J. Plant Biol. (2009) 52:533–542
DOI 10.1007/s12374-009-9069-z

ORIGINAL RESEARCH

Diversity of Novel Glutenin Subunits in Bread Wheat (*Triticum aestivum* L.)

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Received: 6 August 2009 / Accepted: 1 September 2009 / Published online: 30 September 2009

Abstract Glutenin is a major determinant of baking performance and viscoelasticity, which are responsible for high-quality bread with a light porous crumb structure of a well-leavened loaf. We analyzed the diversity of glutenin genes from six wheat cultivars (Korean cvs. Keumgang and Jinpum, Chinese cvs. China-108 and Yeonnon-78, and Japanese cvs. Norin-61 and Kantou-107). Glutenins contain two types of isoforms such as high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW-GS). Glutenin fractions were extracted from wheat endosperm using Osborne solubility method. A total of 217 protein spots were separated on two-dimensional gel electrophoresis with isoelectric focusing (wide range of pH 3–10). The proteins spots were subjected to tryptic digestion and identified by matrix assisted laser desorption/ionization–time of flight mass spectrometry. HMW-GS (43 isoforms) and LMW-GS (seven isoforms) are directly responsible for producing high-quality bread and noodles. Likewise, all the seed storage proteins are digested to provide nutrients for the embryo during seed germination and seedling growth. We identified the diverse glutenin subunits in wheat cultivars and compared the gluten isoforms among different wheat cultivars according to quality. This work gives an insight on the quality improvement in wheat crop.

Keywords Wheat glutenin subunit · Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) · Proteomics · Two-dimensional gel electrophoresis (2-DE)

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Introduction

Gluten protein composition determines the rheological characteristics (strength and extensibility) of flour dough and is the key constituent responsible for differences in end-use suitability (Butow et al. 2003). The gluten proteins consist of the monomeric gliadins and polymeric glutenins, where glutenins contain high and low molecular weight glutenin subunits. Wheat grain research has focused on the detailed analysis of gluten proteins to better understand those aspects of protein composition accounting for the unique properties of flour (Skylas et al. 2005). Variations in

the types of glutenin subunits correlate with quality variations among wheat cultivars, probably by affecting the molecular weight distribution of glutenin polymers (Gupta et al. 1993; Gupta and Shephard 1990a, b). Although the non-gluten protein classes such as albumin and globulin occupy a smaller percentage of endosperm protein, these proteins play critical roles in cellular metabolism, development, and responses to environment. Glutenins are made up of polypeptide chains that are cross-linked by disulfide bonds into higher level polymers. When treated with a reducing agent, glutenins dissociate into subunits of differing molecular weight: the high molecular weight subunits (HMW-GS) and the low molecular weight subunits (LMW-GS) (Payne et al. 1981). The glutenin consisted of two types of subunits containing LMW-GS (10–70 kDa) and HMW-GS (80–130 kDa) (Bietz and Wall 1972). When glutenins are further reduced, two types of subunits are released into high molecular weight subunit of 70–90 kDa and low molecular weight subunit of 20–45 kDa. However, actual molecular weight of glutenin calculated from derived amino acid sequences indicated 60–90 kDa, lower molecular weight rather than ever expected (Anderson and Green 1989; Anderson et al. 1989). Reverse-phase HPLC analysis indicates that HMW-GS to is less hydrophobic than LMW-GS. HMW-GS and LMW-GS are assumed to be cross-linked to form so-called glutenin polymers, which are among the largest molecules in nature, with molecular weights exceeding one million (Wrigley 1996). Using nullisomic–tetrasomic, nullisomic–trisomic, and ditelocentric lines of Chinese Spring, The expression of HMW-GS was controlled by gene loci at the long arms of the chromosomes 1D and 1B (Bietz and Wall 1975). The genetics of HMW-GS and their relationship to bread-making quality were conducted in details (Lawrence and Shepherd 1981; Payne et al. 1981). Recently, studies on the characterization of glutenins have greatly improved toward the understanding of structures and functions of HMW-GS. In particular, proteomic technology centered with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has rapidly developed rapidly and become a powerful tool for identifying storage proteins (Alberghina et al. 2005; Kussmann et al. 1997; Muccilli et al. 2005). Some recent investigations have focused on the tryptic peptide mapping of high molecular weight glutenin subunits (Cozzolino et al. 2001), but little work on measuring accurate molecular weights of the intact HMW-GS within the mixture of HMW subunits has been reported. In order to obtain accurate molecular weights of HMW-GS, we improved the resolving condition of the mixture of HMW glutenin subunits for identification by mass spectrometry, and then established a rapid MALDI-TOF-MS method to determine accurate molecular weights of single HMW glutenin subunits from mixture samples.

Materials and Methods

Materials

Six genotypes of wheat cultivar seeds (two Korean cvs. Keumgang, Jinpum, two Chinese cvs. China-108, Yeonnon-78, and two Japanese cvs. Norin-61, Kantou-107) were used in this study for the identification of novel glutenin proteins by proteomics analysis. The seeds were collected from the field of National Institute of Crop Science, Suwon, Korea. Wheat seeds were grown in the field under low temperature (−20 to −10°C) for 4 months, then slowly increasing temperature and naturally exposed up to 28°C until harvesting. The wheat seeds were harvested for the experiment and kept in −20°C prior to the sample preparation.

