Note

Allelic analysis of low molecular weight glutenin subunits using 2-DGE in Korean wheat cultivars

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Two-dimensional gel electrophoresis (2-DGE) was used as a complement to SDS-PAGE to determine the allelic compositions of LMW-GS in 32 Korean wheat cultivars. Protein patterns generated by 2-DGE from each cultivar were compared to patterns from standard wheat cultivars for each allele. At the *Glu-A3* locus, thirteen *c*, twelve *d*, three *e* (null), two *g* and two new alleles were identified. At the *Glu-B3* locus, one *b*, nineteen *d*, four *h*, one *i* and five *ad* alleles were identified. At the *Glu-D3* locus, twenty-three *a*, four *b*, four *c* and one *l* alleles were identified. When compared to results obtained previously using SDS-PAGE, there were discrepancies in the allelic designations of 10 of 32 cultivars (31%). While SDS-PAGE is a rapid and relatively simple method for assessing LMW-GS composition, the similar mobilities of the proteins makes it difficult to discriminate certain alleles. 2-DGE is a more complicated technique, but provides a more accurate picture of the complement of the LMW-GS in a given cultivar. In addition to providing essential information for wheat breeders, the 2-DGE reference maps generated in this study will make it possible to study the contributions of individual LMW-GS to flour quality.

Key Words: 2-DGE, allelic analysis, flour quality, LMW-GS, *Triticum aestivum* L.

Introduction

The complement of gluten proteins accumulated during wheat grain development determines the viscoelastic properties that make it possible to produce a wide range of breads, noodles and baked goods from wheat flour. The gluten proteins consist of two complex groups of proteins, referred to as gliadins and glutenins. The gliadins are present in the flour as monomers and include three protein types on the basis of sequence, α/β -, γ - and ω -gliadins. In comparison, the glutenins are large polymers consisting of two protein types that are linked together by disulfide bonds, high molecular weight glutenin subunits (HMW-GS) of about 66 to 88 kDa and low molecular weight glutenin subunits (LMW-GS) of about 30 to 40 kDa. Most bread wheat cultivars express three to five different HMW-GS and 20 to 30 different LMW-GS. The HMW-GS are encoded by genes at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci on the long arms of chromosomes 1A, 1B, and 1D, respectively (Payne *et al.*

1980) while the LMW-GS are encoded at the *Glu-A3*, *Glu-B3*, and *Glu-D3* loci on the short arms of the same chromosomes (Gupta and Shepherd 1990). Considerable allelic variation exists at both the *Glu-1* and the *Glu-3* loci and has been associated with differences in the functional properties of wheat flour. As a result, knowledge about the allelic compositions of parental lines is critical for wheat breeding programs aimed at improving flour quality.

The HMW-GS can be separated from other gluten proteins and from each other by SDS-PAGE and their allelic variation often is assessed by this method. A number of methods have been used to characterize the more complex LMW-GS alleles, including SDS-PAGE, two-dimensional gel electrophoresis (2-DGE), polymerase chain reaction (PCR), and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). As summarized by Liu *et al.* (2010), each method has certain limitations. Although SDS-PAGE is a widely used technique, a high level of expertise is required to interpret results because of the complex banding patterns of the LMW-GS. The LMW-GS also overlap considerably with gliadins in SDS-PAGE. Additionally, some alleles are not reliably distinguished by this method. 2-DGE in which proteins are separated according to isoelectric point (pI) and molecular mass

