Allelic Variation and Geographical Patterns of Prolamins in the USDA-ARS Khorasan Wheat Germplasm Collection

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ABSTRACT

Khorasan wheat, *Triticum turgidum* ssp. *turanicum* (Jakubz), is an ancient cultivated wheat possessing several characteristics that may be useful for durum wheat improvement. In this paper, the allelic variation of prolamins, principal determinants of pasta quality, has been studied in 77 accessions from the USDA-ARS germplasm collection. Some prolamin subunits difficult to identify by standard electrophoretic methods were analyzed by molecular and proteomic techniques. These analyses allowed identifying unambiguously the subunits combination HMW-GS 7+16 (usually mistaken for 13+16) and the 13+8 (HMW-GS) not found so far in wheat. In total, 22 different genotypes were found for the entries from 21 countries analyzed. Prolamin alleles related to good quality in durum wheat have been identified. Also, new allelic variants were identified at almost all the loci, providing a new source of genetic variability with a potential use in breeding. Two geographical distribution patterns of prolamin alleles, with contrasting differences in pasta quality, were detected: one for the Southwest of Asia, and other for the Mediterranean zone. Seven accessions, mainly from Ethiopia and not included in any of those two groups, were rather unique. The results obtained in this study can help to select breeding material and in a more efficient use of the collection.

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Abbreviations: A-PAGE, acid polyacrylamide gel electrophoresis; CAPS, cleavage amplified polymorphism sequence; HMW-GS, high molecular weight glutenin subunits; LMW-GS, low molecular weight glutenin subunits; MALDITOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SDS-PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis.

The allotetraploid wheat *T. turanicum* (Jakubz) called Khorasan or orientale wheat, has an AABB genome, similar to durum wheat (*T. turgidum* ssp. *durum*). This species has been described as one of the oldest cultivated wheats in the area of Turkey, Syria, Iran, and Afghanistan. These populations represent a wheat gene pool that can be very useful for improving modern wheat varieties. The cultivation of the cultivar QK-77, also called Kamut, has achieved economical importance. More than 16,600 ha (41,000 acres) of the grain were in production in 2008 in the United States, Canada, and Australia, and approximately 2000 products are made worldwide with this ancient grain (http://www.bigskybusiness.com/index.php?option = com_content&view = article&id = 262:kamut-increases-world-sales&catid = 4:usbusiness&Itemid = 117).

One important target of durum wheat breeding programs is the improvement of pasta quality. There are some interesting parameters related to a good quality suitable to transfer from

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Khorasan wheat to durum wheat. For example, Khorasan wheat has a greater vitreosity and a higher amber color compared to durum wheat varieties (Grausgruber et al., 2005). However, one of the most important parameters of durum wheat quality is gluten strength, mainly determined by the endosperm proteins the prolamins (Ruiz and Carrillo, 1995; Carrillo et al., 2000). In allotetraploid wheats, prolamins, composed of glutenins and gliadins, are coded by complex loci located on chromosomes of homeologous groups 1 and 6. The loci coding for the high molecular weight glutenin subunits (HMW-GS) are located on the long arms of chromosomes 1A and 1B and are called *Glu-A1* and *Glu-B1*, respectively (Payne et al., 1982). Two additional loci, *Glu-A3* and *Glu-B3*, encoding the low molecular weight glutenins (LMW-GS), are located in the short arms of the same chromosomes (Singh and Shepherd, 1988). Closely linked to them, the loci *Gli-A1* and *Gli-B1* encode the ω-and λ-gliadins**,** and some β-gliadins (Singh and Shepherd, 1988). The loci *Gli-A2* and *Gli-B2* encoding α-and β-gliadins respectively, are located on the short arms of homeologous group 6. Other minor loci involved in the expression of LMW-GS, *Glu-B2* and *Glu-B4,* have been identified (Ruiz and Carrillo, 1993; Liu, 1995; Liu and Shepeherd, 1995). Several works have shown the relationship between different prolamin alleles and differences in gluten strength measured by different quality tests (Brites and Carrillo, 2001; Carrillo et al., 1990, 2000). Knowledge of seed storage proteins variability in germplasm collections can be very useful for breeders interested in quality, for broadening the currently narrow genetic basis of modern wheat cultivars. Allelic prolamin variation in wheat has been addressed in several studies (Kudryavtsev et al., 1996; Nieto-Taladriz et al., 1997; Cherdouh et al., 2005; Moragues et al., 2006; Aguiriano et al., 2008).

The identification of LMW-GS and gliadin alleles is sometimes complicated and labor consuming. However, genetic analysis based on alleles offers important advantages with respect to that based on protein profiles: it allows distinguishing biotypes from off-types in heterogeneous accessions (Kudryavtsev et al., 1996); accurate calculation of genetic indexes and distances, which can be compared with similar studies; and enhances the selection of the parental lines for breeding programs. The efficient use of a germplasm collection is improved by the knowledge of the geographical patterns of genetic variation for specific traits of interest, as durum wheat quality.

The aim of this work is to analyze the allelic variation and geographical patterns of prolamins in the USDA-ARS Khorasan wheat germplasm collection.

