo (a new rice cultivar with LGC1-type protein). (B) From left, Koshihikari, LGC-Jun, and LGC1.

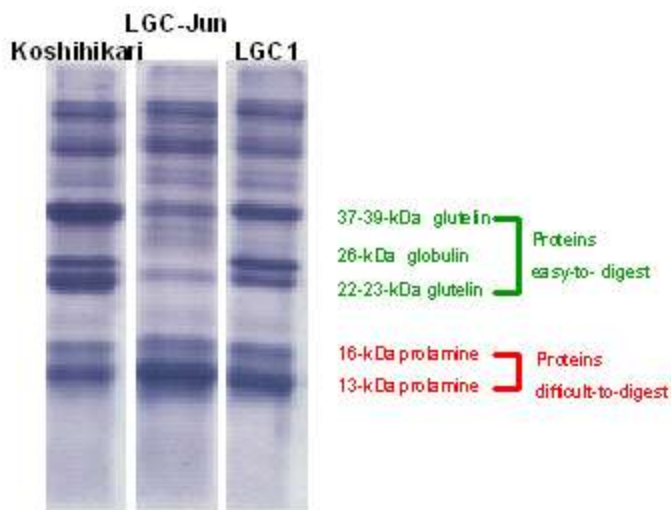


Fig. 3 SDS-PAGE analysis of total proteins in brown rice

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 (0 = standing, 5 = lodged)
LGC1	7 Aug.	78	17.9	45.1	2
LGC-Katsu	30 July	78	17.4	46.6	2

LGC-Jun	7 Aug.	88	18.0	46.0	3
Koshihikari	5 Aug.	100	18.5	52.2	5

**Table 2. Nutritional quality of LGC1, LGC-Katsu, LGC-Jun, and Koshihikari**

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

a: Excluding the 13- and 16-kDa proteins.

## Conclusion

In the near future, Japan will be home to a huge number of elderly people, many in poor health. The LGC1, LGC-Katsu, and LGC-Jun cultivars have been developed as part of a movement to breed new crop cultivars with functional ingredients that improve human health. In the future, such cultivars will also become useful in other countries.

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