Chemical Reagents

All chemicals were of the highest purity and commercially available products and used without further purification. Dithioreitol (DTT), trifluroacetic acid (TFA), acetonitrile (ACN), ethylenediaminetetraacetic acid (EDTA), ammonium bicarbonate (ABC), and iodoacetamide (IAA) were purchased from Sigma (St. Louis, MI). Ultrapure water from Puris Ultrapure Water System (Mirae Co., Ltd, Korea) was used in the preparation of all solutions.

Protein Extraction

We used routinely to fractionate wheat endosperm proteins, taking advantage of the solubility properties of wheat endosperm proteins in KCl, SDS, and acetone with minor modifications (Hurkman and Tanaka 2007; Osborne 1924). Fifty milligrams of flour was suspended in 200 μl of cold potassium chloride (KCl) (50 mM Tris–HCl, 100 mM KCl, 5 mM EDTA, pH 7.8). The suspension was incubated on ice for 5 min with intermittent mixing by vortexing including sonication (Sonics and Materials Inc., Newtown, USA) and centrifuged at 16,000×*g* for 15 min at 4°C (Hanil Science Industrial Co. Ltd. Korea). The pellet or KCl-insoluble fraction was suspended in 800 μl of SDS buffer (2% SDS, 10% glycerol, 50 mM DTT, 40 mM Tris–Cl, pH 6.8) and incubated for 1 h at room temperature. The resultant insoluble materials were removed by centrifugation at 16,000×*g* for 10 min at room temperature. The proteins were precipitated from the SDS buffer by adding 4 volumes of cold (kept at −20°C) acetone and incubating the mixture overnight at −20°C. After the centrifugation, the pellet was rinsed by pipetting cold acetone onto the pellet, subjected to centrifugation at 16,000×*g* for 10 min at room temperature. The supernatant was discarded by careful pipetting. The pellet (proteins including glutenin) was dried

by vacuum centrifugation (Biotron Inc., Puchon Kyunggido, Korea) and solubilized in urea buffer (9 M urea, 4% Triton X-114, 1% DTT, and 2% ampholytes) up to the final volume of 250 µl (Kamal et al. 2009).

Gel Electrophoresis (2-DE)

Soluble proteins responsible for whole seed storage were examined by two-dimensional gel electrophoresis according to the protocol (O'Farrell 1975). Sample solutions (50 µl) were loaded on to the acidic side of the IEF gels for the first dimensional, and anodic and cathodic electrode solutions were filled in the upper and lower electrode chambers, respectively. Wheat grain proteins have been studied using 2-DE composed of the first dimension of IEF over the two pH range of pH 4–7 and 6–11 and the second dimension of SDS-PAGE (Kamal et al. 2009; Laemmli 1970; Woo et al. 2002). With the previous method, however, the separation of protein spots were not satisfactory to resolve at around the neutral (pH 4–7) pH range. Therefore, to avoid the overlapping of protein spots and to increase the resolution capacity, we adopted an IEF gel specific for pH range 3–10 in addition to the acidic and the basic pH range. SDS-PAGE in the second dimension (Nihon Eido, Tokyo, Japan) was performed with 12% separation and 5% stacking gels. Protein spots in 2-DE gels were visualized by Coomassie Brilliant Blue (CBB) R-250 staining (Woo et al. 2002). Each sample was run three times, and the best visualized gels were selected.

In-Gel Digestion and Mass Spectrometry Analysis

According to the previous report (Kamal et al. 2009), selected protein spots were excised from the preparative gels, stained with Coomassie Brilliant Blue (R-250), and then washed with 100 µl distilled water. Each gel piece with protein was dehydrated by 25 mM ABC/50% ACN and washed with 10 mM DTT/0.1 M ABC. Gel pieces were dried under vacuum centrifugation, rehydrated with 55 mM IAA/0.1 M ABC for 30 min under the dark. After removing the solution, the gels pieces were vortexed with 100 mM ABC for 5 min and soaked in ACN for dehydration so that the resulting gel pieces would shrink and become an opaque-white color. The gel pieces were then dried under vacuum centrifugation. For tryptic digestion, trypsin solution (4 µl in a volume) was added in rehydrated gel particles and incubated for 45 min at 4°C and overlaid with 30 µl of 25 mM ABC (pH 8.0) to keep them immersed throughout the digestion. The gel pieces were then incubated overnight at 37ºC. After incubation, the solution was spin down and transferred to a 500-µl siliconized tube. The gel particles were resuspended in 40 µl mixture of ACN/DDW/TFA (660:330:10 µl) three times, and again resuspended in 100% ACN, then vortexed for 30 min, respectively. The supernatant was dried under vacuum centrifugation for 2 h. In MALDI-TOF/MS (AXIMA CFR^+ Plus, Shimadzu, Japan) analysis, the proteins separated by 2-DE were digested in gels according to the method described (Fukuda et al. 2003). The samples were added in 10 µl (0.1% TFA) for the complete digestion. The digests were desalted with C_{18} Zip Tip (Millipore, Boston, MI) and subjected to the analysis by MALDI-TOF-MS.

Bioinformatics Analysis

The proteins were identified by searching NCBI non-redundant database using the MASCOT program (http://www.matrixscience.com,Matrixscienc,UK). The search parameters were allowed for the modification of acetyl (K), carbamidomethyl (C), oxidation (M), propionamide (C) with peptide tolerance (±100 ppm). For MS/MS searches, the fragmentation of a selected peptide molecular ion peak was used to identify with a probability of less than 5%. Thus, MS/MS spectra with a MASCOT score higher than the significant score ($p<0.05$) were assumed to be correct. When more than one peptide sequence was assigned to a spectrum with a significant score, the spectra were manually examined. Sequence lengths, gene names, and protein functions were identified by searching Swiss-Prot/TrEMBL database using UniProtKB (http://www.uniprot.org).

