Development of transgenic rice (*Oryza sativa* L.) expressing wheat high- and low-molecular-weight glutenin subunit proteins

Etsuko Araki^{*1)}, Tatsuya M. Ikeda^{†1)}, Yasunari Ohgihara²⁾, Atsushi Toyoda³⁾ and Hiroshi Yano¹⁾

¹⁾ National Agricultural Research Center for Western Region, 6-12-1 Nishifukatsu, Fukuyama, Hiroshima 721-8514, Japan

²⁾ Kihara Institute for Biological Research of Yokohama City University, 641-12 Maioka, Totsuka, Yokohama, Kanagawa 244-0813, Japan

³⁾ RIKEN Genomic Sciences Center, 1-7-22 Suehiro, Tsurumi, Yokohama, Kanagawa 230-0045, Japan

We isolated transformation-competent artificial chromosome (TAC) clones harboring a high-molecular-weight glutenin subunit (HMW-GS) gene and a low-molecular-weight GS (LMW-GS) gene from wheat genomic DNA, and developed transgenic rice lines with these genes using a transformation system mediated by *Agrobacterium tumefaciens*. We developed two lines of transgenic rice, one expressing the gene coding for HMW-GS and the other expressing the gene coding for LMW-GS. By crossing these transgenic lines, a novel line harboring both genes was developed and the expression of GS proteins was analyzed. This is the first study indicating that the two kinds of wheat GSs, HMW-GS and LMW-GS, accumulated in rice endosperm. In all the transgenic lines, the introduced GS genes were expressed in the rice endosperm, and the expressed proteins were processed at the same site as the mature GS protein in wheat seeds, forming insoluble polymeric proteins similar to those found in wheat. It was suggested that the protein-processing system was conserved between rice and wheat, and that it was possible to produce wheat gluten using the protein-processing system of rice.

Key Words: rice, wheat, high-molecular-weight glutenin subunit, low-molecular-weight glutenin subunit, transformation-competent artificial chromosome (TAC) vector.

Introduction

Storage proteins of rice (*Oryza sativa* L.) seeds are composed of albumins (water-soluble), globulins (saline-soluble), prolamins (alcohol-soluble) and glutelins (residue). These proteins accumulate in special vacuoles referred to as protein bodies. Two kinds of protein bodies occur in rice: endoplasmic reticulum (ER)-derived protein body-I (PB-I) and vacuolar protein body-II (PB-II) (Okita and Rogers 1996, Tanaka *et al.* 1980). PB-I, containing prolamins, accounts for approximately 20% of milled rice proteins, and PB-II, containing glutelins and globulins, for approximately 60% of milled rice proteins (Ogawa *et al.* 1987, Yamagata *et al.* 1982). Many storage protein mutants have been screened from seeds treated with chemical mutagens. Iida *et al.* (1993) produced a mutant line, LGC1, in which several glutelins, controlled by a single dominant gene *Lgc1*, were severely suppressed. However, the levels of prolamins and globulin increased in compensation, and the total amount of seed protein did not differ from that in normal rice (Iida *et al.* 1997).

In wheat (*Triticum aestivum* L.), glutenins and gliadins are the major seed storage proteins which form a continuous proteinaceous matrix surrounding the starch granules (Parker 1982). The gluten network, which shows viscoelastic properties during dough mixing, is derived from this matrix and these properties are required for the processing of a wide variety of foods, such as breads, noodles and cakes. Among the gluten proteins, the glutenins consist of subunits with high (95 to 136 kDa) and low (36 to 44 kDa) molecular weight. The high-molecular-weight glutenin subunits (HMW-GSs) are encoded by the *Glu-A1*, *Glu-B1* and *Glu-D1* genes on the long arm of chromosomes 1A, 1B and 1D, respectively (Payne *et al.* 1980). The HMW-GSs were classified into x-type and y-type based on the amino acid sequence. The low-molecular-weight glutenin subunits (LMW-GSs) are encoded by the *Glu-A3*, *Glu-B3* and *Glu-D3* genes on the short arm of these chromosomes (Gupta and Shepherd 1990, Jackson *et al.* 1983). If gluten proteins could be expressed in rice seeds, a gluten network could be formed from rice flour, and this flour might be used for the processing of various food products.

