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Evaluation of the Storage Protein Variations and Agronomic Performance in Persian Wheat (*Triticum carthlicum* Nevski)

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Abstract: The endosperm storage protein allelic variations and agronomic performance in 85 accessions of persian wheat (*Triticum carthlicum* Nevski) were analyzed for their endosperm storage protein allelic composition. Higher variations of gliadins were observed in the persian wheat accessions and the gliadin variations among persian wheat accessions were associated with their geographic origins. At *Glu-1* loci, 8 alleles were detected by SDS-PAGE analysis. At *Glu-A1* locus, the subunit null was most frequently appeared (96.47%). At *Glu-B1* locus, the most frequent subunits were 7+8 (95.29%). Moreover, the presence of subunits 14+15, 17+18 and 2*, which associated with good quality in bread wheat, were observed. The agronomic traits were evaluated to provide information for its utilization in breeding programs. All accessions were divided into five clusters based on principle component and cluster analysis. Every cluster had its particular characteristics. The cluster 1 had higher spikelet numbers per spike and grains per spike and cluster 2 had higher 1000-grain weight with lower plant height. Most of the accessions in cluster 3 were derived from Turkey.

Key words: *Triticum carthlicum*, gliadin, *Glu-1*, agronomic traits

INTRODUCTION

The seed storage proteins of wheat endosperm could be divided into two main groups, gliadins and glutenins (Wei *et al.*, 2002). Most gliadins are controlled by the genes located on the short arms of group 1 (*Gli-1*) and 6 (*Gli-2*) chromosomes (Lafiandra *et al.*, 1984). The gliadin patterns with great diversity of variations have been used as fingerprints, genotype identification, quality markers and so on (Yan *et al.*, 1992; Liu *et al.*, 1999; Lang *et al.*, 2001). Gliadins were successfully separated into four subfractions: α - (the fastest), β -, γ - and ω -gliadins (the slowest) in according with the mobility in A-PAGE analysis (Woychik *et al.*, 1961; Bushuk and Zillman, 1978). However, later investigation of genetic and chemical studies suggested that only three types of gliadins (α -, γ - and ω -type or α/β -, γ - and ω -type) exist (Bietz *et al.*, 1977; Kasarda *et al.*, 1983). Glutenins could be divided into High Molecular Weight (HMW) and Low Molecular Weight (LMW) fractions (Kreis *et al.*, 1985; Payne *et al.*, 1980). Although accounting for only 10% of the total seed protein, the HMW glutenins are important determinants of wheat grain processing quality (Kreis *et al.*, 1985; Payne *et al.*, 1980). Genetic analysis had shown that, in common wheat, HMW glutenin subunits were specified by genes resided in the *Glu-1A*, *Glu-1B* and *Glu-1D* loci. Within each locus, there were two closely linked genes, which encoded the x- and y-type subunits, respectively. The expression of HMW glutenin genes was

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regulated by gene silencing and allelic variation. Consequently, common wheat varieties often differ in the number and composition of expressed HMW glutenin subunits. In most cases, the 1Ay gene is silenced in common wheats (Forde *et al.*, 1985; Harberd *et al.*, 1987; Halford *et al.*, 1989), however, it do occur occasionally in hexaploid wheats (Margiotto *et al.*, 1993; Johansson and Svensson, 1995) and more widely in the A-genome diploid wheats (Waines and Payne, 1987).

The range of genetic variation of cultivated wheat had decreased drastically during the past several decades because of its interspecific hybridization and modern breeding procedures (Hoisington *et al.*, 1999; Allard, 1996). As a result, the possibility of the further improvement in productivity was reduced and also the world wheat crop increasingly vulnerable to new diseases and to adverse climatic change. Comparing to the exotic wheat varieties, Chinese wheat cultivars showed inferior quality. Ma and Wu (1993) and Zhao *et al.* (1994) proposed that the absence of subunits with good quality should be the reason for worse baking quality of Chinese wheat. Therefore, it is essential to exploit the valuable gene resources from the relative species of genus *Triticum* to improve the yield and quality of common wheat. *T. carthlicum*, with the common name of persian wheat, was of spring habit with early-maturing. And it is also associated with some desirable characters, such as good resistance to powdery mildew, dust brand and stem rust, higher number of tillers and fertility, good fecundity, tolerance to low temperature and preharvest sprouting (Belay *et al.*, 1994; Raut *et al.*, 1984; Gol'denberg, 1984). Up to now, little information about storage protein and agronomic traits of persian wheat is available.