Results

Two-Dimensional Electrophoretic Separation of Proteins

By the conventional method, the separation of protein spots was not satisfactory at around the neutral (pH 4–7) pH range. Therefore, to avoid the overlapping of protein spots and to increase the resolution capacity, finally, we adopted an IEF gel specific for pH range 3–10 in addition to the acidic and the basic pH range. With these methods, we identified more than 250 protein spots among six cultivars by pH 3–10 range gels, which discovered about 45, 32, 38, 40, 26, and 36 protein spots, respectively (Fig. 3). These protein spot patterns were highly reproducible for at least three self-determining protein extractions. Using the 2-DE gels for pH 3–10, the qualitative variations of 36 proteins were revealed from six wheat cultivars (Fig. 1). Among them, protein spots 1, 6, 9, 16, and 17 were found in Jinpum (Fig. 1a) and protein spots 3, 4, 12, 13, 14, 15, 16, 20, 34, 38, 40, and 44 in Keumgang (Fig. 1b). In addition, protein spots 12, 13, 14, 15, 16, 19, 20, 32, 33, 37, 38, and 40 were found in Yeonnon-67 (Fig. 1c), whereas spots 17 and 18 were in China-108 (Fig. 1d). Among the Japanese wheat cultivars, protein spots 5 and 9 were in Norin-61

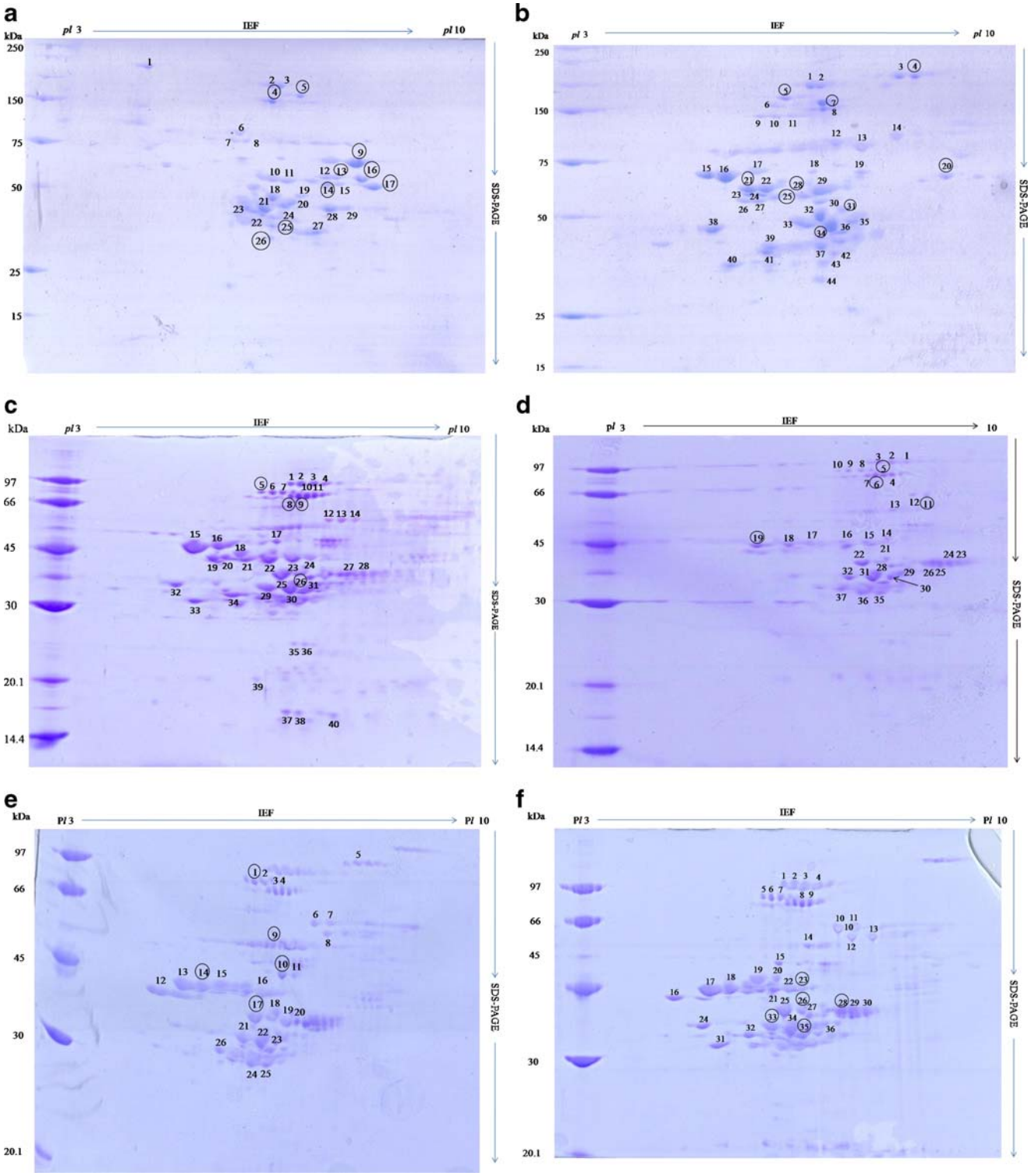

Fig. 1 Two-dimensional (2D) PAGE resolution of glutenin subunits (GSs) of six wheat cultivars (**a** Keumgang, **b** Jinpum, **c** China-108, **d** Yeonnon-78, **e** Norin-61, **f** Kantou-107). The first-dimensional gel electrophoresis (*horizontal*) is represented by isoelectric focusing (IEF) ranging from pH 3–10 (*left* pH 3; *right* pH 10). In the second dimension gels (*vertical*) by 12% SDS-PAGE, protein spots were visualized using Coomassie Brilliant Blue R-250. The protein spots correspondence to glutenin subunits are indicated by *circles*. Molecular weight markers are shown on the *left side* with sizes

