(Korean J. Crop Sci.) 43(4) : 259~263(1998)

Genetic Variation of High Molecular Weight Glutenin (HMW-Glu) Subunit in Korean Wheat

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ABSTRACT

High molecular weight glutenin (HMW-Glu) subunit compositions of 73 Korean wheat cultivars and experimental lines were evaluated by using one dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This method is suitable for obtaining a good resolution of 1Dx2 and 1Ax2* without adverse effects on separation of other HMW-Glu subunits. Korean wheats examined in this study could be divided into 15 different groups on the basis of HMW-Glu subunit compositions. From the wheat lines tested, it was identified that there were three alleles at the *Glu-A1*, five at the *Glu-B1* and three at the *Glu-D1* loci. The null allele of the *Glu-A1* was occurred in high frequency (79. 4%), while low frequencies for 1Ax1 (12.3%) and 1Ax2* (8. 2%) were found. High frequency (75.3%) of the subunit pairs of 1Bx7+1By8 at the *Glu-B1* loci compared with other subunits was found. The frequencies of subunits 1Dx2. 2+1Dy12 and 1Dx2+1Dy12 from the *Glu-D1* loci were 54. 8% and 37.0%, respectively. However, a few Korean wheat lines (8.2%) carried 1Dx5+1Dy10 subunit pair which are responsible for good breadmaking quality. The information of HMW-Glu subunit compositions provide a useful tool to characterize wheat lines, and can be directly used in selection of breeding lines of different end-use properties.

Key words : wheat (*Triticum aestivum* L.), high molecular weight glutenin (HMW-Glu) subunits, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), genetic variations.

Gluten proteins are major seed storage proteins in hexaploid wheat (*Triticum aestivum* L.). Glutens are complex mixtures, and are usually classified into two groups called glutenins and gliadins. Gliadins are defined as readily soluble in aqueous alcohols. Its complex mixture of single polypeptides from 30 to 35 polypeptides are either lack cysteine or have only intra-chain disulfide bonds (Payne, 1987). In contrast, glutenins are insoluble in aqueous alcohol, and contains 12~20 different high molecular weight subunits that are stabilized by inter-chain disulfide bonds (Payne, 1987). Therefore, it is thought that the individual proteins are contributing in some way to the functional properties of whole gluten. Glutenins are largely responsible for gluten elasticity and gliadins are for viscosity. The HMW-Glu subunits may play a key role in determining flour quality because the elasticity of gluten was positively correlated with the proportion of proteins present in high molecular weight polymers (above about 1×10^6 unit) which are enriched in the HMW-Glu subunits (Field et al., 1983; Payne & Corfield, 1979). Each wheat line had three to five HMW-Glu subunits and strong relationship between breadmaking quality and HMW-Glu allelic variation was reported (Shewry et al., 1992; Payne, 1987).

Studies of the genetics and biochemistry of HMW-Glu subunits have shown that they are controlled by genes at three loci, called *Glu-A1*, *Glu-B1* and *Glu-D1*, located on the long arms of chromosomes 1A, 1B and 1D respectively (Payne & Lawrence, 1983). Each locus consists of two genes encoding a high molecular weight x-type subunit and a low molecular weight y-type subunit. These subunits are tightly linked, and inherited as pairs (Payne & Lawrence, 1983; Payne et al., 1981). The variation in HMW-Glu subunits resulted from specific gene silencing, with the 1Ay gene being silent in all hexaploid wheats and the 1By and/or 1Ax genes expressed in only some cultivars (Payne & Lawrence, 1983). Therefore, the *Glu-A1* loci only codes for one subunit (1Ax) or no subunit at all, the *Glu-B1* loci codes for one or two subunits (1Bx or 1Bx+1By), and the *Glu-D1* loci codes for two subunits (1Dx+1Dy)(Payne, 1987). These variations in gene expression have influenced on the total amount HMW-Glu subunit proteins.

Although significant relationship between the presence or absence of specific HMW-Glu subunits and breadmaking quality of wheat varieties, few investigations have been made on the functional properties of the LMW-Glu subunits because of the difficulties of identifying the LMW-Glu subunits (Weegels et al., 1996; Gupta et al., 1995; Shewry et al., 1990; Payne et al., 1987). The identification of HMW-Glu subunits has accomplished for wheat improvement programs that aimed at varietal identification, characterization and selection of cultivars with desirable breadmaking quality. There are reports about describing the HMW-Glu subunit compositions of wheat cultivars grown in different countries whose major crop is wheat (reviewed by Morgunnov et al., 1993). The purposes of this study were to analyze allelic compositions at each of the three loci controlling

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Received 11 Nov. 1998.

