Breeding Science 68: 524–535 (2018)
doi:10.1270/jsbbs.18085

Research Paper

Allelic variation of low molecular weight glutenin subunits composition and the revealed genetic diversity in durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf))

Xin Hu[1,4], Yanchun Peng[1], Xifeng Ren[1], Junhua Peng[2], Eviatar Nevo[3], Wujun Ma[4] and Dongfa Sun*[1,5]

[1] *College of Plant Science and Technology, Huazhong Agricultural University*, Wuhan 430070, Hubei, China
[2] *Science and Technology Center, China National Seed Group Co., Ltd.*, Wuhan, 430075, Hubei, China
[3] *Institute of Evolution, University of Haifa*, Mount Carmel, Haifa 31905, Israel
[4] *Australia-China Joint Centre for Wheat Improvement, State Agriculture Biotechnology Centre, School of Veterinary and Life Sciences, Murdoch University*, WA 6150, Australia
[5] *Hubei Collaborative Innovation Center for Grain Industry*, Jingzhou, 434025, Hubei, China

Low molecular weight glutenin subunits (LMW-GS) play an important role in determining the bread-making characteristics of dough in the end-use quality of wheat. In this study, A total of 149 worldwide-originated durum wheat were used to analyze the composition of LMW-GS using MALDI-TOF-MS. Based on the allelic variation of glutenin subunits, the genetic diversity was evaluated for the 149 durum wheat. Five types of alleles were identified at the *Glu-A3* locus with *Glu-A3e*, *Glu-A3a/c*, *Glu-A3f*, *Glu-A3d* and *Glu-A3b* accounting for 43.0%, 16.1%, 12.8%, 10.1% and 7.4 % of the accessions, respectively. Five types of alleles were identified at the *Glu-B3* locus: *Glu-B3d* (60.4%), *Glu-B3b* (6.0%), *Glu-B3c* (6.0%), *Glu-B3h* (2.7%) and *Glu-B3f* (0.7%). Two novel alleles encoding abnormal subunits 40500 Da and 41260 Da were identified at the *Glu-A3* and *Glu-B3* loci, respectively. Further studies are needed to match these novel alleles to previously discovered novel alleles. Moreover, the genetic diversity analysis indicated that great genetic variation existed in durum wheat among encoding loci of glutenin subunits, released periods of varieties and different geographical origins. The results provide more important information of potential germplasm for the improvement of durum wheat and common wheat.

Key Words: durum wheat, MALDI-TOF-MS, low molecular weight glutenin subunits (LMW-GS), allelic variation, genetic diversity.

Introduction

Glutenin proteins, the compositions of wheat flour, play a key role in determining wheat rheological characteristics including dough strength and extensibility and bread-making performance (Bekes *et al.* 2001, Butow *et al.* 2003, Ma *et al.* 2005). Glutenin fractions consist of aggregated proteins linked by interchain disulfide bonds, and the polymeric glutenin proteins have various sizes ranging in molecular weight from less than 300,000 Da to more than 1,000,000 Da (Liu *et al.* 2010, Wieser *et al.* 2006, Wieser 2007). Glutenin subunits could be divided into high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) (D'Ovidio and Masci 2004, Jackson *et al.* 1983, Payne and Corfield 1979). It has been recognized that the molecular weight (MW) distribution of glutenins mainly determines the properties and baking performance of dough (Weegels *et al.* 1996).

LMW-GS contain a large amount of polypeptides. For the difficult to distinguish LMW-GS from gliadins, the composition, structure of LMW-GS and the relationship between LMW-GS and grain processing quality have not yet been studied to the same level as the HMW-GS (Appelbee *et al.* 2009, D'Ovidio and Masci 2004). LMW-GS, significant components of wheat storage proteins, are important in determining dough properties (including gluten strength and dough extensibility) (Cornish *et al.* 2001, Gianibelli *et al.* 2001). Therefore, identifying the allelic variation of LMW-GS and analyzing the relationships between LMW-GS and grain processing quality have been an attractive research area on quality improvement for the last 20 years, and the successful utilization of specific LMW-GS alleles is foundational and essential for quality breeding programs (Békés

Communicated by Norihiko Tomooka
Received June 13, 2018. Accepted August 6, 2018.
First Published Online in J-STAGE on November 17, 2018.
*Corresponding author (e-mail: sundongfa1@mail.hzau.edu.cn)

et al. 2006, Gupta *et al.* 1994, He *et al.* 2005).

LMW-GS were initially identified from the extracts of wheat flour by gel filtration and starch gel electrophoresis (Elton and Ewart 1966). Most LMW-GS are encoded by the *Glu-A3*, *Glu-B3* and *Glu-D3* loci on the short arms of chromosomes 1A, 1B and 1D, respectively (where, *Glu-A3* and *Glu-B3* in tetraploid wheat), and tightly linked to the complex *Gli-1* loci, which encode γ- and ω-gliadins (Anderson *et al.* 2009, Payne *et al.* 1984, Pogna *et al.* 1990, Singh and Shepherd 1988). A few LMW-GS were encoded by the *Glu-A3* locus on chromosome 1A, however, there is wide variation for LMW-GS encoded by *Glu-B3* locus on chromosome 1B in common wheat (Gupta and Shepherd 1990, Liu *et al.* 2010, Yan *et al.* 2003). Although the *Glu-D3* locus has less variation with five alleles initially reported by Gupta and Shepherd (1990), discrepancy exists among different studies about the alleles (Appelbee *et al.* 2009, Ikeda *et al.* 2006, Jackson *et al.* 1996), suggesting that further studies are necessary to clarify the genetic variation at this locus.

One-dimensional SDS-PAGE, 2-DE (two-dimensional gel electrophoresis (IEF × SDS-PAGE)) and HPLC (High performance liquid chromatography) methods have been generally used to identify and select specific HMW-GS and LMW-GS with superior quality in many breeding programs (Dworschak *et al.* 1998, Yahata *et al.* 2005). Matrix-assisted laser desorption/ionization time-of flight (MALDI-TOF-MS) is an effective and very important approach in rapidly and easily identifying glutenin subunits for its high accuracy and sensitivity in analyzing samples, which has been particularly useful in wheat quality breeding programs (Dworschak *et al.* 1998, Elfatih *et al.* 2013, Liu *et al.* 2009, 2010, Peng *et al.* 2016, Zheng *et al.* 2011). MALDI-TOF-MS has widely been used to identify the HMW-GS compositions of common landraces of bread wheat collected from the Yangtze-River region of China (Zheng *et al.* 2011), to detect the compositions of HMW-GS in durum wheat from different countries (Elfatih *et al.* 2013), to establish an analytical standard for identifying LMW-GS using a set of 19 near-isogenic lines (NIL) of cultivar Aroona (Wang *et al.* 2015).