In the present study, we first screened clones harboring the GS genes from a wheat genomic library constructed with a transformation-competent artificial chromosome (TAC) vector (Liu *et al.* 2000) containing a large, approximately 40 kbp genomic DNA fragment, and analyzed the sequences of

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*Corresponding author (e-mail: earaki@affrc.go.jp)

† Contribution equals to corresponding author

the inserted region. Then, we introduced the genes into LGC1 from selected TAC clones using a transformation system mediated by *Agrobacterium tumefaciens*. Since the GSs are water-insoluble proteins belonging to the prolamin superfamily (Shewry *et al.* 1995), in transgenic rice, they may accumulate within PB-I containing prolamins. We analyzed the expression of the genes in the transgenic lines by 2D gel electrophoresis as well as the N-terminal amino acid sequences of the GS proteins.

Materials and Methods

Selection of wheat TAC clones with glutenin subunit

A wheat genomic library constructed with the TAC vector (pYLTAC17; Liu *et al.* 2000) was used for the cloning of GS genes and transformation. A clone for the HMW-GS gene was selected by PCR using the primers HMW5-1 (TCACTGGCTGGCCGACAATG) and HMW5-2 (GAAGGTGAGGCCTCTGAGCA) which were designed from the HMW-GS gene sequence on GenBank (accession no. X12928). A clone for the LMW-GS gene was selected by PCR using the primers Glu-4.2 (GCAACTTTGATGATGAATCC) and Glu-5.4 (AAACAAAGGTGATCCAATAT) (Ikeda *et al.* 2006). PCR was performed in a total reaction solution of 25 µl containing 1.5 mM of MgCl₂, 0.1 mM of each dNTP, 5 pmol of each primer, 0.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, USA), PCR Gold buffer (Applied Biosystems) and 50 ng of total DNA. The reaction was performed according to the following protocol using the GeneAmp PCR system 9700 (Applied Biosystems): denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min; and a final extension at 72°C for 7 min. PCR products were electrophoresed in 1% agarose gel in a TAE buffer.

Analysis of selected TAC clones

The copy number of the GS genes in the selected clones was estimated by Southern blot analysis using HMW-GS and LMW-GS gene-specific probes. TAC clones were digested with four restriction enzymes, *Bam*HI, *Eco*RI, *Hind*III and *Xba*I. Southern hybridization was conducted using the ECL system (Amersham Biosciences, USA). The loci of the selected individual GS genes were determined using nullisomic-tetrasomic lines of Chinese Spring, CS-N1AT1B, CS-N1BT1D and CS-N1DT1B. PCR was performed using the GS-specific primers HMW 8-1.1 (CTAAGTGCCGTC TCGTC) and HMW 8-2.3 (TGCAGAGAATCTGGGTA GTG) which were designed from the HMW-GS gene sequence on GenBank (accession no. X12928) for the HMW-GS gene, and Glu 24 (GCCGTTGCGCAAATTTTC) and Glu 13 (TTGGGGCTGTTGTTGCTGATA) which were designed from the LMW-GS gene sequence on GenBank (accession no. U86030, Ikeda *et al.* 2002) for the LMW-GS gene, using genomic DNA of these nullisomic-tetrasomic lines. The reactions were performed as described above. The sequence of insert DNA was determined using BigDye ter-

minator v 3.1 (Applied Biosystems).