HMW-Glu subunits in Korean wheat varieties and to provide biochemical-genetic information to wheat breeding programs for varietal identification and their quality improvement.

MATERIALS AND METHODS

Seed samples of Korean wheat varieties and breeding lines were obtained from the National Crop Experiment Station, Suwon, Korea. The wheat samples for analysis of HMW-Glu subunits were listed in Table 1.

To determine the HMW-Glu subunit compositions, total proteins were extracted from crushed single kernel with 200 μl of extraction buffer [0.125 M tris-HCl, pH 6.8, 1% (w/v) SDS, 6.7% (v/v) glycerol, 0.003% (w/v) bromophenol blue, and 5 % (v/v) β-mercaptoethanol] by shaking for 2 hours at room temperature.

Method of SDS-PAGE for HMW-Glu subunit fractionation was based on the procedures described by Laemmli (1970). The separating gel (pH 8.3) was prepared as 10% SDS-polyacrylamide with 1.27% bisacrylamide. After ran for 12 hours at 20mA/gel, gel was stained for overnight with a Commassie Blue R-250 and destained in 10% trichloroacetic acid. The HMW-Glu subunits were evaluated with the scoring system proposed by Payne & Lawrence (1983).

RESULTS AND DISCUSSIONS

For the varietal identification, characterization and selection of lines with desirable breadmaking quality in wheat breeding programs, the precise evaluation of individual HMW-Glu subunits is critical. Genetic variations of HMW-Glu subunit compositions in Korean wheat cultivars by SDS-PAGE were shown in Fig 1. Although the separation of subunit bands 1Dx2 and 1Ax2* on the pH 8.8 of 10% gels were known to be difficult because of their similar mobilities (Zhen & Mares, 1992), it was able to separate 1Dx2 and 1Ax2* subunits with good resolution by adjusting pH of separation gel to 8.3 (Fig. 1). The system also provides no apparent adverse effects on the other HMW-Glu subunits separation. The separation of 1Dx2 and 1Ax2* is crucially important to wheat breeders because wheats possessing 1Ax2* or 1Ax1 subunits on *Glu-A1* loci have a good characteristic of breadmaking quality, while the null allele on *Glu-A1* loci associates with poor quality. An unidentified band, markered on arrow in Fig. 1, was appeared below subunit 1Dy12. This faint band which was previously observed by Lawrence (1986) always presented in all Korean wheat lines tested.

Table 1. Classification of Korean wheat varieties with respect to high molecular weight glutenin (HMW-Glu) subunit compositions.

HMW-Glu subunit†			Varieties
Glu-A1	*Glu-B1*	*Glu-D1*	
N‡,	13 + 16,	2 + 12	Suwon 266
N,	7 + 8,	2 + 12	Chungnamjaerae, Jaeraejong 1, Jaeraejong, Jaeraemil, Jaeraesomaek, Jaraeneulmil, Milyang 27, Shinkwang, Somaekjaerae, Suwon 211, Suwon 234, Suwon 259, Suwon 272, Tongmil,
N,	7 + 9,	2 + 12	Dahongmil, Suwon 85, Suwon 209, Suwon 218
N,	7 + 8,	5 + 10	Keumgangmil, Suwon 236, Tapdongmil
N,	7 + 9,	5 + 10	Suwon 210
N,	7,	5 + 10	Suwon 205
N,	7 + 8,	2.2 + 12	Cheonggeamil, Geukjosaeng 2, Geurumil, Chokwang, Milyang 10, Milyang 11, Milyang 12, Milyang 14, Namhaemil, Olmil, Suwon 86, Suwon 185, Suwon 225, Suwon 229, Suwon 239, Suwon 243, Suwon 244, Suwon 246, Suwon 249, Suwon 258, Suwon 260, Suwon 261, Suwon 264, Suwon 265, Suwon 269, Suwon 271, Urimil
N,	7 + 9,	2.2 + 12	Eunpamil, Milyang 15, Suwon 245, Suwon 252, Suwon 263, Suwon 268, Suwon 270,
1,	17 + 18,	2 + 12	Jinpoong
1,	7 + 8,	2 + 12	Changkwang, Jaraejong 2, Jaraesomaek 1, Yungkwang, Suwon 241
1,	7 + 9,	2 + 12	Gobunmil
2*,	7 + 9,	2 + 12	Youkseong 3
1,	7 + 8,	2.2 + 12	Naemil
2*,	7 + 8,	2.2 + 12	Alchanmil, Kyungkwang, Olgeurumil, Suwon 213, Suwon 230
1,	7 + 9,	5 + 10	Suwon 207

† : Nomenclature according to Payne and Lawrence (1983)
‡ : N = null allele

Table 2. Frequency of high molecular weight glutenin (HMW-Glu) subunit composition in Korean wheats.