The information on characteristics of persian wheat was fundamental for its utilization in breeding programs aimed at improving the performance of this primitive crop, as well as to utilize them as sources of useful genes for the cultivated wheat. The objectives of this study were to evaluate the genetic variations of HMW-glutenin subunits and gliadins, to evaluate the agronomic traits in the selected *T. carthlicum* accessions.

MATERIALS AND METHODS

Plant Materials

The 85 accessions of *T. carthlicum* (Table 1) were collected from 15 countries, which were kindly provided by Dr. H. Bockelman in American National Plant Germplasm System (NPGS) and Dr. D.C. Liu in the germplasm lab of Triticeae Research Institute, Sichuan Agriculture University, China.

A-PAGE and SDS-PAGE

Gliadins and HMW-GSs were extracted from single seeds of each accession. Gliadins was extracted following the procedure of ISTA A-PAGE (pH 3.1) (Draper, 1987). Gliadin bands were scored according to Nei's (1973) and cluster analysis was carried out by NTSYSpc2.1 software. The HMW bands were numbered according to Payne and Lawrence (1983). For glutenins, total protein and selective HMW glutenin were extracted following Ng and Bushuk (1987) and Mackie *et al.* (1996), respectively and were separated by SDS-PAGE. The relative nobilities of these subunits were determined by comparison with the references Chinese Spring (1Bx7, 1By8, 1Dx2, 1Dy12), Chuanyu12 (1Ax, 1Bx7, 1By8, 1Dx5, 1Dy10) and Xiaoyan 6 (1Ax1, 1Bx14, 1By15, 1Dx2, 1Dy11).

Agronomic Trials

Field experiments were carried out at experimental station of Sichuan Agricultural University in 2004. The agronomic performance of PI78813, PI349041, CItr7692 and AS2266 were not evaluated, since no seedlings of these accessions were obtained. Eight agronomic traits (plant height, tillers number per plant, spike number per plant, spike length, spikelet number per plant, grains per spike, heading

Table 1: The origin of 85 *T. carthlicum* accessions in this study

Origin	Accession	No.
Turkey	PI470730, PI470731, PI470732, PI470733, PI470734, PI532475, PI532476, PI532477, PI532478, PI532479, PI532480, PI532481, PI532482, PI532483, PI532484, PI532485, PI532487, PI532488, PI532489, PI532491, PI532492, PI532493, PI532494, PI532495, PI532496, PI532497, PI532498, PI532499, PI532500, PI573178, PI573179, PI573180, PI573181, PI573182	34
Georgia	PI61102, PI78812, PI78813, PI115817, PI251914, PI585018, PI94751, PI94752, PI94753, PI94754, PI94755, PI115816, PI94749, PI94750, PI352278, PI352282, PI499972, PI585017, PI94748	19
Former Soviet Union	PI352279, PI352280, PI352281, PI532501	4
Iran	PI283887, PI283888, PI283889, PI283890	4
Canada	PI532505, PI532509, PI532518, PI532510	4
Poland	PI286070, PI286071, PI532504	3
United States	PI532502, PI532506, PI532507	3
England	PI532513, PI532516, PI532517	3
Russian Federation	Citr7665, Citr7692, PI349041	3
Albania	AS2266*, AS2267, AS2268	3
Iraq	PI70738	1
China	PI168672	1
Hungary	PI272521	1
Armenia	PI349040	1
Ethiopia	PI387696	1

*AS2266, AS2267 and AS2268 were provided by Dr. Deng-Cai Liu of Triticeae Research Institute, Sichuan Agricultural University

date and 1000-grain weight) were investigated in 81 persian wheat accessions. The data of agronomic characters were subjected to principal component and cluster analysis with DPS v.6.01 software (Tang and Feng, 1997).