Durum wheat (*Triticum durum* Desf.) is a tetraploid species containing A and B genomes (2n = 4x = 28, AABB) (Peng *et al.* 2011), and is the main material of semolina for the processing of pasta, bagel and other local end-products of Mediterranean (Fabriani *et al.* 1988, Nachit *et al.* 1992). The quality of durum wheat end-products depends mainly on glutenin composition. Different composition of HMW-GS and LMW-GS and their combinations may result in differences in gluten elasticity and strength (Elfatih *et al.* 2013). Generally, the LMW-GS are associated with resistance and extensibility of dough (Cornish *et al.* 2001, Metakovsky *et al.* 1990), and some allelic forms of LMW-GS present even greater effects than HMW-GS on these characteristics (Gupta *et al.* 1994, Payne *et al.* 1987). LMW-GS are also important for the end-use quality of dough in durum wheat, especially subunits encoded by loci on chromosome 1B (D'Ovidio and Masci 2004, Josephides *et al.* 1987). *LMW-2*, a specific allele encoding typical LMW-GS, is associated with the best pasta making characteristics (Payne *et al.* 1984), and also seems to be significant in determining bread-making properties (D'Ovidio and Masci 2004, Peña *et al.* 1994). Generally, as the genetic basis of modern wheat cultivars is narrow, special durum wheat cultivars, containing unusually useful genes are rich resources for wheat quality improvement (Li *et al.* 2006). The aims of the present study were to: (a) identify the LMW-GS compositions of worldwide-originated durum wheat using MALDI-TOF-MS, and reveal the difference of the LMW-GS compositions in different accessions, and (b) evaluate the genetic diversity in world-wide origin durum wheat based on the allelic variation of LMW-GS and HMW-GS, and genetic diversity in different released periods of varieties and geographical origins, respectively.

Materials and Methods

Plant materials

A total of 149 accessions of worldwide-originated durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.), 2n = 4x = 28, AABB) were used in this study, including 25 from East Asia (EA), 24 from West Asia (WA), 33 from Europe (EU), 16 from Africa (AF), 32 from North America (NA), 12 from South America (SA), and 7 from Australia (AU) (**Table 1**). The accessions used in the present study were also included in the study of Elfatih *et al.* (2013), and were all obtained from USDA (United States Department of Agriculture).

Protein extraction

Proteins were extracted from 20 mg whole meal based on the sequential procedure of Singh *et al.* (1991). The samples were extracted with 1.0 ml of 55% propanol-1-ol (v/v) for 5 min vortexing, followed by incubation for 20 min at 65°C, then continued vortexing for 5 min with a centrifugation at 10,000 × *g* for 5 min. Repeated this step three times to completely remove the gliadins. The glutenin in the pellet was reduced with 55% propanol-1-ol, containing 0.08 M Tris-HCl solution and 1% dithiothreitol (DTT) and incubation for 30 min at 65°C, followed by addition of 1.4% v/v of 4-vinylpyridine, and alkylation and incubation overnight at room temperature. For MALDI-TOF-MS analysis, 80% acetone was used to precipitate the LMW-GS portion.

MALDI-TOF-MS

The dried compounds of LMW-GS samples were dissolved in 60 µl acetonitrile (ACN)/H_2O (v/v, 50:50) containing 0.05% v/v trifluoroacetic acid (TFA) for 1 h at room temperature. Referring to the dried droplet method of Kussmann *et al.* (1997), sample preparation was carried out using sinapinic acid (SA) as matrix. The matrix solution was made by dissolving SA in ACN/H_2O (50:50 v/v) containing 0.05% TFA (v/v) at a concentration of 10 mg/ml. Mixing the extracted LMW-GS solution (a total of 60 µl)

Table 1. The LMW-GS compositions for 149 accessions analyzed by MALDI-TOF-MS

Code	Accession identifier[a]	Accession name	Regions	Place of origin	Year of collection	Type	*Glu-A3*	*Glu-B3*
H45	PI 233213	Sevindz	EA	Azerbaijan	1956	Cultivar	40503 Da	d
