Theor Appl Genet (1997) 94: 177–183

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Mutants lacking glutelin subunits in rice: mapping and combination of mutated glutelin genes

Received: 1 April 1996 / Accepted: 14 June 1996

Abstract Nine mutant lines lacking glutelin subunits were selected from M_2 seeds of about 10 000 M_1 plants mutagenized with gamma rays or EMS and from 1400 mutant lines selected originally for morphological characters. There were three types of mutants, one line lacking the largest subunit among four minor bands of glutelin acidic subunits (Type 1), five lines lacking the second largest subunit band (Type 2), and three lines lacking the third largest subunit band (Type 3). Mutants lacking the smallest subunit band were not found. Type 1 lacked 2 of the 10 spots of glutelin acidic subunits separated by two-dimensional electrophoresis and 1 of the 11 spots of the 57-kDa glutelin precursor. Type 2 lacked 2 spots of acidic subunits and 1 spot of the 57-kDa glutelin precursor, and had low amounts of 1 of the 8 spots of glutelin basic subunits. Type 3 mutants lacked each of 1 spot of the acidic subunits and glutelin precursor and had low amount of 1 spot of the basic subunits. Genetic analysis of the mutated genes showed that these mutant characters were controlled by single recessive genes named *glu-1*, *glu-2*, and *glu-3*, respectively. Mutated genes of different lines of the same type were found to be at the same locus. RFLP analysis of F_2 plants between the mutant lines and cv 'Kasalath' indicated that *glu-1* is on chromosome 2, *glu-2* on chromosome 10, and *glu-3* on chromosome 1. These mutant genes were combined by crossing, and a line lacking the 3 minor bands of the glutelin acidic subunits was developed. However, the total glutelin content of this line was not remarkably reduced, showing a only 13% decrease.

Key words Rice · Mutation breeding · Glutelin subunit · RFLP mapping · Low glutelin

Communicated by G. Wenzel

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Introduction

In breeding rice for improved endosperm protein, the main target has been to increase total protein content. However, rice having a low protein content is also required. Protein content in rice endosperm correlates negatively with taste. Low-protein rice is required in the production of rice wine, sake, and as a diet for patients with kidney disease. The most abundant protein in rice endosperm is glutelin, which accounts for 60% of the total endosperm protein. Glutelin gene expression and the localization of glutelin in endosperm have been intensively studied (Tanaka et al. 1980; Yamagata et al. 1982; Li et al. 1993). Glutelin is synthesized by a multi-gene family, and more than ten glutelin genes have been isolated and sequenced. However, there are few reports on the mapping of the glutelin genes (Suzuki et al. 1991, Nakamura et al. 1995).

Mutation induction by gamma-ray irradiation or chemical mutagen treatment has made it possible to obtain various rice mutants having altered seed protein composition (Kumamaru et al. 1988, Nishio and Iida 1993; Iida et al. 1993). Recently, we found several mutants lacking some glutelin subunits. Genetic analysis of these mutants suggested that the mutated genes are the glutelin genes. Using these mutants, we were able to elucidate members of the acidic subunits, basic subunits and 57-kDa glutelin precursors encoded by each glutelin gene, and the glutelin genes were mapped on the rice chromosome. We also evaluated these mutant lines as breeding materials for developing low-glutelin rice.

Materials and methods

Mutation induction and selection of mutants

Dry seeds of rice, *Oryza sativa* L. cv 'Koshihikari' were irradiated with gamma rays at 200 Gy and 300 Gy or treated with 0.1 *M* and 0.2 *M* ethylmethane sulfonate (EMS) and then sown to grow M_1 plants. M_2 seeds from 10 000 M_1 plants were used for the screening of mutants. Mutant lines of the genetic stock of the Institute of Ra-

diation Breeding were also used for the screening. These mutant lines have been maintained by selfing for more than five generations.

SDS-PAGE for screening of mutants was carried out according to Nishio and Iida (1993).

