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A rice (*Oryza sativa* L.) mutant having a low content of glutelin and a high content of prolamine

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Abstract. Among the mutant lines of rice that have been selected for morphological characters, one line, NM67, was found to have a low content of glutelin and a higher content of prolamine in its seed protein than other Japanese cultivars. This mutant is a semi-dwarf and partially sterile line, and its leaves turn yellow before heading. Genetic analysis after backcross to the original cultivar, 'Nihonmasari', revealed the following: (1) the character of low glutelin content was always accompanied by the character of high prolamine content; (2) the low glutelin (and high prolamine) character seemed to be manifested by a single dominant gene; and (3) semi-dwarfness, low fertility and early yellowing leaf of the mutant, which might also be pleiotropy, were controlled by a single recessive gene independent of the gene for protein content. The protein character of NM67 was genetically separated from semi-dwarfness and low fertility, and a new line having low glutelin content and high prolamine content with normal morphological characters comparable to those of the original cultivar was obtained from the progenies of the cross. The possible use of this line as a low protein rice cultivar is discussed.

Key words: Mutation breeding – Seed protein – Glutelin – Prolamine – Rice – *Oryza sativa*

Introduction

Rice is considered to be a starchy crop because of its relatively low protein content, which ranges from 7% to 10% of the grain weight. In Asian countries, however, the contribution of rice as a protein source is important; the Japanese, for example, consume approximately 19% of their protein in the form of rice. The nutritional value of rice could thus be raised by increasing its protein content. On the other hand, the character of low protein content is also an important target of rice breeding. Low-protein rice is required for the diet of patients with kidney disease. A negative correlation has been found between protein content in rice and eating quality (Yamashita and Fujimoto 1974). In an effort to vary protein content in rice, mutants with high or low levels of seed protein have been selected for (Kataoka 1974; Tanaka 1974). Unfortunately, the numbers of genes controlling the protein content of these mutants have not been studied, and due to high fluctuations in protein content from year to year, these mutants have not been used as breeding materials.

Glutelin is the major storage protein and accounts for 80% of the total protein found in the rice grain (Cagampang et al. 1966); prolamine is the second most important. Mutation of a gene or genes controlling the synthesis of glutelin or prolamine will result in an alteration in the seed-protein content. Using SDS-polyacrylamide gel electrophoresis, Kumamaru et al. (1988) selected several mutants with low amounts of prolamine. Recently, we found a new type of mutant that had a low glutelin content and a high prolamine content. In this report, we describe the characteristics of this mutant, the results of genetic analysis and the possible use of this mutant as a low-protein rice cultivar.

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Materials and methods

Materials

Seed proteins of 1,433 mutant lines of rice (*Oryza sativa* L. var japonica) that had been selected for morphological characters and maintained by selfing for more than five generations were analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE). These mutants had been obtained by treating the seeds of cvs 'Norin 8' and 'Nihonmasari' with gamma radiation from a [^{60}Co] source, or ethyleneimine or ethylmethane sulfonate.

SDS-PAGE

A single grain of brown rice was crushed with pliers and immersed overnight in 700 µl SDS-urea solution, 8 *M* urea, 4% sodium laurl sulfate, 5% 2-mercaptoethanol, 20% glycelin and 50 m*M* Tris-HCl buffer, pH 6.8. After centrifugation at 7,000 *g* for 5 min, 5 µl of supernatant was subjected to SDS-PAGE. SDS-PAGE was carried out according to Laemmli (1970) on a 15% acrylamide gel. After electrophoresis, the gel was stained by Coomassie Brilliant Blue R-250 (CBB-R250). Amounts of polypeptides were measured using a densitometer (Toyo Kagaku, DMC-33C).

Globulin-albumin, prolamine and glutelin were extracted sequentially from seeds by three solvents according to Kumamaru et al. (1988). Polished grains (ca. 93% polished) were crushed and then powdered using a mortar and pestle. For extraction of the globulin and albumin, 100 mg powder was suspended in 1.0 ml of a 50 m*M* KH_2PO_4-NaOH (pH 6.8) solution containing 0.5 *M* NaCl and 1 m*M* EDTA-2Na in a centrifuge tube, and shaken for 6 h at 4 °C. The suspensions were homogenized by a microhomogenizer (Physcotron, Niti-On Co) for 1 min and centrifuged at 10,000 *g* for 15 min at 4 °C. The extraction was repeated 3 times. Prolamine was extracted from the precipitate with 1 ml of 60% *n*-propanol, and the extraction was repeated 3 times at intervals of 6 h. Finally, glutelin was extracted with 1.0 ml of 1% lactic acid containing 1 m*M* EDTA-2Na in the same way as the extraction of prolamine.

