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Characterization and Comparative Analysis of Wheat High Molecular Weight Glutenin Subunits by SDS-PAGE, RP-HPLC, HPCE, and MALDI-TOF-MS

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High molecular weight glutenin subunits (HMW-GS) from 60 germplasms including 30 common wheat cultivars and 30 related species were separated and characterized by a suite of separation methods including sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE), reversed-phase high-performance liquid chromatography (RP-HPLC), high-performance capillary electrophoresis (HPCE), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Comparative analysis demonstrated that each methodology has its own advantages and disadvantages. The main drawback of SDS-PAGE was its overestimation of molecular mass and incorrect identification of HMW-GS due to its low resolution. However, it had the advantages of technical simplicity and low requirements of equipment; thus, it is suitable for large-scale and high-throughput HMW-GS screening for breeding programs, especially when the glutenin composition is clear in the breeding material. MALDI-TOF-MS clearly expressed many technical advantages among the four methods evaluated, including high throughput, high resolution, and accuracy; it was, however, associated with high equipment cost, thus preventing many breeding companies from accessing the technology. RP-HPLC and HPCE were found to be intermediate between SDS-PAGE and MALDI-TOF-MS. Both RP-HPLC and HPCE demonstrated higher resolution and reproducibility over SDS-PAGE but lower detection power than MALDI-TOF-MS. Results demonstrated that MALDI-TOF-MS is suitable for analyzing HMW-GS for routine breeding line screening and for identifying new genotypes.

KEYWORDS: HMW-GS; RP-HPLC; HPCE; MALDI-TOF-MS; wheat

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important grain crops in the world. Its flour is used to make various food products, such as pan bread, noodle, and cakes. It is well-known that the grain endosperm contains storage proteins, mainly consisting of glutenins and gliadins that form the functional gluten through intermolecular disulfide bonds (*1*, *2*). The glutenins, including high molecular weight glutenin subunits (HMW-GS, 70−140 kDa), low molecular weight glutenin subunits (LMW-GS, 30−50 kDa), and gliadins (30−80 kDa), confer dough visco-elasticity and extensibility. In particular, HMW-GS play the most important role in bread-making quality although they represent approximately only 10% of the total seed storage proteins (*3*).

The HMW-GS are encoded by *Glu-1* loci on the long arms of group 1 chromosomes of wheat, including 1A, 1B, and 1D (*1*, *3*, *4*). These loci are named *Glu-A1*, *Glu-B1*, and *Glu-D1* on the basis of their chromosome name. Each locus includes two genes linked together encoding two different types of HMW-GS, x- and y-type subunits (*1*−*4*). The x-type subunits generally have a slower electrophoretic mobility in SDS-PAGE and higher molecular weight than the y-type subunits. Each subunit has been assigned a unique number, which relates to the ascending number of all subunits in SDS-PAGE. When subunits are identified numerically, it is customary to include both the genome from which the subunit is derived and the indication of whether it is an x-type or y-type subunit, for example, Dx5, By9, etc. For simplicity purposes, HMW-GS alleles can be expressed as pure numbers, for example, 5 + 10 (which means Dx5 + Dy10).

Genetic analysis showed that extensive allelic variations in HMW-GS compositions exist. A high number of subunits and

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Table 1. Wheat Materials from Different Species and Their HMW-GS Compositions

				HMW-GS *Glu-B1*			
no.	cultivar and accession no.	species	genome (n)	*Glu-A1*		*Glu-D1*	source
1	Chinese Spring	*T. aestivum* L.	ABD	N	7 + 8	2 + 12	Rothamsted Research, U.K.
2	Jing 411	*T. aestivum* L.	ABD	N	7 + 8	2 + 12	CAAS China
3	Hanno	*T. aestivum* L.	ABD	1	14 + 15	5 + 10	
4	Imbros	*T. aestivum* L.	ABD	1	14 + 15	2 + 12	
5	Xiaoyan 6	*T. aestivum* L.	ABD	1	20x + 20y	2 + 12	
6	Xiaoyan 54	*T. aestivum* L.	ABD	1	20x + 20y	2 + 12	
7	Chuanmai 45	*T. aestivum* L.	ABD	N	20x + 20y	5 + 10	
8	Chuanyu 20	*T. aestivum* L.	ABD	1	20x + 20y	5 + 10	
9	PH-85-16	*T. aestivum* L.	ABD	1	20x + 20y	2 + 12	
10	Linfen 127	*T. aestivum* L.	ABD	1	20x + 20y	2 + 12	
11	Jing 771	*T. aestivum* L.	ABD	1	20x + 20y	5 + 10	
12	Jing 9428	*T. aestivum* L.	ABD	2*	7 + 8	2 + 12	
13	Jimai 19	*T. aestivum* L.	ABD	1	13 + 16	5 + 10	
14	Jimai 20	*T. aestivum* L.	ABD	1	13 + 16	5 + 10	
15	Jinmai 67	*T. aestivum* L.	ABD	2*	7 + 9	5 + 10	
16	Linfen 138	*T. aestivum* L.	ABD	2*	7 + 8	2 + 12	
17	Nongda 3197	*T. aestivum* L.	ABD	N	7 + 8	3 + 12	
18	Ping 4	*T. aestivum* L.	ABD	N	6 + 8	4 + 12	
19	Nei 01	*T. aestivum* L.	ABD	N	7 + 9	5 + 10	
20	Jinmai 67	*T. aestivum* L.	ABD	2*	7 + 9	5 + 10	
21	Chuanmai 39	*T. aestivum* L.	ABD	1	17 + 18	5 + 10	
22	History	*T. aestivum* L.	ABD	1	6 + 8	2 + 12	
23	Kontrast	*T. aestivum* L.	ABD	N	17 + 18	5 + 10	
24	Wanmai 23	*T. aestivum* L.	ABD	1	13 + 19	5 + 10	
25	MG315	*T. aestivum* L.	ABD	N	7 + 9	2.2* + 12	
26	MG7249	*T. aestivum* L.	ABD	2*	7 + 8	2.2 + 12	
27	Jin 45	*T. aestivum* L.	ABD	N	7 + 9	5 + 10	
28	R-1	*T. aestivum* L.	ABD	1	6* + 8	1.5 + 10	
29	R-13	*T. aestivum* L.	ABD	1	7 + 8	1.5 + 10	
30	R-75	*T. aestivum* L.	ABD	N	6* + 8	1.5 + 10	
31	Spelt 137	*T. spelta* L.	ABD	2.1*	13 + 16	2 + 12	TUM Germany
32	Spelt 73	*T. spelta* L.	ABD	1	6.1 + N	5 + 10	
33	Spelt 77	*T. spelta* L.	ABD	1	6.1 + 22.1	2 + 12	
34	Spelt 20	*T. spelta* L.	ABD	N	13* + 19*	2 + 12	
35	Spelt 30	*T. spelta* L.	ABD	1	7 + 9	2 + 12	
36	Spelt 166	*T. spelta* L.	ABD	1	13 + 16	2 + N	
37	Spelt 130	*T. spelta* L.	ABD	2*	13 + 16	2 + 12	
38	Spelt 225	*T. spelta* L.	ABD	1	13 + 16	3 + 12	
39	Club 62	*T. compactum* L.	ABD	2.1*	6 + 8	2 + 12	
40	Club 18	*T. compactum* L.	ABD	N	6	2 + 12	
41	Club 29	*T. compactum* L.	ABD	N	7 + 8	5* + 10	
42	Club 58	*T. compactum* L.	ABD	N	13* + 19*	2 + 12	
43	Bidi 17	*T. durum* L.	AB	N	20x + 20y		Rothamsted Research, U.K.
44	Creso	*T. durum* L.	AB	N	6 + 8		
45	Simeto	*T. durum* L.	AB	N	7 + 8		
46	Y1	*T. dicoccoides* L.	AB	1	7 + 8		TUM Germany
47	Y8	*T. dicoccoides* L.	AB	2*	13 + 22.1		
48	PI481478	*T. dicoccoides* L.	AB	2*	13 + 16		
49	Y12	*T. dicoccoides* L.	AB	1	N + 16		
50	PI554584	*T. dicoccoides* L.	AB	1	13 + 19		
51	PI428097	*T. dicoccoides* L.	AB	N	6* + 22*		
52	PI414718	*T. dicoccoides* L.	AB	1*	1.1 + 15*		
53	D154	*T. dicoccum* L.	AB	1	6.1 + 22.1		
54	HT159	*T. dicoccum* L.	AB	2*	7 + 8		
55	TD81	*T. tauschii* L.	D			5* + 10.1	
56	TD130	*T. tauschii* L.	D			5.1* + 10	
57	TD128	*T. tauschii* L.	D			5.2 + 10	
58	TD132	*T. tauschii* L.	D			2 + 10.1	
59	TD8	*T. tauschii* L.	D			2 + 12.1	
60	TD9	*T. tauschii* L.	D			2 + 12.1	