H61	PI 345707	Sevindz	EA	Azerbaijan	1969	Cultivar	40494 Da	d
H1	CItr 11495	Wash. No. 2628	EA	Heilongjiang, China	1932	Cultivar	b	d
H14	CItr 5077	FHB4495	EA	China	1916	Landrace	e	b
H142	PI 70658	Tulatai Maitai	EA	Heilongjiang, China	1926	Landrace	d	h
H143	PI 70662	Lumanian	EA	Heilongjiang, China	1926	Landrace	d	41300 Da
H146	PI 74830	ICARDA-IG-82496	EA	Jiangsu, China	1927	Landrace	a/c	d
H147	PI 79900	N-85	EA	Heilongjiang, China	1929	Landrace	d	41325 Da
H15	CItr 5083	FHB4501	EA	China	1916	Landrace	a/c	f
H16	CItr 5094	FHB4512	EA	Beijing, China	1916	Landrace	d	41259 Da
H19	CItr 8327	Suifu	EA	Sichuan, China	1924	Landrace	e	d
H23	PI 124292	ICARDA-IG-82575	EA	Jiangsu, China	1937	Landrace	f	d
H54	PI 283853	China 34	EA	China	1962	Cultivar	e	d
H90	PI 435100	Bian Sui	EA	China	1979	Cultivar	e	d
H92	PI 447421	ST-33	EA	Xinjiang, China	1980	Cultivar	f	d
H84	PI 41015	Jalalia	EA	Madhya Pradesh, India	1915	Landrace	b	d
H85	PI 41342	Hansia Broach	EA	Gujarat, India	1915	Landrace	b	d
H133	PI 61351	Medea	EA	Hokkaido, Japan	1924	Landrace	d	41291 Da
H134	PI 61352	Roumania	EA	Hokkaido, Japan	1924	Landrace	d	41289 Da
H130	PI 61112	CItr 7395	EA	Kazakhstan	1924	Landrace	a/c	41248 Da
H131	PI 61123	CItr 7406	EA	Kazakhstan	1924	Landrace	40511 Da	41284 Da
H32	PI 176228	ICARDA-IG-84631	EA	Nepal	1949	Landrace	b	d
H41	PI 210910	T 1	EA	Punjab, Pakistan	1953	Cultivar	a/c	d
H42	PI 210911	T 2	EA	Punjab, Pakistan	1953	Cultivar	a/c	d
H83	PI 388132	FAO 33.268	EA	Punjab, Pakistan	1974	Landrace	a/c	d
H123	PI 591959	DW 1	WA	Cyprus	1994	Cultivar	e	d
H43	PI 210952	Damliko	WA	Cyprus	1953	Landrace	f	d
H47	PI 237632	Tripolitico	WA	Cyprus		Cultivar	e	d
H25	PI 140184	ICARDA-IG-82637	WA	Khuzestan, Iran	1941	Landrace	e	c
H44	PI 222675	ICARDA-IG-85523	WA	East Azerbaijan, Iran	1954	Landrace	a/c	d
H48	PI 243790	ICARDA-IG-85615	WA	Tehran, Iran	1957	Landrace	e	d
H56	PI 289821	ICARDA-IG-97583	WA	Fars, Iran	1963	Landrace	e	c
H144	PI 70736	ICARDA-IG-82459	WA	Iraq	1926	Landrace	e	b
H28	PI 165846	Amarah	WA	Iraq	1948	Cultivar	f	b
H37	PI 208903	Rash Kool	WA	Iraq	1953	Landrace	e	d
H38	PI 208907	Lara	WA	Iraq	1953	Landrace	e	d
H39	PI 208908	Mendola	WA	Iraq	1953	Landrace	a/c	d
H40	PI 208910	Sin El-Jamil	WA	Iraq	1953	Landrace	e	41259 Da
H51	PI 253801	K918	WA	Ninawa, Iraq	1958	Landrace	e	d
H49	PI 249816	N-163	WA	Israel	1958	Cultivar	e	d
H50	PI 249820	Neveh Yaar 51	WA	Israel	1958	Cultivar	e	41269 Da
H57	PI 292035		WA	Israel	1963	Cultivar	e	c
H81	PI 384043	Merarit	WA	Israel	1973	Cultivar	40643 Da	c
H82	PI 388035	Line 76	WA	Israel	1974	Cultivar	e	b
H105	PI 520415	Syrian Durum 27	WA	Syria	1987	Cultivar	e	d
H24	PI 134596	Fere-Alexandrinum	WA	Syria	1939	Landrace	e	d
H33	PI 182697	Nashabie	WA	Dimashq, Syria	1949	Landrace	a/c	d
H36	PI 193391	Aleppo	WA	Halab, Syria	1951	Landrace	b	41267 Da
H26	PI 152567	Aden	WA	Yemen	1945	Cultivar	a/c	h
H109	PI 546462	Gergana	EU	Khaskovo, Bulgaria	1990	Cultivar	40580 Da	d
H60	PI 344743	Apulicum 233	EU	Bulgaria	1969	Cultivar	e	41254 Da
H72	PI 352450		EU	France	1969	Cultivar	d	41283 Da
H12	CItr 2468		EU	Germany	1904	Landrace	40472 Da	d
H58	PI 306664	Heines Hartveizen	EU	Lower Saxony, Germany	1965	Cultivar	f	d
H64	PI 352389	Caravicos	EU	Greece	1969	Cultivar	f	d
H124	PI 593005	V. 433	EU	Latium, Italy	1996	Cultivar	f	d
H68	PI 352408	T-1560	EU	Italy	1969	Cultivar	e	d
H69	PI 352415	Aziziah 17/45	EU	Latium, Italy	1969	Cultivar	b	d
H115	PI 56233	CItr 7041	EU	Lisboa, Portugal	1923	Cultivar	f	d
H74	PI 376498	DF 14/71	EU	Romania	1972	Cultivar	a/c	d
H75	PI 376500	DF 31/71	EU	Romania	1972	Cultivar	a/c	d
H76	PI 376501	DF 42/71	EU	Romania	1972	Cultivar	a/c	41292 Da
H77	PI 376509	DF 4/72	EU	Romania	1972	Cultivar	40617 Da	d
H78	PI 376511	DF 6/72	EU	Romania	1972	Cultivar	a/c	b
H79	PI 376512	DF 7/72	EU	Romania	1972	Cultivar	a/c	d
H13	CItr 3267	Chistunka	EU	Altay, Russian Federation	1911	Landrace	d	41227 Da
H132	PI 61189	CItr 7472	EU	Krasnoyarsk, Russian Federation	1924	Landrace	e	d
H70	PI 352436	T-2114	EU	Former Soviet Union	1969	Cultivar	d	h
H71	PI 352437	T-2115	EU	Former Soviet Union	1969	Cultivar	40503 Da	b