One milliliter of 10% trichloroacetic acid (TCA) was added to 300 µl of the albumin-globulin and glutelin extracts, respectively. Three hundred microliters of the prolamine extract was concentrated by evaporation to 100 µl at 80 °C, then 1.0 ml TCA was added. Each fraction was centrifuged at 10,000 *g* for 15 min. The TCA solution was then removed, and the precipitates were resolved in a 100 µl SDS-urea solution for analysis by SDS-PAGE.

Two-dimensional gel electrophoresis

For detailed analysis, 40 mg of the powdered grain was dissolved in 400 µl lysis buffer solution (8 *M* urea, 2% Triton X-100, 1.6% Pharmalyte pH 5–8, 0.4% Pharmalyte pH 3–10, 5% 2-mercaptoethanol), and this solution was directly subjected to two-dimensional gel electrophoresis according to O'Farrell (1975). In the first dimension, 3% polyacrilamide gels containing 2.5% Pharmalite pH 5–8, 2.5% Pharmalite pH 3–10 and 8 *M* urea were used; 20 µl of each sample was applied on the anode side. In the second dimension, 14% polyacrilamide gels containing 0.1% SDS were used. The gels were stained after electrophoresis by CBB-R250.

Genetic analysis

The mutant line and its original cultivar were crossed reciprocally, and single or half grains of F_1 and F_2 seeds were used for protein analysis. F_1 plants were grown in 1/5000a Wagner pots, and F_2 plants in the field. Fifteen F_3 seeds set on each F_2 plant after self pollination were separately analyzed to identify the genotype of the F_2 plant.

Agronomic characteristics

Agronomic characteristics of the mutant line were investigated using plants grown in a paddy field and compared with those of the original cultivar. Total protein was extracted by boiling the rice powder in 0.1 *N* NaOH and measured according to the method of Lowry et al. (1951).

Results

Selection of a low-glutelin mutant line

The polypeptide bands corresponded to the 13-kDa, 16-kDa, 22- to 23-kDa, 26 kDa, 37- to 39-kDa and 57 kDa polypeptides reported by Yamagata et al. (1982). The polypeptides of 37-39 kDa consisted of four minor bands, and the 22- to 23-kDa polypeptides, of three minor bands. The polypeptide band of 13 kDa was composed of two minor bands. There was no significant variation in the density of each polypeptide among ordinary Japanese cultivars (Kagawa et al. 1988). We designated this type of protein composition as the original type.

Out of 1,433 lines, a mutant, NM67, obtained from cv 'Nihonmasari' treated with 0.2% ethyleneimine, had thin bands of the 37- to 39-kDa and 22- to 23-kDa polypeptides in the glutelin fraction, and dense bands of the 13-kDa polypeptide in the prolamine fraction and 16-kDa and 26-kDa polypeptides in the albumin

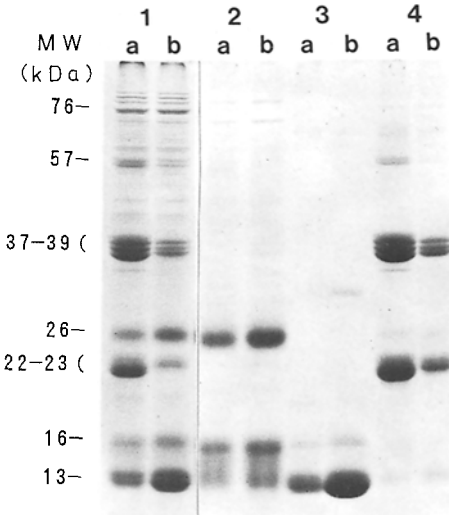

Fig. 1. SDS-PAGE analysis of the rice seed proteins of a mutant line, NM67 (*b*), and the original cultivar, 'Nihonmasari' (*a*). *Lane 1* total proteins, *Lane 2* globulin-albumin fraction extracted by 0.5 *M* NaCl, 50 m*M* KH_2PO_4-NaOH, *Lane 3* prolamine fraction extracted by 60% *n*-propanol, *Lane 4* glutelin fraction extracted by 1% lactic acid

and globulin fraction (Fig. 1). In addition to having a low density of glutelin polypeptides, NM67 lacked the largest polypeptide band of the four minor bands of the 37- to 39-kDa polypeptides and had a reduced density of the smallest polypeptide band of the three minor bands of the 22- to 23-kDa polypeptide. In the glutelin fraction, only a decrease in the amounts of the 37- to 39-kDa and 22- to 23-kDa polypeptides without degraded polypeptide bands was found. Two-dimensional gel electrophoresis showed the deletion of one spot of the highest molecular weight among several spots of the 37- to 39-kDa glutelin subunits in NM67 (Fig. 2).