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(MW) has been used to distinguish many but not all LMW-GS alleles and the increased resolution of 2-DGE has resulted in the identification of new alleles not observed by SDS-PAGE (Ikeda *et al.* 2006, Lee *et al.* 2016, Liu *et al.* 2010, Luo *et al.* 2015, Zhang *et al.* 2012, Zhen *et al.* 2014). PCR is a much simpler technique and primers specific for many alleles at the *Glu-A3* and *Glu-B3* loci have been developed and are currently used in breeding programs (Wang *et al.* 2009, 2010, Zhang *et al.* 2004, 2012, Zhao *et al.* 2006). However, primers are not available for all LMW-GS alleles, particularly those identified recently. Additionally, PCR has not proved to be useful for discriminating alleles at the *Glu-D3* locus because of the few differences in DNA sequences. MALDI-TOF-MS is better at distinguishing alleles at the *Glu-D3* locus but falls somewhat short in discriminating certain alleles at the *Glu-A3* and the *Glu-B3* loci. Additionally, this approach requires expensive instrumentation that is not readily available to many breeding programs (Liu *et al.* 2010).

In Korea, 38 wheat varieties have been developed since the 1970s. Early wheat breeding programs focused on developing high yielding wheat as well as early maturing varieties that were adapted to the Korean rainy season. Around the year 2000, Korean wheat breeders began to consider end-use quality in their breeding programs and focused on the *Glu-1* loci because of well-documented effects on quality (Belton 1999, Payne 1987, Shewry *et al.* 1992). More recently, reports on the importance of LMW-GS in flour quality (Gupta and Shepherd 1990, Gupta and MacRitchie 1994, Maruyama-Funatsuki *et al.* 2004, Zhang *et al.* 2012) made it clear that LMW-GS should also be considered as breeding targets. Thus, it became essential to survey the LMW-GS alleles in Korean germplasm. This was first accomplished using SDS-PAGE (Park *et al.* 2011, Shin *et al.* 2012). In this study, we use 2-DGE to confirm the allelic compositions of LMW-GS in 32 Korean wheat cultivars and to generate proteomic maps that will be used in future studies to link specific LMW-GS to quality.

Materials and Methods

Plant materials

Grain from 32 Korean hexaploid wheat (*Triticum aestivum* L.) cultivars was harvested in 2012 and 2013 by RDA National Institute of Crop Sciences, Jeonju, Korea. Standard wheat cultivars used for the identification of *Glu-3* alleles were kindly provided by National Plant Germplasm System of the USDA-ARS, USA. These included the cultivars Cheyenne, Chinese Spring, Gabo, Glenlea, Insignia, Marquis, Nanbu-Komugi, Norin 61, Orca, Opata and Suneca (Table 1). Grain was crushed with a cyclone sample mill (Udy Corporation, Fort Collins, USA).

Glutenin extraction

The glutenin was extracted using the procedure of Singh *et al.* (1991). Five hundred mg of flour was extracted with 25 ml of 50% (v/v) propanol for 30 min at 65°C. After cen-

Table 1. Standard wheat cultivars used to distinguish alleles at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci by 2-DGE

Alleles	Standard cultivars
<i>Glu-A3a</i>	Chinese Spring
<i>Glu-A3b</i>	Gabo
<i>Glu-A3c</i>	Cheyenne
<i>Glu-A3d</i>	Orca
<i>Glu-A3e</i> (null)	Marquis
<i>Glu-A3f</i>	Insignia
<i>Glu-A3g</i>	Glenlea
<i>Glu-B3a</i>	Chinese Spring
<i>Glu-B3b</i>	Gabo
<i>Glu-B3ab</i>	Nanbu-Komugi
<i>Glu-B3c</i>	Insignia
<i>Glu-B3d</i>	Orca
<i>Glu-B3g</i>	Glenlea
<i>Glu-B3h</i>	Suneca
<i>Glu-B3i</i>	Norin61
<i>Glu-B3ad</i>	Opata
<i>Glu-D3a</i>	Chinese Spring
<i>Glu-D3b</i>	Gabo
<i>Glu-D3c</i>	Insignia

trifugation at 10,000 g for 5 min, the supernatant fraction containing gliadin was removed. This extraction was repeated on the pellet two times. The precipitate was then extracted with 2.5 ml 50% (v/v) propanol, 0.08 M Tris-HCl pH 8.0 containing 1% (w/v) dithiothreitol (DTT) at 65°C for 30 min. After centrifugation at 10,000 g for 5 min, 2.5 ml of the same buffer containing 1.4% 4-vinylpyridine (v/v) instead of 1% DTT was added for alkylation at 65°C for 15 min. After centrifugation at 10,000 g for 2 min, the supernatant was moved to a new 1.5 ml tube and stored at -20°C overnight.