alleles have been identified in common wheat and related species so far (*4*−*9*). The genes coding for HMW-GS were located on the long arms of chromosomes 1A, 1B, and 1D, namely, three complex loci *Glu-1A*, *Glu-1B*, and *Glu-1D*, respectively (*1*). It is

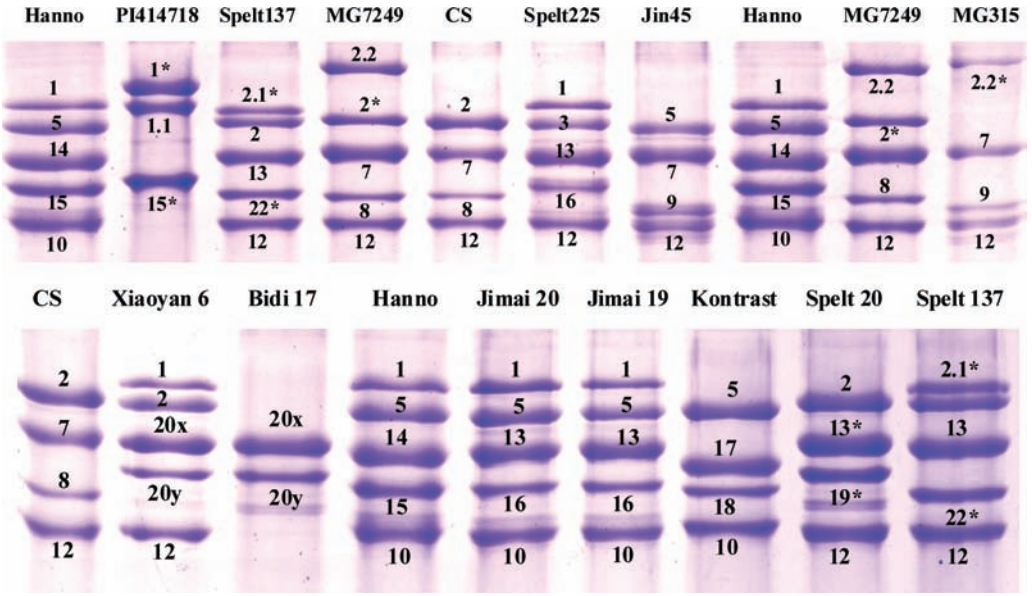

Figure 1. SDS-PAGE patterns of HMW glutenin subunits from some bread wheat cultivars and related species.

well-known that different alleles at the *Glu-1* locus confer different end-use quality. For instance, the allelic pairs of 1Dx5 + 1Dy10 and 1Bx17 + 1By18 have much stronger positive effects on dough properties than other allelic pairs such as 1Dx2 + 1Dy12 (*1*, *10*). Therefore, HMW-GS have become useful protein markers for wheat quality improvement and cultivar identification.

In the past several decades, considerable efforts have been focused on developing powerful techniques for fast differentiation of good- and poor-quality HMW-GS in wheat for germplasm screening and quality improvement (*11*−*15*). Up to now, sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) has been the most popular method to detect the allelic compositions of HMW-GS on the basis of their mobilities (*16*). In the 1980s, reversed-phase high-performance liquid chromatography (RP-HPLC) was developed and used for HMW-GS identification (*11*, *17*). Recently, new technologies, such as high-performance capillary electrophoresis (HPCE) and various mass spectrometry (MS) methods, have been established to characterize HMW-GS (*18*−*23*), which appear to be more effective than the traditional methods. In particular, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has experienced rapid development and recently became a powerful tool for storage protein studies (*24*−*27*).

In this study, HMW-GS compositions of 60 genotypes were analyzed by four methods, SDS-PAGE, RP-HPLC, HPCE, and MALDI-TOF-MS. A key objective was to compare and evaluate these methods in terms of resolution, sensitivity, accuracy, and throughput, which are important factors for application in genetic research and breeding practices.