Table 1. (continued)

Code	Accession identifier[a]	Accession name	Regions	Place of origin	Year of collection	Type	*Glu-A3*	*Glu-B3*
H67	PI 352404	Torcal	EU	Spain	1969	Cultivar	40499 Da	d
H35	PI 192711	Ostpreuss	EU	Gotland, Sweden	1950	Cultivar	e	d
H63	PI 352377	T-357	EU	Switzerland	1969	Cultivar	a/c	d
H111	PI 560702	TU85-008-10-2	EU	Siirt, Turkey	1986	Landrace	e	d
H112	PI 560717	TU85-054-01-2	EU	Bitlis, Turkey	1986	Landrace	e	41267 Da
H113	PI 560718	TU85-054-02	EU	Bitlis, Turkey	1986	Landrace	e	d
H114	PI 560889	TU86-24-02-2	EU	Siirt, Turkey	1989	Landrace	f	c
H21	PI 109588	T-538	EU	Ankara, Turkey	1935	Cultivar	40491 Da	41252 Da
H62	PI 346985	Hacimestan	EU	Turkey	1970	Cultivar	e	d
H52	PI 278223	Gartons Early Cone	EU	England, United Kingdom	1962	Cultivar	e	c
H53	PI 278648	ICARDA-IG-85863	EU	England, United Kingdom	1962	Cultivar	e	b
H59	PI 321702	Nursi	EU	England, United Kingdom	1967	Cultivar	e	d
H91	PI 438973	Har'kovskaja 51	EU	Kharkiv, Ukraine	1980	Cultivar	d	41274 Da
H107	PI 546060	DT367	NA	Saskatchewan, Canada	1990	Cultivar	e	d
H108	PI 546362	DT369	NA	Saskatchewan, Canada	1991	Cultivar	e	d
H11	CItr 17337	Wakooma	NA	Saskatchewan, Canada	1974	Cultivar	e	d
H119	PI 583724	8682-D051-NG	NA	Saskatchewan, Canada	1994	Cultivar	e	d
H120	PI 583731	G8973-AG1-G	NA	Saskatchewan, Canada	1994	Cultivar	e	d
H121	PI 583732	G8973-AG1-NG	NA	Saskatchewan, Canada	1994	Cultivar	e	d
H122	PI 583733	G8973-AQ1-G	NA	Saskatchewan, Canada	1994	Cultivar	e	d
H98	PI 519751	D 31729-2L-OL	NA	Federal District, Mexico	1987	Cultivar	e	41274 Da
H101	PI 519761	Maghrebi'S'	NA	Federal District, Mexico	1987	Cultivar	e	41298 Da
H102	PI 519866	CB 088	NA	Federal District, Mexico	1987	Cultivar	f	d
H103	PI 520053	31814-1L-OC	NA	Federal District, Mexico	1987	Cultivar	e	41287 Da
H104	PI 520173	Tal	NA	Mexico	1987	Cultivar	e	41291 Da
H129	PI 610765	CIGM91.347-6	NA	Federal District, Mexico	1999	Cultivar	f	d
H135	PI 634315	Canelo	NA	Federal District, Mexico	2001	Cultivar	e	d
H136	PI 634318	Afuwan	NA	Federal District, Mexico	2001	Cultivar	e	d
H30	PI 168708	Barrigon Glabrous Selection	NA	Mexico	1948	Cultivar	b	h
H6	CItr 15874	D 19329-28M-11Y	NA	Mexico	1972	Cultivar	a/c	d
H86	PI 422289	Maghrebi 72	NA	Mexico	1978	Cultivar	e	41304 Da
H88	PI 428453	Dommel'S'	NA	Federal District, Mexico	1978	Cultivar	f	d
H99	PI 519752	D 31648-2L-OL	NA	Federal District, Mexico	1987	Cultivar	d	41304 Da
H110	PI 560335	KS91WGRC14	NA	Kansas, United States	1992	Cultivar	e	d
H118	PI 573005	Imperial	NA	Arizona, United States	1988	Cultivar	f	d
H125	PI 600931	D-5003	NA	California, United States	1982	Cultivar	e	d
H126	PI 601250	Westbred Laker	NA	Arizona, United States	1985	Cultivar	e	d
H137	PI 656793	NSGC 19376	NA	California, United States	2009	Cultivar	e	41307 Da
H138	PI 656794	IR51-8	NA	California, United States	2009	Cultivar	e	41325 Da
H139	PI 656795	IR17-47	NA	California, United States	2009	Cultivar	e	41317 Da
H150	PI 9872	Galgalos	NA	Erevan, Armenia	1903	Cultivar	f	b
H18	CItr 6881	Akrona	NA	Colorado, United States	1923	Cultivar	d	41268 Da
H2	CItr 12068	Kubanka 314	NA	North Dakota, United States	1940	Cultivar	40490 Da	41264 Da
H3	CItr 13246	Ramsey	NA	North Dakota, United States	1955	Cultivar	d	41255 Da
H4	CItr 13333	Wells	NA	North Dakota, United States	1957	Cultivar	e	41253 Da
H116	PI 565259	Yurac Mexico	SA	Cochabamba, Bolivia	1991	Landrace	e	d
H117	PI 565266	Mexico	SA	Cochabamba, Bolivia	1991	Landrace	e	d
H100	PI 519759	D 73121	SA	Brazil	1987	Cultivar	e	41214 Da
H34	PI 191645	Timor	SA	Sao Paulo, Brazil	1950	Cultivar	e	d
H10	CItr 17159	CAR 1234	SA	La Araucania, Chile	1972	Cultivar	a/c	d
H7	CItr 17057	CAR 1131	SA	La Araucania, Chile	1972	Cultivar	a/c	d
H8	CItr 17058	CAR 1132	SA	La Araucania, Chile	1972	Cultivar	a/c	d
H9	CItr 17157	CAR 1232	SA	La Araucania, Chile	1972	Cultivar	a/c	d
H55	PI 286546	Morocho Colorado	SA	Pichincha, Ecuador	1963	Cultivar	e	d
H148	PI 91956	Chumpe Negro	SA	Junin, Peru	1931	Cultivar	a/c	d
H149	PI 92024	Candeal	SA	Cajamarca, Peru	1931	Cultivar	d	d
H29	PI 168692	Muestra 2 Barba Blanca Anquipa	SA	Peru	1948	Cultivar	f	d
H22	PI 11715	Marouani	AF	Mascara, Algeria	1904	Landrace	a/c	d
H106	PI 532119	2515	AF	Minufiya, Egypt	1988	Cultivar	f	d
H127	PI 60712	Gawi	AF	Egypt	1924	Landrace	f	c
H128	PI 60742	Sinai No. 8	AF	Sinai, Egypt	1924	Landrace	b	d
H141	PI 7016	Mishriki	AF	Alexandria, Egypt	1901	Landrace	b	c
H145	PI 7422	Girgeh	AF	Sawhaj, Egypt	1901	Landrace	b	c
H27	PI 153774	Durum H	AF	Giza, Egypt	1946	Cultivar	f	d
H66	PI 352395	T-1303	AF	Ethiopia	1969	Cultivar	f	b
H73	PI 352551	Abyssinicum	AF	Ethiopia	1969	Landrace	d	41252 Da
H87	PI 42425	Zwartbaard	AF	South Africa	1916	Landrace	40508 Da	d
H93	PI 45442	ICARDA-IG-98118	AF	Free State, South Africa	1917	Landrace	40546 Da	d
H94	PI 45443	ICARDA-IG-98119	AF	Cape Province, South Africa	1917	Landrace	40552 Da	d

Table 1. (continued)

Code	Accession identifier[a]	Accession name	Regions	Place of origin	Year of collection	Type	*Glu-A3*	*Glu-B3*
H95	PI 46766	Golden Ball	AF	Cape Province, South Africa	1918	Cultivar	e	41308 Da
H65	PI 352390	T-842	AF	Tunisia	1969	Cultivar	e	d
H96	PI 51210	Mahmoudi	AF	Tunisia	1920	Landrace	e	d
H97	PI 519380	BD 1645	AF	Tunisia	1987	Cultivar	e	41258 Da
H140	PI 67341	Huguenot	AU	Western Australia, Australia	1926	Cultivar	40514 Da	d
H17	CItr 5136	Indian Runner	AU	Victoria, Australia	1916	Landrace	40497 Da	d
H20	PI 107606	Cadia	AU	Australia	1934	Cultivar	b	41259 Da
H31	PI 174645	Huguenot	AU	Western Australia, Australia	1949	Cultivar	a/c	d
H46	PI 235159	Giza	AU	New South Wales, Australia	1956	Cultivar	e	41260 Da
H80	PI 377882	Duramba	AU	Australia	1973	Cultivar	e	d
H89	PI 428701	AUS 20299	AU	Australia	1978	Cultivar	e	d

[a] The accession identifier is adopted from the USDA.ARS National Plant Germplasm System-Germplasm Resources Information Network (https://www.ars-grin.gov/npgs/acc/acc_queries.html).

with SA solution (1:10 v/v) for protein-SA mixture, and 2 µl of this mixture was deposited on to a 96-sample MALDI target probe tip, then dried at room temperature. MALDI-TOF-MS experiments were performed on a Voyager DE-PRO TOF mass spectrometer (Applied Biosystems, Foster City, CA, USA) with UV nitrogen laser (337 nm) at the State Agriculture Biotechnology Center, Murdoch University, Australia. Analyses were performed with the following parameters: acceleration voltage 25 kV and delay time 900 ns, mass range 10,000–50,000 Da. The low mass gate value (10,000 m/z) for analysis was chosen to avoid saturation of the sensor. The new standard established with 16 single *Glu-3* allele substitution lines of Aroona, 25 gene deletion lines and 60 wheat lines with known LMW-GS compositions as reference in Wang *et al.* (2015), was used to analyze the composition of LMW-GS alleles. The established standard in Wang *et al.* (2015) for specific MALDI-TOF spectrum patterns corresponding to LMW-GS allele were summarized in **Supplemental Table 1**.

Genetic diversity analysis

The genetic diversity was evaluated based on the allelic variation of LMW-GS in this study and HMW-GS in the study of Elfatih *et al.* (2013) (see **Supplemental Table 2**). POWERMARKER Ver. 3.25 (Liu and Muse 2015) was used to analyze the genetic diversity using the genetic parameters Nei's gene diversity and polymorphism information content (PIC). A phylogenetic NJ tree based on acessions and regions were performed by POWERMARKER Ver. 3.25 with 1000 bootstrap replicates. A consensus tree with bootstrap values was reconstructed by the consensus program of PHYLIP (Plotree and Plotgram 1989) and displayed by FigTree Ver.1.4 (Rambaut 2014).

