Diversity of Low-Molecular-Weight Glutenin Subunit Genes in Asian Common Wheat (*Triticum aestivum* L.)

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We analyzed the diversity of the low-molecular-weight glutenin subunit (LMW-GS) genes of Asian common wheat cultivars. Locus-specific primers for LMW-GSs revealed the presence of four alleles at the *Glu-A3* locus, four at the *Glu-B3* locus and one at the *Glu-D3* locus. Frequency of *Glu-A3* alleles in the Japanese cultivars was very different from that in other Asian cultivars. The combined alleles at the three *Glu-3* loci were classified into 15 genotypes. The frequency of each genotype from the Asian collection or breeding areas varied. One *Glu-3* genotype, *BAA* (consisting of the *Glu-A3* allele *B*, *Glu-B3* allele *A* and *Glu-D3* allele *A*), occurred frequently in many Asian cultivars. *Glu-3* genotypes *BBA*, *CAA* and *DBA* predominated in southern and northern Asia and in Japan, respectively. In addition, the Japanese cultivars with the *BBA* or *CAA* genotypes showed a specific composition of high-molecular-weight glutenin subunits and gliadins. DNA sequencing indicated that the *Glu-A3* allele *B* carried an additional cysteine residue and that the allele *C* showed unusual amino-acid deletions. These mutations were likely to affect dough strength, which is a major characteristic of wheat flour quality.

**Key Words:** *Triticum aestivum* L., high-molecular-weight glutenin subunit, low-molecular-weight glutenin subunit, gliadin, diversity, food preference, Asia.

**Introduction**

Common wheat, *Triticum aestivum* L., appeared about seven thousand years ago in the southeastern coastal area of the Caspian Sea (Tsunewaki 1966, Nishikawa et al. 1980). During its transmission to Europe, Africa and southern and eastern Asia, common wheat became adapted to a wide range of climatic conditions. For instance, since in China, there are high mountainous areas and desert areas under harsh conditions, the eastward spread from the center of origin was not simple.

In the process of transmission to eastern Asia, the mode of wheat consumption changed from baked bread to steamed bread and noodles, each requiring a different processing procedure. The composition of the seed storage proteins, which consist of high-molecular-weight glutenin subunits (HMW-GSs), low-molecular-weight glutenin subunits (LMW-GSs) and gliadins, is the main determinant of the properties corresponding to the different modes of food processing. The HMW-GSs are encoded by genes at homoelogous loci, *Glu-A1*, *Glu-B1* and *Glu-D1*, which are located on the long arm of the homoelogous group-1 chromosomes, i.e., 1AL, 1BL and 1DL, respectively (Payne et al. 1980, 1981, 1982). The gene loci for LMW-GSs (*Glu-3*) are located on the short arm of group-1 chromosomes, namely *Glu-A3* on chromosome 1AS, *Glu-B3* on 1BS and *Glu-D3* on 1DS (Singh and Shepherd 1985, Pogna et al. 1990). The main loci of the genes coding for gliadin, *Gli-1* and *Gli-2*, are located on the short arm of group-1 and group-6 chromosomes, respectively (Payne et al. 1984a, 1984b).

The alleles at the *Glu-A1*, *Glu-B1* and *Glu-D1* loci are highly polymorphic among cultivars in the world, depending on the region of origin (Morgunov et al. 1993, Nakamura 1999, 2000, Nakamura et al. 1999). For example, the *Glu-D1d* allele, which confers superior bread-making quality, appears more frequently in Europe than in eastern Asia (Payne and Lawrence 1983, Payne et al. 1987, Nakamura 2000). On the other hand, *Glu-D1f* is frequently observed in the Japanese cultivars, especially those in the southern part of the country (Nakamura et al. 1990). Nakamura et al. (1999) suggested that Japanese cultivars had been bred mainly for noodle making. The *Glu-D1f* allele might have been introduced from Afghanistan to southern Japan along the so-called ‘silk road’ (Nakamura 2002). Likewise, we previously reported that the patterns of gliadin in the Japanese cultivars differed considerably from those in the cultivars from other countries, and were limited to only 46 patterns (Tanaka et al. 2003). Seven collection or breeding areas in Japan showed different frequencies in the gliadin patterns (Tanaka et al. 2003). This regional specificity of both HMW-GSs and gliadins in common wheat may result from selection based on the modes of food processing or...
from neutral selection.