MATERIALS AND METHODS

Wheat Materials. The wheat materials used included 30 common wheat cultivars and 30 accessions from different related species as listed in **Table 1**, which were mainly collected from Institute of Plant Breeding, Technische Universität München (TUM), Germany, and Crop Research Institute, Chinese Academy of Agricultural Science (CAAS). The common wheat line 'Chinese Spring' and durum wheat line Bidi-17 were used as controls (kindly provided by Dr. Peter Shewry).

HMW-GS Extraction and Sample Preparation. HMW-GS were extracted from wheat grains by using a modified method according to Yan et al. (*5*). One half-seed (about 30−40 mg) was crushed and 120 μL 70% (v/v) aqueous ethanol was added. After 30 min at room temperature with periodic agitation and centrifugation for 10 min at 13000 rpm, the residue was washed with 500 μL of 55% (v/v) isopropanol for 30 min at 65 °C and centrifuged for 10 min at 13000 rpm. The supernatant was discarded. This step removes gliadins and was repeated twice. The pellet was then

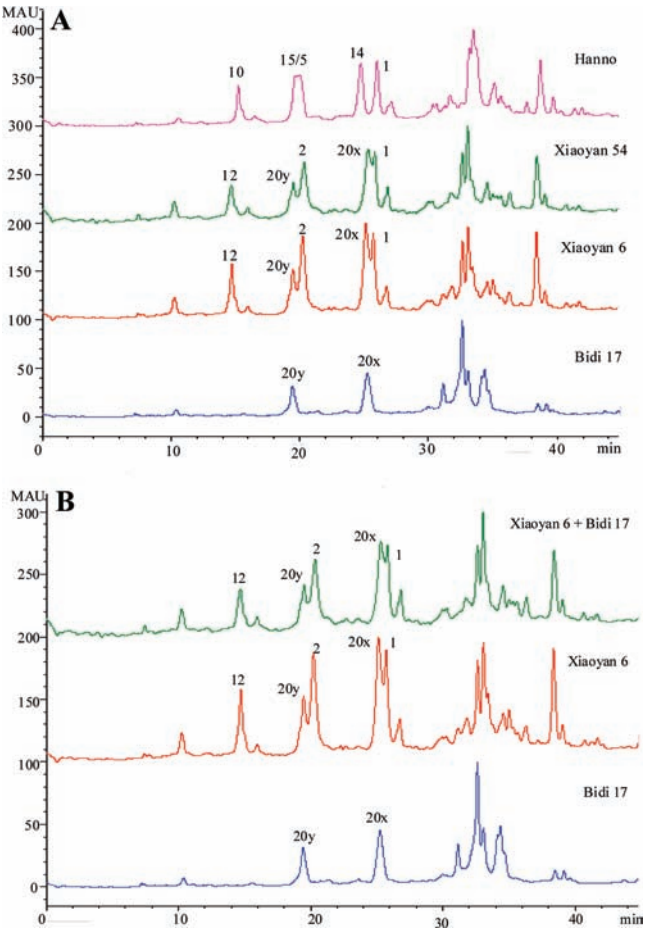

Figure 2. Discrimination between 1Bx20 + 1By20 and 1Bx14 + 1By15 subunits by RP-HPLC: (**A**) RP-HPLC patterns HMW-GS from four cultivars; (**B**) RP-HPLC patterns of HMW-GS from Xiaoyan 6 and Bidi 17 and their mixing sample.

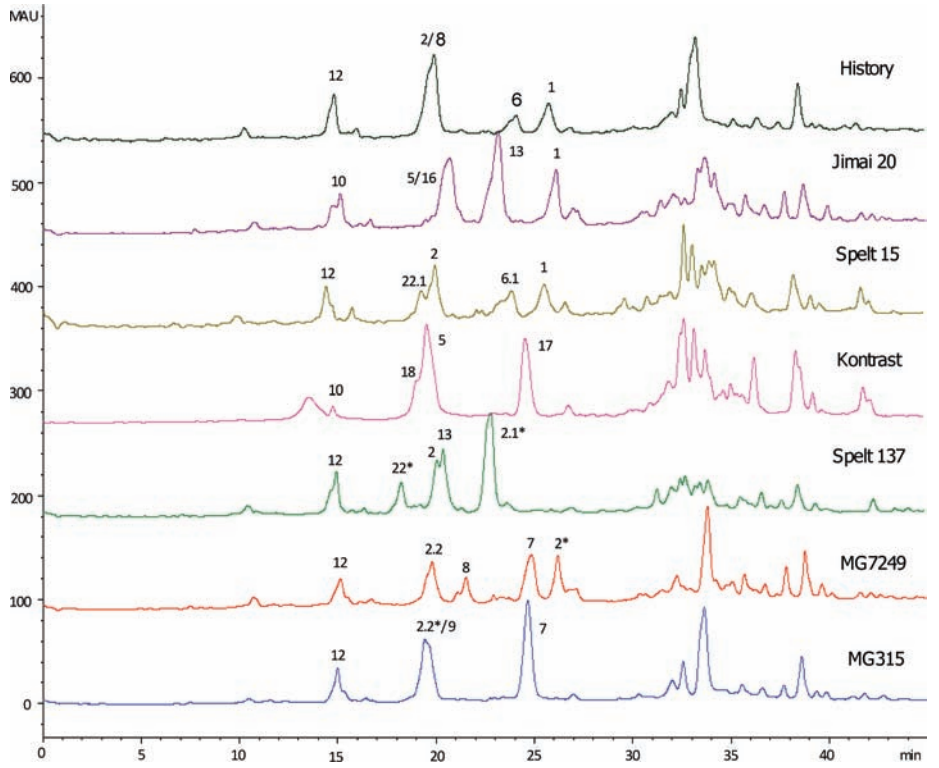

Figure 3. Separation and identification of HMW-GS from seven common wheat cultivars by RP-HPLC.

extracted with 100 μL of extraction buffer (50% isopropanol, 80 mM Tris-HCl, pH 8.0, and 1% DTT) for 30 min at 65 °C. After centrifugation, glutenin fractions were alkylated by adding equal volumes of extraction buffer in which 1.4% 4-vinylpyridine (v/v) replaced 1% DTT and incubated at 65 °C for 30 min. After centrifugation for 15 min at 13000 rpm, the supernatants were transferred into new vials for the following SDS-PAGE, RP-HPLC, HPCE, and MALDI-TOF-MS analyses. To ensure homozygosis, all lines were analyzed with three single seeds.