RESULTS

Variation of Gliadin Patterns

In 85 *T. carthlicum* accessions, a total of 53 gliadin bands were detected, among which all bands were polymorphic. Eleven, 11, 13 and 18 bands were observed in α -, β -, γ - and ω -region, respectively. Each accession had 13 to 24 bands with the mean of 18.5. Consequently, 44, 46, 27 and 44 gliadin patterns were identified in α -, β -, γ and ω -region, respectively, resulting in a total of 66 gliadin patterns in the 85 accessions. Some accessions had the same A-PAGE patterns, thus these accessions could not be differentiated by A-PAGE analysis. The Genetic Similarity (GS) value ranged from 0.176 to 1.000 with the mean of 0.542, indicating that higher genetic diversity existed in the *T. carthlicum* collections. The highest genetic difference existed between PI283887 and PI387696, with the minimum GS of 0.176.

Clustering analysis conducted by UPGMA program indicated that these accessions could be divided into 6 major groups (Fig. 1). Cluster I included all accessions from Georgia (19 accessions). Only one accession (PI387696) from Ethiopia was included in Cluster II. All accessions from America, England, Canada, Albania, Armenia and most accessions from Poland were clustered into cluster III (16 accessions). Cluster V had only one accession (PI70738) from Iraq. The remaining 34 accessions were grouped into Cluster VI, among which 28 accessions were derived from Turkey. These results indicated that the gliadin variations among persian wheat accessions were associated with their geographic origins.

HMW-Glutenin Variations

In the 85 persian wheat accessions, 3 and 5 alleles have been detected on *Glu-A1* and *Glu-B1* loci, respectively. The distribution patterns of HMW-glutenin were listed in Table 2. At *Glu-A1* locus, the