Densitometric analysis of total protein showed that in NM67 the 37- to 39-kDa and 22-to 23-kDa polypeptides comprised 13% and 7.3% of the seven major polypeptides, respectively, while in 'Nihonmasari' they comprised 29% and 23%, respectively. The proportion of the 13-kDa polypeptide increased to 41% in NM67 from 17.6% in 'Nihonmasari'. A small increase in the 26-kDa polypeptide was also found in NM67 (Table 1).

Genetics of mutant characters

Seeds of F_1 hybrids of reciprocal crosses between NM67 and 'Nihonmasari' showed low glutelin and high prolamine characters, suggesting that the mutant gene had a dominant nature. In F_2 seeds, the low glutelin character was always accompanied by the high prolamine character. This might be due to pleiotropy or a compensatory metabolic reaction, although further investigation is needed. The mutant character of low glutelin (and high prolamine) and original type were segregated in the proportions of approximately 3:1 [167:73 in 240 F_2 seeds, $\chi^2(3:1) = 3.76$]. These results might indicate that the low glutelin (and high prolamine) character of NM67 is controlled by a single dominant gene.

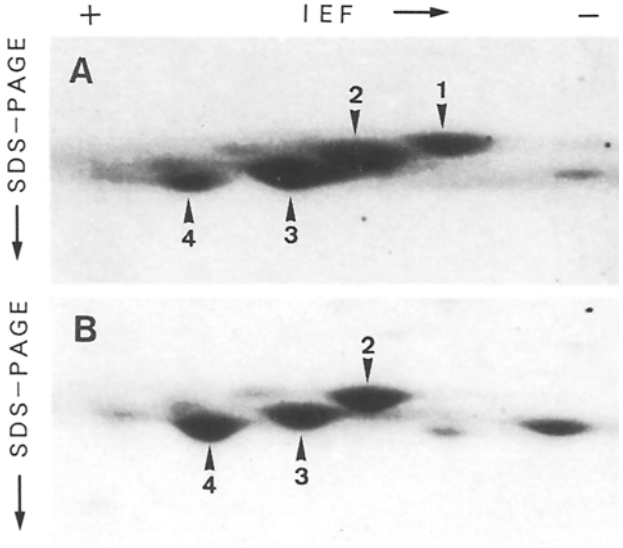

Fig. 2A, B. Two-dimensional electrophoresis of glutelin-α subunits of cv 'Nihonmasari' (**A**) and NM67 (**B**). The spots were numbered in the order of their decreasing molecular weights

NM67 also had a character of yellowing leaves before heading (early yellowing), as NM67 had been selected as a semi-dwarf mutant with low fertility. Our investigation of the F_1 and F_2 plants indicated that the early yellowing character is controlled by a single recessive gene. All early-yellowing plants in the F_2 were semi-dwarf and partially sterile, while green plants were without exception normal in plant height and seed fertility. The semi-dwarfness and low fertility of NM67 are considered to be an expression of pleiotropism of the gene that controls early yellowing.

The genotypes of the F_2 plants were examined by analyzing each F_3 seed. This progeny test revealed that the segregation ratio of low glutelin (and high prolamine) homozygotes, their heterozygotes and homozygotes of the original type were 1:2:1 in both green and yellowing plants (Table 2). These results suggest that the gene for low glutelin (and high prolamine) is independent of, or not linked to, that of early yellowing ($\chi^2 = 1.85$).

Selection of LGC-1 and its agronomic characteristics

In the course of this genetic analysis, 41 lines homozygous for the low glutelin (and high prolamine) gene were selected. Out of these 41 lines, 7 were homozygous for normal green leaf. We named 1 of these lines LGC-1.

In addition to the traits mentioned above, NM67 was characterized by having fewer panicles per plant, a lower seed weight and a higher protein content than its original cultivar, 'Nihonmasari'. However, LGC-1 had a similar level of productivity and protein content as 'Nihonmasari', even though LGC-1 was slightly shorter than 'Nihonmasari' (Table 3). Most of the seeds of NM67 were immature and their surface was rough.