Two dimensional electrophoresis

After removal of the embryos, the rice grains were crushed with pliers. The protein was extracted from 40 mg of rice powder with 400 µl lysis buffer containing 8 *M* urea, 2% Triton X-100, 1.6% Pharmalyte 5-8, 0.4% Pharmalyte 3-10, 5% 2-mercapthoethanol. After centrifugation at 7000 *g* for 5 min., the supernatant was loaded onto polyacrylamide gel containing 1.7% Ampholine pH 3.5–10, 3.3% Pharmalite pH 8.5–10, and 8 *M* urea in a glass tube for non-equilibrium pH gradient gel electrophoresis (NEPHGE). The protein was run from the anode side under a voltage of 200 V for 20 min., 300 V for 20 min. and 400 V for 3 h. After electrophoresis, the gel was removed from the glass tube, and equilibrated for 30 min in 0.062 *M* TRIS-HCl buffer containing 10% glycerol, 5% mercapthoethanol, and 2.3% SDS. The gel was placed horizontally on a stacking gel of 4% acrylamide made on a separation gel of 14% acrylamide (acrylamide: *N*,*N*′-methylenebisacrylamide=38.9:1.1) containing 0.1% SDS. After electrophoresis under a constant current at 50 mA/gel, the gel was stained with Coomassie Brilliant Blue R250.

Genetic analysis and gene mapping

F_1 and F_2 seeds of the crosses between the mutant lines and their original cultivars or between mutant lines were used for genetic analysis of mutated genes.

In order to map the mutated genes using restriction fragment length polymorphism (RFLP) analysis, the mutant lines were crossed with an indica cultivar, 'Kasalath', and proteins in half grains of the F_2 seeds were analyzed. The other half grains with embryos were sown in petri dishes and genomic DNA was extracted from leaves of the plantlets grown in pots for 1 month using the CTAB method (Murray and Thompson 1980). DNA markers for gene mapping were provided by Dr. A. Saito of the Kyushu National Agriculture Research Station and Dr. N. Kurata of the Rice Genome Team at the National Institute of Agrobiological Resources. RFLP was analyzed by the Dig-labeling procedure according to the manufacturer's instructions (Boeringer).

DNA samples from 15 plants grown from the F_2 seeds lacking glutelin subunits were analyzed to find a linked marker. The DNA markers which gave segregation rates deviating from 1:2:1 in this analysis were used in an RFLP analysis of 181–288 F_2 plants. The distance between the mutated gene and the DNA marker was estimated using the MAPL program (Ukai 1989).

Analysis of the glutelin gene

The glutelin multi-gene family has been classified into two subfamilies, GluA and GluB (Takaiwa et al. 1989). A cDNA clone of GluA-1, pREE61, and a cDNA clone of GluB-1, pREEK1, were used as probes for the RFLP analysis of genomic DNA. After screening the restriction endonucleases which give different band patterns between 'Koshihikari' and 'Kasalath', we analyzed the RFLPs of homozygotes of the mutated genes in the F_2 between the mutant and 'Kasalath'.

Combining the mutated genes

The mutant line lacking the largest acidic subunit was crossed with that lacking the second largest acidic subunit. The seeds of the F_2 lacking both glutelin subunits were selected by protein analysis of the half grain, and the other half seeds having embryos were sown to produce a line homozygous for the mutated genes. This line was again crossed with the line lacking the third largest acidic subunits to develop a line lacking these three glutelin subunits. Total protein content of the seeds of this line and of that of the original cultivar were compared using the method of Lowry et al. (1951).

Results

Selection of mutant lines lacking glutelin subunits

In our SDS-PAGE analysis, the acidic subunits of glutelin in common *japonica* rice cultivars, e.g. 'Koshihikari', were separated into 4 minor bands ranging from 37 kDa to 39 kDa, which were named a-1, a-2, a-3, and a-4 starting from the largest subunit band (Fig. 1). In the screening of M_2 seeds from about 10000 M_1 plants, 8 mutants lacking a-1, a-2, and a-3 were selected, with 1 mutant lacking a-1 (Type-1), 4 lacking a-2 (Type-2), and 3 lacking a-3 (Type-3) (Table 1). One mutant lacking a-2 was also found in the 1400 mutant lines of the genetic stock.

By conventional two-dimensional electrophoresis, isoelectric focusing in the first dimension and SDS-PAGE in the second dimension, acidic subunits of glutelin in 'Koshihikari' were separated into 10 spots, but basic subunits were not detected. In this electrophoresis system, isoelectric focusing using carrier ampholite ranging from pH 3 to pH 10 was used. The basic subunits may have run right through the polyacrylamide gel or may not have entered the gel. Therefore, non equilibrium pH gradient gel electrophoresis was used for the separation of the first dimension. The basic subunits of glutelin were separated into 8 spots in this analysis. Acidic subunits were separated into 10 spots, and the glutelin precursor of 57 kDa into 11 spots (Fig. 2A-C).