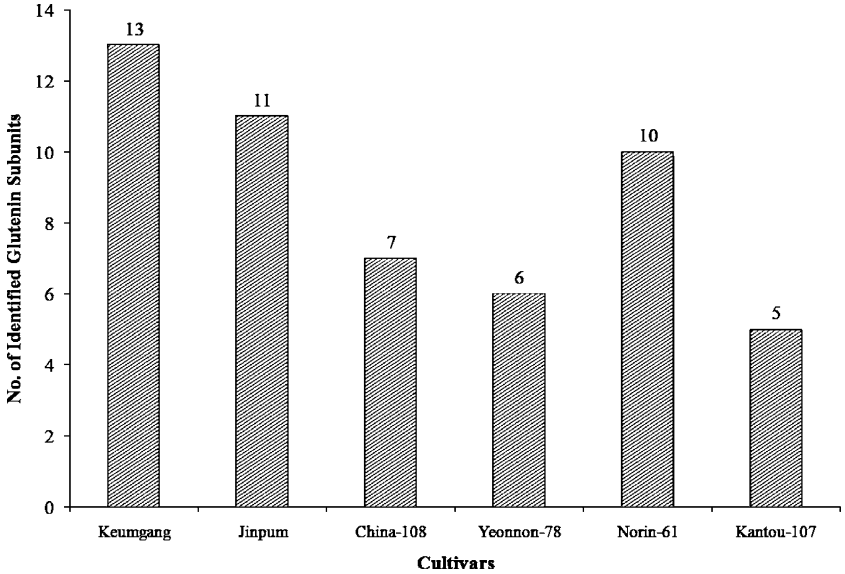

Fig. 2 Distribution of total identified glutenin subunits (GSs) protein among wheat cultivars

(Fig 1e), and protein spots 16, 19, 24, and 31 were in Kantou-107 (Fig. 1f). We identified varietal variation according to protein expression by two-dimensional electrophoresis. Out of 37 analyzed protein spots by MALDI-TOF-MS, nine expressed protein spots revealed glutenin subunits (HMW-GS and LMW-GS) in Jinpum, ten in Keumgang, four in Yeonnon-78 and China-108, and five in Norin-61 and Kantou-107, respectively (Fig. 1).

Protein Identification

The 217 protein spots were analyzed by the tryptic digestion followed by MALDI-TOF-MS. A number of proteins could not be identified by this procedure due to factors such as low resolution of mass spectrometry and the lack of genome sequence about wheat. Out of 52 glutenin proteins, using peptide fragmentation method, 13 proteins were identified in Keumgang followed by 11 in Jinpum, seven in China-108, six in Yeonnon-78, ten in Norin-61, and five in Kantou-107 (Fig. 2). Out of these, 37 selected protein spots were detected by 2-DE, and five proteins were revealed as high molecular weight glutenin subunit (HMW-GS) in Jinpum, nine in Keumgang, four in Yeonnon-78, three in China-108, and five in Norin-61 and Kantou-107 using MALDI-TOF-MS (Table 1). Some proteins identified as multiple spots differed from each other in their p*I*s and/or M*r*. Additionally, 4, 1, and 1 spots were identified as low molecular weight glutenin subunit (LMW-GS) in Jinpum, Keumgang, and China-108, respectively (Table 1). HMW-GS was identified in Keumgang (spots 31 and 34), Yeonnon-78 (spot 8) and Norin-61 (spot 1), which is directly associated with baking performance of wheat. HMW-GS was encoded by the structural gene *Glu-1* loci such as *Glu-1-2* (14–15 kDa) in Yeonnon-78 and China-108. Furthermore, HMW-GS was encoded by structural gene *Glu-1* loci as *Glu-1B* (79.4 kDa) and *Glu-1B* (77.2 kDa) and *Glu-1R* (77.3 kDa) in Yeonnon-78 and Norin-61, respectively. LMW-GS proteins were encoded by LMW-GS gene (26–40 kDa) in Jinpum, Keumgang and China-108 (Table 1). Interestingly, we identified the different types of glutenin subunits in wheat such as y-type (19.6 kDa from *Aegilops ventricosa*, 1.5 kDa from *Leymus racemosus*, and 17.2 kDa from *Triticum aestivum*) and s-type (27.7 kDa from *Triticum aestivum* and 34.7 kDa from *Aegilops tauschii*) in our experiment (Table 1 and Fig. 3).

Discussion

Throughout the world, many Asian countries have recently launched programs to quality development with balanced nutrients and good bread and noodles made of wheat. Our research has focused on identifying new proteins to affect the wheat quality. Using 2-DE and MALDI-TOF/MS technique, the proteomic profiles of seeds were analyzed from the relatively high-quality cultivars among Jinpum, Keumgang, Yeonnon-78, China-108, Norin-61, and Kantou-107. Two thousand seventeen protein spots were chosen based on protein spot intensity among six wheat cultivars. By the database search, the selected proteins were separated into two functional groups. The first contains HMW-GS proteins to directly affect the baking performance and viscoelasticity. The second group contains LMW-GS proteins to affect the bread-making quality, which are associated with HMW-GS as major storage proteins.