SDS-PAGE. SDS-PAGE was performed according to the method of Yan et al. (*5*) with some modifications in the electrophoresis apparatus and conditions: 10 μL of supernatant protein sample described above was mixed with an equal volume of sample buffer (2% SDS, 0.02% bromophenol blue, 0.08 M Tris-HCl, pH 8.0, 40% glycerin) and placed in a water bath at 65 °C for 15 min. After centrifugation, samples were electrophoresed with 12% gel concentration and mini-cell apparatus (Bio-Rad) at 15 mA for 3 h and stained with Coomassie brilliant blue G-250 for 24 h. A GS-800 calibrated densitometer (Bio-Rad) was used to scan the gel.

RP-HPLC. The RP-HPLC for HMW-GS separation, based on the method of Dong et al. (*28*), was performed on an Agilent 1100 by using a Zorbax 300SB-C18 column (300 Å pore size, 5 μm particle size). Solvents were composed of (A) water and (B) acetonitrile (ACN), both containing 0.06% (v/v) trifluoroacetic acid (TFA) and filtered (0.45 μm) and degassed prior to use. For each sample, 5 μL was injected for analyses. Proteins were eluted at 1 mL/min using a gradient from 21 to 48% B over 65 min, running for 20 column volumes. The column was maintained at 50 °C, and the proteins were monitored at 210 nm.

HPCE. HPCE was performed on the basis of the method of Yan et al. (*38*). The extracted HMW-GS were precipitated with the addition of acetone to a final concentration of 40% (v/v). Precipitated HMW-GS were redissolved in 0.2 mL of 25% (v/v) ACN + 0.1% (v/v) TFA and centrifuged for 10 min at 13000*g*. All samples were analyzed within 24 h of extraction.

A BioFocus-3000 instrument was used for capillary electrophoretic separations of the HMW-GS according to the method of Yan et al. (*13*) with minor modification in the analysis time. Acidic CE buffer, namely, 0.1 M phosphate−glycine (pH 2.5), containing 20% (v/v) acetonitrile (ACN) and 0.05% (w/v) hydroxypropylmethylcellulose (HPMC), and uncoated fused-silica capillaries (BioFocus-3000 accessory) of 25.5 cm length (20 cm to detector) and 50 μm inner diameter (i.d.) were used. CE was performed at 12.5 kV and 40 °C. All samples were injected at 10 kV for 8 s and analyzed for 24 min. HMW-GS were detected by UV absorbance at 200 nm.

MALDI-TOF-MS. As described for CE analysis, the precipitated protein sample was dried at room temperature, and the dried HMW-GS were dissolved in the mixture of TFA, ACN, and H_2O, according to the optimized method of Zhang et al. (*15*).

MALDI-TOF-MS experiments were carried out on the basis of the method of Zhang et al. (*15*) by using an AXIMA-CFRTMPlus MS apparatus (Shimadzu Corp., Kyoto, Japan) equipped with delayed extraction technology operating in the linear mode (*15*). A mass range of 10000−100000 Da was used and about 80−120 laser shots were averaged to improve the signal-to-noise level. Internal calibration was performed by using the standard albumin−alderase calibration kit for the AXIMA-CFRTMPlus MS apparatus (Shimadzu Corp.). The matrix solution was prepared by dissolving sinapinic acid (SA) in 50% ACN at the concentration of 10 μg/μL. One microliter of 1:10 sample/matrix solution mixture (v/v) was deposited onto a stainless steel gold-plated 384-sample MALDI probe tip and dried at room temperature.

RESULTS AND DISCUSSION

The HMW-GS compositions of the 60 common wheat cultivars and related accessions identified by SDS-PAGE are listed in **Table 1**, and some major subunits are shown in **Figure 1**. Some subunits and alleles previously characterized by Yan et al. (*5*−*7*) and Li et al. (*29*) were confirmed, such as 1Ax2.1*, 1Bx6.1, 1Bx13*, 1By22.1, 1By19*, 1Dy10.1, 1Dy12.1 etc., which were mainly presented in spelt wheat, einkorn, and *Ae. tauschii*. In a previous study (*30*), a pair of subunits 1Bx14 + 1By15 encoded by *Glu-B1* locus in common wheat was incorrectly identified in Chinese Xiaoyan series cultivars such as Xiaoyan 6, Xiaoyan 54, PH85-16, Chuanmai 45, which were previously considered to be related to good gluten quality (*31*, *32*). As shown in **Figure 1**, this subunit pair had the same *Glu-B1* patterns as those from Bidi-17 that contained 1Bx20 + 1By20 (*33*). This was further confirmed by RP-HPLC, HPCE, and MALDI-TOF-MS (see below). In fact, two bread wheat cultivars, Hanno and Imbros from

