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Allelic variation of glutenin and gliadin genes in Iranian einkorn wheat

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Abstract

The glutenin and gliadin characteristics of wheat germplasm have a potential value and understanding the allelic distribution in glutenin and gliadin loci is very important for any wheat breeding program. In the present study, we reported the allelic status at *Glu-A3* and *Gli-A2* loci among a set of 40 accessions of *T. boeoticum* and *T. urartu* collected from different regions of Iran, based on diagnostic polymerase chain reaction assays. With respect to *Gli-A2*, 93% of the accessions carried a 210 bp allele. However, 5 and 13% of the accessions carried two new alleles with size of 490 and 700 bp, respectively. For *Glu-A3* gene, 11 allelic variants were detected using the locus-specific primers set *Glu-A3.1*, which among them nine alleles identified as new alleles. Distribution of alleles in different origins and species showed high frequency in some of alleles in accessions of *T. boeoticum* than *T. urartu* species. In general, we characterized nine new alleles for *Glu-A3* as well as two new alleles for *Gli-A2* locus which it indicated that the higher genetic variations exist in the species of Iranian Einkorn wheat. Moreover, the results revealed a remarkable potential in accessions collected from west and central of Zagros Mountains especially, parts of Kurdistan and 'Kermanshah' as well as 'Chaharmahal & Bakhtiari' and 'Kohgiluyeh & Buyer-Ahmad' provinces, which carried different alleles. Therefore, these germplasm could be used as rich gene pool to broaden the genetic base of bread wheat.

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Introduction

One of the primary needs for wheat improvement is the estimation of diversity in wild relatives of wheat for breeding purposes (Moradkhani *et al.,* 2012). The wild relatives of crop plants constitute an increasingly important genetic resource for improving agricultural production and maintaining sustainable agro-ecosystems (Maxwell *et al.,* 2009). Wild species of *Aegilops* L. and *Triticum* L. provide a useful source of new genetic variation for wheat improvement. Einkorn wheat is a diploid (2n = 2x =14, AA) and self-pollinated species. Three species comprising domesticated *T. monococcum* and wild *T. urartu* and *T. boeoticum* belong to the einkorn wheat. *T. boeoticum* and *T. urartu* are separated by crossing barriers (Johnson and Dhaliwal, 1976), differ slightly in plant morphology (Dorofeev *et al.,* 1979), biochemical and molecular marker loci (Dvorak *et al.,* 1997; Kilian *et al.,* 2007). It has been widely recognized that the diploid einkorn wheat *T. monococcum* was among the first crops domesticated in the Fertile Crescent starting from the wild progenitor *T. boeoticum* (Heun *et al.,* 1997). Although *T. urartu* was never domesticated but it has played an important role in wheat evolution, so that this species donated the A genome to all tetraploid and hexaploid wheats (Kilian *et al.,* 2007). Primary origins of Einkorn wheat situate in the central and eastern parts of the Fertile Crescent (Zohary and Hopf, 2000) Such as; Armenia, Azerbaijan, Iran, Iraq, Lebanon, Syria and Turkey (Miller, 1988).