Two-dimensional gel electrophoresis

For two-dimensional gel electrophoresis (2-DGE), the extracted glutenin fractions were precipitated using 15% (v/v) TCA/acetone at -20°C for 3 hours and the resulting glutenin pellets were mixed completely in 70 µl of rehydration buffer (7 M urea, 2 M thiourea, 2% CHAPS, 0.5% IPG buffer) (GE Healthcare Life Sciences, USA) containing 12.8 µl 1 M DTT. Protein was quantified by the method of Bradford (1976). Fifty µg of glutenin in 350 µl rehydration buffer containing 12.8 µl 1 M DTT was loaded onto an 18 cm IPG strip (pI 6–11 or 3–11) (GE Healthcare Life Sciences, USA) and rehydrated in-gel for 15 hours at 20°C using the IPGphor system (Amersham Biosciences, GE Healthcare Life Sciences, USA). IEF was carried out for a total of 80 kVh. The IPG gel strips were equilibrated with 6 M urea, 75 mM Tris-HCl pH 8.8, 29.3% glycerol, 2% SDS and 1% DTT for 15 min and then incubated with the same buffer containing 2.5% iodoacetamide instead of DTT for 15 min. 2-DGE was performed using a 12.5% gel. After electrophoresis, gels were stained with Coomassie Brilliant Blue R-250 for 3 hours followed by destaining with 10% methanol and 10% acetic acid for 3 hours. Gels were analyzed using Image Master Platinum 6.0 (GE Healthcare Life Sciences, USA).

Mass spectrometry

For the identification of proteins by mass spectrometry (MS), spots were excised from the Coomassie-stained 2-D gels and subjected to in-gel digestion using chymotrypsin. The chymotryptic peptides were subsequently analyzed using nanoAcquity UPLC coupled to a Synapt G1 HDMS mass spectrometer (Waters, Milford, MA). MS spectra were searched against a database containing 63,245 *Triticum* protein sequences downloaded from the NCBI non-redundant database (www.ncbi.nlm.nih.gov) and 151,173 *Triticum* protein sequences downloaded from UniProt (www.uniprot.org).

MS/MS analyses were conducted at the Korea Basic Science Institute, Seoul, Korea.

Results

The allelic compositions of LMW-GS in 32 Korean cultivars were previously determined by SDS-PAGE (Park *et al.* 2011, Shin *et al.* 2012). Three alleles (c, d, e) were reported at the *Glu-A3* locus, four alleles (b, d, h, i) were found at the *Glu-B3* locus, and three alleles (a, b, c) were reported at the *Glu-D3* locus in these cultivars.

Fig. 1. LMW-GS profiles of standard wheat cultivars determined by 2-DGE. Only the regions of the gels containing LMW-GS are shown. Black boxes highlight LMW-GS proteins that are found in all cultivars and used as reference points among gels. Cultivar names and allelic designations are indicated on the lower left and right of each gel, respectively. A) *Glu-A3* alleles. Characteristic proteins in each cultivar are shown in red circles. B) *Glu-B3* alleles. Characteristic proteins in each cultivar are shown in blue circles. Blue arrows point to additional spots in the boxed areas. C) *Glu-D3* alleles. Characteristic proteins in each cultivar are shown in green circles.