Results

Allelic variation of LMW-GS at Glu-A3 and Glu-B3

According to the established standard in Wang *et al.* (2015) for specific MALDI-TOF spectrum patterns corresponding to LMW-GS alleles (**Supplemental Table 1**), the mass spectra of the LMW glutenin subunits showed well-separated peaks in the spectrum of each material, and the mass spectra of the LMW glutenin subunits for some materials were shown in **Fig. 1**. The LMW-GS compositions for 149 accessions analyzed by MALDI-TOF-MS are listed in **Table 1**. A total of 12 alleles (ten previously reported and two unreported alleles) of LMW-GS were found in the MALDITOF-MS profile and their frequencies were presented in **Table 2**. A total of 23 types of LMW-GS compositions were detected during 149 accessions at *Glu-A3* and *Glu-B3* loci (**Table 3**).

At the *Glu-A3* locus, five previously reported alleles were identified. *Glu-A3e* showed the highest frequency that was detected in 43.0% of the 149 accessions, followed by the *Glu-A3a/c* (16.1%), *Glu-A3f* (12.8%), *Glu-A3d* (10.1%) and *Glu-A3b* (7.4%) (**Tables 1**, **2**). However, alleles *Glu-A3a* and *Glu-A3c* have identical molecular masses, and were difficult to be distinguished by MALDI-TOF-MS (Wang *et al.* 2015). Moreover, one previously unreported allele was detected at *Glu-A3* locus in sixteen (10.7%) accessions encoding a novel subunit with a molecular weight of approximately 40,500 Da (ranging from 40,472 Da to 40,580 Da).

At the *Glu-B3* locus, five previously reported alleles were identified. Out of 149 accessions, 60.4% (90) of them were identified with *Glu-B3d*, indicating that *Glu-B3d* was the most frequent allele at *Glu-B3* locus. *Glu-B3b* and *Glu-B3c* each accounted for 6.0% of the accessions. *Glu-B3h* was detected in 4 accessions and *Glu-B3f* was detected only in one accession. Moreover, a new LMW glutenin subunit was identified with the molecular weight of around 41,260 Da (ranging from 41,214 Da to 41,325 Da) in 36 accessions (24.2% of the accessions examined) (**Tables 1**, **2**).

A total of 23 types of LMW-GS compositions were detected in this study. The most common combination type is *Glu-A3e* + *Glu-B3d* (26.2%), followed by *Glu-A3a/c* + *Glu-B3d* (12.8%), *Glu-A3e* + a new subunit with molecular weight of about 41260 Da (11.2%), moreover the combination of a new subunit with a molecular weight of about 40,500 Da and *Glu-B3d* was detected in 11 accessions

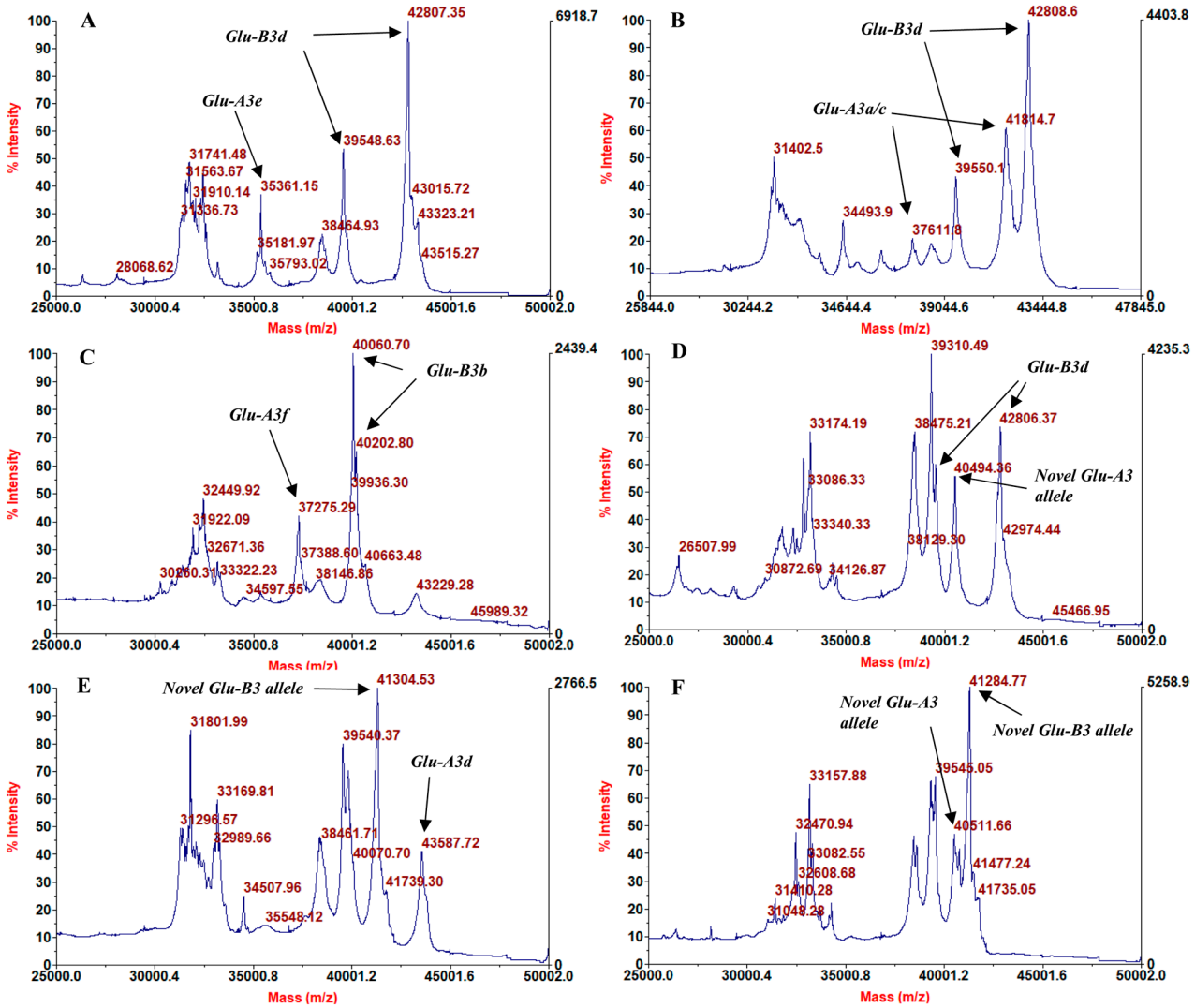

Fig. 1. Detection of LMW-GS for some durum accessions by MALDI-TOF-MS. Accessions code: (A) H24, (B) H39, (C) H66, (D) H61. (E) H99, (F) H131.

Table 2. Allele frequencies of LMW-GS revealed by MALDI-TOF-MS

Locus	LMW-GS	Number	Frequency %
GluA3	40500 Da	16	10.7
	a/c	24	16.1
	b	11	7.4
	d	15	10.1
	e	64	43.0
	f	19	12.8
GluB3	41260 Da	36	24.2
	b	9	6.0
	c	9	6.0
	d	90	60.4
	f	1	0.7
	h	4	2.7

(**Table 3**). Different subunits and different combinations of subunits have different effects on the quality and processing quality of the dough.