Wheat gluten is composed of a protein complex of polymeric glutenins and monomeric gliadins, and this complex also plays an important role in determining processed food quality (Shewry *et al.,* 2003). The gliadins and glutenins are major components of the storage proteins in wheat endosperm. Glutenins mainly include two types of subunits: high molecular-weight glutenin subunits (HMW-GS) and low molecular-weight glutenin subunits (LMW-GS). The genes coding for HMW-GS (*Glu-1*) and LMW-GS (*Glu-3*) are located respectively on the long and short arms of 1A, 1B and 1D chromosomes (Kawaura *et al.,* 2005). Gliadin proteins were separated into three groups based on electrophoretic mobility: α/β-gliadin, γ-gliadin, and ω-gliadin. The α/β-gliadin are located on the *Gli-2* loci of the short arm of the homoeologous group 6 chromosomes and the γ-gliadin, and ω-gliadin are tightly linked and are located on the *Gli-1*/*Gli-3* loci of the short arm of the homoeologous group 1 chromosomes (Jackson *et al.,* 1983, Payne *et al.,* 1984; Payne, 1987; Kawaura *et al.,* 2005). Gluten subunits as genetic markers are considered as an applied tool which allows more rapid evaluation and introduction of efficient traits in new selected wheat. Promising sources of genes coding for novel gluten subunits are indicated by diploid relatives of wheat such as *T. boeoticum* and *T. urartu* (Waines and Payne, 1987; Long *et al.,* 2005). Iran is mainly distribution center of wild wheat (Kimber and Feldman 1987) and the associated compositions of *T. boeoticum* and *T. urartu* as the richest wheat gene pool has been found in this region (Fakhre-Tabatabaei and Ramak-Massoumi, 2001). The origins of wild wheat in the west of Iran (East of the Fertile Crescent) are potentially ideal areas for discover suitable genes to further transfer into cultivated wheat (van Slageren, 1994). However, it seems that little information is available about these wild relatives of wheat from different parts of Iran. Thus, the aim of this study was evaluate the *T. boeoticum* and *T. urartu* populations collected from different origins of Zagros Mountains in Iran, in terms of coding *Glu-A3.1*, *Glu-A3.2* and *Gli-A2* genes using specific molecular markers.

Materials and methods

Plant materials and DNA extraction

Thirty two accessions of *T. boeticum* along with eight accessions of *T. urartu* which located in different parts of Zagros Mountains in northwestern to southwestern Iran were selected for this study (Fig. 1). A summary of geographical distributions of these accessions are shown in Table 1. Plant materials provided by the Gene Bank of Ilam University (Ilam province is located in western Iran). For each accession, after the seed germination and growth,

genomic DNA was extracted from the leaves of greenhouse-grown plants according to the method of Piccolo *et al.* (2012) with minor modification. DNA quantity and quality was determined using NanoDrop-2000c spectrophotometer as well as 0.8% agarose gel. PCR were performed in total volumes of 15 µl, including 150 mM Tris-HCl pH 8.5, 40 mM $(NH_4)_2S0_4$, 4.0 mM of $MgCl_2$, 0.4 mM of each dNTPs, 0.05 units/µl of ampliqon Taq DNA polymerase and 2 µl of DNA. Sequences of primers synthesized according to Long *et al.* (2005) and Kawaura *et al.* (2005), and their details are shown in Table 2. PCR cycling conditions were first denaturation at 95 °C for 5 min, followed by 35 cycles consisted of denaturation at 95 °C for 40 s, annealing at 56 - 58.3 °C (varied for each primer set) for 40, extension at 72 °C for 60 s, and final extension at 72 °C for 7 min. The amplification products were separated on 1.5% agarose gels. Finally, PCR products were stained with ethidium bromide and visualized using UV light.

