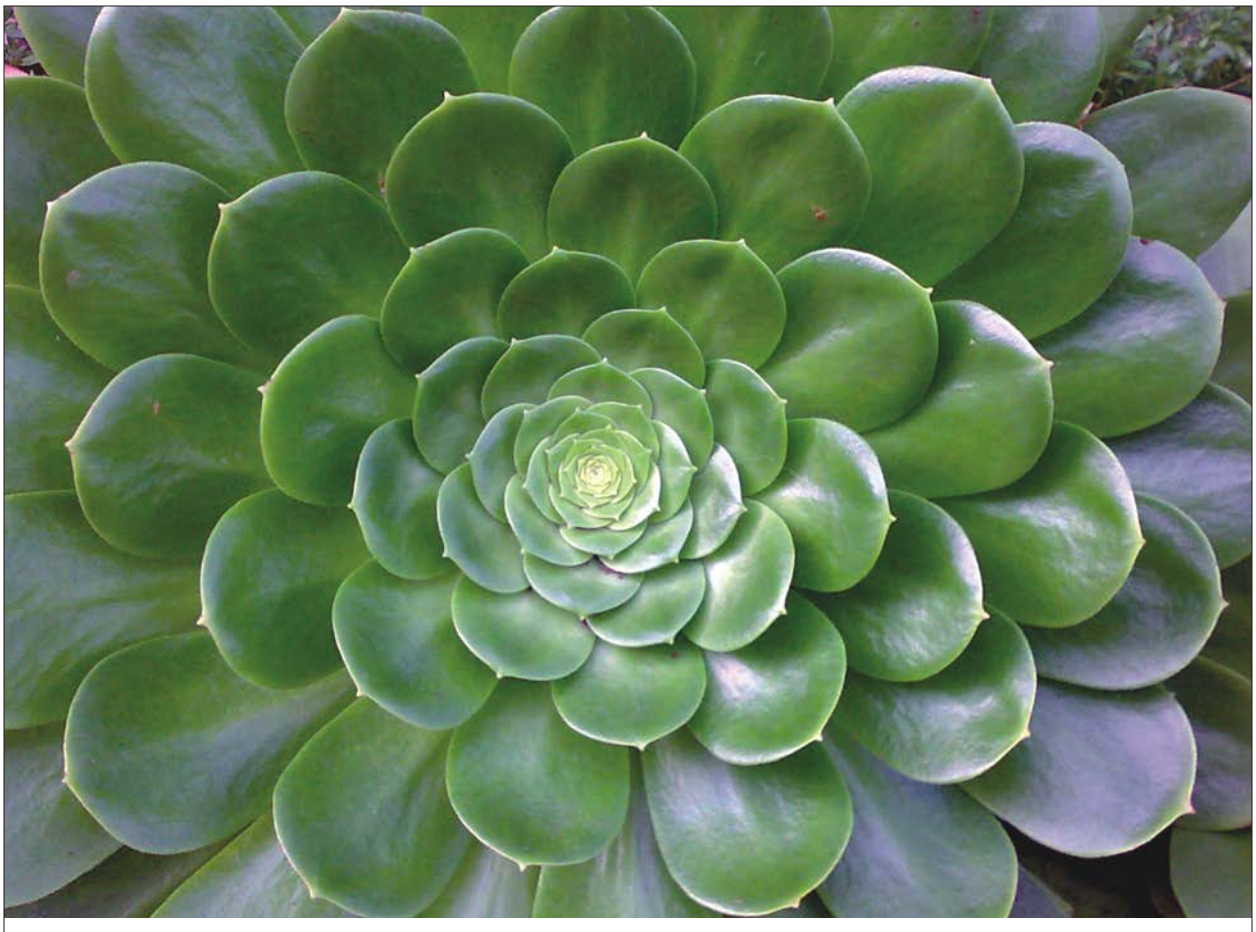

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Genetic Diversity of Storage Proteins in *Triticum polonicum* L.