Plant materials and transformation

Calli were induced from the embryos of mature seeds of LGC1 according to the method of Fukuoka *et al.* (2000). Genes for HMW-GS and LMW-GS were introduced into the calli derived from LGC1 using the *Agrobacterium* strain EHA101 with the selected TAC clones, 3-113 for HMW-GS and 4-228 for LMW-GS. Six T₀ plants with the HMW-GS gene and four T₀ plants with the LMW-GS gene were grown in a greenhouse and mature seeds were obtained individually from each plant. Progenies in the homozygous state for the HMW-GS gene or the LMW-GS gene were repeatedly selected by PCR and 2D-PAGE in each generation until the T₅ generation.

Since there were no differences in the growth vigor among the homozygous plants expressing HMW-GS or LMW-GS, each of the T₄ plants, in which the expression of HMW-GS or LMW-GS was confirmed, was designated as HMW-113 and LMW-228, respectively, and used in this analysis. To develop a rice line with both HMW-GS and LMW-GS genes, these lines were artificially crossed in the greenhouse. Then, the F₂ population was grown in the greenhouse and homozygote lines with both HMW-GS and LMW-GS genes were selected from the segregating F₃ lines using the PCR method described above.

2D-PAGE and N-terminal amino acid sequence analysis

Total storage proteins were extracted from rice seeds using 50 mM of Tris-HCl (pH 8.0) containing 2% of SDS and 50% of 1-propanol in the presence of the reducing agent dithiothreitol (1% DTT). For the extraction of the glutenin fraction, milled seeds were washed using 50% of 1-propanol. The glutenin fraction was extracted using 50 mM of Tris-HCl (pH 8.0) containing 50% of 1-propanol and 1% of DTT. Each fraction was precipitated with 80% of acetone, and the resulting pellets were dissolved in 250 µl of IEF sample buffer containing 16% of isopropanol, 8.5 M of urea, 4% of CHAPS, 25 mM of DTT, and 0.5% of IEF buffer, pH 6–11 (Amersham Biosciences), according to the method of Ikeda *et al.* (2005). After mixing for 30 min at room temperature, the samples were applied to Immobiline Dry-Strip, pH 6–11 (Amersham Biosciences), and the 13-cm strip was cut on the acidic side to 11 cm to fit the Protean IEF Cell (Bio-Rad Laboratories, USA). After the samples were allowed to stand overnight in-gel rehydration, IEF was performed for a total of 18 kVh. The strip gels were equilibrated with 6 M of urea, 2% of SDS, 20% of glycerol, 10 mM of DTT, and 50 mM of Tris-HCl (pH 6.8), and stored at -25°C. For the second dimension, SDS-PAGE was performed using a 14% gel (14 cm × 14 cm × 1 mm, acrylamide : bis-acrylamide = 29.6 : 0.4) at 30 mA for 2.5 h. The gels were stained with Coomassie Brilliant Blue G-250, according to the method of Neuhoﬀ *et al.* (1988). To analyze the N-terminal amino acid sequence, the gels were blotted onto a Sequi-Blot PVDF Membrane (Bio-Rad Laboratories) with a Trans-Blot SD

Semi-Dry Transfer Cell (Bio-Rad Laboratories). The N-terminal amino acid sequences of individual spots were determined with a PPSQ-21A protein sequencer (Shimadzu, Japan).

Results

Selection of wheat TAC clones with glutenin subunits and their characterization

From the TAC library constructed by Liu *et al.* (2000), we selected two TAC clones by PCR using GSs-specific primers. Clone 3-113 was selected using the HMW-GS gene-specific primer sets and clone 4-228 was selected using the LMW-GS gene-specific primer sets. The complete sequences of clones 3-113 and 4-228 were determined, and the

restriction enzyme sites of *Bam*HI, *Eco*RI, *Hind*III and *Xba*I were identified in the sequences of each clone. The insert sizes of clones 3-113 and 4-228 were 53,888 and 46,433 bp, respectively. The copy number of the GS gene in each TAC clone was confirmed by Southern analysis with probes specific for GSs. In 3-113, a single band was detected in DNA digested with *Bam*HI, *Eco*RI and *Xba*I, and two bands were found in cloned DNA digested with *Hind*III (Fig. 1A). Since the sequence of HMW-GS included the *Hind*III and *Bam*HI restriction enzyme sites (Fig. 2A), two bands found in DNA digested with *Hind*III were derived from one HMW-GS gene. Based on the sequence of 3-113, the DNA including HMW-GS was divided into two fragments, 2.9 and 2.5 kbp long, using *Bam*HI. These two fragments may not have been distinguishable in Southern analysis, due to their similar