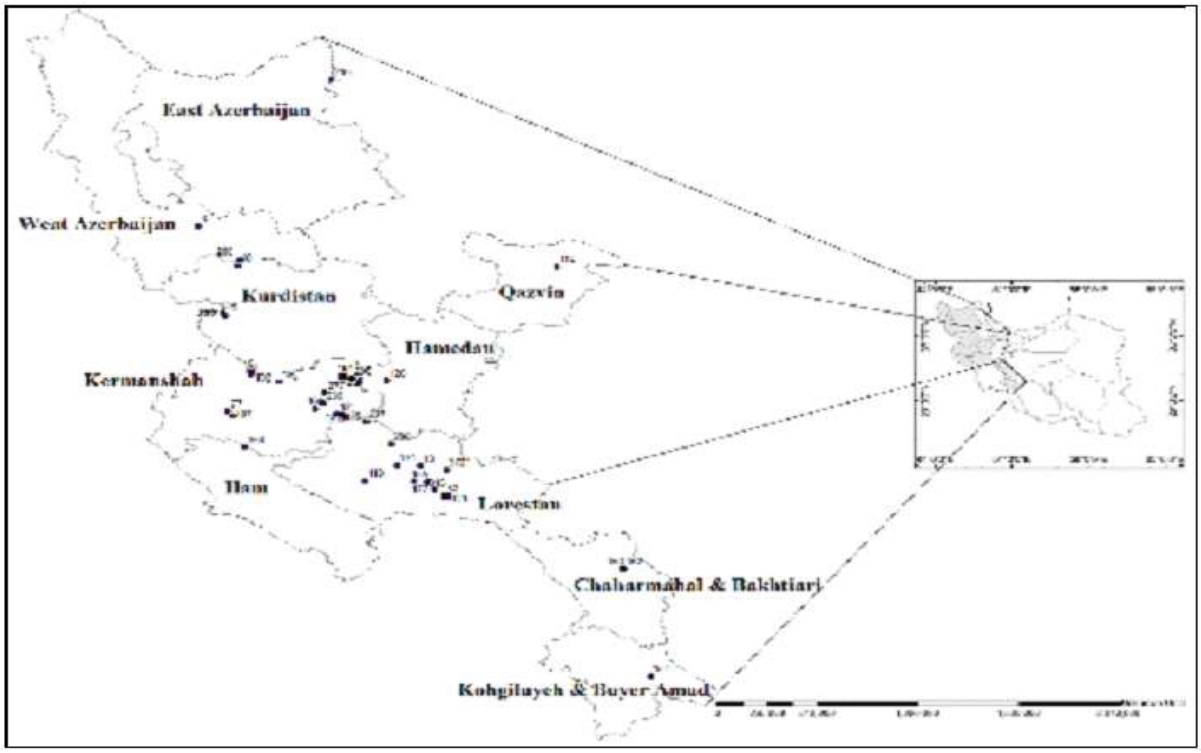

Fig. 1. Origin of *T. boeticum* and *T. urartu* populations examined in this study. The numbers correspond to the accessions in Table 1.

Results

Detection of corresponding genes

For *Glu-A3* gene 11 allelic variants were detected using the locus-specific primers set *Glu-A3.1*. The size of amplified alleles ranged from approximately 300 bp to 1000 bp, and among them 9 alleles identified as new alleles. According to *Glu-A3.2* primers set one allele was identified, and the size of amplified allele was 350 bp. Moreover, at *Gli-2A* locus three allelic variants were detected by *Gli-2A-s* primer pairs. The sizes of PCR products for these alleles were 700-bp, 500-bp, and 210-bp.

The allelic state at Glu-3A and Gli-As loci

The allelic state at *Glu-A3* and *Gli-2A* for each of the accessions is given in Table 3. At *Glu-A3.1*, five of the 40 accessions (12%) carried 330 bp allele, seven accessions (17%) carried 400 bp allele, 10 accessions (25%) carried 430 bp allele, 11 accessions (27%) carried 450 bp allele, 3 accessions (8%) carried 490 bp allele, 17 accessions (42%) carried 500 bp allele, 16 accessions (40%) carried 510 bp allele, nine accessions (23%) 550 bp allele, 15 accessions (38%) carried 600 bp allele, four accessions (10%) carried 650 bp allele, and two accessions (5%) carried the

1000 bp allele. 13 of the 40 accessions (33%) did not any PCR product with the locus-specific primer set *Glu-A3.1*. On the other hand, 11 accessions (27%) did not generate any PCR product by *Glu-A3.1* primer pairs. Amplification profile for *Glu-A3.1* for some accessions are shown in the Fig.2. At *Glu-A3.2* locus, 29 accessions (73%) only carried 210 bp allele (Fig. 3). With respect to the allelic status at *Gli-2A* locus, the only five accessions (13%) and two accessions (5%) produced 700 and 490 bp fragments when their DNA was amplified with the *Gli.As.2* primer pair diagnostic for *Gli-2A*. However, 37 of the 40 accessions (93%) carried 210 bp allele, and three accessions (8%) did not any product for this marker. Amplification profiles for *Glu3A.2* and *Gli-2A* for some accessions are presented in the Fig.4.

Table 1. The origin and site description of the 40 accessions of *Triticum boeoticum* and *Triticum urartu*.