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Abstract: The gliadins and high molecular weight glutenin subunits (HWM-GSs) variations of 72 *Triticum polonicum* accessions derived from 23 countries were characterized by A-PAGE and SDS-PAGE, respectively, for the purpose of evaluating the genetic diversity *T. polonicum* accessions at the level of proteins. Higher genetic variabilities were observed for both gliadins and HWM-GSs. Forty-eight gliadin bands and 65 gliadin patterns were detected. The average of genetic similarity coefficient based on gliadin bands was 0.5123. The cluster analysis indicated that all the accessions could be divided into 5 groups and the gliadin variations among *T. polonicum* accessions were associated with their geographic origins. Three and five HWM-GS alleles were detected at *Glu-A1* and *Glu-B1* loci, respectively. A total of 10 HWM-GS combinations were observed. The genetic diversity indices (*H*) at *Glu-B1* loci (0.659) were much higher than that at *Glu-A1* loci (0.271).

Key words: *Triticum polonicum* L., gliadin, HWM-GS, A-PAGE, SDS-PAGE

INTRODUCTION

Compared with common wheat, *Triticum polonicum* L. (2n = 28, AABB) has some typical agronomic traits, such as large grains, more spikes and higher tillering ability (Zheng, 1989). *T. polonicum* is mainly cultivated in Mediterranean and Ethiopia and often mixed grown with durum wheat (Dong and Zheng, 2000). *T. polonicum* had been widely evaluated from its classification and distribution (Dong and Zheng, 2000; Jin *et al*., 1996), origin and evolution (Belea, 1971; Belea *et al*., 1975; Gorgidze and Zhizhilashvili, 1983; Orel *et al*., 1990; Yang *et al*., 1992), genetics (Puzyreva, 1971; Prasad, 1972; Zhangaziev, 1990; Watanabe *et al*., 1998; Efremova *et al*., 2001; Deng *et al*., 2005), bio-chemistry marker (Zahoor *et al*., 1986; Yang *et al*., 2000; Liu *et al*., 2001; Sissons and Batey, 2003) and molecular marker (Akond *et al*., 2007; Liao *et al*., 2007)

Wheat storage proteins are mainly composed of gliadins and glutenins and the glutenins could be subdivided into high and low molecular weight glutenin subunits (HMW- and LMW- GSs) (Wei *et al*., 2002). Gliadins encoded by *Gli-1* and *Gli-2* loci (Lafiandra *et al*., 1984) are generally considered to contribute to the viscosity and extensibility of gluten (Gianibelli *et al*., 2001; Khatkar *et al*., 2002; Clarke *et al*., 2003). HMW-GSs, controlled by three gene loci identified as *Glu-A1*, *Glu-B1* and *Glu-D1* (Payne *et al*., 1982), have closely associated with bread-making quality (Payne, 1987; Yahata *et al*., 2006; Chen *et al*., 2007). In addition, storage proteins are also reliable bio-chemical markers for the evaluation of genetic diversity in wheat (Branlard *et al*., 2001).

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In this study, the genetic variations of gliadins and HMW-GSs in 72 *T. polonicum* accessions, which were widely collected from various areas, were investigated using A-PAGE and SDS-PAGE methods, respectively. The objective of this study was to evaluate the genetic diversity of *T. polonicum* accessions at the level of proteins with reference to the improvement of common wheat.

MATERIALS AND METHODS

Plant Materials

A total of 72 *T. polonicum accessions* received from American national resource information net (GRIN) (Table 1) were derived from 6 main regions, including North America, South America, Europe, East Africa and West Asia, Center and South Asia and Australia. Common wheat cultivar, Chines Spring (CS) was used as reference in A-PAGE analysis. The relative motilities of the HMW glutenin 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).

A-PAGE

Gliadin proteins were extracted from single seeds with a solution of 25% (v/v) α-ethanol and 0.05% (w/v) methyl green and fractionated by a standard acid-plyacrylamide-ger electrophoresis (A-PAGE) at pH 3.1 (Draper, 1987).