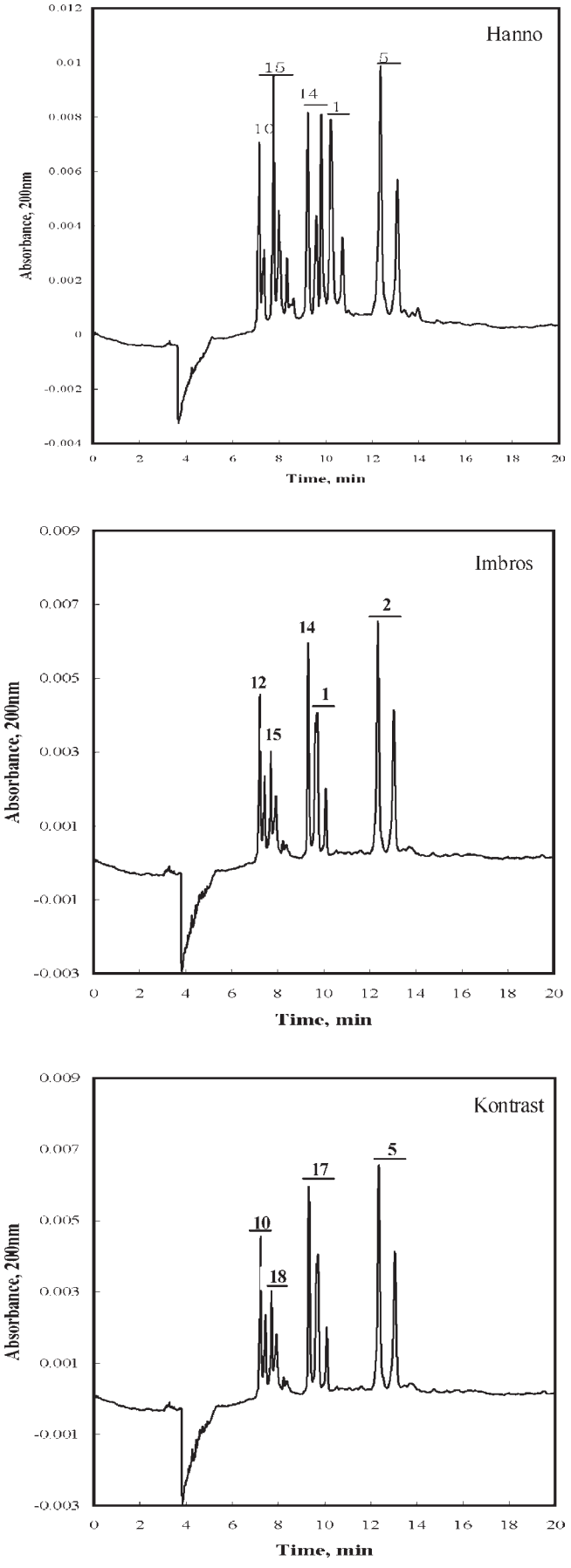

Figure 4. Separation and identification of HMW-GS from three bread wheat cultivars (Hanno, Imbros, and Kontrast) by HPCE.

Germany, had 1Bx14 + 1By15 subunits. Therefore, the *1Bx14* gene separated by Li et al. (*30*) should be *1Bx20* reported by Shewry et al. (*33*). Furthermore, both genes reported shared high identity. According to previous investigations, 1Bx14 + 1By15 subunits were mainly present in a few European bread wheat cultivars (*4*), which appeared to be rare in Asia and other countries. The pedigree and origin of 1Bx14 + 1By15 subunits and their encoding sequences remain unknown so far.

Because the electrophoretic apparatus is simple and easy to perform in most of the laboratories, the routine SDS-PAGE has become a widely used method for screening desirable subunits and for gluten quality prediction across the world. However, its disadvantages are still obvious and sometimes it may result in wrong identification such as that described above. In general, due to its relatively poor resolution and low reproducibility, it is usually difficult to correctly identify some subunits with very similar mobilities (*34*) such as 1Ax2* and 1Dx2, let alone new variations. Furthermore, gel electrophoresis for HMW-GS is relatively slow and labor-intensive, and the quantitation for different subunits is especially difficult (*12*−*14*).

To further confirm the SDS-PAGE identifications, two pairs of *Glu-B1* subunits, 1Bx14 + 1By15 and 1Bx20 + 1By20, were separated by RP-HPLC as shown in **Figure 2**. Apparently, Xiaoyan 6, Xiaoyan 54, and Bidi 17 had the same patterns at the *Glu-B1* locus but differed from Hanno (**Figure 2A**). Furthermore, sample mixing between Xiaoyan 6 and Bidi 17 showed the same *Glu-B1* pattern (**Figure 2B**), confirming that the Xiaoyan series developed in China possess 1Bx20 + 1By20 rather than 1Bx14 + 1By15 at the *Glu-B1* locus. The HMW-GS of some common wheat cultivars identified by RP-HPLC are shown in **Figure 3**. Compared to the SDS-PAGE separation that is mainly based on protein molecular mass and charge, RP-HPLC separation is based on the hydrophobicities of proteins. Our results demonstrated that most 1Dx and 1By subunits, for example, 1Dx2 and 1By15, 1Dx2 and 1By22.1, 1Dx5 and 1By15, 1Dx5 and 1By16, 1Dx5 and 1By18, 1Ax2.2* and 1By9 shown in **Figures 2** and **3**, and certain 1Ax and 1Bx subunit such as 1Ax1 and 1Bx20, 1Ax2.1* and 1Bx13 were not well discriminated. In addition, subunits 1Dx2 and 1Dx5 and 1Dy10 and 1Dy12 were also difficult to discriminate. These results suggest that subunits having similar hydrophobicity cannot be identified by RP-HPLC. Although RP-HPLC possesses many advantages over SDS-PAGE, such as high reproducibility, automatic separation, and relative quantitation for different subunits, its separation ability for some HMW-GS is still limited. Additionally, its apparatus and analysis costs are much higher than those of SDS-PAGE, which could restrict its wide application.

Figure 4 shows the results of some HPCE separated HMW-GS from three bread wheat cultivars. It was obvious that all subunits studied, including 1Dx5 + 1Dy10 and 1Dx2 + 1Dy12, could be well separated in < 14 min, much faster than RP-HPLC, which generally needs 30 min. The HMW-GS elution order under acidic buffer CE condition was 1Dy → 1By → 1Bx → 1Ax → 1Dx. As indicated by previous investigations (*13*, *14*, *35*), the peaks of individual subunits displayed isoforms under acidic separation condition and similar results were obtained by two-dimensional gel electrophoresis (data not shown), which probably resulted from sample treatment or post-translational modifications (PTMs). In most cases, each subunit had one major peak and one or two minor peaks. This is true for subunits 1Dx5, 1Dx2, 1Ax1, 1Bx14, 1By15, 1Bx17, and 1By18 (**Figure 4**).

Some subunits related to different gluten properties could be easily discriminated by HPCE. As shown in **Figure 5**, both 1Dx5 and 1Dx2 had two peaks (a major peak with a minor one) and could be well separated on the basis of their eluting times. Similar