MATERIALS AND METHODS

Plant Material

A total of 82 accessions of Khorasan wheat (Jakubz) from the USDA-ARS National Small Grains Collection (http://www.ars-grin.gov) were analyzed for prolamins composition. Seed samples were kindly provided by National Small Grains Collection (Aberdeen, ID).

Protein Extraction and Electrophoresis

Glutenin proteins were extracted according to Singh et al. (1991). Extracted proteins were fractionated by SDS-PAGE using 12% polyacrylamide gels as described by Payne et al. (1980). Gliadins were fractionated at acidic pH 3.1 in 7.5% polyacrilamide gel (A-PAGE) as described by Lafiandra and Kasarda (1985). HMW-GS were designated according to the nomenclature of Payne and Lawrence (1983) and B-LMW-GS according to Nieto-Taladriz et al. (1997). The standard cultivars for the HMW-GS used were the bread wheats Chinese Spring (standard for 7+8 HMW), Lancota (standard for 13+16 HMW), Federation (standard for 20x+20y HMW), Hope (standard for 6+8 HMW) and Dawbull (standard for 6★+8★ HMW), as described in Payne and Lawrence (1983); and the durum wheat PI 61189 (standard for *Glu-A1*V). The standard varieties for the LMW-GS were described by Nieto-Taladriz et al. (1997). Identification of *Gli-* alleles in the protein spectra was performed following the catalog and nomenclature proposed by Kudryavtsev et al. (1996) and Aguiriano et al. (2006). The standard cultivars for the gliadins were the durum wheats Jabato (standard for λ-42) and Mexicali (standard for λ-45). The new alleles, which were found in the present work but not cataloged in durum wheat, were termed as 'new- ^{turanicum}'.

Polymerase Chain Reaction Screening

Isolation of genomic DNA from leaves was performed as previously described (Saghai-Maroof et al., 1984). The primers BxTaqF (5'-GCCTCTGGACAACTACAATGTG-3') and BxTaqR (5'-GCAGGTATTCCCCAAAATATCA-3') were designed with the Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) from the sequences available in GeneBank for the *GluB1*-Bx7 y *GluB1*-Bx13 glutenin subunits (DQ119142 and EF540764). Sequence aligment and restriction map construction was performed with CLC Free Workbench (http://www.clcbio.com). Polymerase chain reaction (PCR) amplification was performed in a 25μL volume containing 100 ng of genomic DNA, 1x Certamp Complex Buffer (Biotools), 2 mM $MgCl_2$, 200 μM dNTPs, 0.5 μM primers and 1μL de Certamp Complex Enzyme Mix (Biotools). The PCR was conducted in a MyCycler thermocycler (BioRad) as follows: heat denaturation at 94°C for 2 min, followed by six cycles of touchdown PCR (94°C for 30 s, an annealing step starting at 61°C for 1 min and decreasing 1°C per cycle, and 72°C for 1min), then 35 more cycles of PCR (94°C for 30 s, 56°C for 1 min, and 72°C for 1min) and a final extension step 72°C for 10 min. Five microliters of PCR amplification products were incubated with 10 units of TaqI enzyme (Fermentas) for 1 h at 65°C. The results were analyzed in 4% NuSieve agarose gels (Cambrex) staining with GelRed (Biotium).

Mass Spectrometry Analysis of Protein

The gel bands of interest were manually excised from micropreparative gels using biopsy punches. Proteins selected for analysis were in-gel reduced, alkylated, and digested with trypsin according to

Sechi and Chait (1998). After digestion, the supernatant was collected and analyzed in a 4800 Proteomics Analyzer MALDI-TOF/TOF mass spectrometer (Applied Biosystems, Framingham, MA) at the Genomics and Proteomics Center, Complutense University of Madrid. Proteins ambiguosly identified by peptide mass fingerprint, were subjected to MS/MS sequencing analyses. For protein identification, the nonredundant NCBI database (12 Aug. 2007; 7463447 sequences; 2570742364 residues) was searched using MASCOT 2.1 (matrixscience.com) through the Global Protein Server v3.6 from Applied Biosystems. Search parameters were: Taxonomy restrictions: (i) Viridiplantae, (ii) fixed modification: carbamidomethyl cysteine, (iii) variable modifications: methionine oxidation; (iv) peptide tolerance: 50 to 80 mg/kg; (v) MS/MS tolerance: 0.3 Da; and (vi): allowed number of missed cleavages: 1. In all protein identification, the probability scores were greater than the score fixed by mascot as significant with a p value minor than 0.05. *Denovo* sequencing from fragmentation spectra of peptides was performed using DeNovo explorer v.3.6 software (Applied Biosystems) and homology search of the sequences was obtained by Blast (http://www.ncbi.nlm.nih.gov/BLAST).

Data Analysis

Glutenin and gliadin alleles were subjected to the multivariate statistical procedure Multiple Correspondence Analysis (Benzecri and Benzecri, 1980). Total gene diversity (Ht) was calculated according to Nei (1973).