Table 1: The geographical origin and HMW-GSs of 72 *T. polonicum* accessions

No.	Accession	Origin	HMW-GSs	No.	Accession	Origin	HMW-GSs
1	PI29447	Ukraine	Null, 7	37	PI272566	Hungary	Null, 7
2	PI42209	Australia	Null, 6+8	38	PI272567	Hungary	Null, 20
3	PI56261	Portugal	Null, 20	39	PI272569	Hungary	Null, 20
4	PI56262	Portugal	Null, 20	40	PI272570	Hungary	1, 7
5	CItr13919	Ethiopia	Null, 6+8	41	PI272572	Hungary	Null, 20
6	CItr14139	Unknown	Null, 7	42	PI278647	England	Null, 7
7	CItr14140	Unknown	Null, 7	43	PI286547	Ecuador	Null, 6+8
8	CItr14803	Ethiopia	*Glu-A1-I,* 7+8	44	PI289606	England	Null, 6+8
9	CItr14869	Ethiopia	Null, 20	45	PI290512	Portugal	Null, 20
10	CItr14892	Ethiopia	Null, 7	46	PI298572	Ethiopia	Null, 20
11	CItr17442	USA.	Null, 6+8	47	PI306548	Romania	1, 7
12	PI134945	Portugal	Null, 20	48	PI306549	Romania	1, 7
13	PI167622	Turkey	Null, 20	49	PI330554	England	Null, 7
14	PI185309	Argentina	Null, 20	50	PI330555	England	
15	PI190951	Spain	Null, 7	51	PI349051	Georgia	Null, 7
16	PI191808	Portugal	*Glu-A1-I,* 7+8	52	PI349052	Azerbaijan	Null, 7
17	PI191810	Portugal	Null, 7+8	53	PI352487	Germany	1, 7
18	PI191823	Portugal	Null, 13+19	54	PI352488	Italy	*Glu-A1-I,* 7
19	PI191826	Portugal	Null, 7+8	55	PI352489	Cyprus	1, 7
20	PI191852	Portugal	Null, 7	56	PI361757	Denmark	1, 6+8
21	PI191881	Portugal	Null, 7	57	PI366117	Egypt	Null, 20
22	PI191890	Portugal	Null, 7	58	PI384266	Ethiopia	Null, 20
23	PI191893	Portugal	Null, 7	59	PI384267	Ethiopia	Null, 20
24	PI191903	Portugal	*Glu-A1-I,* 7	60	PI384268	Ethiopia	Null, 20
25	PI192666	Portugal	Null, 20	61	PI384337	Ethiopia	Null, 20
26	PI208911	Iraq	Null, 20	62	PI384338	Ethiopia	Null, 20
27	PI210845	Iran	Null, 7	63	PI384339	Ethiopia	Null, 20
28	PI223171	Jordan	Null, 20	64	PI384341	Ethiopia	Null, 20
29	PI225334	Iran	Null, 7	65	PI384342	Ethiopia	Null, 20
30	PI225335	Iran	Null, 20	66	PI384343	Ethiopia	Null, 20
31	PI245663	Afghanistan	Null, 7	67	PI384344	Ethiopia	Null, 20
32	PI254214	India	Null, 20	68	PI387457	Ethiopia	Null, 20
33	PI254215	Iraq	Null, 20	69	PI387479	Ethiopia	Null, 20
34	PI266846	England	Null, 7	70	PI566593	USA.	Null, 7
35	PI272564	Hungary	Null, 7	71	PI608017	USA.	Null, 7
36	PI272565	Hungary	*Glu-A1-I,* 6+8	72	PI629119	USA.	Null, 7

SDS-PAGE

The HMW-GS extractions from single seeds were performed as described by Mackie *et al.* (1996). According to the procedure of Ng and Bushuk (1987), HMW-GSs were separated by polyacrylamide-gelelectrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE).

Allele Identification

Based on the classification of Payne and Lawrence (1983), the HMW-GSs were identified. The new subunits and alleles were designated according to Wang *et al.* (2006).

Data Analysis

The gliadin genetic similarity coefficient (GS) among the accessions was calculated by Nei and Li (1979) genetic similarity

$$GS = 2N_{ij}/(N_i+N_j)$$

where, N_i is the bands of accession i, N_j is the bands of accession j and N_{ij} is the bands in both accessions i and j. The genetic similarity matrix of all accessions was analyzed by using Unweighted Pair Group Method with Arithmetic Average (UPGMA) algorithm and the result was used to construct a clustering dendrogram under NTSYS-pc2.1.

The gene diversity at *Glu-A1* and *Glu-B1* loci was calculated by Nei (1973) genetic variation index,

$$H = 1-\sum p_i^2$$

where, H is Nei's genetic variation index and p_i is the frequency of a particular allele at that locus.