In 85KG30-756 (hereafter abbreviated to 756), a Type-1 mutant, 1a and 1b spots of the acidic subunits (Fig. 2A-1) and glutelin precursor spot No. 4 (Fig. 2C-1) were deleted, while the deletion of basic subunit spots was not detected (Fig. 2B-1). M539, a Type-2 mutant, lacked acidic subunit 2a and 2b spots (Fig. 2A-2) and the glutelin precursor spot No. 8 (Fig. 2C-2); basic subunit spots 1a and 1c were decreased in this mutant (Fig. 2B-2). In 90WPKE0.1–561 (hereafter abbreviated to 561), a

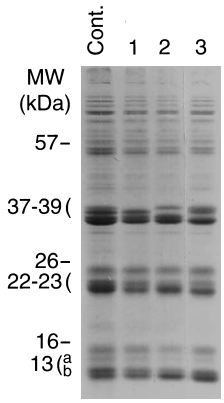

Fig. 1 SDS-PAGE analysis of the seed proteins in mutant lines. The 37-to 39-kDa glutelin acidic subunits in the original cultivar 'Koshihikari' were separated into 4 minor bands. These were named a-1, a-2, a-3, and a-4 starting from the largest subunit band. *Lane 1* a-1 deletion (Type 1), *lane 2* a-2 deletion (Type 2), *lane 3*: a-3 deletion (Type 3)

Table 1 List of rice mutants lacking glutelin subunits

Name of lines	Original variety	Mutagen	Fertility of homozygote[a]	Other characteristics
Type-1: a1 deletion				
1. 88KG30-756	Koshihikari	γ-ray	+	
Type-2: a2 deletion				
2. M539	Norin8	γ-ray	+	Hindered heading
3. 88KG30-913	Koshihikari	γ-ray	+	
4. 88KG30-958	Koshihikari	γ-ray	+	
5. 88KG30-993	Koshihikari	γ-ray	+	
6. 87KE0.1-397	Koshihikari	EMS	+	
Type-3: a3 deletion				
7. 87KG20-130	Koshihikari	γ-ray	–	Endosperm with a small white core
8. 89WPKG30-16	Koshihikari	γ-ray	+	Dwarf
9. 90WPKE0.1-561	Koshihikari	EMS	+	Semi-dwarf
Total 9 lines				

[a] +, fertile; –, sterile

Type-3 mutant, deletion of acidic subunit spot 3a (Fig. 2A-3) and glutelin precursor spot No. 6 (Fig. 2C-3) was found. A decrease in basic subunit 2a spot was also detected (Fig. 2B-3).

The visual characters of 756, Type-1, and 88KG30-913, 88KG30-958 and 87KE0.1–397 (hereafter abbreviated to 913, 958, and 397, respectively), Type-2, were not different from those of the original cultivar, 'Koshihikari', while the panicle of M539, Type-2, protrudes incompletely from the leaf sheath and the seed fertility of 89WPKG30-16 (hereafter abbreviated to 16) and 561, Type-3 was low. These characters of M539, 16, and 561 were separated from the protein character by crossing with their original cultivars, 'Norin 8' for N539 and 'Koshihikari' for 16 and 561. In 87KG20-130 (hereafter abbreviated to 130), seeds lacking a-3 gave completely sterile plants (Table 1).

Genetic analysis of mutant characters

In the F_2 seeds of all the crosses between mutant lines and their original cultivars seeds having thin bands of glutelin were found as well as those lacking the bands. The segregation ratio of normal seeds, the seeds having the thin bands, and those lacking the bands corresponded to 2:1:1, which is expected from the assumption that the normal seeds have the genotype *AAA* or *AAa*, the seeds having the thin bands have the genotype *Aaa*, and the seeds lacking the glutelin bands have the genotype *aaa* (Table 2).

Different mutant lines of the same type were crossed with each other to elucidate allelic relationships between the mutated genes. Five mutant lines belonged to Type-2. All F_1 seeds of reciprocal crosses 913×958, 958×397, and 958×993 lacked a-2. All F_2 seeds of the crosses M539×913, 913×M539, M539×397, 958×913, 993×958, 397×913, and 958×397 also lacked a-2. Hence, all 5 lines were considered to have the mutation at the same locus.