Overall, 12 alleles (ten previously reported and two unreported alleles) of LMW-GS were found in the MALDI TOF-MS at the two loci in durum wheat. Two unreported alleles were observed at loci *Glu-A3* and *Glu-B3*, with 10.7% for *Glu-A3* and 24.2% for *Glu-B3*. Furthermore, we also detected, in some materials, the spectrum peaks of approximately 43,267 Da and 41,758 Da , which were reported to be associated with novel subunits in Wang *et al.* (2015). However, these peaks were not novel in the current study.

Genetic diversity

The genetic diversity is listed in **Table 4**. For LMW-GS coding loci, a higher genetic diversity was detected at *Glu-A3* locus with Nei's gene diversity, and PIC values of 0.245 and 0.208, respectively, while 0.225 and 0.186 for *Glu-B3* locus, respectively. For HMW-GS coding loci, the genetic diversity of *Glu-A1* (with Nei's gene diversity, and

Table 3. Allele combinations and variants at *Glu-A3* and *Glu-B3* loci in durum wheat

	GluA3	*GluB3*	Number	Frequency %
1	40500 Da	41260 Da	3	2.0
2	40500 Da	b	1	0.7
3	40500 Da	c	1	0.7
4	40500 Da	d	11	7.4
5	a/c	41260 Da	2	1.3
6	a/c	b	1	0.7
7	a/c	d	19	12.8
8	a/c	f	1	0.7
9	a/c	h	1	0.7
10	b	41260 Da	2	1.3
11	b	c	2	1.3
12	b	d	6	4.0
13	b	h	1	0.7
14	d	41260 Da	12	8.1
15	d	d	1	0.7
16	d	h	2	1.3
17	e	41260 Da	17	11.4
18	e	b	4	2.7
19	e	c	4	2.7
20	e	d	39	26.2
21	f	b	3	2.0
22	f	c	2	1.3
23	f	d	14	9.4

Table 4. The genetic diversity of *GluA3*, *GluB3*, *GluA1* and *GluB1* based on LMW-GS and HMW-GS alleles

Locus	Genetic Diversity	PIC
GluA3	0.245	0.208
GluB3	0.225	0.186
GluA1	0.309	0.249
GluB1	0.153	0.134

PIC values of 0.309 and 0.249, respectively) was higher than *Glu-B1* (with Nei's gene diversity, and PIC values of 0.153 and 0.134, respectively).

The genetic diversity for the 7 geographical regions is shown in **Table 5**. European accessions showed the highest values of both Nei's gene diversity (0.216) and PIC (0.181), followed by African (AF: 0.213, 0175), East Asian (EA: 0.206, 0172) and North American accessions (NA: 0.195, 159), while the lowest level of Nei's gene diversity and PIC were detected in South American accessions (SA: 0.156, 0.128). West Asian (WA) and Australian (AU) accessions had a moderate level of Nei's gene diversity and PIC (with the values of 0.191, 0.160 and 0.180, 0.145, respectively).

The difference of genetic diversity between landrace and cultivar, and the release time is shown in **Table 6**. The higher genetic diversity was detected in the cultivars with Nei's gene diversity and PIC values of 0.215 and 0.180, than values in the landrace. Therefore, according to Ren *et al.* (2013), the cultivars were also further divided into three temporal groups: OC (old cultivars before 1965), EGR (early green revolution, 1966–1980), PGR (post green revolution, 1980–2009), to compare the genetic difference. The genetic diversity parameters of three temporal groups of cultivars are shown in **Table 6**. Loss of genetic diversity

Table 5. The genetic diversity of the accessions from 7 ecogeographic regions based on LMW-GS and HMW-GS alleles

Origin	Genetic Diversity	PIC
AF	0.213	0.175
AU	0.180	0.145
EA	0.206	0.172
EU	0.216	0.181
NA	0.195	0.159
SA	0.156	0.128
WA	0.191	0.160

Table 6. Comparison of genetic diversity generated by the allelic variation of LMW-GS and HMW-GS between landraces and cultivars

Group	Genetic Diversity	PIC
Cultivar	0.215	0.180
Landrace	0.210	0.175
Time group of Cultivar		
Before 1965	0.239	0.200
1965–1980	0.211	0.177
1981–2009	0.165	0.135

was observed from OC to EGR (Nei's gene diversity: 0.239 *vs.* 0.211 and PIC values: 0.200 *vs.* 0.177). However, the decrease of genetic diversity was observed from EGR to PGR (Nei's gene diversity: 0.200 *vs.* 0.177 and PIC values: 0.165 *vs.* 0.135).

Cluster analysis

The allelic variation of LMW-GS and HMW-GS loci was used for the cluster analysis. The consensus NJ tree of accessions based on Nei's genetic distance (Nei 1972) is shown in **Fig. 2**. The durum wheat accessions were divided into two major groups.

Group I contained the American accessions (North America and South America), this group was dominated by landraces and cultivars released during OC, EGR and PGR. Group II was further divided into 7 subgroups, grouping of some accessions appeared to be associated with the release period of varieties to some extent (**Fig. 2**, **Supplemental Table 3**).

The consensus NJ tree was constructed based on geographical regions of accessions (**Fig. 3**). The result indicated that the accessions of AU was different from the other regions. The accessions from other regions were divided into two group, EA, EU and AF were clustered in one group, WA, SA and NA were in the other group.

Discussion

Allelic variation of LMW-GS at Glu-A3 and Glu-B3 and the novel subunits

The allelic variation of glutenin subunits can provide a more direct, reliable and efficient tool for the conservation and management of germplasm. In this study, the compositions and allelic variation of low molecular weight glutenin subunit (LMW-GS) in 149 worldwide-originated durum wheat were analyzed using MALDI-TOF-MS.