RESULTS

Gliadin Variations

A total of 48 gliadin bands were detected in the 72 *T. polonicum* accessions (Fig. 1) and 8 to 24, with an average of 16.2, gliadin bands were identified in each accession. The α, β, ω and γ gliadin zones

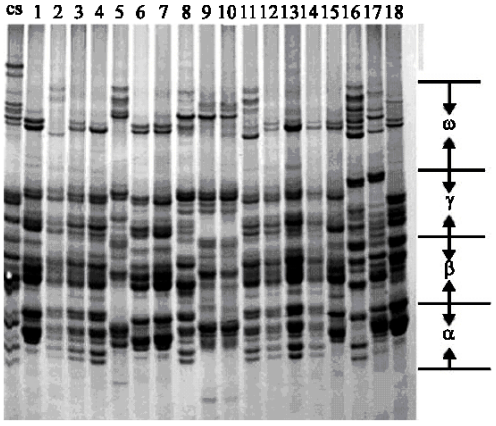

Fig. 1: Gliadin patterns in the representative *T. polonicum* accessions. 1 = PI29447, 2 = PI42209, 3 = PI56261, 4 = PI56262, 5 = CItr13919, 6 = CItr14139, 7 = CItr14140, 8 = CItr14803, 9 = Citr14869, 10 = Citr17442, 11 = Citr134945, 12 = PI134945, 13 = PI167622, 14 = PI185309, 15 = PI190951, 16 = 191808, 17 = PI191810, 18 = PI191823

were identified by 11, 9, 13 and 15 bands, respectively. The frequency of each band ranged from 1.4 to 90.3%, with the mean of 33.7%. A total of 65 gliadin patterns were identified from 72 accessions, among which 58 accessions had the unique gliadin genotypes. The gliadin patterns in seven pairs of accessions (i.e., CItr14869 and CItr14892, PI306548 and PI306549, PI384338 and PI384339, PI384337 and PI384342, PI384337 and PI384344, PI384342 and PI384344 and PI629119 and PI608017) were identical, thus, these accessions could not be distinguished by A-PAGE.

The GS among the *T. polonicum* accessions varied from 0.1429 to 1.000, with the mean of 0.5132, indicating that higher genetic diversity existed in these *T. polonicum* accessions. The mean GS in the accessions from North America (0.6782) was the highest, followed by Europe (0.5802). The accessions from East Africa and West Asia and Center and South Asia, which were the origin center of wheat, had the higher genetic diversity, with the mean GS of 0.5473 and 0.4165, respectively. Due to the lack of enough accessions, the mean GSs among the accessions from South America and Australia were not calculated.

All the *T. polonicum* accessions could be divided into 5 groups (Fig. 2). Only one accession PI349051 from Georgia were included in Group I. Twelve accessions from Ethiopia and 1 accession from Egypt were clustered into Group II. Group III consisted of 4 accessions from Ethiopia and Group IV had 5 accessions from Portugal. The remaining 49 accessions were clustered into Group V, which included the all accessions from Hungary, England, the United States, Iraq and Australia and some accessions from European. These results suggested that most of the accessions with close geographic origins had the tendency to cluster together, indicating that the gliadin variations among *T. polonicum* accessions were associated with their geographic origins.

HWM-GS Variations

The HMW-GSs and their frequencies were identified in 72 *T. polonicum* accessions (Table 2 and Fig. 3). A total of 8 allelic variants at two *Glu-1* loci were observed. At the *Glu-A1* locus, only three HWM-GS alleles were detected and the genetic variation index (*H*) was 0.271. The frequency of *Glu-A1c* was 84.7%, indicating that *Glu-A1c* was the most frequent allele. Six accessions had the allele a (subunit 1) at the frequency of 8.3%. A novel subunit (allele *Glu-A1-I*), moving slightly slower than the subunit 5 but slightly faster than the subunit 2*, was present in five accessions (i.e., PI91808, PI191903, PI272565, PI352488 and CItrl14892) at the frequency of 7.0%.