Table 1 List of identified glutenin subunits (GSs) protein from six wheat cultivars including name of gene by using MALDI-TOF-MS coupled to bioinformatics

SN	Identified Protein	Mr	Pl Value	Species	Gene identifier	Score	SC (%)	GN	CN
04	Y-type HMW- glutenin subunit	19,683	8.64	*Aegilops ventricosa*	gi|7188718	55	18	–	Jinpum
	HMW- glutenin subunit	14,991	9.17	*Triticum aestivum*	gi|32328619	52	37	HMW-GS	
05	HMW-glutenin subunit	19,908	8.85	*Triticum aestivum*	gi|24474926	73	23	HMW-GS	
	Y-type HMW glutenin subunit	19,683	8.64	*Aegilops ventricosa*	gi|7188718	71	24	–	
09	LMW- glutenin subunit group 3 type II	26,718	8.21	*Triticum aestivum*	gi|17425184	32	20	LMW-GS	
13	Glutenin, high molecular weight subunit PC237	4,058	8.20	*Triticum aestivum*	gi|121451	18	33	–	
14	Low-molecular-weight glutenin subunit	30,679	8.69	*T. turgidum* subsp. *polonicum*	gi|124109356	17	6	LMW-GS	
16	LMW-glutenin subunit -S13 precursor	34,733	9.08	*Aegilops tauschii*	Q6J6U8_AEGTA	20	14	–	
17	LMW-Glutenin subunit	40,994	9.04	*Triticum aestivum*	GLTA_WHEAT	16	3	–	
25	LMW- glutenin	32,501	8.82	*T.turgidum* subsp. *dicoccoides*	gi|53854906	39	25	–	
26	HMW-glutenin PC237	4,058	8.20	*Triticum aestivum*	GLT2_WHEAT	13	61	–	
4	High-molecular-weight glutenin subunit	15,006	8.95	*T. aestivum* subsp. *spelta.*	Q7XZA8_WHEAT	25	58	GLU-1–2	Keumgang
5	Y-type high molecular weight glutenin subunit	19,683	8.64	*Aegilops ventricosa*	gi|7188718	38	17	–	
	HMW glutenin subunit 1By16	79,420	8.75	*Triticum aestivum*	gi|146261042	34	7	–	
	HMW glutenin subunit Dty10	27,040	8.20	*Aegilops tauschii*	gi|46981764	33	12	–	
7	Y-type high molecular weight glutenin subunit	19,683	8.64	*Aegilops ventricosa*	Q9M5N3_AEGVE	33	11	–	
20	Y-type HMW- glutenin subunit	1,572	8.53	*Leymus racemosus*	gi|71159594	14	93	–	
21	LMW-glutenin subunit group 4 type II	38,417	8.89	*Triticum aestivum*	gi|17425188	33	15	LMW-GS	
	S-type low molecular weight glutenin L4-55	27,777	8.51	*Triticum aestivum*	Q6J160_WHEAT	30	15	–	
25	Y-type HMW-glutenin subunit	1,572	8.53	*Leymus racemosus*	Q1G7F6_9POAL	14	93	–	
28	HMW-Glutenin subunit PC237	4,058	8.20	*Triticum aestivum*	GLT2_WHEAT	13	41	–	
31	High molecular weight glutenin	1,007	8.53	*Triticum aestivum*	Q308Z8_WHEAT	12	87	GLU-DY	
34	Glutenin high molecular weight subunit	19,908	8.85	*Triticum aestivum*	Q8H0L3_WHEAT	20	34	HMW-GS-DY	
36	Glutenin, high molecular weight subunit PC237	4,058	8.20	*Triticum aestivum*	GLT2_WHEAT	10	41	–	
5	Glutenin, high molecular weight subunit PC237	4,058	8.20	*Triticum aestivum*	GLT2_WHEAT	11	12	–	Yeonnon-78
8	High-molecular-weight glutenin subunit	14,973	9.17	*Triticum aestivum* subsp. *spelta*	gi|32328625	29	30	GLU-A1-2	
	High-molecular-weight glutenin subunit	14,991	9.17	*Triticum aestivum*	Q7XZB8_WHEAT	44	51	GLU-A1-2	
	HMW glutenin subunit Dty10	27,040	8.20	*Aegilops tausch*	Q6PMI8_AEGTA	33	28	–	
9	Y-type high molecular weight glutenin subunit	19,683	8.64	*Aegilops ventricosa*	Q9M5N3_AEGVE	30	19	–	
26	High-molecular-weight glutenin subunit	14,991	8.95	*Triticum aestivum* subsp. *spelta*	Q7X6P8_WHEAT	15	19	GLU-A1-2	
	High-molecular-weight glutenin subunit	14,991	9.17	*Triticum aestivum*	Q7XZB8_WHEAT	44	51	GLU-A1-2	
	HMW glutenin subunit Dty10	27,040	8.20	*Aegilops tausch*	Q6PMI8_AEGTA	33	28	–	
9	Y-type high molecular weight glutenin subunit	19,683	8.64	*Aegilops ventricosa*	Q9M5N3_AEGVE	30	19	–	
26	High-molecular-weight glutenin subunit	14,991	8.95	*Triticum aestivum* subsp. *spelta*	Q7X6P8_WHEAT	15	19	GLU-A1-2	
5	High-molecular-weight glutenin subunit	15,046	8.70	*Triticum aestivum* subsp. *spelta*	gi|32328663	16	12	GLU-A1-2	China-108