Table 2 Genetic analysis of the mutant character using F_2 seeds

Type	Mutant lines	Segregation ratio of thickness of bands[a]	χ^2 (2:1:1)
1	88KG30-756	82:42:36	0.55
2	88KG30-913	78:46:36	1.35
2	M539	47:22:11	5.48
2	88KG30-958	53:35:32	1.78
2	88KG30-993	123:51:66	1.2
2	87KE0.1-397	40:23:17	0.9
3	88KG20-130	84:38:38	0.4
3	90WPKG30-16	63:32:25	1.12
3	90WPKE0.1-561	53:37:31	2.45

[a] Normal: low glutelin subunit: glutelin subunit deletion

Among 3 mutant lines lacking a-3, 16 and 561 were shown to have a mutation at the same locus based on an analysis of F_1 seeds of their reciprocal crosses. Homozygotes of the mutated gene of 130 were sterile, and therefore this mutant line was maintained by selfing of the heterozygotes. The heterozygote was crossed with 16, and the seeds on the F_1 panicles were analyzed. About half of the F_1 plants set only seeds lacking a-3, suggesting that the mutated gene of 130 is also at the same locus as that of 16.

The mutant characters of the a-1, a-2, and a-3 deletion were found to be controlled by a single recessive gene, respectively, and the mutated genes of different mutant lines of the same type were at the same locus. These genes were named *glu-1* for the a-1 deletion, *glu-2* for the a-2 deletion, and *glu-3* for the a-3 deletion.

Mapping of the mutated genes using RFLP markers

The *indica* cultivar 'Kasalath' showed the same electrophoretic profile of seed protein by SDS-PAGE as the com-

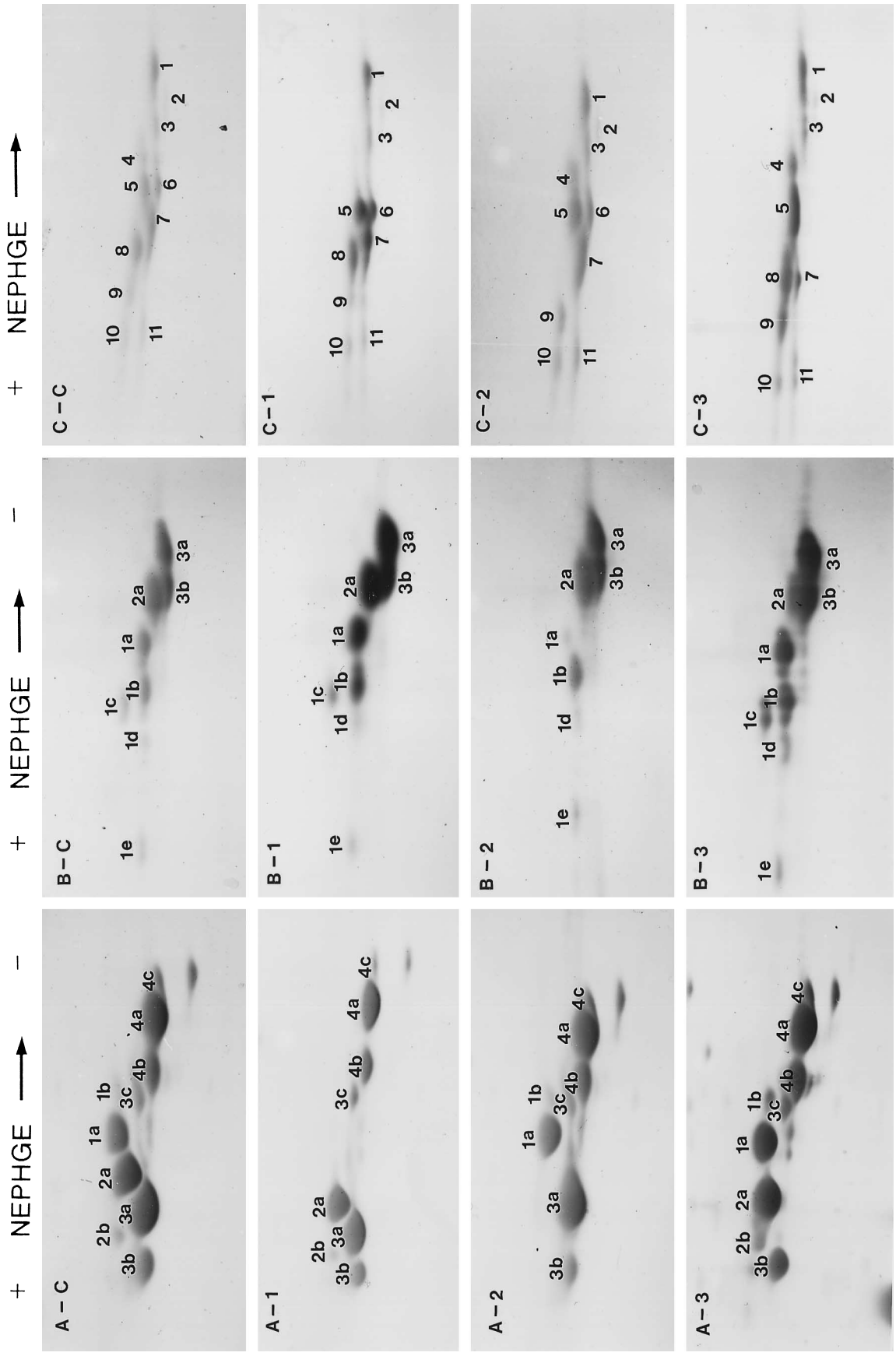
+ NEPHGE −
+ NEPHGE −
+ NEPHGE
SDS-PAGE
A – C
B – C
C – C
A – 1
B – 1
C – 1
A – 2
B – 2
C – 2
A – 3
B – 3
C – 3