At the *Glu-B1* locus, five alleles were observed and the *H* (0.659) was much higher than that of *Glu-A1* loci. *Glu-B1a* (subunit 7) and *Glu-B1e* (subunit 20) were the most frequent subunits with the frequencies of 40.3%, while the other two alleles (*Glu-B1b* and *Glu-B1d*) appeared at lower frequencies of 8.3 and 9.7%, respectively. Only one accession, PI19183, had the *Glu-B1g* (13+19) allele.

A total of 10 HMW-GS combinations (Table 1) were observed at the *Glu-A1* and *Glu-B1* loci. Two major genotypes (Null + 20 and Null + 7) were observed in higher frequencies with 41.67 and 30.56%, respectively. The remaining 8 patterns were observed at lower frequencies. The HWM-GS combinations among the accessions from different geographic regions were also analyzed. All the HWM-GS combinations detected in this study were also appeared in Europe. The HMW-GS

Table 2: Allelic variations and frequencies of HMW glutenin subunits and genetic diversity indices (*H*) at *Glu-1* in 72 *T. polonicum* accessions

			Frequency					Frequency	
Locus	Allele	Subunits	No.	(%)	Locus	Allele	Subunits	No.	(%)
Glu-A1	*a*	1	6	8.3	*Glu-B1*	*a*	7	29	40.3
	c	Null	61	84.7		*b*	7+8	6	8.3
	Glu-A1-I		5	7.0		*d*	6+8	7	9.7
						e	20	29	40.3
						g	13+19	1	1.4
H	0.271				*H*	0.659			