Fig. 1. (continued)

2-DGE of standard cultivars

LMW-GS profiles from the 32 cultivars were generated by 2-DGE and compared with profiles generated from a set of standard cultivars with known allelic compositions. Standard cultivars used for this analysis are shown in Table 1 and 2-DGE profiles of glutenin fractions from the cultivars are shown in Fig. 1. Interestingly, there are four spots with molecular weights of about 43 kDa and isoelectric points of about 9.2 that are found in nearly all of the standard cultivars as well as most of the Korean cultivars. These spots are shown in black boxes in Fig. 1 and Fig. 2 and were used as reference points among the gels. Previous analysis of spots within the black box by tandem mass spectrometry in the Korean cultivar Keumkang demonstrated that all four spots correspond to m-type LMW-GS beginning with the N-terminal amino acid methionine. The two spots in the lower right corner of the box correspond to proteins encoded by the *Glu-B3* locus while the two spots in the upper left portion of the box correspond to proteins encoded by the *Glu-D3* locus (Lee *et al.* 2016).

These common LMW-GS spots were also reported by Ikeda *et al.* (2006). Only two Korean cultivars, Hanbaek and Uri, do not contain all four spots in this region (Fig. 2A, 2B, black arrows).

The cultivars Chinese Spring, Gabo, Cheyenne, Orca, Marquis, Insignia and Glenlea were used as standards for the *Glu-A3* alleles a, b, c, d, e, f and g, respectively (Table 1). *Glu-A3e* is a null allele. With the exception of *Glu-A3a* and *Glu-A3b*, the patterns of spots corresponding to the *Glu-A3* alleles were readily distinguishable in the standard cultivars (Fig. 1A). The cultivars Chinese Spring, Gabo, Cheyenne, Orca, Glenlea, Suneca, Norin 61, Nanbu-Komugi, and Opata were used to represent the *Glu-B3* alleles a, b, c, d, g, h, i, ab and ad, respectively (Table 1). With the exception of *Glu-B3d* and *Glu-B3i*, all alleles could be distinguished by 2-DGE (Fig. 1B). The *Glu-D3* alleles a, b and c were represented by the cultivars Chinese Spring, Gabo and Insignia, respectively (Table 1). *Glu-D3a* and *Glu-D3b* alleles are not readily distinguished by 2-DGE (Fig. 1C).

Allelic analysis of Korean wheat cultivars at *Glu-3* loci by 2-DGE

At the *Glu-A3* locus, 13 of the 32 Korean cultivars contained 2-DGE spots characteristic of the *Glu-A3c* allele (Fig. 2A, red circles; Table 2). Twelve cultivars contained spots characteristic of the *Glu-A3d* allele (Fig. 2B, red circles; Table 2) and two cultivars (Jinpoom, Seodun) contained three spots that are characteristic of the *Glu-A3d* allele, but did not contain the two higher MW spots in the acidic portion of the gel (Fig. 2C, red circles). Since the 2-DGE profile does not match that from any of the seven

standard *Glu-A3* cultivars, Jinpoom and Seodun probably contain a new unidentified *Glu-A3* allele, referred to here as *UI*. Two cultivars, Jonong and Sinnichal 1 contained the five spots that were indicative of the *Glu-A3g* allele (Fig. 2D, red circles; Table 2). Three cultivars, Anabaek, Joeun and Ol, contained the null allele, *Glu-A3e*, and did not have any spots corresponding to proteins encoded at the *Glu-A3* locus (Fig. 2A, Table 2).

At the *Glu-B3* locus, 20 Korean cultivars contained the five spots characteristic of the *Glu-B3d* and *Glu-B3i* alleles (Fig. 2A, 2B, 2C, blue circles; Table 2). Four cultivars

Fig. 2. LMW-GS profiles of Korean wheat cultivars determined by 2-DGE. Only the regions of the gels containing LMW-GS are shown. Black boxes highlight LMW-GS proteins that are found in most cultivars and used as reference points among gels. Spots corresponding to *Glu-A3*, *Glu-B3* and *Glu-D3* alleles are shown in red, blue and green circles, respectively. Black arrows point to spots that are missing in the boxed areas. Blue arrows point to additional spots in the boxed areas. Cultivars are arranged in alphabetical order from left to right within each panel. Cultivar names and allelic designations are indicated on the lower left and right of each gel, respectively. Panels B and D include spots located in the acidic portion of the gels that are characteristic of the *Glu-A3d* and *Glu-B3g* alleles, respectively while Panel C demonstrates that cultivars Jinpoom and Seodun do not contain spots in the acidic portion of the gel that are characteristic of the *Glu-A3d* allele.