Table 3 Mapping of the mutant genes using RFLP markers

Combination of mutant genes and RFLP markers	Segregation mode in F_2[a]						Recombination value (%)	Genetic map distance (cM)
	A-B^j B^j	*A-B^j B^i*	*A-B^i B^i*	*aaB^j B^j*	*aaB^j B^i*	*aaB^i B^i*		
glu-1 – XNpb243	6	97	46	43	9	1	8.4 ± 2.0	8.5 ± 2.0
glu-1 – G365	33	85	45	24	23	4	32.7 ± 3.8	39.1 ± 3.8
glu-2 – G1082	6	77	49	36	5	0	6.7 ± 2.0	6.8 ± 2.0
glu-2 – G1084	9	82	44	29	11	1	13.4 ± 2.5	13.7 ± 2.5
glu-3 – XNpb370	21	124	72	52	15	1	14.0 ± 2.2	14.4 ± 2.2
glu-3 – C250 (got)	6	138	75	47	21	0	10.1 ± 1.9	10.2 ± 1.9

[a] *A*, +; *a*, *glu-1*; *B^i*, *indica*-type RFLP; *B^j*, *japonica*-type RFLP. *A*- means *AA* or *Aa*. All parental mutant lines had *japonica*-type RFLP

mon *japonica* cultivars and can be used as a parent for gene mapping by RFLP analysis. The RFLPs of F_2 plants of the crosses 'Kasalath'×756, 'Kasalath'×M539, and 16×'-Kasalath' were analyzed using RFLP markers after an analysis of the seed protein, and the distances of the mutated genes from the markers were estimated.

The mutated genes of 765, lacking a-1, was assumed to be on chromosome 2 from the significantly high segregation ratio of the *japonica* band detected with probes on chromosome 2, G365 and XNpb243, in the 15 *glu-1* homozygotes. RFLP analysis of 220 F_2 plants showed the distance of *glu-1* from the marker XNpb243 to be 8.5 cM toward G365 (Fig. 3, Table 3).

The gene of M539, *glu-2*, was found to be linked to RFLP markers on chromosome 10 by the analysis of 15 *glu-2* homozygotes, and the distances of *glu-2* from G1082 and G1084 were estimated to be 6.8 cM and 13.7 cM, respectively, by the analysis of 181 F_2 plants (Fig. 3, Table 3).

The analysis of 288 F_2 plants of the cross 16×'Kasalath showed that *glu-3* of 16 was on chromosome 1, and the distances of *glu-3* from C250 and XNpb370 were estimated to be 10.2 cM and 14.4 cM, respectively (Fig. 3, Table 3).