Accession code	Geographical position in Zagros area	Province	Altitude (m)	Longitude (E)	Latitude (N)
IUGB-0003	Northwest	West Azerbaijan	1649.2	45° 45′	36° 39′
IUGB-0004	Central	Kermanshah	2267.6	46° 35′	34° 51′
IUGB-0010	Central	Kurdistan	1588.2	46° 28′	36° 19′
IUGB-0012	Central	Kermanshah	1936.0	47° 32′	34° 19′
IUGB-0015	Northwest	Kurdistan	1509.0	46° 16′	34° 27′
IUGB-0016	Central	Kermanshah	1760.4	46° 34′	35° 41′
IUGB-0018	Central	Lorestan	2431.8	48° 26′	34° 53′
IUGB-0052	Central	Lorestan	1679.4	48° 48′	34° 50′
IUGB-0077	Central	Kermanshah	1971.4	46° 19′	33° 17′
IUGB-0102	Northwest	Kurdistan	1291.8	46° 44′	33° 12′
IUGB-0113	Northwest	Qazvin	1291.8	*	*
IUGB-0114	Northwest	Qazvin	1709.2	50° 04′	34° 23′
IUGB-0118	Central	Lorestan	1573.6	47° 54′	35° 25′
IUGB-0120	Central	Lorestan	1367.2	48° 51′	36° 17′
IUGB-0125	Central	Kermanshah	1153.3	47° 37′	33° 19′
IUGB-0126	Northwest	Hamadan	1270.2	48° 08′	33° 11′
IUGB-0127	Central	Kermanshah	1851.2	46° 46′	34° 18′
IUGB-0154	Central	Kohgiluyeh & Boyer-Ahmad	1796.2	51° 11′	34° 47′
IUGB-0155	Central	Lorestan	1613.6	48° 41′	34° 45′
IUGB-0162	Central	Chaharmahal and Bakhtiari	1673.2	50° 51′	33° 53′
IUGB-0165	Central	Kermanshah	1961.8	47° 22′	33° 31′
IUGB-0171	Central	Lorestan	2249.4	48° 13′	30° 44′
IUGB-0176	Central	Lorestan	1652.0	48° 41′	33° 23′
IUGB-0177	Central	Lorestan	2105.0	48° 29′	32° 12′
IUGB-0179	Central	Kermanshah	2047.0	47° 50′	34° 44′
IUGB-0181	Central	Kermanshah	2152.8	47° 37′	32° 13′
IUGB-0206	Central	Kermanshah	1637.2	47° 52′	34° 16′
IUGB-0216	Northwest	East Azerbaijan	1706.8	47° 33′	38° 40′
IUGB-0230	Central	Lorestan	2096.0	48° 10′	33° 17′
IUGB-0257	Central	Kermanshah	1522.2	47° 33′	33° 24′
IUGB-0372	Central	Lorestan	1757.4	48° 45′	33° 55′
IUGB-0277	Central	Kermanshah	2093.8	47° 32′	34° 52′
IUGB-0285	Central	Kermanshah	1551.0	47° 25′	36° 25′
IUGB-0316	Central	Lorestan	1967.8	48° 40′	34° 48′
IUGB-0320	Central	Kermanshah	2044.6	47° 33′	34° 12′
IUGB-0368	Central	Ilam	1072.2	46° 31′	33° 47′
IUGB-0407	Central	Kermanshah	1895.0	46° 23′	34° 47′
IUGB-0484	Unknown	Unknown	*	*	*
IUGB-0200	Northwest	Kurdistan	1518.4	42° 19′	35° 37′
IUGB-0300	Northwest	Kurdistan	1692.2	46° 28′	34° 39′

*** data not available**

Distribution of alleles in different origins and species

Large differences in frequency distributions for LMW-GS subunit studied genes were found in the accessions from different origins in Zagros areas (Tables 4 and 5). In all, high frequencies of all alleles in three studied loci were present in the accessions from the central of Zagros Mountains, whereas the corresponding frequencies were much lower in the accessions collected from the north and northwest parts of Zagros Mountains. Moreover, among the 40 accessions of tested Einkorn wheat, there were some differences in allelic variation in LMW-GS. At *Glu-A3.2* and *Gli-A2* loci, the difference for the frequency distribution of 350 bp allele was not significant. However, out of 40 accessions, only two and three accessions of *T. boeoticum* and *T. urartu* carried 700