We expected that the LMW-GSs, accounting for 40% of the total seed storage proteins, would exert a significant effect on the dough strength of wheat flour. However, the analysis of LMW-GSs is difficult, since during SDS-PAGE, the bands may overlap, because the molecular weight of LMW-GSs is similar to that of gliadins. Furthermore, the genes at the Glu-3 locus for LMW-GSs consist of a multigene family, including 30–40 variable genes (Sabelli and Shewry 1991, Cassidy et al. 1998).

In the present study, we reported the diversity of the LMW-GS genes of Asian common wheat cultivars based on PCR fragment size polymorphisms. Furthermore, we analyzed the deduced amino-acid sequences of the LMW-GSs that may affect the dough strength of wheat flour. The frequencies of the LMW-GS genes and the amino-acid changes in different cultivars enabled us to assume the existence of a relationship between their diversity and food preference. The observed combinations of HMW-GSs, LMW-GS genes and gliadins were also reported.

Materials and Methods

Plant materials

We examined 233 cultivars of common wheat from Asia. Japan was divided into two regions, northeastern Japan (Hokkaido, Tohoku, Kantō and Hokuriku/Nagano) and southwestern Japan (Tokai/Kinki, Chugoku and Shikoku/Kyushu) (Nakamura et al. 1999). China was grouped into seven regions, eastern China (Shandong, Jiangsu, Anhui, Zhejiang and Fujian Provinces), northwestern China (Shaanxi, Gansu and Ningxia Provinces), northeastern China (Heilongjiang, Jilin and Liaoning Provinces), Sichuan, Inner Mongolia, Xinjiang and Tibet (Tsujimoto et al. 1998). The cultivars were maintained at Tottori University as a part of the National Bioresources Project-Wheat, Japan.

Analysis of LMW-GS genes

Total DNA was extracted from young leaves by the CTAB method (Murray and Thompson 1980) and used as template for PCR. The PCR primers used to amplify the LMW-GS genes were those reported by Van Campenhout et al. (1995) as follows: 5′-CGCCGTTGTGGCGACAAATTA-3′ and 5′-GTCTTGTAGGATGATGGAGTAGG-3′ for the amplification of Glu-A3 on chromosome 1A; 5′-GTACCAACAAACCAACC-3′ and 5′-GGTGGCTGTGAGGTGGTGGT-3′ for the amplification of Glu-B3 on chromosome 1B; and 5′-GACCATCTCTAGTTTGAGGA-3′ and 5′-ATGTATTTTAGTTGTTGCGGA-3′ for the amplification of Glu-D3 on chromosome 1D. PCR amplification was performed using TaKaRa Ex Taq™ DNA polymerase (2.5 U, TaKaRa), in 100 μl of reaction buffer (TaKaRa, 2 mM MgCl2) containing 200 ng of genomic DNA, 200 μM of each dNTP and 50 pmol of each primer. The PCR conditions were 94°C for 1 min followed by 35 cycles of 94°C for 60 s, 59°C for 60 s, 72°C for 90 s. The products were separated in 5% polyacrylamide gels to classify the cultivars by the fragment size polymorphism for each Glu-3 allele. Three cultivars were randomly chosen from the cultivars in each Glu-3 allele group. Each major fragment from the selected cultivars was inserted into the cloning site of the plasmid vector pGEM-T Easy (Promega). The DNA sequences of the inserts were determined on a DNA sequencer (ABI PRISM 3100 Genetic Analyzer) using a BigDye Terminator v3.0 Cycle Sequencing Kit (Applied Biosystems).

Seed storage proteins

The genotypes of the HMW-GSs were designated by three lowercase letters, of which the first, second and third characters indicated the alleles at the Glu-A1, Glu-B1 and Glu-D1 loci, respectively; for example, the genotype cba of HMW-GS refers to the c allele at Glu-A1, b allele at Glu-B1 and a allele at Glu-D1 (Payne and Lawrence 1983).