6	Glutenin, high molecular weight subunit PC237	4,058	8.20	*Triticum aestivum*	GLT2_WHEAT	11	61	–	
	High molecular weight gluteni y-type	17,281	8.91	*Triticum aestivum*	gi|220900283	20	9	–	
11	Glutenin, high molecular weight subunit PC237	4,058	8.20	*Triticum aestivum*	GLT2_WHEAT	21	69	–	
	High-molecular-weight glutenin	17,860	5.35	*Triticum aestivum*	JC4966	19	24	–	
19	S-type low molecular weight glutenin L4-55	27,777	8.51	*Triticum aestivum*	Q6J160_WHEAT	38	7	–	
	LMW-glutenin subunit group 4 type II	33,456	8.71	*Triticum aestivum*	Q8W3W3_WHEAT	36	6	LMW-GS	
1	HMW glutenin subunit 1By8	77,297	8.76	*Triticum turgidum* subsp. *durum*	Q84TG6_TRITU	36	11	GLU-1B	Norin-61
	Glutenin high molecular weight subunit	19,908	8.85	*Triticum aestivum*	gi|24474926	56	33	HMW-GS-DY	
	Glutenin, high molecular weight subunit 12	70,824	7.64	*Triticum aestivum*	GLT3_WHEAT	25	9	–	
	HMW glutenin subunit Dty10	27,040	8.20	*Aegilops tauschii*	Q6PMI8_AEGTA	41	17	–	
	High molecular weight glutenin subunit y	77,325	8.94	*Triticum aestivum*	gi|14329763	44	13	GLU-1R	
	Y-type high molecular weight glutenin subunit	19,683	8.64	*Aegilops ventricosa*	Q9M5N3_AEGVE	48	24	–	
9	Glutenin, high molecular weight subunit PC237	4,058	8.20	*Triticum aestivum*	GLT2_WHEAT	20	41	–	
10	Glutenin, high molecular weight subunit PC237	4,058	8.20	*Triticum aestivum*	GLT2_WHEAT	11	12	–	
14	High-molecular-weight glutenin	17,860	5.35	*Triticum aestivum*	JC4966	18	37	–	
17	Glutenin, high molecular weight subunit PC237	4,058	8.20	*Triticum aestivum*	GLT2_WHEAT	10	41	–	
23	Glutenin, high molecular weight subunit PC256	10,889	8.18	*Triticum aestivum*	GLT1_WHEAT	19	29	–	Kantou-107
26	High-molecular-weight glutenin	17,860	5.35	*Triticum aestivum*	JC4966	17	25	–	
28	High-molecular-weight glutenin subunit	3,053	9.98	*Aegilops kotschyi*	gi|225380772	14	82	HMW-GS	
33	Glutenin, high molecular weight subunit PC256	10,889	8.18	*Triticum aestivum*	GLT1_WHEAT	8	23	–	
35	High-molecular-weight glutenin	17,860	5.35	*Triticum aestivum*	JC4966	22	34	–	

Criteria: *SN* Spot number, *Mr* mass range, *Pl* iso-electric points, *SC* sequence coverage, *GN* gene name, *CN* cultivar name

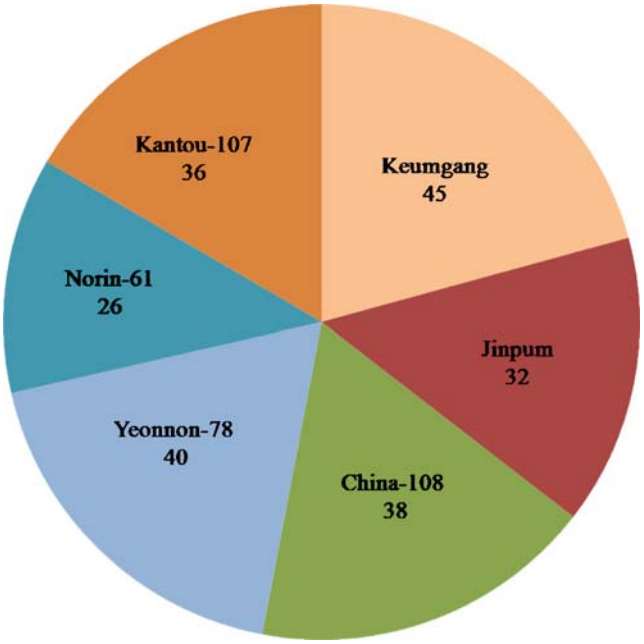

Fig. 3 Distribution of total detected protein spots by two-dimensional electrophoresis from six wheat cultivars

Most of wheat seed proteins are accumulated as protein bodies in the endosperm and embryo or in the aluerone layer. Wheat seed storage proteins, composing of HMW-GS, LMW-GS, and gliadin, are the main contributors to wheat flour quality to affect food preference (Tanaka et al. 2005). The HMW-GSs are encoded by genes at the homologous loci, *Glu-A1*, *Glu-B1*, and *Glu-D1*, which are located in the long arm of the homologous group 1 chromosome, which are 1AL, 1BL, and 1DL, respectively (Payne et al. 1982). HMW-GS plays critical roles in bread-making quality (Payne et al. 1984), and allelic variations existed at each of the *Glu-1* loci with tightly linked genes encoding x-type and y-type HMW-GS (Payne 1987).