Fig. 2. (continued)

contained the six spots characteristic of the *Glu-B3h* allele (Fig. 2A, blue circles; Table 2). Only one cultivar, Sukang, contained the four spots typical of the *Glu-B3b* allele (Fig. 2B, blue circles; Table 2). Two cultivars (Jonong, Sinmichal 1) contained the same *Glu-B3* spots as Sukang,

but also contained the higher MW protein on the acidic front of the gel that is characteristic of the *Glu-B3g* allele (Fig. 2D, blue circles; Table 2). Finally, five cultivars (Anbaek, Dajoong, Geuru, Suan and Jopoom) had 2-DGE profiles that were similar to those with the *Glu-B3d* and

Table 2. Allelic compositions of LMW-GS at the *Glu-A3*, *Glu-B3* and *Glu-D3* loci in Korean cultivars determined by SDS-PAGE and 2-DGE. Cultivars are listed in alphabetical order along with the pedigree for each cultivar

Cultivar	Pedigree	LMW-GS alleles						Reference Figure	Breeding years
		SDS-PAGE ^a			2-DGE ^b				
		<i>Glu-A3</i>	<i>Glu-B3</i>	<i>Glu-D3</i>	<i>Glu-A3</i>	<i>Glu-B3</i>	<i>Glu-D3</i>		
Alchan	Suwon210/Tapdong	d	d	b	d	d ^c	b ^d	2B	1994
Anbaek	Sae/Geuru	e	i	a	e	ad	a ^d	2A	2001
Baekjoong	Keumkang/Olgeuru	c	d	a	c	d ^c	a ^d	2A	2007
Chungkye	Norin4/Sharbatisonora	d	d	c	d	d ^c	c	2B	1979
Dabun	Suwon234//sw76039/Suwon220/3/Keumkang	c	d	a	c	d ^c	a ^d	2A	2006
Dahong	Norin72/Wonkwang	c	i	a	c	i ^c	a ^d	2A	1979
Dajoong	SW992114-NM-131-7/Gobun	c	i	a	c	ad	a ^d	2A	2010
Eunpa	Chugoku81/3/Tob-CNO// Yuksung3/Suwon185	d	d	a	d	d ^c	a ^d	2B	1982
Geuru	Strampelli/69D-3607//Chokwang	c	i	a	c	ad	a ^d	2A	1980
Gobun	Eunpa/Tapdong/Eunpa/Shannung6521	d	d	b	d	d ^c	b ^d	2B	1995
Goso	Gobun/Ol	d	d	a	d	d ^c	a ^d	2B	2010
Hanbaek	Shann7859/Keumkang//Guamuehill	c	h	c	c	h	l	2A	2008
Jeokjoong	Keumkang/Tapdong	c	d	a	c	d ^c	a ^d	2A	2007
Jinpoom	Geuru/Genaro81	d	d	c	UI ^e	d ^c	c	2A	1998
Joeun	Eunpa/Suwon242	e	h	a	e	h	a ^d	2A	2000
Jokyung	Seri82/Keumkang	c	h	a	c	h	a ^d	2A	2004
Jonong	Suwon234/SW80199	c	h	a	g	g	a ^d	2C	2003
Jopoom	Kanto75//OR8500494P/Bezostaya	d	i	a	d	ad	a ^d	2B	2001
Keumkang	Geuru/Kanto75//Eunpa	c	h	a	c	h	a ^d	2A	1996
Milsung	Shirogane//Norin43/Sonalika	c	d	a	c	d ^c	a ^d	2A	1998
Namhae	Ol/Calidad	d	d	a	d	d ^c	a ^d	2B	1988
Ol	Norin72/Norin12	e	d	a	e	d ^c	a ^d	2A	1976
Olgeuru	Geuru/Chokwang//Saikai143	d	d	a	d	d ^c	a ^d	2B	1993
Saeol	Shirogane//Norin43/Sonalika	d	d	b	d	d ^c	b ^d	2B	1997
Seodun	Geuru/Genaro81	d	d	c	UI ^e	d ^c	c	2A	1997
Sinmichal	Olgeuru//Kanto107/BaiHuo	c	d	a	c	d ^c	a ^d	2A	2002
Sinmichal1	Alchan//Kanto107/BaiHuo	c	b	a	g	g	a ^d	2C	2006
Suan	Keumkang/Eunpa//Keumkang	c	i	a	c	ad	a ^d	2A	2009
Sukang	Suwon266/Asakaze	d	b	a	d	b	a ^d	2B	2008
Tapdong	Chugoku81//Suwon158/Toropi	d	d	b	d	d ^c	b ^d	2B	1986
Uri	Geuru/Ol	d	d	c	d	d ^c	c	2B	1992
Younbaek	Keumkang/Tapdong	c	d	a	c	d ^c	a ^d	2A	2005