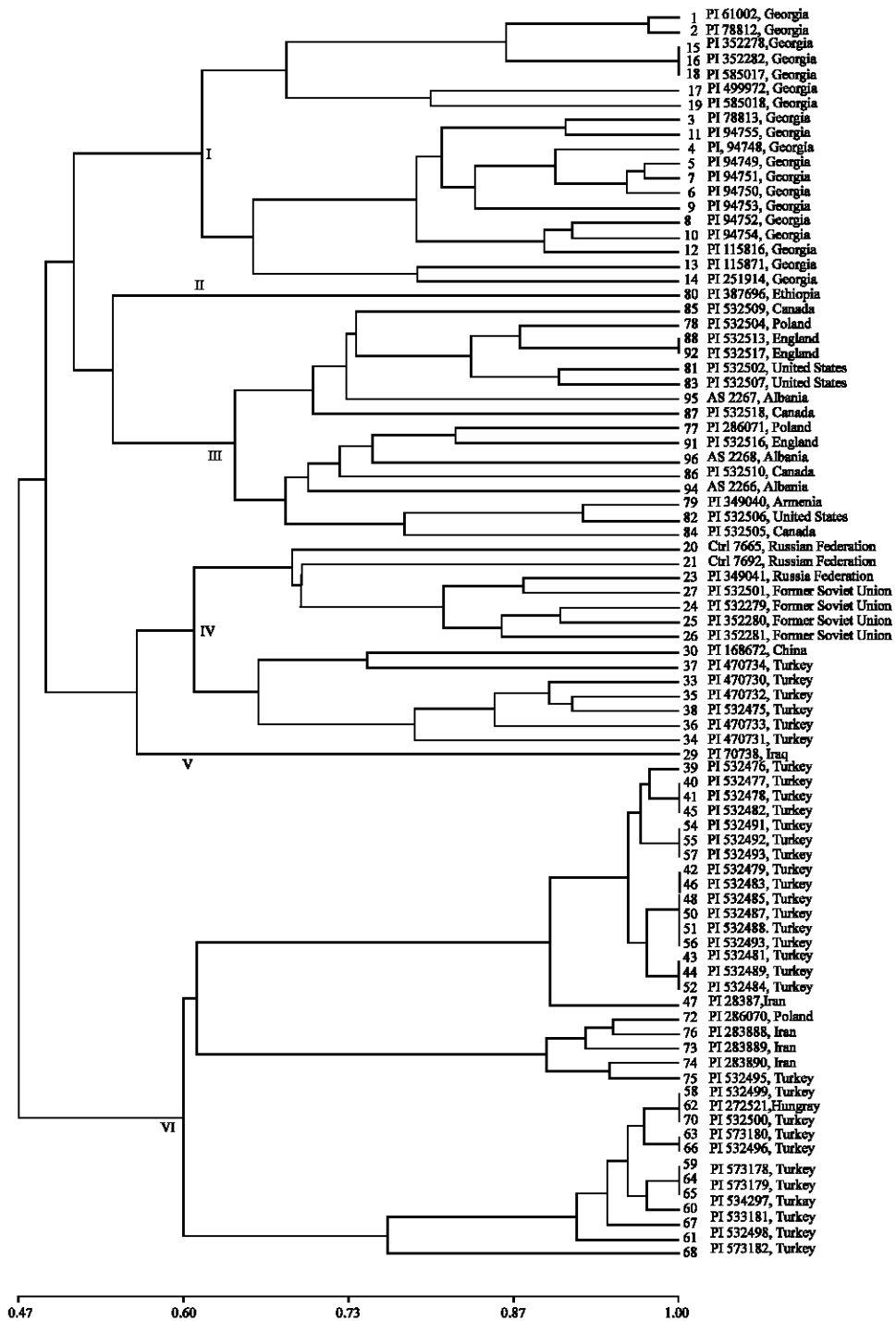


Fig. 1: Dendrogram for clustering analysis based on the genetic similarity coefficient of gliadin in 85 accessions of *T. carthlicum* Nevski

Table 2: Allele Frequencies for *Glu-1* in *T. carthlicum*, *T. dicoccoides*, *T. polonicum*, *T. turanicum*, *T. turgidum*, *T. dicoccum*, *T. durum* and *T. aestivum*.. The frequencies of dominant subunits were in bold. 2. (a) 85 *T. carthlicum* of 15 countries in this study, (b) 172 *T. dicoccoides* from Israel (Li *et al.*, 2002); (c) 72 *T. polonicum* from 24 countries (Liu, 2005); (d) 87 *T. turanicum* from more than 20 countries (Xu *et al.*, 2005); (e) 123 *T. turgidum* from China (Zhang *et al.*, 2003); (f) 167 *T. dicoccum* from 23 countries (Vallega and Waines, 1987); (g) 502 *T. durum* from 23 countries (Branlard *et al.*, 1989); (h) 195 *T. aestivum* of a world collection (Payne *et al.*, 1981)

		Frequency (%)								
Locus	Alleles	Subunits	(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)
			<i>T. carthlicum</i>	<i>T. dicoccoides</i>	<i>T. polonicum</i>	<i>T. turanicum</i>	<i>T. turgidum</i>	<i>T. dicoccum</i>	<i>T. durum</i>	<i>T. aestivum</i>
<i>Glu-A1</i>	a	1	---	44.77	8.3	---	0.81	56	7.0	28.00
	b	2*	3.53	15.70	---	10.34	59.35	16	4.6	28.00
	c	null	96.47	9.30	84.7	89.66	29.27	21	83.5	44.00
<i>Glu-B1</i>	a	7	1.18	---	40.3	---	1.63	2	0.8	19.00
	b	7+8	95.29	2.91	8.3	10.34	15.45	11	25.9	20.00
	c	7+9	---	2.91	---	---	---	---	---	27.00
	d	6+8	1.18	11.63	9.7	21.84	8.94	18	26.3	19.00
	e	20	---	---	40.3	4.60	2.44	rare	33.5	5.00
	f	13+16	---	1.74	---	60.92	---	---	5.5	1.50
	g	13+19	---	---	---	2.30	---	rare	---	0.50
	h	14+15	1.18	6.40	---	---	1.63	---	5.5	1.50
	i	17+18	1.18	29.65	---	---	---	---	---	6.50
	j	21	---	---	---	---	0.81	1	---	rare
	k	22	---	---	---	---	---	2	---	rare

Table 3: Agronomic performance of 81 persian wheat accessions

Characters	Min.	Max.	Mean	Standard error	Variation coefficient (%)
Plant height (cm)	73.8	136.2	110.0	13.49	12.26
Tiller number per plant	5.0	49.0	19.5	9.28	47.49
Spike number per plant	3.0	29.0	12.6	5.77	45.70
Spike length (cm)	8.7	16.2	12.6	1.69	13.45
Spikelet number per spike	20.0	29.0	25.2	2.21	8.79
Grains per spike	18.0	65.0	42.4	9.75	23.00
Heading date (d)	159.0	211.0	185.5	8.56	4.62
1000-grain weight (g)	11.5	33.5	17.3	4.00	23.10

subunit null was most frequently appeared (96.47%). At *Glu-B1*, the subunits associated with good quality had been identified. Two accessions (PI 168672 and PI 532510) with the subunit combination of 2* and 7+8, one accession (AS2268) with 14+15 and one accession (AS2266) with 2* and 17+18 subunit combinations were observed. In addition, the subunits 7 and 6+8 were only detected in PI349041 and CItr7665, respectively.