Fig. 1. Southern blot analysis of TAC clones with probes specific for the HMW-GS (A) and LMW-GS (B) genes. TAC clones were digested with the following restriction enzymes: 1, *Bam*HI; 2, *Eco*RI; 3, *Hind*III; 4, *Xba*I.

Fig. 2. Structure of the regulatory region of the TAC clone 3-113 harboring the HMW-GS gene (A) and of the TAC clone 4-228 harboring the LMW-GS gene (B). Restriction enzyme sites are indicated by black (*Bam*HI) and white (*Hind*III) arrowheads.

size. These results indicated that clone 3-113 contained a single copy of the HMW-GS gene. In clone 4-228, a single band was detected in the cloned DNA digested with *Bam*HI and *Eco*RI, and a major band and a minor band were found in the DNA digested with *Xba*I and *Hind*III (Fig. 1B). Since none of these restriction enzyme sites were included in the sequence of the LMW-GS gene (Fig. 2B), it was considered that the minor band was not derived from the LMW-GS gene. These results indicated that clone 4-228 also contained a single copy of the LMW-GS gene.

The deduced N-terminal amino sequences of these GSs are shown in Fig. 3. The expected amino acid sequence of HMW-GS in clone 3-113 was the same as that of B1y9 (Fig. 3A). In the PCR analysis using the nullisomic-tetrasomic lines of Chinese Spring with the HMW-GS gene-specific primer (HMW 8-1.1/HMW 8-2.3), the PCR products were not detected in N1BT1D (Fig. 4A), indicating that the HMW-GS gene-specific primer could detect the gene on chromosome 1B of Chinese Spring. The expected amino acid sequence of LMW-GS in clone 4-228 showed a high similarity to that of Group 11 or Group 12 in the classification of Ikeda *et al.* (2002) (Fig. 3B). In the PCR analysis using the nullisomic-tetrasomic lines of Chinese Spring with the LMW-GS gene-specific primer (Glu 24/Glu 13), the PCR products were not detected in N1AT1B (Fig. 4B), indicating that the LMW-GS gene-specific primer could detect the gene on chromosome 1A of Chinese Spring.

Fig. 3. Comparison of deduced N-terminal amino acid sequences of HMW-GSs (A) and LMW-GSs (B). Processing sites are indicated by black and white (Group3 and Group4) arrowheads. GenBank accession numbers are as follows: Glu-A1x1 (CAA43331), Glu-B1x7 (AAZ23584), Glu-B1y9 (S18733), Glu-D1x5 (CAA31395), Glu-D1y10 (P10387), Group1 (BAB78737), Group2 (BAB78738), Group3 (BAB78739), Group4 (BAB78750), Group5 (BAB78751), Group6 (BAB78754), Group7 (BAB78758), Group8 (BAB78759), Group9 (BAB78760), Group10 (BAB78761), Group11 (BAB78762), Group12 (BAB78763).