HMW-Glu subunit†	Varieties percentages	HMW-Glu subunits†	Varieties percentages
Glu-A1			
N‡	769.4	1, 5 + 10	1.4
1	12.3	1, 2.2 + 12	1.4
2*	8.2	2*, 2 + 12	1.4
		2*, 2.2 + 12	6.8
Glu-B1			
17 + 18	1.4	*Glu-B1 & Glu-D1*	
13 + 16	1.4	13 + 16, 2 + 12	1.4
7 + 8	75.3	7 + 8, 2 + 12	26.0
7 + 9	20.5	7 + 9, 2 + 12	8.2
7	1.4	17 + 18, 2 + 12	1.4
		7 + 8, 5 + 10	4.1
Glu-D1		7 + 9, 5 + 10	2.7
2 + 12	37.0	7, 5 + 10	1.4
5 + 10	8.2	7 + 8, 2.2 + 12	45.2
2.2 + 12	54.8	7 + 9, 2.2 + 12	9.6
Glu-A1 & Glu-B1		*Glu-A1 & Glu-B1 & Glu-D1*	
N, 13 + 16	1.4	N, 13 + 16, 2 + 12	1.4
N, 7 + 8	60.3	N, 7 + 8, 2 + 12	19.2
N, 7 + 9	16.4	N, 7 + 9, 2 + 12	5.5
N, 7,	1.4	N, 7 + 8, 5 + 10	4.1
1, 17 + 18	1.4	N, 7 + 9, 5 + 10	1.4
1, 7 + 8	8.2	N, 7, 5 + 10	1.4
1, 7 + 9	2.7	N, 7 + 8, 2.2 + 12	37.0
2*, 7 + 8	6.8	N, 7 + 9, 2.2 + 12	9.6
2*, 7 + 9	1.4	1, 17 + 18, 2 + 12	1.4
		1, 7 + 8, 2 + 12	6.8
Glu-A1 & Glu-D1		1, 7 + 9, 2 + 12	1.4
N, 2 + 12	26.0	2*, 7 + 9, 2 + 12	1.4
N, 5 + 10	6.8	1, 7 + 8, 2.2 + 12	1.4
N, 2.2 + 12	46.6	2*, 7 + 8, 2.2 + 12	6.8
1, 2 + 12	9.6	1, 7 + 9, 5 + 10	1.4

† : Nomenclature according to Payne & Lawrence (1983)
‡ : N = null allele

However, its origin and nature were remained obscure (Zhen & Mares, 1992).

The distribution of HMW-Glu subunits for the 73 Korean wheat cultivars and experimental lines was shown in Table 1. The 73 Korean varieties examined in this study were divided into 15 different groups on the basis of the HMW-Glu subunit composition (Table 1). Among the 73 varieties, three alleles were identified at the *Glu-A1*, five at the *Glu-B1* loci and three at *Glu-D1* loci. The percentages of HMW-Glu subunit compositions in Korean wheats were shown in Table 2. The high frequency (79.4%) of null allele on *Glu-A1* loci were found. Normally 1Ay gene was not expressed by gene silencing. Therefore, lack of 1Ax gene expression and with absence of 1Ay gene expression in Korean wheat resulted quality depreciation. The subunits of 1Bx7+1By8 were present at high frequence (75.3%) in *Glu-B1* loci. The extremely low frequency (1.4%) was found in *Glu-B1* loci for 1Bx17+1By18, 1Bx13+1By16, and 1Bx7.

Payne et al. (1983b) evaluated the relationship of breadmaking quality to allelic variation among HMW-Glu subunits. The *Glu-1* scoring system which was proposed by Payne et al. (1987) was used to predict breadmaking quality of each wheat variety. The allelic subunits of 1Dx5+1Dy10 were reported that they were associated with better quality than the other subunits followed by the 1Ax subunits 1Ax1 or 1Ax2*, and the 1B pairs 1Bx17+1By18 and 1Bx7+1By8 (Shewry et al.,