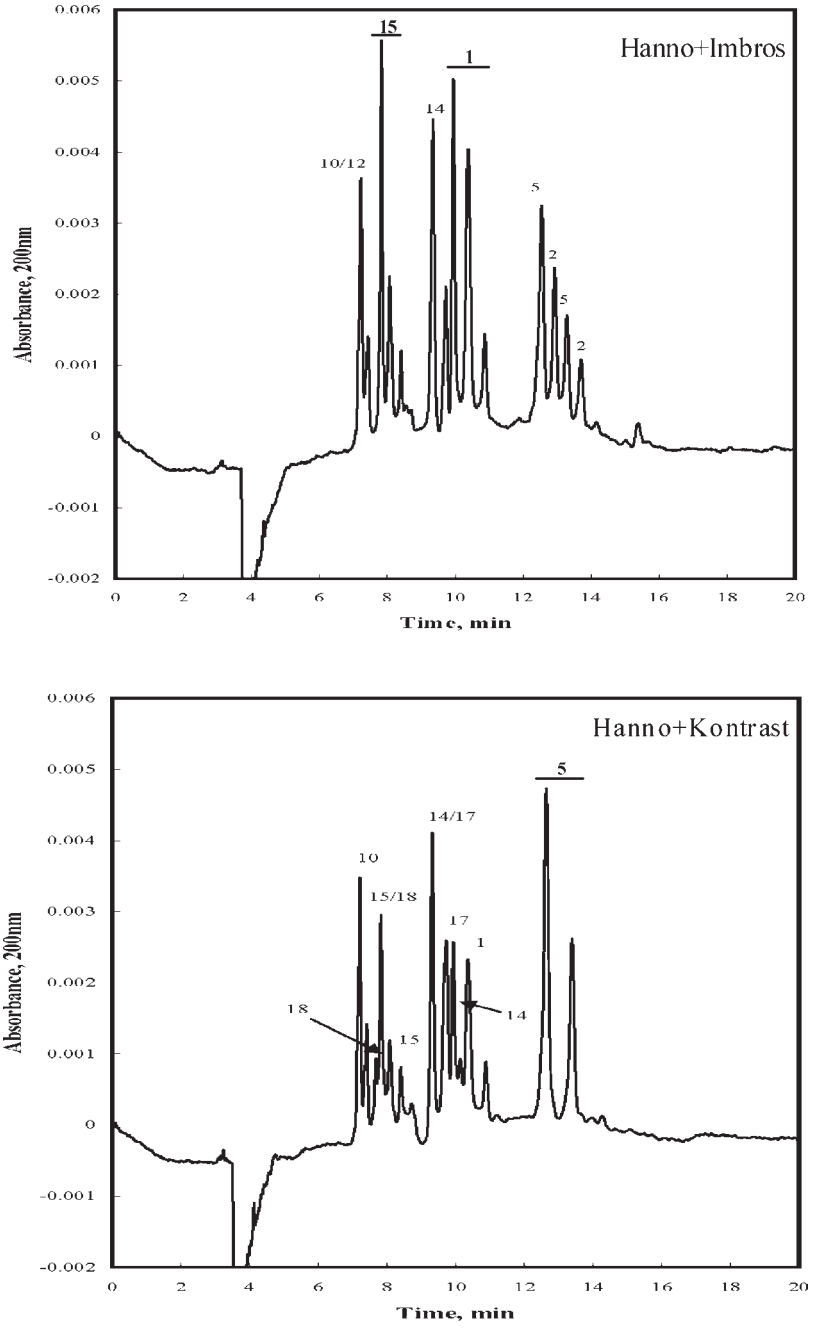

Figure 5. HPCE identification of HMW-GS from mixing samples of Hanno + Imbros and Hanno + Kontrast.

results were also obtained for 1Bx14 + 1By15 and 1Bx17 + 1By18 subunits. However, 1Dy10 and 1Dy12 subunits could not be differentiated. Because 1Dx5 + 1Dy10 and 1Dx2 + 1Dy12 always appear as two allelic subunit pairs, they can be discriminated on the basis of the identification of 1Dx5 and 1Dx2 subunits. According to our studies and previous work (*5*−*7*, *13*, *14*), most of the HMW-GS can be well separated and readily identified through HPCE, including new subunits in related species. Thus, HPCE appears to be a rapid, high-resolution, and automated separation method for HMW-GS (*35*−*40*). The narrow i.d. capillaries are intrinsically anticonvective, thus avoiding the use of gels to obtain high-resolution separations (*41*).

In this work, in addition to SDS-PAGE, RP-HPLC, and HPCE analyses, MALDI-TOF-MS procedures were used to accurately identify and determine molecular masses of a total of 40 HMW-GS from common wheat and related species (**Table 2**).

Table 2. Comparison of HMW-GS Molecular Masses Deduced from Coding Gene Sequences with Those Determined by MALDI-TOF-MS

HMW-GS	deduced M_r from coding gene (Da)	M_r by MALDI-TOF-MS (Da)	origin	difference (Da)	error (%)
1Ax1	87678	87779	Spelt 166	−101	−0.12
		87860	R-61	−182	−0.21
		88099	Jing 771	−421	−0.48
		88056	Chuanyu 20	−378	−0.43
		87507	D154	171	0.19
		87539	Y12	139	0.16
1Bx1.1	unknown	87345	PI414718		
1Ax1*	unknown	87122	PI414718		
1Ax2*	86335	86843	Jing 9428	−508	−0.59
		86975	Jinmai 67	−640	−0.74

Table 2. Continued

HMW-GS	deduced M_r from coding gene (Da)	M_r by MALDI-TOF-MS (Da)	origin	difference (Da)	error (%)
		86900	Linfen 138	−565	−0.65
		86984	HT159	−649	−0.75
1Ax2.1*	unknown	87308	Spelt 137		
		87254	Club 62		
1Bx6	86414	86458	History	−44	0.05
		86554	Club 62	−140	−0.16
		86675	Club 18	−261	−0.30
		86310	Ping 4	104	0.12
1Bx6*	unknown	87324	PI428097		
1Bx6.1	unknown	87258	Spelt 73		
		87190	Spelt 77		
1Bx7	82524	82658	Jing 411	−134	−0.16
		82749	Chinese Spring	−225	−0.27
		82974	Simeto	−450	−0.55
		82686	Club 29	−162	−0.20
		82363	Nei 01	161	0.19
		82298	Jing 9428	226	0.27
1Bx13	83249	83264	Spelt 30	−15	−0.02
		83356	Spelt 130	−107	−0.13
		83314	Spelt 137	−65	−0.07
		83334	Spelt 166	−85	−0.10
		83101	Y8	148	0.18
		83091	PI481478	158	0.19
1Bx13*	unknown	83922	Spelt 20		
		83884	Club 58		
1Bx20	83913	83567	Bidi 17	346	0.41
		83776	Xiaoyan 6	137	0.16
		83618	Chuanmai 45	295	0.35
		83605	Chuanyu 20	308	0.37
		83602	PH-85-16	311	0.37
		83906	Linfen 127	7	0.01
1Bx14	unknown	82505	Hanno		
		82423	Imbros		
1Bx17	78607	78679	Kontrast	−72	−0.09
		78451	Chuanmai 39	156	0.20
1By8	75156	75591	Jing 411	−435	−0.58
		75172	Simeto	−16	−0.02
		75545	Club 29	−389	−0.52
		75488	Chinese Spring	−332	−0.44
		75483	Ping 4	−327	−0.44
		75384	Jing 9428	−228	−0.30
1By9	73515	73489	Nei 01	26	0.04
		73679	Jinmai 67	−164	−0.22
		73550	Floreal	35	0.05
		73570	Liocorno	55	0.07
1By15	unknown	75282	Hanno		
		75302	Imbros		
1By15*	unknown	75618	PI414718		
1By20	75733	75709	Bidi 17	24	0.03
		75534	Xiaoyan 6	199	0.26
		75374	Chuanmai 45	359	0.47
		75407	Chuanyu 20	326	0.43