^a Allelic determinations from Park *et al.* (2011) and Shin *et al.* (2012).^b Allelic designations determined by 2-DGE that differ from those determined by SDS-PAGE are indicated in underlined boldface type.^c *Glu-B3d* and *Glu-B3i* alleles were not readily distinguished by 2-DGE, but can be distinguished by PCR (Wang *et al.* 2009).^d *Glu-D3a* and *Glu-D3b* alleles were not readily distinguished by 2-DGE, but can be distinguished by SDS-PAGE.^e denotes new unidentified allele.

Glu-B3i alleles, but contained two additional minor spots in the lower right corner of the black box (indicated by blue arrows in **Fig. 2A, 2B**). These profiles were similar to standard cultivar Opata containing *Glu-B3ad*.

Because the spot that distinguishes the *Glu-B3b* allele in the standard cultivar Gabo and the Korean cultivar Sukang from the *Glu-B3g* allele found in the standard cultivar Glenlea and the Korean cultivars Jonong and Sinmichal 1 migrates at the acidic front of the gel, 2-DGE was conducted using a pI range of 3–11 instead of 6–11 (**Fig. 3**). A single spot of ~63 kDa was observed between the HMW-GS and gliadins in the acidic region of the gel in Glenlea, Jonong and Sinmichal 1, but not in Gabo or Sukang. The 63 kDa spot in Glenlea was excised from the gel, digested with chymotrypsin, analyzed by tandem mass spectrometry and identified as a D-type LMW-GS (UniProtKB Accession B6ETS0) on the basis of the peptides SQQQISQQPQQ LPQQQQIPQQPQQF, QQYPQQQPSGSDVISICGL, and PQQPQQF. D-type LMW-GS have sequences like omega

gliadins but contain a single cysteine residue and are encoded at the closely linked *Gli-B1* locus.

At the *Glu-D3* locus, 27 of the Korean cultivars contained the four spots that are characteristic of the *Glu-D3a* and *Glu-D3b* alleles. Because these two alleles could not be distinguished by 2-DGE, the allelic designations determined by SDS-PAGE were considered to be confirmed. As shown in **Fig. 4**, these alleles can be distinguished by SDS-PAGE. Four cultivars contained the three spots characteristic of the *Glu-D3c* allele (Jinpoom, Seodun, Chungkye, Uri) (**Fig. 2B, 2C**, green circles; **Table 2**). The 2-DGE profile of one cultivar, Hanbaek, did not correspond to any of the *Glu-D3* alleles shown in **Fig. 1**. This cultivar contained two spots that are consistent with the *Glu-D3l* allele described in the cultivar Amadina by Liu *et al.* (2010). However, Liu *et al.* (2010) suggested that a third spot with a lower MW and more acidic pI was also characteristic of this allele. While Hanbaek contains a spot in a similar position, the protein in the spot has an N-terminal sequence corresponding to a gamma gliadin