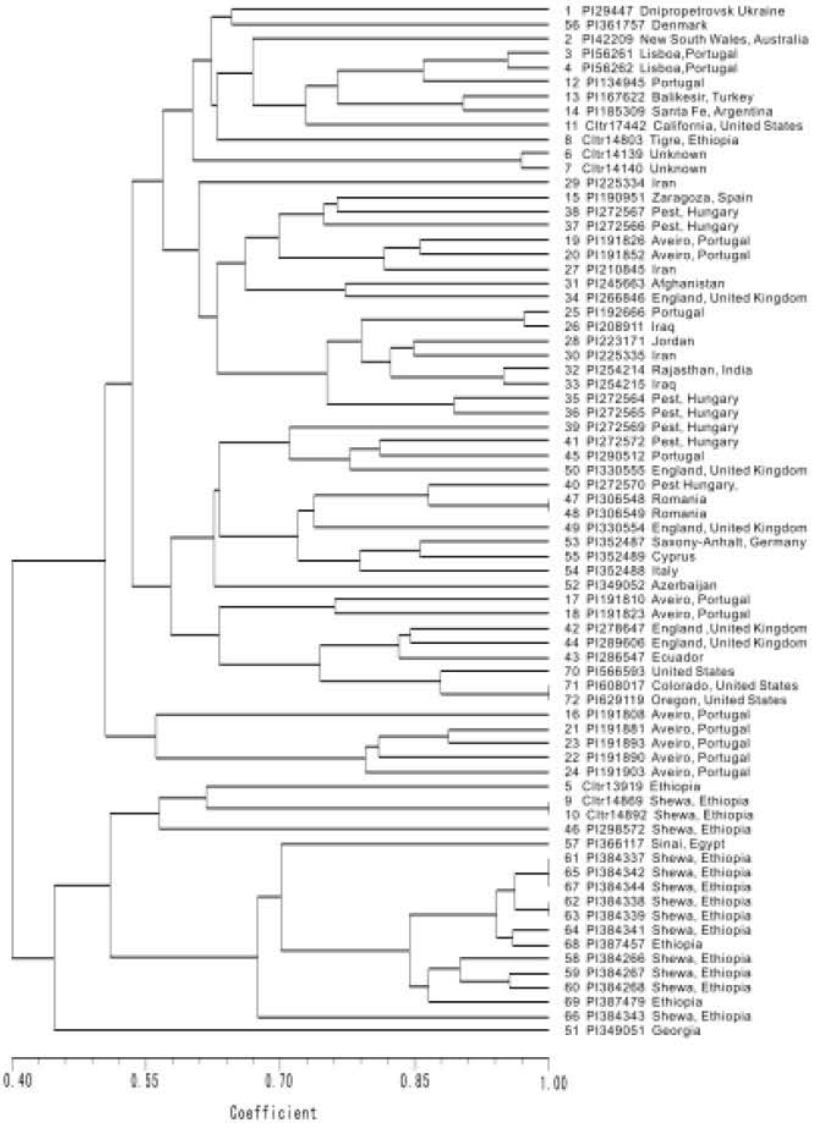

Fig. 2: Clustering results of 72 *T. polonicum* accessions based on UPGMA method

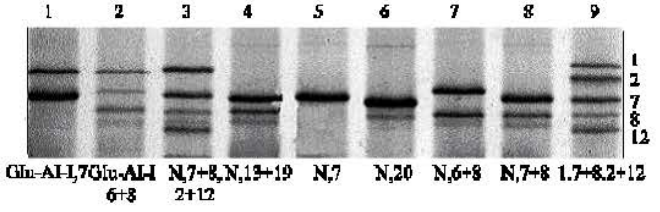

Fig. 3: HMW-GS patterns in some *T. polonicum* accessions. 1 = PI191903, 2 = PI272565, 3 = Chinese Spring (CS), 4 = PI191823, 5 = CItr14139, 6 = PI134945, 7 = CItr139919, 8 = PI191826, 9 = Chuan Yu 12

combination N + 7 was the most frequent combination with the frequency of 30.0%, followed by the combination N + 20 (24.2%). Four HMW-GS combinations were observed among 25 accessions from East Africa and west Asia and the combination N + 20 was also the most frequent combination (76%).

In Australia only one subunit combination (N, 6+8) was detected and 2 combination were observed in other three regions.

DISCUSSION

It has been proposed that gliadin bands are an easy, cheap and dependable marker for the evaluation of wheat genetic resources. Wang *et al*. (2006) reported that the gliadin patterns could reflect the genetic diversity in durum wheat. Zhang *et al*. (1995), Hou *et al*. (2004) and Lan *et al*. (1999) suggested that A-PAGE method could be used for identifying and evaluating the wheat germplasm resources. In this study, higher variations of gliadins were detected in the *T. polonicum*. A total of 65 gliadin patterns were identified in 72 *T. polonicum* accessions.

Li *et al*. (2002) found that the genetic relationships of wild emmer wheat were associated with their geographical distribution. The similar results were also obtained in *T. compactum* (Zhang *et al*., 2005a), *T. turanicum* (Xu *et al*., 2005a) and *T. durum* (Wang *et al*., 2006). In this study, also it was observed that most of the accessions with close geographic origins had the tendency to cluster together, indicating that the gliadin variations among *T. polonicum* accessions were also associated with their geographic origins. Meanwhile, cluster analysis indicated that the genetic similarity was the lowest in the West Asia and Center Asia, which were the origin center of wheat.

It was reported that the genes encoding HWM-GS were a resertively restable and dependable marker (Nevo and Payne, 1987). In present study, the subunit null, coded by *Glu-A1* locus, was the predominant subunit with the highest frequency of 84.7%, which is similar as in *T. astivum* (Zeng *et al*., 2005), *T. macha* (Xiong *et al*., 2005), *T. turanicum* (Xu *et al*., 2005b), *T. durum* (Wang *et al*., 2006) and *T. compactum* (Zhang *et al*., 2005b), whereas the predominant subunit in *T. dicoccoides* (Li *et al*., 2002), *T. turgidum* ssp. *dicoccum* (Li *et al*., 2006), *T. carthlicum* (Zhuang *et al*., 2006) and *T. spelt* (Xueli *et al*., 2005) was subunit 1 and subunit 2* was the most frequent subunit in *T. turgidum* landraces (Zhang *et al*., 2003). At *Glu-B1* loci, subunits 7+8 were the predominant subunits in *T. compactum* (Zhang *et al*., 2005b), *T. carthlicum* (Zhuang *et al*., 2006), *T. macha* (Xiong *et al*., 2005) and *T. astivum* (Zeng *et al*., 2005), whereas *T. turanicum* (Xu *et al*., 2005b) and *T. spelt* (Xueli *et al*., 2005) had the predominant subunits 13+16. In this study, 7 and 20 subunits were the most frequent subunits with the frequency of 40.3%. Therefore, the predominant subunits in different species or subspecies of wheat germplasm were not identical.

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