RESULTS AND DISCUSSION

Five grains for each one of the 82 landraces of Khorasan wheat, from USDA-ARS collection, were analyzed for HMW-GS and LMW-GS. Five accessions had admixtures for glutenins (PI 182717, PI 192658, PI 225328, PI 256034, and PI 627211) and they were not analyzed for gliadins. Seventy accessions were homogeneous and seven possessed a more frequent biotype, which was analyzed for gliadin alleles. These last heterogeneous accessions were from Morocco (PI 185192, PI 185193, PI 191599, PI 192641, PI 525355, and PI 559976) and one from Russia (PI 349055). These accessions did not correspond with the eight found by Piergiovanni (2009) for gliadins and albumins in 74 entries from the same collection. Although the homogeneity of the collection was high in both works, the different result for these accessions indicates that intra-accession variability should be considered for the utilization of the collection. The Table 1 showed the HMW-GS, LMW-GS, and gliadin composition of the 77 landraces analyzed.

HMW-GS Variation

Three different HMW-GS encoded by *Glu-A1* locus were found (Table 1). The 2 ⋆ and null subunits (*b* and *c* alleles, respectively, according to Payne and Lawrence, 1983) found with a frequency of 3.9 and 93.5%, respectively (Table 2), and the rare subunit V (allele *o*), only found in two accessions from Ethiopia (Fig. 1C). High frequencies for the Null *Glu-A1* subunit (between the 60.32 and the 90%) have been also described in other studies with durum wheat varieties from different countries (Carrillo et al., 1990; Raciti et al., 2003; Cherdouh et al., 2005; Moragues et al., 2006). The V subunit had been previously described in landraces of *T. dicoccum* and durum wheat (Vallega and Mello-Sampayo, 1987).

Seven different pairs of HMW-GS (Table 1) were found for the *Glu-B1* locus, the 7+8, 6+8, 13+16, 14+15 pairs (alleles *b*, *d*, *f*, and *g*, respectively, according to Payne and Lawrence, 1983), the pair of subunits 20x+20y (allele *e* in Margiotta et al., 1993), and the two rare combinations 7+16 and 13+8. The 7+16 pair (allele *ao*) was found in 51 landraces (66.20%), being the most frequent combination (Fig. 1A, Table 2). This result was in contrast with the result of a previous study analyzing the HMW-GS composition of a wide collection of tetraploid wheats, including 87 accessions (Xu et al., 2009). In that study**,** the most frequent combination found at *Glu-B1* locus was the 13+16 pair (60.92%)**,** and the authors did not describe any landrace with the 7+16 combination. This disagreement led us to perform a complementary analysis by PCR and restriction analysis. A novel cleavage amplified polymorphism (CAPS) marker was designed to discriminate the 7 subunit from the 13 subunit. The 5' gene region was targeted for primer design, the BxTaqF and BxTaqR primer efficiently amplified a 223pb fragment in all landraces. The presence of an additional TaqI restriction site in the seven subunit sequence with respect to the 13 subunit made possible to easily discriminate between them (Fig. 2A). Additionally, one sample with the 7+16 pair was analyzed by MALDI-TOF-MS. The peptide mass fingerprint spectrum obtained from spots of HMW-GS-7 and 16 (from PI124494 accession and standards) hydrolyzed using trypsin, gave numerous peptides. Many peaks were clearly resolved. The spectra of measured peptide masses of HMW-GS-16 from standard had the same pattern than the one of HMW-GS-16 from PI124494. The same result was obtained with HMW-GS-7 samples (Fig. 2B). Moreover, the ms/ms fragmentation of some peptides has been made and the sequences obtained confirmed the identity of the proteins through database interrogation. Both analyses confirmed our results identifying undoubtedly the 7+16 pair. This rare combination was first described in Spanish landraces of bread wheat (Rodríguez-Quijano et al., 1990) and, so far**,** has only been described in a durum wheat landrace, in a durum wheat cultivar, in four accessions of Khorasan wheat (Sissons and Batey, 2003), and in five accessions of *T. dicoccum* (Li et al., 2006). The 13+8 HMW-GS pair was found only in five landraces, all of them from Morocco (Fig. 1B, Table 1). To discard confusion with the 7+8 pair (much more frequent in wheat) the identity of the 13 subunit was confirmed by PCR with the CAPS marker previously described (data not shown). It is interesting to note that this novel combination had not been previously described in wheat.

Nine different combinations were found for the *Glu-A1* and *Glu-B1* loci. The most frequent was the Null, 7+16 combination (63.6%), this combination was also found in the five Khorasan wheat analyzed by Sissons and

Table 1. Composition in high molecular weight glutenin subunits (HMW-GS), low molecular weight glutenin subunits (B-LMW-GS), and gliadins in a collection of 77 *Khorasan* wheat landraces from different countries.