We identified *Glu-1-2* alleles in Yeonnon-78 and China-108, which originated from Chinese wheat cultivars. Five *Glu-A1-2* alleles were identified in 19 informative sites, which suggested a polyphyletic origin of the A- and B-genomes of hexaploid wheat from Asian wheat (Blatter et al. 2004). The cultivars such as Keumgang, Yeonnon-78, and Norin-61 have Glu-Dy alleles similar to the previous report (Giles and Brown 2006), which has undergone relatively rapid change since polyploidization. Duplications and deletions of these motifs are responsible for the allelic variation at the Glu-1R locus. Orthologous genes (from different genomes) were more close than paralogous genes (x- and y-type), supporting the hypothesis of gene duplication before *Triticeae* speciation. Differences in the number and position of cysteine residues identified alleles in which the wheat cultivars are associated with good dough quality (De Bustos and Jouve 2003).Two tightly linked genes encode a lower molecular mass y-type subunit and a higher molecular mass x-type subunit at each locus, respectively. These two genes share a similar primary structure: a signal peptide (cleaved off during maturing), conservative N- and C-terminal domains, which contain most of the cysteine residues present in the HMW glutenin subunits, and a central repetitive domain constituted of tripeptides (only in x-type), hexapeptides, and nonapeptides, which are similar to our experiment (Shewry et al. 1992).

Low molecular weight (LMW) GSs ranging from about 30 to 60 kDa are associated with bread-making quality (Payne 1987). LMW-GSs are encoded by the Glu-3 loci on the short arms of homologous group 1 chromosomes. The Glu-3 loci consist (Gupta and Shephard 1990a, b) of a multigene family, estimated to include 30–40 genes (Cassidy et al. 1998).However, little is known about the roles of LMW-GSs in bread-making quality. Almost all LMW-s are, however, not associated with any cloned gene. The characterization of gene(s) encoding the LMW glutenin components associated with good bread-making quality of "Keumgang" would be helpful for elucidating how the components improve dough properties. Thus, we demonstrated that the detailed characterization of glutenin components encoded by alleles from six cultivars is possible to separate on 2D-gel. The technique is a good tool for the characterization of HMW and LMW glutenin complexes in genetic analyses such as segregation of products encoded by *Glu-1* alleles. Finally, the selection of seeds carrying both of HMW-GS and LMW-GS by wheat breeders should lead to the development of new varieties of wheat with improved bread-making qualities.

In conclusion, rapid and accurate differentiation of glutenin subunits is important in wheat quality improvement. To date, 2D-PAGE has been the most popular method to detect glutenin proteins compositions of wheat cultivars. In addition, MALDI-TOF/MS technology has been established for characterization of wheat storage proteins accompanying the development of wheat proteomics (An et al. 2006; Pei et al. 2007; Wrigley 1996). The *Glu-B1* proteins are highly variable, and the variants are often related to different quality attributes and represent a group of biochemical factors that are not yet fully utilized for wheat quality improvement. Since these variants often have similar molecular weights, it is usually difficult to differentiate them by traditional SDS-PAGE methods. The current study indicated that MALDI-TOF-MS is a powerful technique for rapid identification of HMW-GS allele diversity at the *Glu-B1* locus (Li et al. 2009). Therefore, the accurate molecular masses of the particular glutenin subunit could be obtained rapidly as performed throughout this study. Combining the traditional gel with MALDI-TOF/MS may provide an alternative tool to accelerate

quality-improvement procedures of wheat cultivars. In addition, this basic proteomic tool can be particularly useful for accurately identifying desirable glutenin subunits in the early generations of hybridization when some candidate subunits are expected to transfer from one wheat cultivar to others.

Acknowledgments This work was supported by a grant from the BioGreen 21 Program (20070301034043), Rural Development Administration, Korea (Republic of).

References

Alberghina G, Cozzolino R, Fisichella S, Garozzo D, Savarino A (2005) Proteomics of gluten: mapping of the 1Bx7 glutenin subunit in Chinese Spring cultivar by matrix-assisted laser desorption/ionization. Rapid Comm Mass Spectrom 19:2069–2074

An X, Zhang Q, Yan Y, Li Q, Zhang Y, Wang A, Pei Y, Tian J, Wang H, Hsam SLK, Zeller FJ (2006) Cloning and molecular characterization of three novel LMW-i glutenin subunit genes from cultivated einkorn (*Triticum monococcum* L.). Theor Appl Genet 113:383–395

Anderson OD, Green FC (1989) The characterization and comparative analysis of high-molecular-weight glutenin genes from genomes A and B of a hexaploid bread wheat. Theor Appl Genet 77:689–700

Anderson OD, Green FC, Yip RE, Halford NG, Shewry PR, Malpica-Romero JM (1989) Nucleotide sequences of the two high-molecular weight glutenin genes from the D-genome of a hexaploid bread wheat, *Triticum aestivum* L. cv Cheyenne. Nucleic Acids Res 17:461–462

Bietz JA, Wall JS (1972) Wheat gluten subunits: molecular weights determined by sodium sulfate-polyacrylamide gel electrophoresis. Cereal Chem 49:416–430

Bietz JA, Wall JS (1975) The effect of various extractants on the subunit composition and association of wheat glutenin. Cereal Chem 52:145–155

Blatter RH, Jacomet S, Schlumbaum A (2004) Allelic differentiation of the HMW glutenin B1-1 and A1-2 subunit genes and the origin of European Spelt (*Triticum spelta* L.). Theor Appl Genet 108:360–367