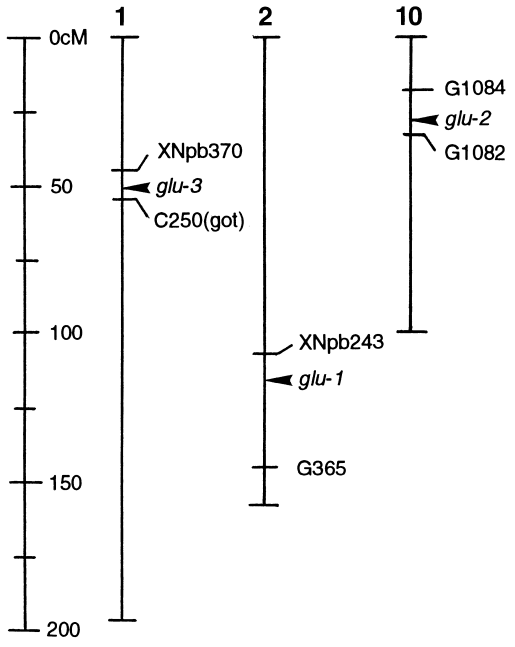

Fig. 3 Location of *glu-1*, *glu-2*, and *glu-3* in an RFLP linkage map. The *number* on each linkage line indicates the chromosome number

Analysis of the glutelin gene

The *Bgl*II fragments of 'Koshihikari' and 'Kasalath' genomic DNA detected by the GluA probe, pREE61, varied in size. *Dra*I also gave polymorphism between 'Koshihikari' and 'Kasalath'. One of the two dense bands had different sizes. The GluB probe, pREEK1, showed polymorphism after digestion of the genomic DNA with *Hap*II and with *Bst*PI.

In RFLP analysis using the GluA probe, all 15 plants homozygous for *glu-2* in the F_2 between the Type-2 mutant and 'Kasalath' showed the *japonica* band , suggesting that *glu-2* is a mutated gene of one of the glutelin genes in the GluA subfamily. Digestion with *Bgl*II and that with *Dra*I gave the same result. Homozygous plants of *glu-1* and *glu-3* did not show significant deviation from the segregation ratio 1:2:1 between *japonica* type, hybrid type, and *indica* type.

Using the GluB probe, we found significant deviation of the segregation ratio from 1:2:1 in the Type-1 mutant. Among 30 *glu-1* homozygotes in the F_2 analyzed after digestion with *Bst*PI, 26 plants were *japonica* type and 4 were hybrid type, suggesting that *glu-1* links to a glutelin gene in the GluB subfamily. Evidence suggesting the linkage of *glu-2* and *glu-3* to the GluB gene was not found.

Fig. 2A–C Two-dimensional electrophoresis of glutelin acidic subunits (**A**), glutelin basic subunits (**B**), and the 57-kDa glutelin precursor (**C**) in mutant lines. *Numbers* and *letters* to the *right* of hyphens show: *1* a-1 deletion (Type 1), *2* a-2 deletion (Type 2), *3* a-3 deletion (Type 3), *C* 'Koshihikari'. The spots were *numbered* in the order of their decreasing molecular weight and *alphabetized* in the order of decreasing spot size