HMW-GS		B-LMW-GS			Gliadins				
Glu-A1	Glu-B1	Glu-A3	Glu-B3	Glu-B2	Gli-A1	Gli-B1	Gli-A2	Gli-B2	Accessions
Null	6+8	*a*	*a*	*a*	*b*	*c*	*b/k*	*h*	CI *tr*11390; PI 166308; PI 166450; PI 184526; PI 306665; PI 317495; PI 337643; PI 341414; PI 347132; PI 362067; PI 561075
Null	13+8	*a*	*a*	*a*	*b*	*c*	*b*	*h/new-9turanicum*	PI 191599; PI 192641; PI 525355; PI 559976;
Null	13+16	*a*	*a*	*a*	*b*	*c*	*new-2turanicum*	*h*	PI 352514
Null	7+16	*b*	*b*	*b*	*b*	*a*	*b/k/new-3turanicum*	*h/new-2 turanicum/ new- 4 turanicum/ new- 8turanicum*	PI 10391; PI 257544; PI 67343; PI 68104; PI 68293; PI 113392; PI 113393; PI 115814; PI115815; PI 125351; PI 127106; PI 166554; PI 166959; PI 167481; PI 225331; PI 251925; PI 254201; PI 254202; PI 254203; PI 254205; PI 254207; PI 254208; PI 254209; PI 254210; PI 254211; PI 254212; PI 254213; PI 272601; PI 286069; PI 290530; PI 317494; PI 349055; PI 352515; PI 576854; PI 623629; PI 623641; PI 623656; PI 624207; PI 624217; PI 624420; PI 624421; PI 624422; PI 624893; PI 625164; PI 625187; PI 625189; PI 625214; PI 625401.
Null	6+8	*b*	*b*	*b*	*b*	*a*	*k*	*new-8turanicum*	PI 68287
2*	7+16	*b*	*b*	*b*	*b*	a	f	new-7turanicum	PI 210386
2*	7+16	*d*	*h*	*b*	*b*	*new-1*	*f*	*new-7turanicum*	PI 537992
2*	14+15	8*	*f*	*a*	*new-2turanicum*	c	new-1turanicum	new-5turanicum	PI 321737
Null	20x+20y	*a*	*c*	*b*	*b*	*c*	*b*	*h/new-9turanicum*	PI 185192; PI 283795
Null	13+8	*a*	*c*	*b*	*b*	c	b	new-9turanicum	PI 185193
Null	20x+20y	8*	9*+14+18	*a*	*g*	*new-2*	*new-4turanicum*	*new-6turanicum*	PI 273985
Null	7+16	8*	*a*	*b*	*k*	c	k	new-3turanicum	PI 124494
V	7+8	*a*	*f*	*b*	*new-1turanicum*	*new-3turanicum*	*f*	*new-10turanicum*	CI*tr* 14598
V	20x+20y	8*	2+4+14+18	*b*	*k*	b	k	new-1turanicum	CI*tr* 14599
Null	6+8	*a*	*c*	*b*	*b*	*c*	*k*	*h*	PI 190973; PI 278350

Batey (2003). The Null, 6+8 combination was the second most frequent (18.2%), this combination is quite common in durum wheat (Carrillo et al., 1990; Cherdouh et al., 2005). The combination of subunits 2★, 7+16 was only found in two landraces (Table 1).

The *Glu-A1b* allele and the *Glu-B1b,d* and *e* alleles have been described to have a good influence in durum wheat quality parameters (Raciti et al., 2003; Martínez et al., 2005; Aguiriano et al., 2009).

Very few works have analyzed HMW-GS polymorphism in Khorasan wheat. Xu et al. (2009) obtained an Ht = 0.083 for *Glu-A1* and *Glu-B1* in a sample of 87 accessions. Here, the corresponding value for these two loci was 0.322, indicating that much higher variability was detected in the present work.

LMW-GS Variation

In the *Glu-A3* locus, four different types of B-LMW-GS were found, the six and five subunits, the 6+11 pair (alleles a, b, and d, respectively, in Nieto-Taladriz et al., 1997), and the rare subunit 8★ (Table 1). The 8★ glutenin subunit was found in four landraces (two from Ethiopia, one of Afghanistan, and one from India; Table 1). Up to now, this subunit had been described only in a durum wheat landrace called Mourisco Fino from Portugal (Brites and Carrillo, 2000). So, its presence in other accessions provides more sources to study its relationship with quality. In the 77 landraces analyzed, the most frequent alleles at *Glu-A3* locus were the *b* (64.9%) and *a* (28.6%) alleles (Table 2). In contrast, the *Glu-A3a* allele was the most frequent in previous analysis of durum wheat varieties (Nieto-Taladriz et al., 1997), and durum wheat landraces from Algeria (Cherdouh et al., 2005) and Spain (Aguiriano et al., 2008).

Seven different combinations of B-LMW-GS were encoded by the *Glu-B3* locus (Table 2). Five out of these corresponded to the *a, b, c, f,* and *h* alleles, as described by Nieto-Taladriz et al. (1997) in durum wheat varieties. The novel *Glu-B3* combination 2 +4 +14 +18 was only found in the CI*tr*14599 accession from Ethiopia (Tables 1 and 2, Fig. 1C). A new B-LMW subunit which had a slightly higher mobility than the nine subunit of the standard 'Langdon', was found in the PI 273985 accession also from Ethiopia. It

Table 2. Allelic frequency at *Glu-A1*, *GluB1*, *Glu-A3*, *Glu-B3*, and *Glu-B2* loci in a collection of 77 *Khorasan* wheat landraces.