The structure of the flanking region of the HMW-GS gene is shown in Fig. 2A. In the 5' upstream region of HMW-GS, a HMW prolamin promoter (Thomas and Flavell 1990) (-246 to -209) and a TATA box (-91 to -85) were identified. In the 3' downstream region of HMW-GS, a polyA signal (2267 to 2273) was found as the terminator. Other sequences detected in the 5' upstream region of HMW-GS consisted of two GCN4 boxes (Takaiwa *et al.* 1996) (-628 to -619 and -582 to -573) and an AACA motif (Takaiwa *et al.* 1996) (-529 to -524). The structure of the flanking region of the LMW-GS gene is shown in Fig. 2B. In the 5' upstream region of LMW-GS, LMW prolamin boxes (Wu *et al.* 2000) (-577 to -570, -301 to -294 and -113 to -107) and a TATA box (-79 to -73) were identified. In the 3' downstream region of LMW-GS, polyA signals (1206 to 1211, 1275 to 1281 and 1427 to 1435) were found as the terminator. Other sequences observed in the 5' upstream region of LMW-GS included an ACGT motif (Schmidt *et al.* 1992) (-976 to -969) and three GCN4 boxes (Takaiwa *et al.* 1996) (-563 to -554, -311 to -302 and -288 to -279).

Development of transgenic rice harboring wheat HMW- and LMW-GS genes

To select plants harboring either the HMW-GS gene or the LMW-GS gene in the homozygous state, progenies were repeatedly selected by PCR using specific primer sets and 2D-PAGE in each generation until the T₅ generation. The PCR products in the transgenic lines (T₅ generation) are shown in Fig. 5. LGC1 did not harbor the HMW-GS and LMW-GS genes (lanes 7 and 8), whereas the transgenic rice HMW-113 with clone 3-113 exhibited a HMW-GS gene-specific PCR product (lanes 5 and 6). The transgenic rice LMW-228 with clone 4-228 showed a LMW-GS gene-specific PCR product (lanes 3 and 4).

To develop rice plants harboring both HMW-GS and LMW-GS genes, F₁ plants were obtained from a cross between the HMW-113 (T₅) and LMW-228 (T₅) lines. PCR

Fig. 4. PCR products in nullisomic-tetrasomic lines of Chinese Spring (CS) using primers specific for the HMW-GS (A) and LMW-GS (B) genes. 1, CS; 2, CS-N1AT1B; 3, CS-N1BT1D; 4, CS-N1DT1B.

Fig. 5. HMW-GS and the LMW-GS genes in transgenic rice. Lanes 1 and 2, HLMW-GS; lanes 3 and 4, LMW-228; lanes 5 and 6, HMW-113; lanes 7 and 8, LGC1. The gene product of 0.8 kb is derived from the HMW-GS gene and that of 0.9 kb is derived from the LMW-GS gene.

analysis showed that these F_1 plants harbored both HMW-GS and LMW-GS genes (Fig. 5, lanes 1 and 2). Homozygous plants with both HMW-GS and LMW-GS genes were selected by PCR using primer sets specific for the HMW-GS and LMW-GS genes in the F_3 generation, and these were designated as HLMW-GS as a line. In the transgenic rice lines, the estimation of the copy number of the GS gene by Southern analysis with probes specific for GSs was not carried out, because there were no appropriate restriction enzyme sites in the introduced large wheat DNA fragment to detect restriction fragments containing the neighboring rice genome sequence, which should vary among the copies. However, in the PCR analysis of the F_2 generation from a cross between the HMW-113 and LMW-228 lines, plants with and without the GS gene were segregated in the ratio of 3 : 1 (HMW-GS; 35 : 13, $\chi^2 = 0.111$, $P = 0.74$, LMW-GS; 33 : 15, $\chi^2 = 1.000$, $P = 0.32$). This result indicated that each HMW-GS and LMW-GS gene was integrated into the rice genome as a single locus in the transgenic rice lines.