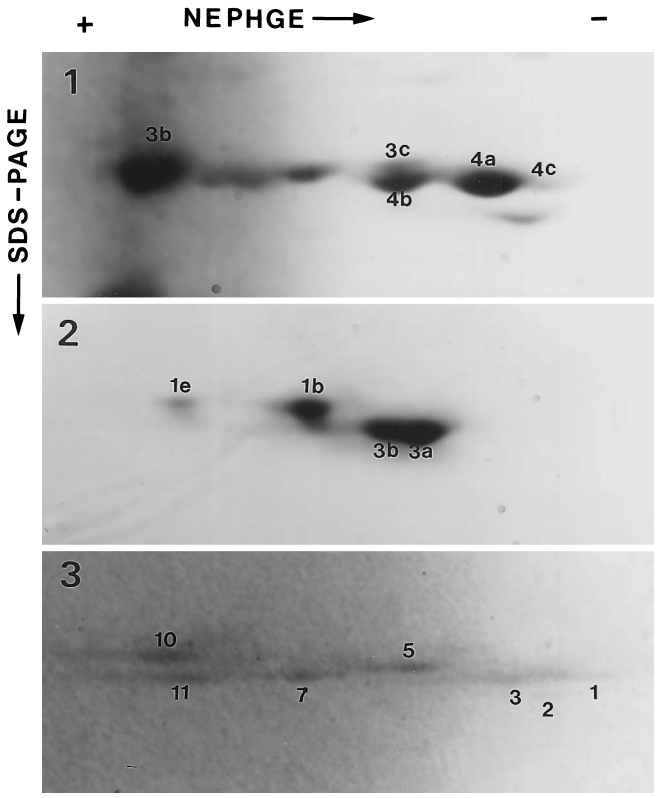

Fig. 4 Two-dimensional electrophoresis of glutelin acidic subunits in a-1,2,3less homozygous for *glu-1*, *glu-2*, and *glu-3*. *1* Glutelin acidic subunits, *2* glutelin basic subunits, *3* 57-kDa glutelin precursors. The spots were *numbered* as shown in Fig. 2

Table 4 Contents of the major polypeptides in the rice grain of a-1,2,3less and the original cultivar

Lines	Molecular weight (kDa) of polypeptide							Total (%)[a]
	76	57	37–39	26	22–23	16	13	
Koshihikari	0.32	0.72	1.87	0.48	1.29	0.33	0.79	5.8
a-1,2,3less	0.29	0.81	1.73	0.66	1.02	0.51	1.18	6.2

[a] Percentage in brown rice (w/w)

Combination of the mutated genes

From F_2 seeds of the cross between 756 and 993, seeds lacking both a-1 and a-2 were selected by SDS-PAGE analysis of half grains, while the other half grains bearing embryos were sown to develop breeding lines homozygous for both *glu-1* and *glu-2*. This breeding line was crossed with 561, and a new breeding line, a-1,2,3less, lacking 3 glutelin subunit bands, a-1, a-2, and a-3, was developed. The deletion of glutelin acidic subunits 1a, 1b, 2a, 2b, and 3a, the deletion of 57-kDa glutelin precursor 4, 6, and 8, and the deletion of glutelin basic subunits 1a, 1c, 1d, and 2a were confirmed in this line a-1,2,3less, by two dimensional electrophoresis (Fig. 4).

Total protein content in brown rice of a-1,2,3less was 6.2%, not significantly different from that of 'Koshihikari'. Glutelin content in a-1,2,3less was reduced to 2.75% from 3.16% of that of 'Koshihikari', a 13% decrease, while the 26-kDa globulin, 16-kDa albumin, and 13-kDa prolamine subunits increased in a-1,2,3less (Table 4).

Discussion

Mutation of glutelin genes

Glutelin is encoded by a multi-gene family, about ten genes per haploid genome, which is classified into two sub-families, GluA and GluB (Takaiwa et al. 1987). Many glutelin genes have been isolated and sequenced (Takaiwa et al. 1987, 1989; Masumura et al. 1989; Okita et al. 1989). N-terminal amino acid sequences of several acidic subunit spots separated by two-dimensional electrophoresis have been determined, and some of these spots have been correlated with glutelin genes (Hirano et al. 1991). However, the relationships between basic subunits and glutelin genes are not known. Glutelin is first synthesized in the form of a 57-kDa glutelin precursor, and after removal of the signal peptide it is cleaved to an acidic subunit and a basic subunit (Yamagata et al. 1982).

In the study presented here, 1 mutant lacking the acidic subunit a-1, Type 1, 5 mutants lacking a-2, Type 2, and 3 mutants lacking a-3, Type 3, were obtained. It was found that each mutant character is controlled by a single recessive gene and that mutated genes of lines of the same type are at the same locus. In F_2 seeds between mutants and their original cultivar, the densities of the glutelin subunit bands segregated in a ratio of 2:1:1 of normal: thin: none, suggesting an effect of gene dosage on the contents of the glutelin acidic subunits. The RFLP of *glu-2* homozygotes probed with the GluA gene was always the mutant type (*japonica* type) in the F_2 between the Type-2 mutant and a normal *indica* cultivar. These results suggest that the mutated genes of these mutants are glutelin genes. These mutated genes of Type 1, Type 2, and Type 3 were named *glu-1*, *glu-2*, and *glu-3*, respectively.