Locus	Allele	Glutenin subunits	No.	Percentage, %
Glu-A1	*b*	2*	3	3.9
	c	Null	72	93.5
	o	V	2	2.6
Glu-B1	*b*	7+8	1	1.3
	d	6+8	14	18.2
	e	20x+20y	4	5.2
	f	13+16	1	1.3
	g	14+15	1	1.3
	ao	7+16	51	66.2
		13+8	5	6.5
Glu-A3	*a*	6	22	28.6
	b	5	50	64.9
	d	6+11	1	1.3
		8*	4	5.2
Glu-B3	*a*	2+4+15+19	17	22.1
	b	8+9+13+16	50	64.9
	c	2+4+14+15+19	5	6.5
	f	2+4+15+17	2	2.6
	h	1+3+14+18	1	1.3
		(9*) 14, 18	1	1.3
		2+4+14+18	1	1.3
Glu-B2	*a*	12	18	23.4
	b	Null	59	76.6

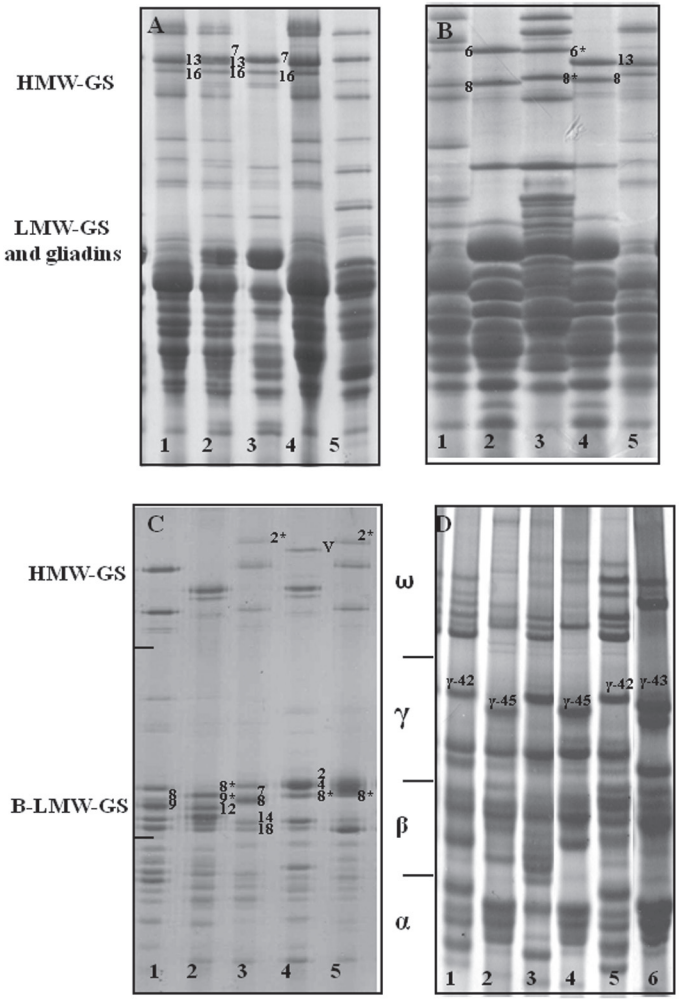

Figure 1. High molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS). (A) 'Lancota' (lanes 1 and 4), 'Lancota' + PI 123394 (lane 2), PI 123394 (lane 3), 'Chinese Spring' (lane 5). (B) 'Hope' (lane 1), PI 347132 (lane 2), 'Dawbull' (lane 3), PI 559976 (lane 4), and 'Lancota' (lane 5). (C) 'Langdon' (lane 1), PI 273985 (lane 2), 'Andalucia 344' (lane 3), CI 14599 (lane 4), and 'Mourisco Fino' (lane 5). (D) Gliadin patterns of PI 166959 (lane 1), PI 185193 (lane 2), PI 210386 (lane 3), 'Mexicali' (lane 4), 'Jabato' (lane 5), and PI 273985 (lane 6).

appeared in combination with the 14 and 18 subunits (Fig. 1C). We have called this new subunit as 9 ⋆. Although we have not been able to determine the genetic control of this subunit, we have postulated that was controlled by the *Glu-B3* locus due to the similar electrophoretic mobility compared to the nine subunit. In all the landraces analyzed, the most frequent allele of *Glu-B3* locus was the *b* allele (64.9%), followed by the *a* allele (22.1%) (Table 2). Other authors have described the *a* allele as the most frequent in durum wheat varieties (Nieto-Taladriz et al., 1997), in different allotetraploid wheat subspecies (Sissons and Batey, 2003), and in Algerian and Spanish wheat landraces (Cherdouh et al., 2005; Aguiriano et al., 2008).

For the *Glu-B2* locus, only two allelic variants were found, the *a* and *b* alleles coding for the 12 and null subunits, respectively (Nieto-Taladriz et al., 1997), being the *b* allele the most frequent (76.6%) (Table 2).