Table 1. Percentage of major polypeptides contained in brown rice of 'Nihonmarari' (NM) and NM67

MW (kDa)	76	57	37–39 (glutelin-α)	26 (globulin)	22–23 (glutelin-β)	16	13	Total
NM	4.8	8.8	29.0	8.0	23.0	8.8	17.8	100(%)
NM67	4.0	7.6	13.0	16.5	7.3	10.5	41.1	100(%)

Table 2. Segregation of low-glutelin trait and early yellowing in F_3 of the cross between NM67 and 'Nihonmasari'

Leaf type	Homozygotes of low glutelin	Heterozygotes of low glutelin	Homozygotes of original type	Total	χ^2 (1:2:1)
Normal	41	81	49	171	1.22
Early yellowing	14	28	10	52	0.92
Total	55	109	59	223	0.26
χ^2(3:1)	0.01	0.03	2.04	0.89	

Table 3. Characteristics of the low-glutelin mutant (NM67), its improved line (LGC-1) and the original cultivar ('Nihonmasari')

	Characters	NM67	LGC-1	Nihonmasari
	Plant height (cm)	71.8**	91.3**	96.1
	Culm length (cm)	50.9**	69.1**	73.4
	Protein content (%)	10.5**	7.6	7.5
A	Number of panicles in a plant	7.3**	12.9	13.1
B	Number of flowers in a panicle	76.9*	88.5	88.2
C	Number of fertile seeds in a panicle	27.3**	81.3	81.5
D	Seed fertility (%) (C/B)	43.9**	92.1	92.4
E	Weight of 1,000 grains (g)	17.1**	21.4	22.2
	Estimate of yield per plant (g) (A × B × D × E)/100,000	4.2**	21.4	23.7

* Significant at 5% level in t-test between 'Nihonmasari' and the mutant line; ** significant at 1% level in t-test

Seeds of LGC-1 were as normal as those of the original cultivar. Several undesirable characters of NM67 were considered to be caused by genes other than that of the low glutelin (and high prolamine) gene.

Discussion

A new type of mutant, NM67, which had a low glutelin content, high prolamine content and a slightly higher globulin content, was found by screening lines using SDS-PAGE. Besides a low content of total glutelin, NM67 lacked the largest polypeptide of the 37- to 39-kDa subunits of glutelin. NM67 is a mutant line previously selected as a semi-dwarf and partially sterile mutant in progenies of cv 'Nihonmasari' treated with ethyleneimine. On the basis of genetic analysis we suggest that a single dominant gene seems to control both the low glutelin and high prolamine characters and that a single recessive gene governs early-yellowing leaf, which might be pleiotropy with semi-dwarfness and low fertility. These genes were transmitted independently to progenies. It can be inferred that two mutation events occurred at different sites of chromosomes by ethyleneimine treatment and that both mutant genes have been maintained in NM67 as a homozygote.

Most of the mutant phenotypes observed in seed protein are controlled by a recessive gene: for example, in maize, *opaque-2* (Mertz et al. 1964), *floury-2* (Nelson et al. 1965; Hansel et al. 1973), *opaque-7*, *brittle-2* (Misra et al. 1972) and *opaque-6* and *floury-3* (Ma and Nelson 1975). Only a few dominant genes have been reported so far: for example, *Mc* and *De*-30*, which alter the content of zein (Salamini et al. 1983a, b). The low glutelin (and high prolamine) gene is one of the rare examples of dominant mutated genes controlling seed-protein content.

In rice endosperm, glutelin is stored in protein body II (PB II), while prolamine is stored in protein in body I (PBI) (Tanaka et al. 1980). PBI is resistant to autoclaving and to degradation by proteolytic enzymes such as pepsin in the human body (Ogawa et al. 1987), while PB II can be easily digested in the human body. The presence of rice protein bodies in human feces have been observed by electron microscopy, and these protein bodies are considered to be PBI (Tanaka et al. 1975a, b). This evidence suggests that prolamine in PBI is nutritionally less meaningful for human consumption than glutelin in PB II. Although the proportion of prolamine in cooked rice digested in the human body has not been evaluated, the low glutelin and high prolamine mutant line may be used as a low protein genetic resource in rice. An improved low glutelin and high prolamine line (LGC-1) has been developed from a backcross between NM67 and the original cultivar, 'Nihonmasari'. The productivity of this line is comparable to that of 'Nihonmasari', which is still widely

planted. Provided that no 13-kDa polypeptides of prolamine can be digested by humans, digestible protein in LGC-1 is estimated to be 72% of that found in 'Nihonmasari'. A limited intake of protein is required for patients with kidney disease. Whether this mutant rice could be used as a dietary measure for patients with kidney disease is now under investigation.

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