The Type-3 mutants lacked 1 acidic subunit spot, 3a, and 1 glutelin precursor spot, 6, and had a low amount of 1 basic subunit spot, 2a. These spots are considered to be encoded by the glutelin gene that is a wild-type gene of *glu-3*. In the Type-2 mutants, the deletion of the a-2 band in SDS-PAGE corresponded to the lack of 2a and 2b acidic subunit spots, the lack of glutelin precursor 8 , and the decrease of 1c and 1a basic subunit spots in the two-dimensional gels. These acidic subunits, 2a and 2b, the glutelin precursor, 8, and the basic subunits,1a and 1c, would be single-gene products, and post-translational modification, such as glycosilation (Hirano et al. 1991), may give 2 acidic subunit spots and 2 basic subunits having different pI values. The Type-1 mutant lacked 2 spots, 1a and 1b, of the acidic subunits and 1 glutelin precursor, 4, when separated by two dimensional electrophoresis, but there was no detectable change in the basic subunit spots. A basic subunit

must be missing in this mutant, but it may be overlapped with another basic subunit. By means of this analysis of glutelin subunits of the mutant lines, the genetic relationship between the acidic subunits, basic subunits, and the precursor polypeptide was revealed.

Mapping of the glutelin genes

Using in situ hybridization with GluA gene, pREE61, Suzuki et al. (1991) found that a glutelin gene is located on the middle of the short arm of chromosome 2. They also detected a gene that hybridized with a glutelin gene isolated by Masumura et al. (1989) on chromosome 1. The mutant genes, *glu-1* and *glu-3*, which were mapped on chromosome 2 and chromosome 1, respectively, in the present study, may correspond with the glutelin genes detected by in situ hybridization.

Nakamura et al. (1995) reported that a glutelin gene, *Glu-1*^{a} which encodes a_{5a}, was linked with a semi-dwarf gene, *sd-1*, on chromosome 1, and that the recombination value between them was 17.1%. The a_{5a} subunits are inferred to be the 3a spot on the basis of its molecular size and isoelectric point. Since the mutant gene of 3a spot is *glu-3*, which was mapped at the site of similar distance from *sd-1*, *glu-3* would be the same locus as *Glu-1*^{a}.

The mutant gene, *glu-2*, was found to be at the site 6.8 cM from G1082 and 13.7cM from G1084 on chromosome 10. This gene is at the locus of GluA or linked closely with GluA gene.

Combination of the mutated glutelin genes

Glutelin is accumulated in protein body II (PB-II), and prolamine in protein body I (PB-I) (Tanaka et al. 1980). PB-I is known to be indigestible in the human body (Ogawa et al. 1987) and, therefore, rice having higher prolamine-to-glutelin ratios would be equivalent to low-protein rice. Digestion by rats of the seed protein of the LGC-1 line, the low-glutelin character of which is controlled by a single dominant gene (Iida et al. 1993), was found to be lower than that of the original cultivar (Ishii et al. in preparation), and the former is used as a breeding material for low-protein rice.

We crossed the mutant lines to combine the mutated genes of glutelin. The developed line, a-1,2,3less, which is homozygous for *glu-1*, *glu-2*, and *glu-3*, had a low amount of glutelin, but the total protein content of this line was not low being a little higher than the original cultivar. Instead of a decrease in glutelin content, prolamine and globulin increased, as in LGC-1.

The densities of the 4 minor bands of the acidic subunits separated by SDS-PAGE are similar to each other, and hence combination of the three mutant genes was expected to give a 75% decrease in glutelin. However, the decrease in glutelin in a-1,2,3less plant was only 13%, as the loss of function of the three glutelin genes was compensated for by the other glutelin genes. Other mutant genes should be found in order to obtain low-glutelin rice by combination with these three mutated genes.

The line a-1,2,3less would be useful in for selecting mutants lacking other glutelin subunits. We are trying to combine *glu-1*, *glu-2*, and *glu-3* with the mutant gene of LGC-1 to develop a line having a much lower amount of glutelin.

Acknowledgement The authors wish to express their sincere thanks to Dr. F. Takaiwa for providing the cDNA clones of glutelin and to Dr. A. Saito and the Rice Genome Research Program in NIAR/STAFF for providing the RFLP markers. We gratefully acknowledge the kindness of Dr. Y. Ukai for providing the MAPL computer program and his advice on genetic analysis. We also thank Mr. M. Tobita for his help in developing the method for growing plants from the half seeds.

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