Expression of HMW- and LMW-GS in transgenic rice lines

Fig. 6 shows the total protein composition in the transgenic rice lines. In the HMW-113 and LMW-228 lines, protein bands around 75 and 37 kDa, respectively, were identified by SDS-PAGE (lanes 2 and 3). In the HLMW-GS line, both protein bands were detected (lane 4), unlike in the LGC1. It was expected that these transgenic-specific protein bands corresponded to the GS proteins. We analyzed the expression of the wheat glutenin genes in the three transgenic lines by 2D-PAGE and determined the N-terminal amino acid sequence of the proteins. Fig. 7 shows the expression of wheat GSs in the transgenic rice seeds, as revealed by 2D-PAGE analysis. In the LGC1, neither HMW-GS nor LMW-GS was detected (Fig. 7A). In the HMW-113 and the LMW-228 lines, protein spots around 75 and 37 kDa, respectively, were identified by 2D-PAGE (Fig. 7B and C). In the HLMW-GS line, both protein spots were detected (Fig. 7D). The N-terminal amino acid sequences of the 75 and 37 kDa proteins corresponded to the mature HMW-GS

Fig. 6. Total protein composition of transgenic rice lines. 1, LGC1, 2, HMW-113, 3, LMW-228, and 4, HLMW-GS. Protein bands around 75 and 37 kDa are indicated by white arrowheads and white circles, respectively.

(EGEASGQLQ) and LMW-GS (ISQQQQQ) proteins, which were processed at the same positions as those of the mature GS proteins within wheat seeds (Fig. 3).

HMW-GS in the HMW-113 line and LMW-GS in the LMW-228 line were extracted either as glutenin fraction with 50% of 1-propanol or as total storage protein with 2% of SDS in the presence of 1% DTT (Fig. 8A-D). In the LMW-228 and HLMW-GS lines, the amount of LMW-GS extracted with only 50% of 1-propanol (Fig. 9, lanes 3 and 4), was smaller than that of LMW-GS extracted with 50% of 1-propanol in the presence of 1% DTT (Fig. 9, lanes 7 and 8).

Discussion

We isolated two TAC clones, 3-113 and 4-228, harboring a HMW-GS gene and a LMW-GS gene, respectively. Based on our results, we were able to characterize these GS genes in the selected clones. The expected N-terminal amino acid sequence of HMW-GS of clone 3-113 was identical to that of B1y9 (Fig. 3A). The HMW-GS gene-specific primer detected the gene on chromosome 1B in Chinese Spring (Fig. 4A). The *Glu-B1* gene on chromosome 1B that encoded a y-type HMW-GS in Chinese Spring corresponded to that of B1y8 (Payne *et al.* 1980). Therefore, the HMW-GS gene of clone 3-113 corresponded to that of B1y8 encoded by the *Glu-B1* gene on chromosome 1B in Chinese Spring. The expected N-terminal amino acid sequence of LMW-GS of clone 4-228 had showed a high similarity to that of Group 11 or Group 12 (Fig. 3B), and the LMW-GS gene-specific primer detected the gene on chromosome 1A in Chinese Spring (Fig. 4B). Therefore, the LMW-GS gene of clone 4-228 belonged to Group 11 encoded by the *Glu-A1* gene on chromosome 1A in Chinese Spring (Ikeda *et al.* 2006).

Clones 3-113 and 4-228 also contained some motifs

Fig. 7. 2D gel electrophoresis of total storage proteins in transgenic rice lines. (A) LGC1, (B) HMW-113, (C) LMW-228 and (D) HLMW-GS. The LMW-GS and HMW-GS proteins are indicated by the letters L and H, respectively.

Fig. 8. 2D gel electrophoresis of glutenin fractions and total storage proteins in HMW-113 (A, C) and LMW-228 (B, D). A and B, glutenin fractions extracted with 50% of 1-propanol in the presence of 1% DTT. C and D, total storage proteins extracted with 2% of SDS in the presence of 1% DTT. The LMW-GS and HMW-GS proteins are indicated by the letters L and H, respectively.