The allelic diversity of the LMW-GSs found at the Glu-3 loci was first reported by Jackson et al. (1983). Several studies have been carried out to describe the general variability of the LMW-GS alleles (Gupta and Shepherd 1990, Jackson et al. 1996, Igrejas et al. 1999, Flæte 2000). However, due to the complexity of the LMW-GS patterns, which result from the encoding a multigene family, no classification system or description for LMW-GSs has yet been universally accepted. In the present study, we observed fragment size polymorphisms at the Glu-A3 and Glu-B3 loci. These differences in fragment sizes reflected the differences in gene length at the Glu-3 loci because of the lack of introns in the LMW-GS genes. Therefore, we could easily deduce the amino-acid sequences from the nucleic-acid sequences of the amplified genes. The other genes tightly linked to the amplified gene at the Glu-3 loci, which consist of a multigene family, may be different from each other, even if the fragments with the same size were amplified. Although the actual number of alleles at each Glu-3 locus might exceed the number found in the present study, polymorphism of each fragment size was tentatively assigned a capital letter for the allele name at the Glu-3 loci. The genotypes were designated by three capital letters, of which the first, second and third characters indicated the alleles at the Glu-A3, Glu-B3 and Glu-D3 loci, respectively; for example, the genotype BBA refers to the B allele at Glu-A3, A allele at Glu-B3 and A allele at Glu-D3.

Similarly, gliadin is encoded by a multigene family. No classification system or description for the gliadins has yet been universally accepted, either. In the present study, the electrophoretic patterns of the gliadins were designated by three capital letters, of which the first, second and third characters indicated the electrophoretic fractions of α-gliadin, β-γ-gliadin and α-gliadin; for example, the gliadin pattern FHD refers to pattern F for the α-gliadin fraction, pattern H for the β, γ-gliadin fraction and pattern D for the α-gliadin fraction (Tanaka et al. 2003).
Results

Classification of the LMW-GS alleles

The locus-specific primers for the LMW-GS genes generated amplified fragments with length polymorphisms (Fig. 1). The sizes of the major fragments from 233 Asian cultivars were classified into four alleles at the Glu-A3 locus, four at the Glu-B3 locus, including an unamplified allele, and one at the Glu-D3 locus. The composition of the Glu-A3 alleles in the Japanese cultivars was quite different from that in the other Asian cultivars (Table 1). The frequency of the B alleles at Glu-A3 was lower than that in the other Asian cultivars. On the other hand, alleles C and D at Glu-A3 frequently appeared in the Japanese cultivars.

Classification of the LMW-GS genotypes

The composition of the alleles at the three Glu-3 loci was classified into 15 types. The frequency of the genotypes varied depending on the area (Table 2). The genotype BAA (38.6% of the cultivars) appeared frequently in all the regions of Asia. On the other hand, the genotype DBA tended to be present more frequently in the Japanese cultivars. Genotypes CA4 and BBA predominated in northern (northeastern China) and southern Asia (Pakistan, Nepal, Bhutan, Tibet and Sichuan), respectively (Fig. 2).

Combination of HMW-GSs, LMW-GSs and gliadins

We next analyzed the combination of two of the above genotypes at the Glu-3 loci (BBA predominating in southern Asia and CA4 predominating in northern Asia) with the genotypes of HMW-GSs at the Glu-1 loci and the electrophoretic patterns of gliadins (Tanaka et al. 2003) in 107 Japanese cultivars. We expected that these might differ, because the

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Table 1. Frequency of Glu-3 alleles in Japanese and other Asian common wheat cultivars

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele</th>
<th>No. of cultivars (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Japan</td>
<td>Other Asia</td>
</tr>
<tr>
<td>Glu-A3</td>
<td>A</td>
<td>3 (3)</td>
<td>6 (5)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>49 (45)</td>
<td>95 (75)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>21 (20)</td>
<td>11 (9)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>34 (32)</td>
<td>14 (11)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>107 (100)</td>
<td>126 (100)</td>
</tr>
<tr>
<td>Glu-B3</td>
<td>A</td>
<td>64 (60)</td>
<td>82 (66)</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>29 (27)</td>
<td>37 (29)</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>8 (7)</td>
<td>4 (3)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>6 (6)</td>
<td>3 (2)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>107 (100)</td>
<td>126 (100)</td>
</tr>
</tbody>
</table>

$^1$ Statistics could not be analyzed because of the small number of samples.