Agronomic Performance

Agronomic performance of 81 persian wheat accessions were investigated (Table 3). The persian wheat accessions had higher plant height with the mean of 110.0 cm. The grains per spike were 42.4, whereas the 1000-grain weight was lower, only 17.3 g. The mean of heading date (a relative measure of maturity) was 185.5 days, in which 2 accessions with early maturity were found. On the coefficient of variations, it indicated that tiller and spike numbers per plant had the highest variations in persian wheat accessions, whereas heading date had the lowest variability.

Principle Component and Cluster Analysis

Principle component analysis was used prior to cluster analysis to determine the relative importance of classification variables (Jackson, 1991). Eigenvalues from the first, second, third and fourth principal component axes, respectively, accounted for 42, 25, 9 and 9% of the total variance present. The relative magnitude of eigenvectors from the first principal component axis (Table 4) indicated that plant height, tiller number per plant and spike number per plant were important traits for classifying accessions into clusters. From the second principal component axis, 1000-grain weight was the most important classification variable. Grains per spike and heading date had relatively high eigenvectors in the third and fourth principle component axis, respectively.

The entries in this study were grouped into 5 clusters (Fig. 2). Cluster 1 was the main group with 40 accessions from various origins, in which they had higher spikelet number per spike and grains per

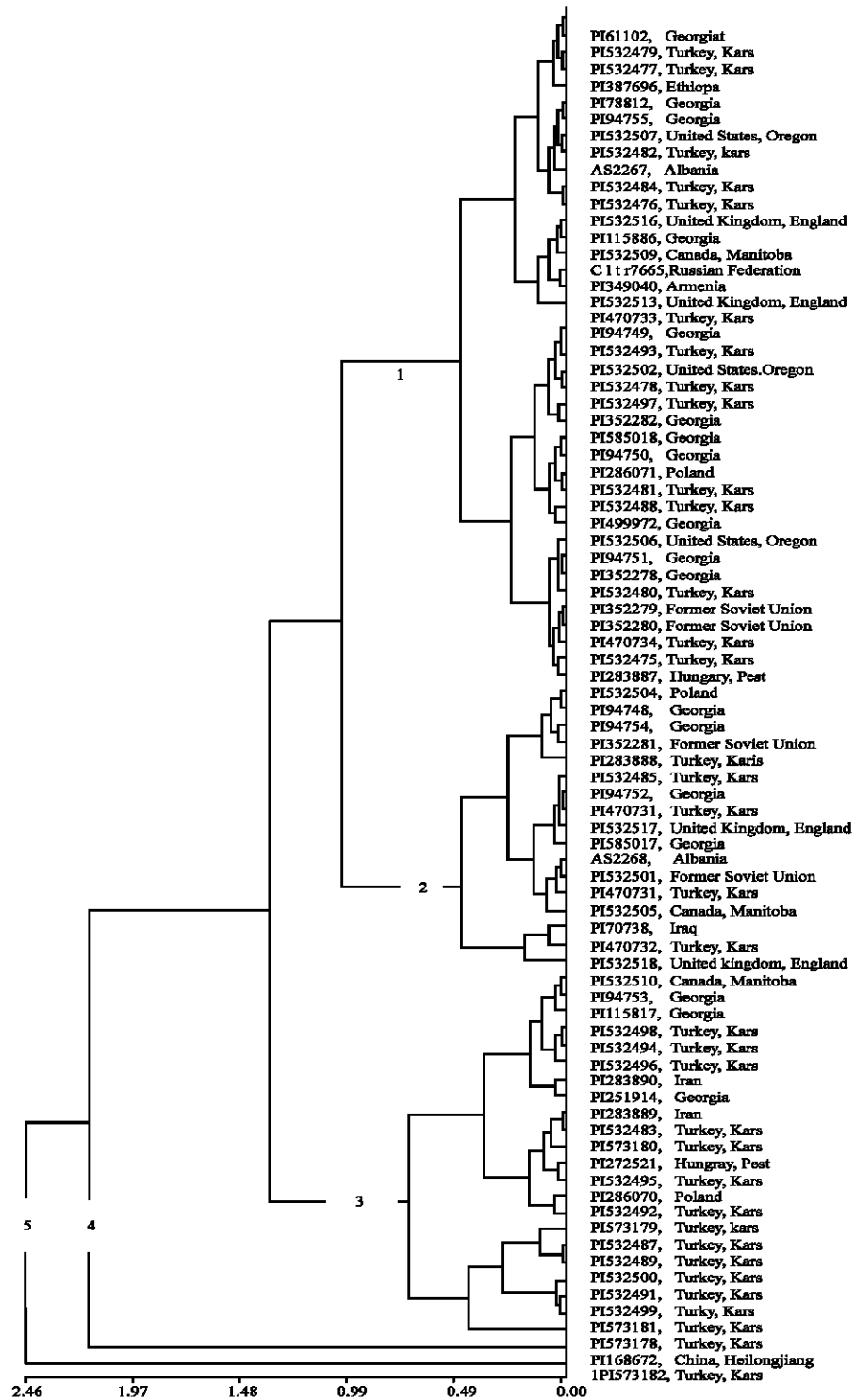


Fig. 2: Dendrogram for 81 accessions of *Triticum carthlicum* obtained by the analysis of 8 agronomic characterization variables based on agronomic traits

Table 4: Eigenvector from the first four principle component axes for traits used to classify 81 accessions into 5 clusters

Principal component	Index of character	Tiller factor	Grain weight factor	Spike grain factor	Heading date factor
Eigenvalues		3.357	2.007	0.758	0.726
Contribution (%)		41.964	25.089	9.479	9.075
Cumulative contribution (%)		41.964	67.053	76.533	85.608
Eigenvector	Plant height	0.420	-0.195	-0.027	0.463
	Tiller number per plant	0.487	0.041	-0.454	-0.060
	Spike number per plant	0.483	-0.067	-0.423	-0.134