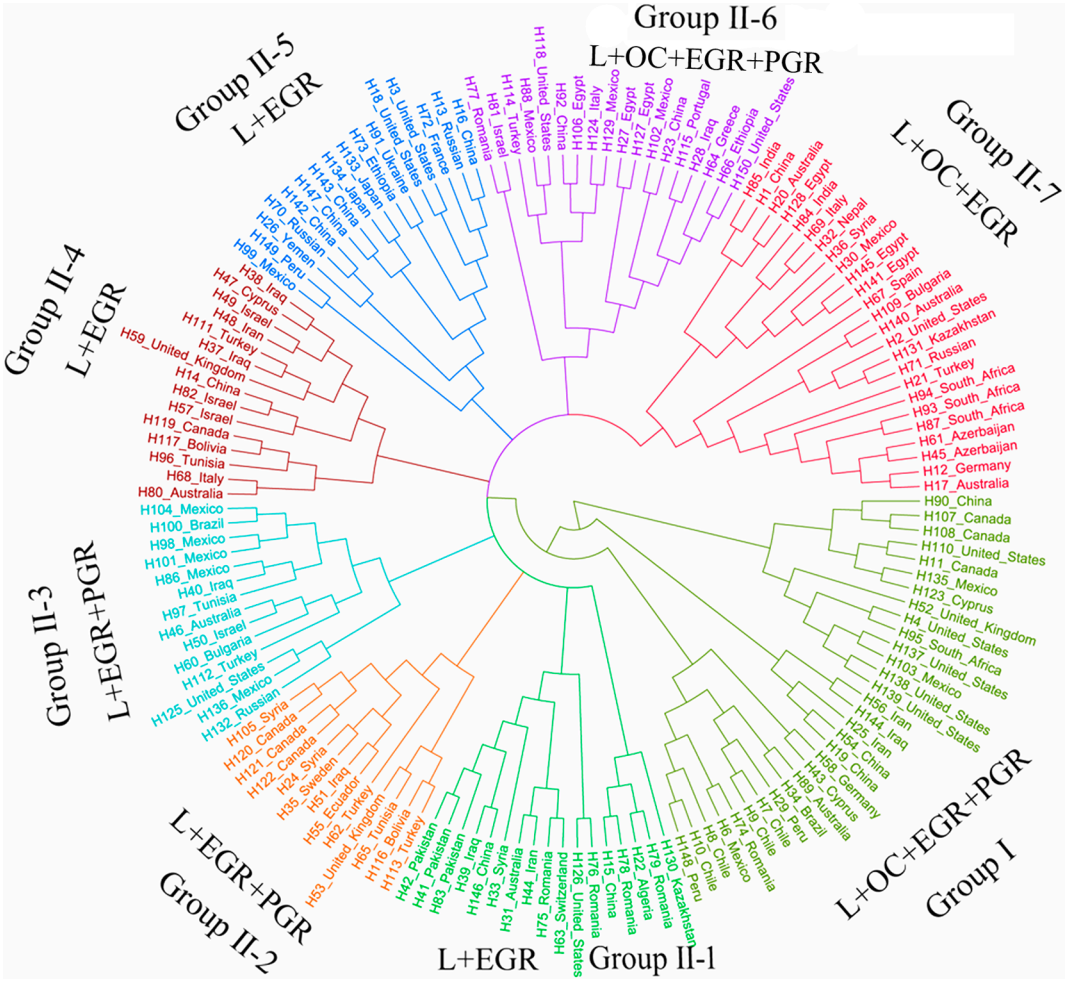

Fig. 2. The NJ tree of 149 durum accessions based on the Nei's genetic distance calculated from the alleles of LMW-GS and HMW-GS. The allelic variation data of HMW-GS was from the study of Elfatih *et al.* (2013), L: Landrace, OC: Old cultivars before 1965, EGR: Early green revolution, 1966–1980, PGR: Post green revolution, 1980–2009.

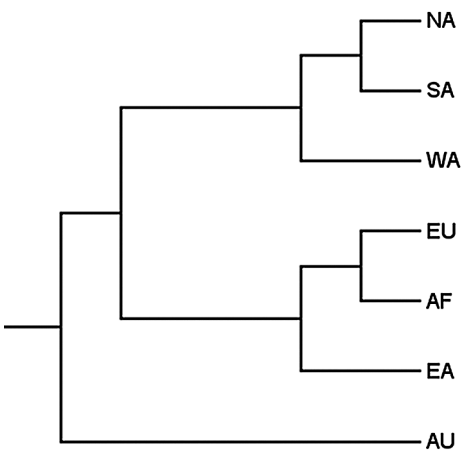

Fig. 3. The consensus NJ tree for the accessions from 7 ecogeographic regions based on the Nei's genetic distance calculated from the alleles of LMW-GS and HMW-GS. The allelic variation data of HMW-GS was from the study of Elfatih *et al.* (2013).

For the *Glu-A3* locus, the most frequent allele was *Glu-A3e* accounting for 43.0%, while the frequency of *Glu-A3a/c* alleles was lower (16.1%). This is different from some previous studies. Bellil *et al.* (2012), Bradová and Štočková (2010), and Nieto-Taladriz *et al.* (1997) reported that *Glu-A3a/c* was the predominant alleles in wheat, while *Glu-A3e* was relatively low. *Glu-A3a* and *Glu-A3c* appeared to be world widely predominant among bread wheat in previous studies, whereas, *Glu-A3e* was predominant among durum wheat in our collections. However, low frequency of *Glu-A3c* was found in the Algerian local and old durum wheat cultivars (Cherdouh *et al.* 2005, Hamdi *et al.* 2010). Different species (common wheat and durum wheat), different sources and distributions of materials should lead to the differences in allele frequencies of LMW-GS reported by different scientists. It seems that the frequency of *Glu-A3a* and *Glu-A3c* were higher in common wheat than in durum wheat, while the frequency of *Glu-A3e* was relatively low. A previous study discovered that *Glu-A3e* reduced the maximum resistance and extensibility of dough in relative to

other alleles of *Glu-A3* (Appelbee 2007). It is worthy of noting that the *Glu-A3d* is a desirable allele for gluten quality and pan bread quality (He *et al.* 2005) and presented in 15 landraces. Moreover, a novel allele, encoding a subunit with a molecular weight of approximately 40,500 Da (ranging from 40,472 Da to 40,580 Da) located at *Glu-A3*, was detected in 20 accessions.

Allelic variation at the *Glu-A3* locus did not significantly affect gluten strength, whereas the *Glu-B3* locus had a significant influence on gluten strength, as measured by sedimentation volume on durum wheat (Vazquez *et al.* 1996). For the *Glu-B3* locus, five previously reported alleles were identified in our study. The most frequent allele was *Glu-B3d* (60.4%). The similar result was reported in Saharan bread wheat and Durum wheat from Algerian Oases by Bellil *et al.* (2012). However, *Glu-B3d* had medium to weak dough properties, and should be avoided at the early stages of a bread wheat breeding program (Luo *et al.* 2001). *Glu-B3b* was rare and only detected in 9 accessions accounting for 6%, which is consistent with the studies of Bellil *et al.* (2010, 2012). It is worthy of noting that a novel allele, expressing a subunit with a molecular weight of approximately 41,260 Da (ranging from 41,214 Da to 41,325 Da) at *Glu-B3*, presented in 60 accessions.