bp allele diagnostic for *Gli-2A*. On the other hand at *Glu-A3.1* locus, the frequencies of 330, 400, 430 bp alleles were higher in *T. boeoticum* accessions than in *T. urartu* accessions. The difference for the frequency distribution of 450, 500, 510 and 600 bp alleles were not significant. As a result of the comparison between two species, in *T. urartu* the highest allelic frequency belonged to 450, 500, 510 and 600 bp alleles, while in *T. boeoticum* species it was to 400, 430, 500, 510 and 600 bp alleles. Additionally, the 330, 400, 430, 490, 650 and 1000 bp alleles were only carried in the accessions of *T. boeoticum*. In order to assess the relationships among allelic status and geographical parameters such as; altitude, longitude and latitude firstly we converted "+ and -" pattern to binary codes (0 and 1). Results of correlation coefficients revealed that, altitude had significantly positive correlation with *Glu-A3.2* banding pattern. With regards to these finding, it seems that, banding pattern detected by means of *Glu-A3.2* primer pairs significantly affected by altitude above sea, because the accessions of *T. urartu* which did not produced fragment had a similar altitude.

Table 2. Specific primer pairs for study of glutenin and gliadin subunits in *T. boeticum* and *T. urartu* accessions.

Set	Chromosome	Marker	Sequence	Product size (bp)	Reference
Glu-3A.1	1AS	F	GCCGTTGCGCAAATTTCACAG	450 and 600	Long *et al.* 2005
		R	AACAGATGGATGAATAACTGGTAT		
Glu-3A.2	1AS	F	AGTGCCATTGCGCAGATGAAT	350	Long *et al.* 2005
		R	AACGGATGGTTGAACAATAGA		
Gli-As.2	6A	F	CAACGACCAAACCATGGACTAAGAGC	300	Kawaura *et al.* 2005
		R	GCCCAGGGCTTTGTCCAACC		

Table 3. The allelic state at *Glu-A3* and *Gli-2A* for each of the accessions studied in this experiment.

	Glu-3A.1 alleles											*Glu-3A.2* allele	*Gli-As.2* alleles		
Accession code	330	400	430	450	490	500	510	550	600	650	1000	**210**	700	490	210
IUGB-0003	-	-	+	-	-	-	+	-	+	-	-	+	-	-	+
IUGB-0004	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
IUGB-0010	-	-	-	+	-	+	-	+	+	-	-	-	-	-	+
IUGB-0012	+	-	-	+	+	+	-	+	+	-	-	+	-	-	+
IUGB-0015	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+
IUGB-0016	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
IUGB-0018	-	-	-	-	-	+	-	+	-	-	-	+	-	-	+
IUGB-0052	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+
IUGB-0077	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-
IUGB-0102	-	-	+	+	-	-	-	+	-	-	-	-	-	-	+
IUGB-0113	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
IUGB-0114	-	-	+	+	-	+	-	-	+	-	-	+	-	-	+
IUGB-0118	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+
IUGB-0120	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
IUGB-0125	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
IUGB-0126	-	-	+	-	-	-	+	-	+	-	-	+	+	-	+
IUGB-0127	+	-	+	-	-	-	-	-	-	-	-	+	-	-	+
IUGB-0154	-	-	+	-	-	-	+	-	+	+	-	+	+	+	+
IUGB-0155	-	-	+	-	-	-	+	-	+	-	-	+	+	+	+
IUGB-0162	-	-	-	+	-	+	+	-	+	+	-	+	+	-	+
IUGB-0165	-	-	-	-	-	-	+	-	-	-	-	+	+	-	+