Among the 77 landraces of Khorasan wheat analyzed, nine different patterns for the B-LMW glutenin encoded by the *Glu-A3, Glu-B3,* and *Glu-B2* loci were found. The higher frequencies were for the combinations of the alleles *Glu-A3b, Glu-B3b, Glu-B2b* (64.9%), and *Glu-A3a, Glu-B3a, Glu-B2a* (20.8%). The alleles *Glu-A3a, Glu-B3c,* and *Glu-B2b,* were present in only five landraces (6.5%), while the remaining six patterns were only found in a landrace each one (Table 1).

The *Glu-A3a* and *d* alleles and the *Glu-B3a,c and f* alleles, have been related to good gluten strength with several quality tests (Carrillo et al., 2000) and some studies have found significant, but small, differences in quality related to the *Glu-B2a* alelle (Brites and Carrillo, 2001; Martínez et al., 2005; Aguiriano et al., 2009).

Gliadins Variation

Fifteen gliadin genotypes were identified in the 77 accessions analyzed (Table 1). A similar result was obtained by Piergiovanni (2009) who found 14 patterns in the 74 accessions analyzed from the same collection. The different alleles detected at the gliadin loci and their frequency is showed in Table 3. Seventeen new alleles, not previously cataloged in durum wheat, were found at the four gliadin loci (designated as new-^{turanicum}). These new alleles were rare with a frequency below 5%, except for *Gli-B2new-8*^{turanicum} and *Gli-B2new-9*^{turanicum} (Table 3). The most frequent

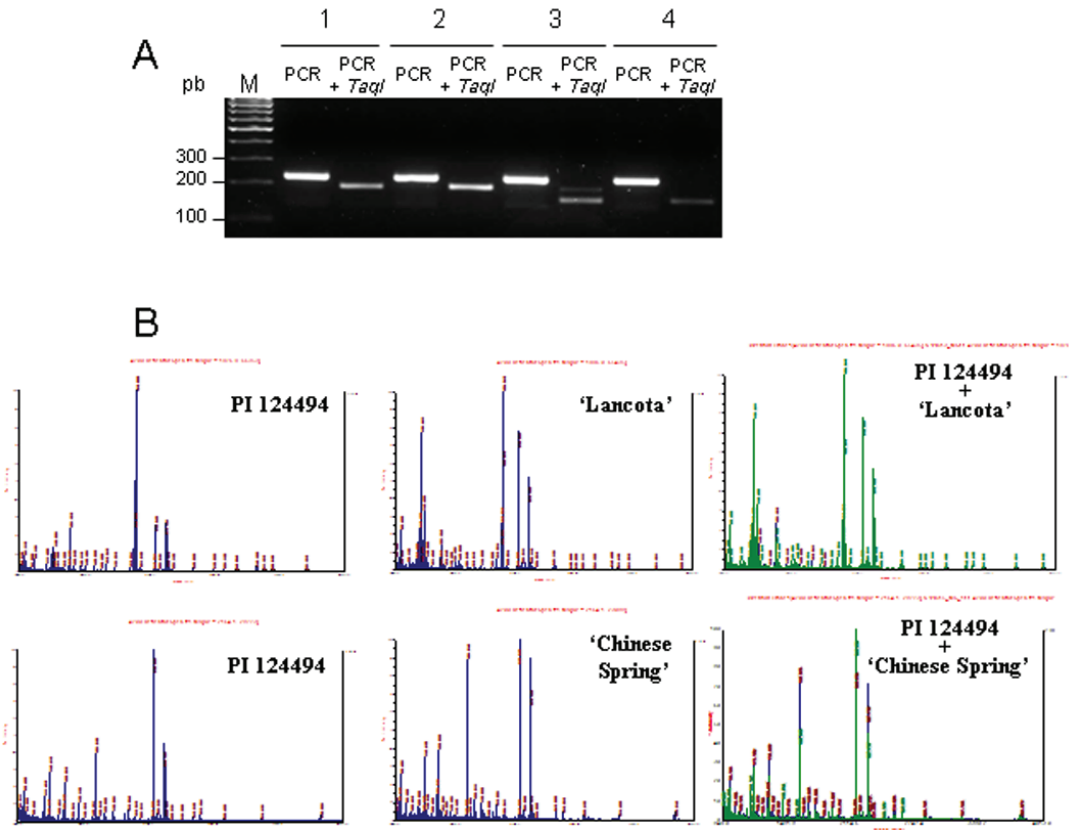

Figure 2. (A) PCR screening for discrimination between high molecular weight (HMW) glutenin subunits 7 and 13. One and 2 are samples carrying the HMW-7 subunit; 3 and 4 are samples carrying the HMW-13 subunit. For each sample, the first lane shows the PCR product amplified with BxTaqF and BxTaqR primers and the second one shows the same PCR product after digestion with TaqI restriction enzyme. After Taq I digestion, samples with HMW-7 subunit show a 189pb fragment and samples with HMW-13 show a 153pb fragment (the additional fragments of 34 and 34+36 pb are not visible in the gel). Lane identified as "M" contains a 100 bp size marker. (B) Peptide mass fingerprint spectrum obtained by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF-MS) of HMW-GS. In upper line, from left to right: HMW-16 sample from Khorasan wheat PI 124494 HMW-16 sample from bread wheat 'Lancota', and the overlapping spectrum to show that both are virtually identical, presenting the same peak pattern. In lower line, from left to right: HMW-7 sample from Khorasan wheat PI 124494, HMW-7 sample from bread wheat 'Chinese Spring' and the overlapping spectrum to show that both are virtually identical.