Butow BJ, Ma W, Gale KR, Cornish GB, Rampling L, Larroque O, Morell MK, Bekes F (2003) Molecular discrimination of Bx7 alleles demonstrates that a highly expressed high-molecular-weight glutenin allele has major impact on wheat flour dough strength. Theor Appl Genet 107:1524–1532

Cassidy BG, Dvorak J, Anderson OD (1998) The wheat lowmolecular-weight glutenin genes: characterization of six new genes and progress in understanding gene family structure. Theor Appl Genet 96:743–750

Cozzolino R, Di Giorgi S, Fisichella S, Garozzo D, Lafiandra D, Palermo A (2001) Matrix-assisted laser desorption/ionization mass spectrometric peptide mapping of high molecular weight glutenin subunits 1Bx7 and 1Dy10 in Cheyenne cultivar. Rapid Comm Mass Spectrom 15:778–787

De Bustos A, Jouve N (2003) Characterisation and analysis of new HMW-glutenin alleles encoded by the Glu-R1 locus of *Secale cereale*. Theor Appl Genet 107:74–83

Fukuda M, Islam N, Woo SH, Yamagishi A, Takaoka M, Hirano H (2003) Assessing matrix assisted laser desorption/ionization-time of flight-mass spectrometry as a means of rapid embryo protein identification in rice. Electrophoresis 24:1319–1329

Giles RJ, Brown TA (2006) GluDy allele variations in *Aegilops tauschii* and *Triticum aestivum*: implications for the origins of hexaploid wheats. Theor Appl Genet 112:1563–1572

Gupta RB, Shephard KW (1990a) Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutelin. 2. Genetic control of the subunits in species related to wheat. Theor Appl Genet 80:183–187

Gupta RB, Shepherd KW (1990b) Two-step one-dimensional SDS-PAGE analysis of LMW subunits of glutelin. 1: variation and genetic control of the subunits in hexaploid wheat's. Theor Appl Genet 80:65–74

Gupta RB, Khan K, MacRitchie F (1993) Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. J Cereal Sci 18:23–41

Hurkman WJ, Tanaka CK (2007) Extraction of wheat endosperm proteins for proteome analysis. J Chromatography 849:344–350

Kamal AHM, Kim KH, Shin DH, Seo HS, Shin KH, Park CS, Heo HY, Woo SH (2009) Proteomics profile of pre-harvest sprouting wheat by using MALDI-TOF mass spectrometry. Plant Omics J 2:110–119

Kussmann M, Nordhoff E, Rahbek-Nielsen H, Haebel S, Rossel-Larsen M, Jakobsen L, Gobom, Mirgorodskaya E, Kroll-Kristensen A, Palm L, Roepstorff P (1997) MALDI-MS sample preparation techniques designed for various peptide and protein analytes. J Mass Spectrometry 32:593–601

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lawrence GJ, Shepherd KW (1981) Inheritance of glutenin protein subunits of wheat. Theor Appl Genet 60:333–337

Li L, Aili W, Rudi A, Junhong M, Xianchun X, Zhonghu H, Bekes F, Yan Y, Wujun M (2009) A MALDI-TOF based analysis of high molecular weight glutenin subunits for wheat breeding. J Cereal Sci 50:295-301. doi:10.1016/j.jcs.2009.05.006

Muccilli V, Cunsolo V, Saletti R, Foti S, Masci S, Lafiandra D (2005) Characterization of B- and C-type low molecular weight glutenin subunits by electrospray ionization mass spectrometry and matrix assisted laser desorption/ionization mass spectrometry. Proteomics 5:719–728

O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. J Biol Chem 250:4007–4021

Osborne TB (1924) The vegetable proteins. Longmans Green, London, pp1–151

Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread making quality. Annu Rev Plant Physiol 38:141–153

Payne PI, Corfield KG, Blackman JA (1981) Correlation between the inheritance of certain high-molecular-weight subunits of glutenin and bread making quality in progenies of six crosses of bread wheat. J Sci Food Agric 32:51–60

Payne PI, Holt LM, Worland AJ, Law CN (1982) Structural and genetical studies on the high molecular weight subunits of wheat glutenin. Part. 3. Telocentric mapping of the subunit genes on the long arms of the homeologous group 1 chromosomes. Theor Appl Genet 63:129–138

Payne PI, Jackson EA, Holt LM (1984) The association between y-gliadin 45 and gluten strength in durum wheat varieties: a direct casual effect or the result of genetic linkage? J Cereal Sci 2:73–81

Pei YH, Wang AL, An XL, Li XH, Zhang YZ, Huang XQ, Yan YM (2007) Characterization and comparative analysis of three low molecular weight glutenin C-subunit genes isolated from *Aegilops tauschii*. Can J Plant Sci 87:273–280

Shewry PR, Halford NG, Tatham AS (1992) The high molecular weight subunits of wheat glutenin. J Cereal Sci 15:105–120

Skylas DJ, Van Dyk D, Wrigley CW (2005) Proteomics of wheat grain. J Cereal Sci 41:165–179

Tanaka H, Toyoda S, Tsujimoto H (2005) Diversity of low molecular weight glutenin genes in Asian common wheat (*Triticum aestivum* L.). Breeding Science 55:349–354

Woo SH, Fukuda M, Islam N, Takaoka M, Kawasaki H, Hirano H (2002) Efficient peptide mapping and its application to identification of embryo proteins in the rice proteome analysis. Electrophoresis 23:647–654

Wrigley CW (1996) Giant proteins with flour power. Nature 381:738–739