$^2$ Significant at 1% level.

$^3$ Significant at 5% level.

Table 2. Frequency of Glu-3 genotypes in Asian common wheat cultivars

<table>
<thead>
<tr>
<th>Collection/Breeding area</th>
<th>Glu-3 genotypes$^1$</th>
<th>BAA</th>
<th>BBA</th>
<th>CA4</th>
<th>DBA</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northeast Japan</td>
<td>15</td>
<td>5</td>
<td>10</td>
<td>3</td>
<td>19</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Southwest Japan</td>
<td>16</td>
<td>6</td>
<td>4</td>
<td>10</td>
<td>19</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Korea</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>China (Northeast)</td>
<td>3</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>China (Inner Mongolia)</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>China (East)</td>
<td>14</td>
<td>13</td>
<td>1</td>
<td></td>
<td>3</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>China (Sichuan)</td>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>China (Northwest)</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>China (Xinjiang)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>China (Tibet)</td>
<td>14</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Bhutan</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nepal</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pakistan</td>
<td>3</td>
<td>2</td>
<td></td>
<td></td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Afghanistan</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>42</td>
<td>24</td>
<td>16</td>
<td>61</td>
<td>233</td>
<td></td>
</tr>
</tbody>
</table>

$^1$ Glu-3 genotypes are indicated in the order of loci Glu-A3, Glu-B3 and Glu-D3, respectively.

Fig. 1. Polymorphisms of PCR amplicons and their classified alleles at each of the three Glu-3 loci on homoeologous group-1 chromosomes. Expected amplicons and their sizes from ‘Chinese Spring’ are indicated by arrows. Chromosome-specific primers for Glu-3 loci were used (Van Campenhout et al. 1995).
food preference in southern China (Sichuan and Tibet) is quite different from that in northeastern China. Tanaka et al. (2003) previously reported that both the *cba* genotype of HMW-GS and the FHD pattern of gliadin appeared most frequently in the Japanese cultivars. In the present study, both the *cba* genotype of HMW-GS and the FHD pattern of gliadin were also observed most frequently in the cultivars with the LMW-GS genotypes *BBA* and *CAA*. The other genotypes of HMW-GS and patterns of gliadin were observed in the cultivars with either the genotype *BBA* or *CAA* of LMW-GS. In the combinations of *Glu-3* genotypes and HMW-GSs, the cultivars with the genotype *BBA* of LMW-GS showed the HMW-GS genotype *cbf* or *ccf*, and these subunits were not observed in the cultivars with the genotype *CAA* of LMW-GS. On the other hand, cultivars with the genotype *CAA* of LMW-GS displayed the HMW-GS genotypes *abc*, *acc*, *adc*, *bga* or *caa*, and these subunits were not observed in the cultivars with the genotype *BBA* of LMW-GS. This tendency was also observed in the combinations of *Glu-3* genotypes and gliadin patterns (data not shown).

Cloning and characterization of the amplified LMW-GS genes

The sequences of the *Glu-A3* and *Glu-B3* alleles were classified in ‘Norin 61’ into groups 6 and 2, respectively (Fig. 3, Ikeda et al. 2002). The deduced insertions or deletions in the alleles at the *Glu-A3* and *Glu-B3* loci were mainly observed in the repetitive domain. However, the *Glu-A3* allele *C* showed 4 unusual amino-acid deletions within the C-terminal conserved domain. All the clones from the *Glu-A3* and *Glu-B3* loci exhibited six cysteine residues conserved

Fig. 2. Frequency of *Glu-3* genotypes *BBA* and *CAA* in Asian common wheat cultivars.