	Spike length	0.317	0.344	0.242	0.303
	Spikelet number per spike	0.307	0.424	0.465	-0.073
	Grains per spike	0.334	-0.342	0.547	0.004
	Heading date	-0.196	0.444	-0.198	0.660
	1000-grain weight	-0.096	-0.586	0.036	0.481

spike. The accessions in Cluster 2 had higher 1000-grain weight with lower plant height. The accession PI 70738 with highest grain weight was fell into Cluster 2. The Cluster 3 contained 22 accessions, most of which were from Turkey. There was only one accession in Cluster 4 and Cluster 5, respectively.

DISCUSSION

Gliadin pattern, a useful marker for evaluating genetic variation (Lafiandra *et al.*, 1990), could provide valuable and exact information for the identification and utilization of germplasm, since the gliadin pattern of a cultivar was rarely affected by the area of plant growth (Zillman and bushuk, 1979). In this study, the higher gliadin variations were observed in persian wheat accessions and their gliadin patterns were obviously different from common wheat. Therefore, it is valuable to utilize these materials in the improvement of the quality and adaptation of common wheat. Additionally, it was found that the gliadin variation among *T. carthlicum* were associated with their geographic origin.

The allele null, encoded by *Glu-A1* locus, was the predominant subunit with the highest frequency in *T. carthlicum*, which was similar as those in *T. durum*, *T. polonicum*, *T. turanicum* and *T. aestivum*, but different from those in *T. dicoccoides*, *T. dicoccum*, *T. spelta* and *T. turgidum* (Table 2). At *Glu-B1* locus, the subunits 7+8 were found at the frequency of 95.29% in *T. carthlicum*, whereas much lower frequency presented in other tetraploid wheats (Table 2). It was well known that HWM-glutenin subunits attribute greatly to wheat grain processing quality (Kreis *et al.*, 1985; Payne *et al.*, 1980). Consistent efforts have been made to improve wheat quality by accumulating good HMW-glutenin subunits. The presence of subunits, such as 7+8, 14+15, 17+18 and 2* associated with good quality, were observed in *T. carthlicum*. This information could be useful to use these accessions as a source of genes to develop new lines in the improvement of wheat quality.

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