Fig. 3. 2-DGE patterns of glutenin fractions of standard wheat cultivars Gabo (*Glu-B3b*) and Glenlea (*Glu-B3g*) and Korean wheat cultivars Sukang, Jonong and Sinmichal 1 using a pI range of 3–11. Protein spots corresponding to *Glu-B3* are shown in blue circles. Gliadins are indicated with orange circles. The position of the protein spot that distinguishes the *Glu-B3g* allele from the *Glu-B3b* allele is indicated with an arrow.

Fig. 4. Comparison of profiles of standard cultivars and Korean cultivar Jonong by SDS-PAGE. Red, blue and green arrows indicate the positions of bands characteristic of *Glu-A3*, *Glu-B3* and *Glu-D3* alleles, respectively. *Glu-B3* bands that are common to most cultivars are shown in the black dotted box.

rather than a typical LMW-GS (data not shown).

There were discrepancies between the LMW-GS allelic designations determined by SDS-PAGE and by 2-DGE for ten of the 32 Korean cultivars (31%) (**Table 2**). Four alleles at the *Glu-A3* locus were scored incorrectly (13%). In two cultivars, the *Glu-A3c* allele should have been scored as the *Glu-A3g* allele. In another two cultivars, the *Glu-A3d* allele should have been scored as a new unidentified allele. Seven cultivars were scored incorrectly at the *Glu-B3* locus by SDS-PAGE (30%). In five cultivars, 2-DGE revealed that the *Glu-B3i* allele should have been scored as the *Glu-3ad* allele. In two other cases, either the *Glu-B3h* or the *Glu-B3b* allele should have been scored as a *Glu-B3g* allele. Because the *Glu-B3h* and *Glu-B3g* alleles should be readily distinguished by SDS-PAGE, a separate sample from Jonong was analyzed by SDS-PAGE along with standard cultivars for all of the *Glu-B3* alleles (**Fig. 4**). This analysis demonstrated that the *Glu-B3* allele reported by Park *et al.* (2011) and Shin *et al.* (2012) in Jonong was incorrect and that the correct designation was *Glu-B3g*. Since the SDS-PAGE analysis of LMW-GS in Jonong was not shown in either report, it is impossible to determine whether the discrepancy was due to misidentification of gel bands or contamination of seed. Only one allele at the *Glu-D3* locus was scored incorrectly. 2-DGE revealed that a *Glu-D3c* allele should have been scored as a *Glu-D3l* allele.

Discussion

2-DGE was used to provide accurate information about the allelic compositions of LMW-GS in 32 Korean wheat cultivars. The study revealed five additional *Glu-3* alleles not detected previously in these cultivars by SDS-PAGE. These included two alleles at the *Glu-A3* locus (*g* and an unidentified allele, *UI*), two at the *Glu-B3* locus (*g*, *ad*) and one at the *Glu-D3* locus (*l*). The correct identification of the *Glu-B3* alleles is important for breeding efforts to improve wheat quality since certain alleles are associated with favorable characteristics. Previous studies using near isogenic lines (NILs) demonstrated that *Glu-A3d*, *Glu-B3b*, *Glu-B3g*, and *Glu-B3i* alleles are associated with superior bread-making quality, while *Glu-A3e* and *Glu-B3c* alleles are related to inferior properties. *Glu-D3* alleles made only slight differences in quality (Zhang *et al.* 2012). The *Glu-A3d* allele was found in 12 cultivars while the *Glu-B3b* was present in one, the *Glu-B3g* in two, and *Glu-B3i* in one of the 32 Korean cultivars. Three cultivars contained the inferior *Glu-A3e* allele.