Table 2. Continued

HMW-GS	deduced M_r from coding gene (Da)	M_r by MALDI-TOF-MS (Da)	origin	difference (Da)	error (%)
		75328	PH85-16	405	0.54
		75596	Linfen 127	137	0.18
1By16	77282	77205	Spelt 30	77	0.10
		77199	Spelt 130	83	0.11
		77243	Spelt 137	39	0.05
		77544	Spelt 166	−262	−0.34
		77520	PI481478	−238	−0.31
1By18	unknown	75254	Kontrast		
		75407	Chuanmai 39		
1By19	unknown	75305	PI554584		
		75565	Wanmai 23		
1By19*	unknown	75614	Spelt 20		
		75607	Club 58		
1By22*	unknown	75631	Spelt 137		
		75014	PI428097		
1By22.1	unknown	75372	Spelt 77		
		75015	Y8		
		75152	D154		
1Dx1.5	86807	86579	R-1	228	0.26
		86739	R-13	68	0.08
		86628	R-75	179	0.21
1Dx1.6[i]	87778	87565	TD16	213	0.24
1Dx2.2*	107000	86620	MG315	20380	19.05
1Dx2.2	101000	86340	MG7249	14660	15.52
1Dx2	87022	87190	Jing 411	138	0.16
		87120	Chinese Spring	−98	−0.11
		87095	Jing 9428	−73	−0.08
		87251	Spelt 20	−229	−0.26
		86941	PH85-16	81	0.09
1Dx3	86664	86448	TD13	216	0.25
1Dx4	87655	87702	TD38	−47	−0.05
		87742	T198	−87	−0.10
		87519	Ping 4	136	−0.16
1Dx5	88128	88056	Chuanmai 45	72	0.08
		88058	Chuanmai 39	70	0.08
		88075	Chuanyu 20	53	0.06
		88086	Nei 01	42	0.05
		88094	Jing 771	34	0.04
1Dx5*	85782	85678	TD81	104	0.12
1Dx5.1*	87663	87480	TD130	183	0.21
1Dx5.2	86779	86651	TD128	128	0.15
1Dy10	67473	67751	Chuanmai 45	−278	−0.41
		67738	Chuanmai 39	−265	−0.39
		67783	Chuanyu 20	−310	−0.50
		67719	Nei 01	−246	−0.36
		67949	Jing 771	−476	−0.71
1Dx10.1	68611	68407	TD81	204	0.30
		68509	TD132	102	0.15

Table 2. Continued

HMW-GS	deduced M_r from coding gene (Da)	M_r by MALDI-TOF-MS (Da)	origin	difference (Da)	error (%)
1Dy12	68652	69017	Jing 411	−365	−0.53
		68935	Chinese Spring	−283	−0.41
		69017	Spelt 20	−365	−0.53
		68996	Club 18	−344	0.50
		68724	Jing 9428	−72	−0.11
		68754	Ping 4	−102	−0.15
		68781	PH-85-16	−129	−0.19
1Dy12.1	67518	67627	TD8	−109	−0.16
		67345	TD9	173	0.26

The MS spectra of some subunits are shown in **Figure 6**. Most subunits detected appeared in two to seven genotypes and generally displayed similar molecular masses. Except the two largest subunits identified so far, 1Dx 2.2* and 1Dx2.2, all other subunits could be readily discriminated through their molecular masses determined by MALDI-TOF-MS. In particular, some subunits that could not be discriminated by the other three methods described above displayed clear MS spectra differences, such as 1Dx2 (87095 Da) and 1Ax2* (86843 Da) in Jing 9428, 1Dy10 (67751 Da in Chuanmai 45), and 1Dy12 (69017 Da in Jing 411 and Spelt 20) as shown in **Table 2**. In general, the mass spectrum for each sample could be obtained in < 5 min, much faster than the other three methods.

To date, the encoding genes for the most HMW-GS named by Payne and Lawrence (*4*) and some new subunits identified in