Following the standard for LMW-GS of common wheat varieties reported by Wang *et al.* (2015), we were able to identify the alleles of LMW-GS in most of the durum wheat accessions. Most LMW-GS compositions of durum wheat materials can be detected rapidly and easily according to the characteristic peaks of standard samples in Wang *et al.* (2015). Several novel alleles were identified in landraces collected from Yangtze-River region of China in our research and in Peng *et al.* (2016) and Wang *et al.* (2015) at *Glu-A3* and *Glu-B3* loci. It should be mentioned that Peng *et al.* (2016) and Wang *et al.* (2015) found two novel subunits associated with the spectrum peaks 41,758 Da at *Glu-A3* and 40,499 Da at *Glu-B3*. In our research, we also detected the spectrum peaks with similar masses of approximately 41,758 Da and 40,499 Da. However, compared with the results of Wang *et al.* (2015), our data tended to indicate the spectrum peak of approximately 41,758 Da present with the characteristic spectrum peak (37,600 Da) of *Glu-A3a/c*. This might suggest that the spectrum peak 41,758 Da was another characteristic spectrum peak for *Glu-A3a/c* (**Fig. 1B**). The spectrum peak 40,499 Da was identified as a characteristic spectrum peak for subunit of a novel allele at *Glu-B3* in Peng *et al.* (2016) and Wang *et al.* (2015), however, this characteristic peak can be confidently treated as a new allele located at *Glu-A3* in our study (**Fig. 1D**, **1F**). Furthermore, another novel allele encoding a subunit with a molecular weight of approximately 41,260 Da at *Glu-B3* locus was detected in our study, which was not reported in their studies (**Fig. 1E**, **1F**). A more detailed study is needed to identify the novel alleles in the landraces collected from the Yangtze-River region in China and worldwide-originated durum wheat. Recently, a set of PCR primers have been developed and effectively used to amplify the coding region of the HMW-GS and LMW-GS genes, and numerous LMW-GS genes have been identified in the *Glu-A3*, *Glu-B3* and *Glu-D3* coding regions (Lan *et al.* 2013, Si *et al.* 2014, Wang *et al.* 2012). Using the conserved primers, the novel LMW-GS gene sequences may be amplified from genomic DNA of wheat accessions to match the novel alleles to previously reported alleles.

Genetic diversity

The genetic diversity of *Glu-A3* was higher than *Glu-B3* in this set of durum wheat, similar results were reported in the study of Moragues *et al.* (2006) for the accessions from North Africa, South Europe and West Asia. However, the genetic diversity of *Glu-A1* was higher than *Glu-B1* in this study, which was opposite to the result of Moragues *et al.* (2006). This could be due to different materials. In the study of Moragues *et al.* (2006), only 63 durum wheat landraces from the Iberian Peninsula and other Mediterranean countries were analyzed, while in our study, more world-wide originated accessions (including landraces and cultivars released in different period) were used.

The genetic diversity of durum wheat from 7 ecogeographic regions revealed by the allelic variation of LMW-GS and HMW-GS indicated the genetic diversity of durum wheat from ecogeographic origins was different. Generally, great genetic variation should exist in the center of origin and domestication. It was reported that "Fertile Crescent" is the centers of origin and diversification of durum wheat (Vavilov 1951). However, in this study, the highest genetic diversity of durum wheat was found in EU accessions, followed by AF and EA accessions, while WA accessions showed moderate levels of genetic diversity. Similar result was reported by Ren *et al.* (2013) based on SNP markers. One of the reasons should be uneven distribution of landraces or cultivars among countries and different genetic diversity levels between landraces and cultivars used in this study as discussed by Ren *et al.* (2013). Moreover, the genetic diversity, revealed by the allele variation of LMW-GS and HMW-GS loci, should be different to the genetic diversity evaluated by SNP markers around genome, this should be another reason.

The difference of genetic diversity between landrace and cultivar had been reported by Ren *et al.* (2013) based on SNP markers. In our study, the difference of genetic diversity based on the allele variation of LMW-GS and HMW-GS loci showed similar results to Ren *et al.* (2013) on some extent. The higher genetic diversity was detected in cultivar than landrace. Decrease of genetic diversity was observed from OC (before 1965) to EGR (1965–1980), which was consisted to Ren *et al.* (2013). As discussed in Ren *et al.* (2013), the low level diversity of varieties released in 1965–1980 (EGR) might be due to the "Early Green Revolution", which resulted from widely use of the semi-dwarf varieties and the high yield breeding target. While, a continuous loss of genetic diversity was observed from EGR (1965–1980)

to PGR (1981–2009), which is opposite to the result of Ren *et al.* (2013). During PGR, CIMMYT have realized the danger of narrowing down genetic diversity, they changed the breeding strategy for increasing genetic diversity of wheat and durum wheat, which increased genetic diversity (Reeves 1999). However, meanwhile, CIMMYT started to focus on the quality breeding, although, the genetic diversity was generally increased considering the whole genome. While the quality related loci or regions of chromosome were suffered selection pressure in breeding programs, and single germplasms with high-quality subunits was selected by breeder for breeding and promoting, these result in the decreasing of genetic diversity observed from EGR (1965–1980) to PGR (1981–2009) on the allele variation of LMW-GS and HMW-GS loci in this study.

Cluster analysis

Cluster analyses for accessions and their geographical originations were performed based on the allelic variation of LMW-GS and HMW-GS loci (**Figs. 2**, **3**). some of accessions from the same geographic region and release period were clustered together though into different groups corresponding to their geographical regions of collection, release period and accession type (Landrace or Cultivar) (**Fig. 2**, **Supplemental Table 3**). For example, Group I contained 11 accessions (Cultivar) from NA (North America), most of which (8/11) were released during PGR (**Fig. 2**, **Supplemental Table 3**), and Group II-7 contained 6 landraces, all of which were from AF (Africa) and collection before 1924 (**Fig. 2**, **Supplemental Table 3**). These results indicated that many of the accessions were clustered corresponding to their geographical regions, collection time and accession type, which may be due to the similar environmental conditions or the utilization of single elite germplasm in breeding or agronomical practices.

The NJ tree for the origination regions of the durum accessions showed that the accessions from EA, EU and AF were close to each other, and accessions from WA, SA and NA have close relationship (**Fig. 3**). The accessions of AU were much difference from the others. A close relationship of between EA, EU and AF accessions align well the discussion of Moragues *et al.* (2006) based on the accepted theory of wheat cultivation spreading across the Mediterranean basin (Feldman and Millet 2001, Zohary *et al.* 2012), the theory reported *T. monococcum* spread west from the Fertile Crescent by two ways: North, through the Balcan Peninsula, Greece and Italy, and south through ancient Egypt. This explained the close relationship among the accessions of EA, EU and AF. The close relationship among the accessions of WA, SA and NA indicated that the three geographic regions maybe share some similar origin germplasms with similar allelic variation of LMW-GS and HMW-GS. Moreover, the germplasm exchange through cultural diffusion or historical human dispersal could also play an important role. As we known, between the Old and New World after Columbus' voyages, not only the European culture, but also many crops (including durum wheat landraces and cultivars) were introduced from Europe to the America (Capparelli *et al.* 2005). Besides, trade routes and immigration between WA, SA and NA, new varieties of wheat were transported or shared. This maybe also explain the closer relationship among the accessions of WA, SA and NA on some aspect.

In conclusion, the results of allelic variation of LMW-GS provide useful information for wheat breeder to explore germplasm resources for end-use quality improvement. Further studies of the two novel alleles are currently underway to match them with previously reported alleles and to evaluate their potential utility value in improving the bread-making quality. The genetic diversity indicated that despite strict selection pressures on cultivar purity and related breeding practices, there is still a significant level of genetic variation on LMW-GS and HMW-GS alleles in the modern varieties of durum wheat. And there existed abundant genetic variation among loci, released periods of varieties and different geographical origins. The results provide useful information of potential germplasm for the improvement of durum wheat and common wheat.

Acknowledgments

This work is supported by China National Key Project Grant No. 2016YFD0100102 and National Natural Science Foundation of China (No.31701506).

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