alleles at each locus were *Gli-A1b*, *Gli-B1a*, *Gli-A2k* and *Gli-B2new-8*^{turanicum}. About 65% of accessions presented the allele *a* at *Gli-B1* (coding for the gliadin λ-42, Fig. 1D, lane1), whereas 31.2% of them had the *b* and *c* alleles (both coding for the gliadin λ-45, Fig. 1). Only three accessions possessed *Gli-B1* alleles coding for none of these two typical gliadins. Two of them had the alleles *new-1* and *new-2,* coding for λ-44 and λ-43–46, respectively (Fig. 1D, lane 6). Both alleles had been previously described in durum wheat (Aguiriano et al., 2006). The other accession possessed a new allele, *new-3*^{turanicum}, which controlled the gliadin block ω-34–37 λ-47. It is known that *Gli-B1* alleles usually mark a different quality of gluten (Payne et al., 1984), and the new alleles may be associated with some untypical qualitative characteristics. All the accessions with the λ-gliadin 42 had the *b* allele at the *Glu-B3* locus, while the gliadin λ −45 was frequently linked to alleles *a* and *c* at the *Glu-B3*.

In this work, in all the prolamin loci analyzed, alleles related to good quality have been identified, but also have been identified some new alleles that can be a source of alternative, potentially useful alleles with application in durum wheat breeding.

Geographical Variation

The Multiple Correspondence Analysis performed on the 77 accessions and based on the nine prolamin loci, explained 100% of the total variation (35, 34, and 31% for axis 1, 2, and 3, respectively). *Gli-B2*, *Glu-B3*, *Gli-A1*, and *Gli-B1* loci provided the highest contribution to the variance. Most of the accessions were grouped in two main groups (I and II) in the three- dimensional projection mode (Fig. 3A). Group I included 49 accessions whose origin was Afghanistan, Australia, Azerbaijan, Egypt, Hungary, Iran, Iraq, Poland, Russia, Turkey, Turkmenistan, and Ukraine. The accessions from Australia and Egypt were identical in prolamins, confirming that the former was a selection from the latter. All the accessions of Group I possessed the alleles *Glu-B3b* and *Gli-B1a*, and more than

the 96% of the landraces had the *Glu-B1ao* (subunits 7+16), *Gli-A2k*, and *Gli-B2new-8*^{turanicum}. The genetic diversity of this group was low (Ht = 0.069), being *Glu-B2* and *Gli-B2* the loci with the largest genetic diversity (0.348 and 0.117, respectively). Group II comprised 21 accessions from Afghanistan, Azerbaijan, France, Italy, Morocco, Portugal, Rumania, Spain, Turkey, and the United States. The two accessions from the United States, one of them the cultivar QK-77, had the same prolamin composition. These landraces had the alleles *Glu-B3a* or *c, Gli-B1c*, and 17 of them possessed the *Gli-A2b* and *Gli-B2h*. The genetic diversity was larger than that of Group I (Ht = 0.253), and *Glu-B1* was the most polymorphic locus (Ht = 0.586). Figure 1D shows the gliadin pattern of one landrace of each Group I and II (lanes 1 and 2, respectively). Seven landraces were not included in any of these two groups. Three came from Ethiopia, and the others came from Iran, Turkey, Afghanistan, and India (Fig. 3A). They had rare alleles, mainly at the *Glu-A1*, *Glu-B1,* and *Gli-B2* loci. Only India and Ethiopia had not accessions in any of the two Groups I and II. Also, the three landraces from Ethiopia were the most different (Fig. 3A and Fig. 1D, lanes 3 and 6).The overall gene diversity of these seven landraces was 0.660, larger than those of Groups I and II.

In durum wheat, the gliadins λ-42 (*Gli-B1b*) and λ-45 (*Gli-B1a* or *c*) have served as markers for poor and good gluten strength, respectively, although it has been determined that the LMW glutenins, closely linked to them (λ-42-*Glu-B3b* and λ-45-*Glu-B3a* or *c*), are the ultimately responsible for the strength (Payne et al., 1984; Pogna et al., 1990, Ruiz and Carrillo., 1995; Carrillo et al., 2000). Consequently, the accessions of Group I that had the allele *Glu-B3b* possibly have poorer gluten strength than the accessions of Group II, which had the allele *Glu-B3a* or *c*. Most of the landraces of Group I came from Middle East, and countries from the former Soviet Union or in its sphere of influence (Fig. 3B). Group II was mainly composed by Mediterranean countries (Fig. 3B). Landraces from Afghanistan, Azerbaijan, and Turkey were present in the two groups. This result indicated that there are two geographical patterns for prolamin alleles with important differences in gluten quality in the accessions analyzed. Piergiovanni (2009) also observed a similar grouping for gliadin profiles in this collection, and Moragues et al.