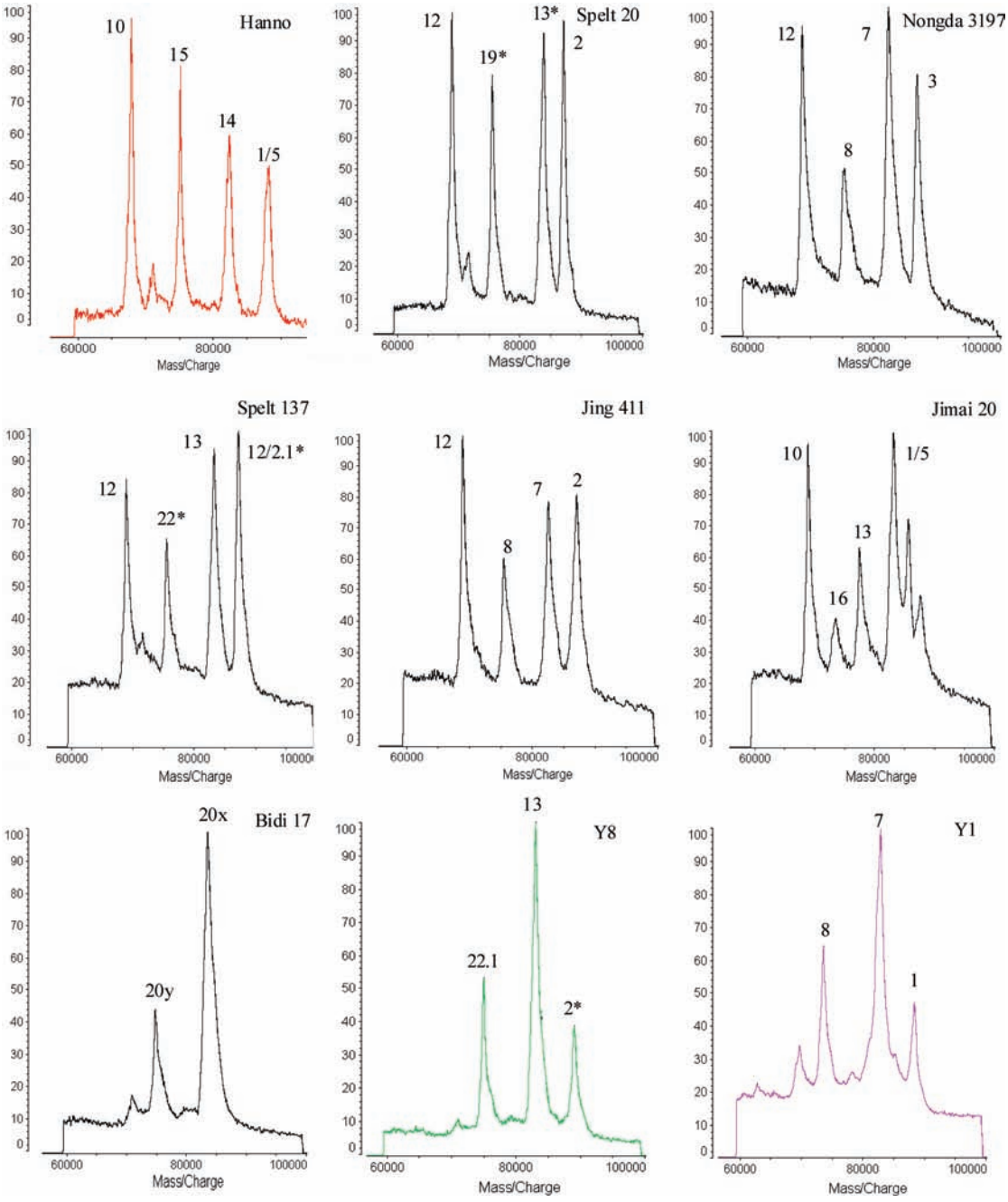

Figure 6. MS spectra of HMW-GS from nine cultivars and accessions of common wheat and related species by MALDI-TOF-MS.

related species have been cloned, allowing a comparative analysis between deduced molecular masses from gene sequences and those determined by MALDI-TOF-MS. As shown in **Table 2**, among 40 subunits measured, the gene sequences of 26 subunits were available. In most cases, the molecular mass of a particular subunit from different genotypes determined by MALDI-TOF-MS was consistent with that predicted from its gene sequence, and the difference was generally $<1\%$. The differences for some frequent subunits in bread wheat were <100 Da such as 1Dx2, 1By9, and 1Bx13 in three cultivars and 1Dx5 in four cultivars. Particularly, there were eight subunits for which the differences were <50 Da: 1Dx5 in Nei 01(42 Da), 1By9 in Nei 01 (26 Da) and Floreal (35 Da), 1By8 in Simeto (16 Da), 1Bx6 in History (44 Da), 1Bx13 in Spelt 30 (15 Da), 1By16 in Spelt 137 (39 Da), 1Bx20 in Linfen 127 (7 Da), and 1By20 in Bidi 17 (24 Da). The exceptions were for 1Dx2.2* and 1Dx2.2 subunits; their deduced molecular masses were $>100{,}000$ Da. Because MS spectra in this study were obtained by using a predefined mass range of 10,000−100,000 Da, out of this range is the reason for incorrect lower molecular mass determination for 1Dx2.2* and 1Dx2.2.

The above results further support previous studies (*15*, *20*−*23*, *42*) in which post-translational modifications (PTMs) for HMW-GS are uncommon. The germplasms used in this study generally expressed good match between the MALDI-TOF-MS determined molecular masses and these deduced from the coding sequences. However, anomalies do exist. For example, the 1Ax2* subunit in this study expressed a molecular mass difference of >500 Da between MS spectra and gene sequences in four genotypes (**Table 2**). Previously, we have also encountered a 2000 Da difference (*46*). These discrepancies were most likely caused by PTMs. Tilley et al. (*43*) reported that certain HMW-GS were extensively glycosylated, causing up to 26% difference in molecular weight. Phosphotyrosine in HMW-GS and N-glycosylation with xylose in LMW-GS were also detected (*44*, *45*). This suggests that the difference between the MS spectra and coding sequence is a reflection of the protein molecular variation rather than errors in MALDI-TOF measurement.

For the subunits studied, their molecular mass order revealed by SDS-PAGE differed from that by MALDI-TOF-MS. For SDS-PAGE, the mass order revealed by mobility is 1Ax1 > 1Ax2* > 1Dx2 > 1Dx5 > 1Dy10 > 1Dy12, whereas for MALDI-TOF-MS the mass order is 1Dx5 > 1Ax1 > 1Dx2 > 1Ax2* > 1Dy12 > 1Dy10. The mass order based on MS spectra was consistent with coding gene sequences. It is known that SDS-PAGE generally leads to overestimation of molecular mass for HMW-GS, which is probably due to high repetitive structures of glutenins and the SDS molecules attached to the protein subunits.

The present work clearly showed some technical advantages of MALDI-TOF-MS in analyzing HMW-GS, including high resolution and sensitivity. Moreover, the protein sample preparation time does not differ significantly for the four methods. This means that the MALDI-TOF method is capable of high-throughput identification and screening of desirable HMW-GS, which is particularly important for wheat breeding programs. However, the drawback of the MALDI-TOF MS methods is also obvious: the equipment cost is by far higher than those for SDS-PAGE, HPCE, and RP-HPLC, which may potentially prevent breeding programs from accessing this technology. On the other hand, the HMW-GS knowledge base was largely built based on SDS-PAGE results, and such knowledge works well in breeding programs. Furthermore, SDS-PAGE had the advantages of technical simplicity and low requirement of equipment; thus, it is suitable for large-scale and high-throughput HMW-GS screening for breeding programs, especially when the glutenin composition is clear in the breeding material.

ABBREVIATIONS USED

HMW-GS, high molecular weight glutenin subunits; HPCE, high-performance capillary electrophoresis; HPMC, hydroxypropylmethylcellulose; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; RP-HPLC, reversed-phase high-performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis;

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