In this study, 31% of the cultivars were scored incorrectly using SDS-PAGE. It is noteworthy that a number of the alleles associated with good quality were misidentified by SDS-PAGE. Seven cultivars were incorrectly scored by SDS-PAGE as having good quality alleles. Two cultivars scored as *Glu-A3d* by SDS-PAGE (Jinpoom, Seodun) had new unidentified alleles by 2-DGE and five cultivars scored as *Glu-B3i* by SDS-PAGE (Anbaek, Dajoong, Geuru, Jopoom, Suan) had *Glu-B3ad* alleles by 2-DGE. Additionally,

Table 3. Frequency of alleles for LMW-GS in 32 Korean wheat cultivars

Locus	Allele	Number of cultivars	Frequency (%)
<i>Glu-A3</i>	<i>c</i>	13	40.6
	<i>d</i>	12	37.5
	<i>e</i>	3	9.3
	<i>g</i>	2	6.3
	<i>UI</i>	2	6.3
<i>Glu-B3</i>	<i>b</i>	1	3.1
	<i>d</i>	19	59.4
	<i>g</i>	2	6.3
	<i>h</i>	4	12.5
	<i>i</i>	1	3.1
	<i>ad</i>	5	15.6
<i>Glu-D3</i>	<i>a</i>	23	71.9
	<i>b</i>	4	12.5
	<i>c</i>	4	12.5
	<i>l</i>	1	3.1

one cultivar (Jonong) was scored incorrectly by SDS-PAGE as *Glu-B3h*, but was found to have the good quality allele *Glu-B3g* by 2-DGE. In this case, incorrect scoring of the LMW-GS allele may have been due to a mix-up in the seed sample.

Overall, the predominant LMW-GS alleles were the *Glu-A3c* and *Glu-A3d*, found in 41 and 38% of cultivars, respectively, the *Glu-B3d* found in 59% and the *Glu-D3a* found in 72% of the cultivars (**Table 3**). The study suggests that Korean wheat cultivars have a very narrow genetic base with regards to the LMW-GS, probably because there was little selection for these genes in the past. *Glu-A3c* is also a predominant allele in wheat from the US, Australia and Argentina while the *Glu-A3d* allele is the predominant allele in wheat from China and also is prevalent in wheat from Japan and France (Branlard *et al.* 2003, Eagles *et al.* 2002, Lerner *et al.* 2009, Li *et al.* 2009, Shan *et al.* 2007, Tanaka *et al.* 2005). The *Glu-B3d* allele is moderately abundant in Chinese wheat, but rarely found in wheat from the US, Australia, Japan, France or Argentina. The most frequent *Glu-D3* allele in Korean cultivars, *Glu-D3a*, is also moderately abundant in US wheat.

Eight of the Korean cultivars analyzed in this study are frequently found in the pedigrees of other Korean cultivars (Alchan, Eunpa, Geuru, Gobun, Keumkang, Ol, Olgeuru, Tapdong). Of these, Alchan, Keumkang and Tapdong contain the *Glu-D1d* allele encoding HMW-GS subunits 1Dx5 and 1Dy10 that are associated with gluten strength. Alchan and Tapdong also contain the favorable *Glu-A3d* allele. It is interesting that more than half of the cultivars released since 2000 contain Alchan, Keumkang or Tapdong in their backgrounds.

While the current work focused solely on Korean cultivars, it is likely that the lessons learned, particularly the high rate of misidentification of alleles resulting from SDS-PAGE alone, also apply to cultivars being used in the breeding programs of other countries. The 2-DGE patterns generated in this study serve as reference maps of LMW-GS for

most Korean cultivars that are not only important for wheat breeders but also will facilitate further study on the contributions of individual LMW-GS to flour functional properties. For example, it should be possible to analyze individual spots from these maps by tandem mass spectrometry and determine differences in the sequences of specific LMW-GS proteins that might relate to flour functional properties.

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