Table 3. Allelic frequency at the *Gli-* loci in a collection of 77 *Khorasan* wheat landraces.

Locus	Allele	No.	Percentage, %
Gli-A1	*b*	72	93.5
	g	1	1.3
	k	2	2.6
	new-1 ^{turanicum} and-*2*^{turanicum}	1+1	1.3+1.3
Gli-B1	*a*	50	64.9
	b	1	1.3
	c	23	29.9
	new-1	1	1.3
	new-2	1	1.3
	new-3^{turanicum}	1	1.3
Gli-A2	*b*	18	23.4
	f	3	3.9
	k	52	67.5
	new-1 ^{turanicum},*-2* ^{turanicum}, *-3* ^{turanicum}, and-*4*^{turanicum}	1+1+1+1	1.3+1.3+1.3+1.3
Gli-B2	*h*	18	23.4
	new-8^{turanicum}	46	59.7
	new-7^{turanicum}	2	2.6
	new-9^{turanicum}	4	5.2
	new-1 ^{turanicum},*-2* ^{turanicum}, *-3* ^{turanicum},*-4* ^{turanicum}, *-5* ^{turanicum},*-6* ^{turanicum}, and-*10*^{turanicum}	1+1+1+1+1+1+1	1.3+1.3+1.3+1.3+ 1.3+1.3+1.3

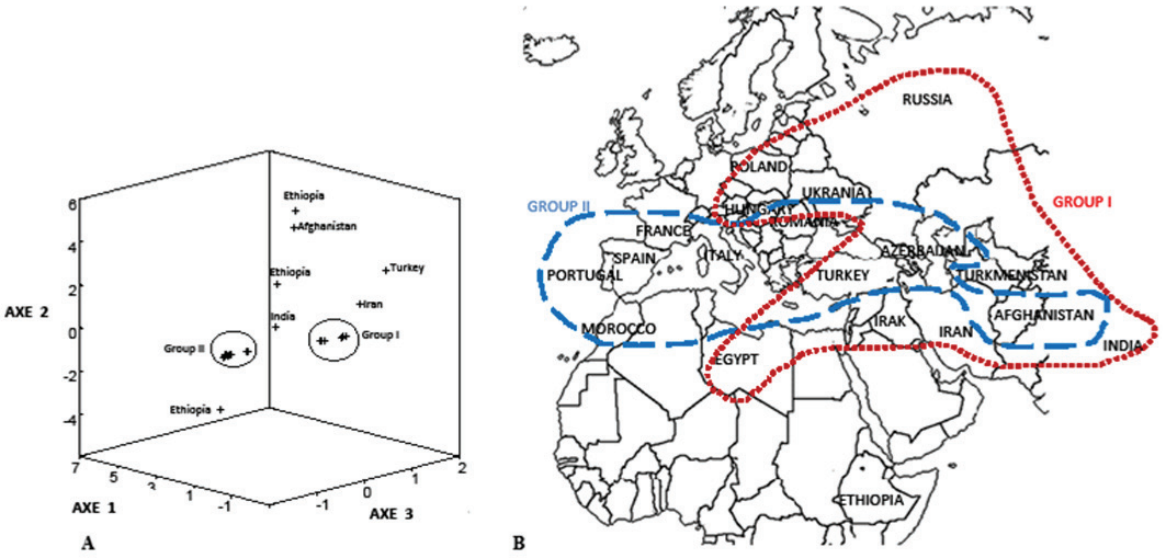

Figure 3. (A) Representation of the 77 accessions in the first three factors of the Multiple Correspondence Analysis using glutenin and gliadin alleles. (B) Geographical map showing the countries included in Group I (in red) and II (in blue). Only Australia in Group I and the United States in Group II are not represented.

(2006) found different patterns of glutenin alleles in durum wheat landraces from Southwest Asia and from the Mediterranean region. The origin of Khorasan wheat is located in South West Asia, so, similar to *T. monococcum*, Khorasan wheat could spread west from the Fertile Crescent to the Mediterranean countries; in fact some countries of Southwest Asia presented the two prolamin patterns (Turkey, Afghanistan, and Azerbaijan). The introduction of wheat in the Iberian Peninsula and North Africa could occur through the south of Italy. Therefore, it should be expected more similarities between landraces of the western Europe and North Africa than with those from Southwest Asia.

CONCLUSIONS

There are two geographical distribution patterns of prolamin alleles, with contrasting differences in gluten strength, in the landraces of Khorasan wheat analyzed: one for the Southwest of Asia (Group I), and other for the Mediterranean Zone (Group II). Some alleles related to good gluten quality have been identified at different loci. Additionally, some novel alleles of prolamins not previously described have been found, providing a new source of genetic variability. It would be interesting to analyze the relation to quality of these novel alleles to transfer them to the much more productive durum wheat in a quality breeding program.

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