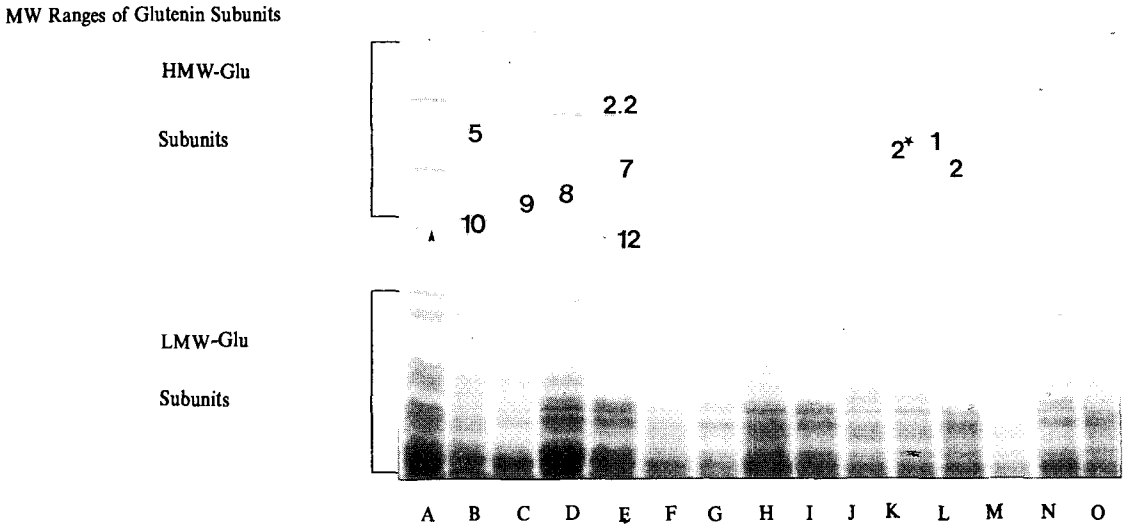

Fig. 1. One-dimensional SDS-PAGE(10%) patterns of HMW-Glu subunit compositions for a number of Korean wheat cultivars to illustrate the alleles. A, Urimil(2.2, 7, 8, 12); B, Tapdongmil(5, 7, 8, 10); C, Eunpamil(2.2, 7, 9, 12); D, Greumil(2.2, 7, 8, 12); E, Chokwang(2.2, 7, 8, 12); F, Namheamil(2.2, 7, 8, 12); G, Cheonggemil(2.2, 7, 8, 12); H, Dahongmil(2, 7, 9, 12); I, Olmil(2.2, 7, 8, 12); J, Alchanmil(2.2, 2*, 7, 8, 12); K, Olgeurumil(2.2, 2*, 7, 8, 12); L, Changkwang(1, 2, 7, 8, 12); M, Gobunmil(2.2, 1, 7, 9, 12); N, Keumgangmil(5, 7, 8, 10); O, Shinkwang(2, 7, 8, 12). Note : lane F and L were lightly loaded. Arrow is unidentified band.

1992). Shewry et al. (1992) proposed that wheat varieties with a good breadmaking quality may require allelic subunits 1Ax1 or 1Ax2* on *Glu-A1* locus, 1Bx17+1By18 or 1Bx7+1By8 on Glu-B1 locus, and 1Dx5+1Dy10 on *Glu-D1* locus. However, there was no such subunits combination in Korean wheats studied in this study. HMW-Glu subunits that were most frequently found in wheats with good breadmaking quality were 1Dx5+1Dy10. On the other hand, subunit 1Dx2+1Dy12 were related to poor rheological properties (Shewry et al., 1992). Although limited number of Korean wheats were evaluated, the result of low frequency (8.2%) of 1Dx5+1Dy10 subunits related to high frequency (37.0%) of 1Dx2+1Dy12 subunits indicated that other uses rather than breadmaking quality should be considered when selection was made in Korean wheat breeding programs. Thus, since most of Korean wheats showed the low frequency of the allelic subunits 1Dx5+1Dy10, introduction of the subunits 1Dx5+1Dy10 should be highly considered in Korean wheat breeding programs for better breadmaking quality.

Payne et al. (1983a) have reported that Japanese wheat cultivars contained high frequency of the 1Dx2.2+1Dy12 subunits and concluded their presence might be associated with flour texture. Most of Japanese cultivars which possessed 1Dx2.2+1Dy12 subunits showed fine flours compared with the cultivars lacking those subunits (Nakamura et al., 1990; Oda et al., 1992). Although limited number of samples were tested, this study also found high frequency (54.8%) of 1Dx2.2+1Dy12 subunits in Korean wheat lines. The reason for the frequent occurrence of 1Dx2.2+1Dy12 subunits in Korean and Japanese wheat has not been studied yet. However, the fact that co-occurrence (50%) of 1Dx2.2+1Dy12 subunits and friabilin, the water-soluble proteins surrounding the starch granules strongly associated with controlling in kernel hardness, in Korean wheat indicated that these subunits may be related to kernel hardness.

ACKNOWLEDGMENTS

We thank our colleague at the National Crop Experiment Station for providing experimental materials.

We wish to acknowledge the financial support of the Korea Research Foundation made in the program year of 1997 (997-001-G00085).

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