in the 5' upstream region, which might be associated with endosperm-specific gene expression (Fig. 2). In clone 3-113, a HMW prolamin promoter (Thomas and Flavell 1990), two GCN4 boxes (Takaiwa *et al.* 1996) and an AACA motif (Takaiwa *et al.* 1996) were identified in the 5' upstream

region of the HMW-GS gene (Fig. 2A). Clone 4-228 contained LMW-GS, LMW prolamin boxes (Wu *et al.* 2000), an ACGT motif (Schmidt *et al.* 1992) and three GCN4 boxes (Takaiwa *et al.* 1996) in the 5' upstream region of the LMW-GS gene (Fig. 2B). Wu *et al.* (2000) reported that the

Fig. 9. SDS-PAGE analysis of GS in transgenic rice lines. 1 and 5, LGC1, 2 and 6, HMW-113, 3 and 7, LMW-228, 4 and 8, HLMW-GS. 1, 2, 3 and 4, 50% 1-propanol-soluble fractions, 5, 6, 7 and 8, 50% 1-propanol- and 1% DTT-soluble fractions. The LMW-GS and HMW-GS proteins are indicated by the arrows.

GCN4 motif acted as an essential element determining the endosperm-specific expression and that AACAA and ACGT and the prolamin box were involved in quantitative regulation of the endosperm-specific gene. Therefore, we expected that the selected clones contained *cis*-elements necessary for the expression of the GS genes in the wheat endosperm. Using these clones, we developed two lines of transgenic rice, namely HMW-113 harboring the gene coding for HMW-GS, and LMW-228 harboring the gene coding for LMW-GS. Moreover, by crossing these transgenic lines, a novel line, HLMW-GS harboring both genes was also developed. In the transgenic rice lines HMW-113 and LMW-228, the HMW-GS and LMW-GS proteins accumulated within the endosperm, respectively (Fig. 7), confirming that these clones contained all the *cis*-elements for the proper expression of GS (Fig. 2), as expected from the complete sequences.

The expressed GS proteins in the transgenic lines were processed at the same site as that of the mature GS protein in wheat seeds, and accumulated as mature GS proteins within the transgenic rice seeds. This indicated that HMW-GS and LMW-GS were expressed within transgenic rice seeds, and were properly processed, as in the case of the wheat seeds. Disulfide bonding among HMW-GSs and LMW-GSs is important for the formation of a glutenin network with a large amount of polymeric GS proteins. In the transgenic rice line HLMW-GS, similar amounts of HMW-GS and LMW-GS to those in HMW-113 and LMW-228 accumulated, respectively (Fig. 7). These results suggested that HMW-GS and LMW-GS did not compete with each other for accumulation within rice seeds. This is the first study showing that the two kinds of wheat GSs, HMW-GS and LMW-GS, could accu-

mulate in the rice endosperm.

These GS proteins were preferentially extracted by either 50% of 1-propanol or 2% of SDS in the presence of 1% DTT (Fig. 8). The results suggested that these proteins had formed insoluble polymeric proteins within the transgenic rice endosperm and that they may be located with prolamins in PB-I.

In the present study, we showed that wheat GSs were processed and accumulated within the rice endosperm, although wheat GSs accumulate as a continuous proteinaceous matrix surrounding the starch granules in wheat seeds (Parker 1982), in contrast to the two kinds of protein bodies for storage in rice seeds (Okita and Rogers 1996, Tanaka *et al.* 1980). In the transgenic rice lines expressing LMW-GSs, a small amount of LMW-GS was found in the 50% 1-propanol-soluble fraction, while most of LMW-GS was extracted in the insoluble fraction (Fig. 9), suggesting that most of the GSs expressed in the transgenic lines formed insoluble polymeric proteins. In the transgenic rice lines expressing soybean glycinins, unprocessed proglycinins had been detected in the endosperm (Katsube *et al.* 1999). The results obtained in the present study suggested that the protein-processing system was conserved between rice and wheat, and that it was possible to produce wheat gluten using the protein-processing system of rice.

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Note added to the proof

- Oszvald *et al.* reported the expression of another HMW-GS protein in transgenic rice in *Cereal Research Communications*, in December, 2007.
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