Fig. 3. Comparison of the deduced amino-acid sequences of each allele of the LMW-GS genes amplified by PCR. Identical residues are denoted by a black color. The positions of the cysteine residues are indicated by asterisks (*). The new sequences have been deposited at DDBJ under the Accession Nos. indicated in parentheses as follows: *Glu-A3* allele *A* (AB209927), *C* (AB209928), *D* (AB209929), *Glu-B3* allele *B* (AB209930) and *C* (AB209931). Accession Nos. of *Glu-A3* allele *B* and *Glu-B3* allele *A* from ‘Chinese Spring’ are X84959 and X84960, respectively.
among both previously published LMW-GS sequences and those we obtained in the present study, and the relative positions of the cysteine residues were also conserved. However, allele B at the Glu-A3 locus showed an additional cysteine residue due to a missense change within the C-terminal conserved domain.

**Discussion**

Wheat seed storage protein, composed of HMW-GS, LMW-GS and gliadin, is one of the main contributors to wheat flour quality that affects food preference. In the present study, emphasis was placed on the diversity of the LMW-GS genes in Asian common wheat cultivars, because LMW-GS affects the dough strength, which is a major characteristic of wheat flour quality, and is expected to display a wide diversity because it is encoded by a multigene family. We observed the region-dependent composition of the genes. The polymorphisms of the fragment size of the LMW-GS genes depended on the presence of various deletions within the repetitive-sequence domain.

The frequency of the alleles C and D at Glu-A3 in the Japanese cultivars was higher than that in the other Asian cultivars, while that of the allele B was lower, presumably because the population size may have been reduced at one time during the transmission of common wheat to Japan. Tsujimoto et al. (1998) argued that the low variation between the Japanese and eastern Asian wheat cultivars was due to a decrease in the population size during the transmission of common wheat to these areas, and that plants with less genetic variation became the ancestors of the present common wheat cultivars in the region. Tanaka et al. (2003) who also reported that the gliadin patterns of the Japanese common wheat cultivars differed significantly from those of European countries, suggested that the limited and specific variation in the seed storage protein composition of the Japanese cultivars may simply be attributable to the limited variability in the germplasm used in the breeding program.

Food preference may possibly affect the frequency of Glu-3 alleles. In other words, some LMW-GS genes may be associated with selective advantages for food preference in each region. In all the Asian cultivars, Glu-A3 allele B, Glu-B3 allele A and combined Glu-3 genotype BAA appeared frequently. Allele B at the Glu-A3 locus bore an additional cysteine residue, which may be related to glutenin polymer formation by disulfide bonds. Therefore, this allele may have been advantageous in selection in terms of Asian food culture, typified by noodles, as opposed to bread in Europe. Glu-A3 allele C and the combined Glu-3 genotype CAA were not observed in Pakistan, Nepal, Bhutan or Tibet. Although the Glu-A3 allele C was observed in Sichuan, the Glu-3 genotype combination CAA was not observed there. In contrast, although the Glu-A3 allele B was observed in northeastern China, the Glu-3 genotype BBA was not observed there, while the Glu-3 genotype CAA was frequently observed. The Glu-A3 allele C exhibited 4 unusual amino-acid deletions within the C-terminal conserved domain. Therefore, these deletions may affect the dough strength, which may be suitable for food preference in these regions, as evidenced by chapati.

For the possibilities mentioned above, wheat adaptation to a wide range of climatic conditions and the frequency of the Glu-3 alleles could have resulted in individual selection through climatic effects and the founder effect, respectively. Therefore, it is possible that after wheat cultivars had been selected by adaptation to the climatic conditions in various regions, they might have been selected further by preference for foods in each region. These wheat cultivars subjected to double selection might have been land races in each region in Asia.

**Acknowledgments**

The present study was partly supported by the Iijima Memorial Foundation for the Promotion of Food Science and Technology. DNA sequence analysis was conducted at the CREST-Akita Plant Molecular Science Satellite Laboratory at the Life Research Support Centre established at Akita Prefectural University.

**Literature Cited**