IUGB-0171	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+
IUGB-0176	-	+	-	-	-	-	+	-	-	-	-	+	-	-	+
IUGB-0177	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+
IUGB-0179	-	-	-	-	-	+	-	+	-	-	-	-	-	-	+
IUGB-0181	-	-	+	-	-	-	-	-	-	-	-	+	-	-	+
IUGB-0206	-	-	-	+	-	+	-	+	-	-	-	-	-	-	+
IUGB-0216	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
IUGB-0230	+	-	+	-	-	+	-	-	-	-	-	+	-	-	-
IUGB-0257	-	+	-	-	-	+	-	-	+	-	+	+	-	-	+
IUGB-0372	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
IUGB-0277	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+
IUGB-0285	-	-	-	+	-	+	-	-	+	-	-	-	-	-	+
IUGB-0316	-	-	-	+	-	-	+	-	+	+	-	+	-	-	+
IUGB-0320	-	-	+	-	-	-	+	-	-	+	+	+	-	-	+
IUGB-0368	+	+	-	-	-	+	+	-	-	-	-	-	-	-	-
IUGB-0407	-	+	-	-	-	+	+	-	+	-	-	+	-	-	+
IUGB-0484	-	+	-	+	+	+	-	-	+	-	-	+	-	-	+
IUGB-0200	-	+	-	-	+	+	-	+	-	-	-	+	-	-	+
IUGB-0300	-	+	-	-	-	+	+	-	+	-	-	-	-	-	+

Discussion

Wheat endosperm storage proteins are the major components of gluten, and they play a key role in dough properties and in bread making quality in various wheat cultivars (Branlard *et al.,* 2003). On the other hand, the LMW-GS and HMW-GS proteins along with gliadin are important parts of the gluten complex, and they are encoded by a highly variable gene family.

Table 4. Allelic frequencies for glutenin and gliadin subunits in *T. boeticum* and *T. urartu* accessions.

Locus	Allele	Frequency	%	Fragment size
Glu-3A.1	*1*	5	12.5	330
	2	7	17.5	400
	3	10	25.0	430
	4	11	27.5	450
	5	3	7.5	490
	6	17	42.5	500
	7	16	40.0	510
	8	9	22.5	550
	9	15	37.5	600
	10	4	10.0	650
	11	2	5.0	1000
Glu-3A.2	*1*	29	72.5	350
Gli-As.2	*1*	5	12.5	700
	2	2	5.0	500
	3	37	92.5	210

The presence of glutenin and gliadin genes can be monitored directly at every stage of wheat growth. In this regard, the various LMW-GS and HMW-GS as well as gliadin genes were detected with respect to their effect on quality and other aspects of bread making (Metakovsky *et al.,* 1991; Zhao-cai *et al.,* 2006; Wang *et al.,* 2010; Sharma *et al.,* 2013).

Table 5. Allelic frequencies for glutenin and gliadin subunits in *T. boeticum* and *T. urartu* accessions from central and northwest of Zagros Mountains.

Locus	Allele	Northwest (n = 9)	Central (n = 31)	*T. boeticum* (n = 32)	*T. urartu* (n = 8)
Glu-3A.1	*a*	0	5	5	0
	b	2	5	7	0
	c	4	6	10	0
	d	3	8	6	5
	e	1	2	3	0
	f	3	14	12	5
	g	4	12	10	6
	h	3	6	6	3
	i	4	11	10	5
	j	0	4	4	0
	k	0	2	2	0
Glu-3A.2	*a*	5	24	26	4
Gli-As.2	*a*	1	4	2	3
	b	0	2	1	1
	c	9	28	29	7

Table 6. Simple correlation coefficients among banding patterns and eco-geographical parameters.

		Altitude	Longitude	Latitude
Altitude		1	-0.16	-0.14
Longitude		-0.16	1	0.19
Latitude		-0.14	0.19	1
Glu-3A.1 alleles	*1*	-0.05	-0.04	0.14
	2	-0.25	-0.03	-0.01
	3	0.01	-0.12	-0.09
	4	-0.11	0.06	0.17
	5	-0.01	0.09	-0.05
	6	0.01	0.08	0.001
	7	0.03	-0.27	-0.22
	8	0.04	0.05	-0.08
	9	-0.10	0.16	-0.005
	10	0.13	0.006	-0.05
	11	0.02	-0.09	0.24
Glu3A.2 allele	*1*	0.32*	-0.31	-0.29
Gli2A-s alleles	*1*	-0.10	-0.11	-0.17
	2	-0.03	-0.06	-0.16
	3	0.03	0.19	0.05

*; significant at 0.05 probability level.

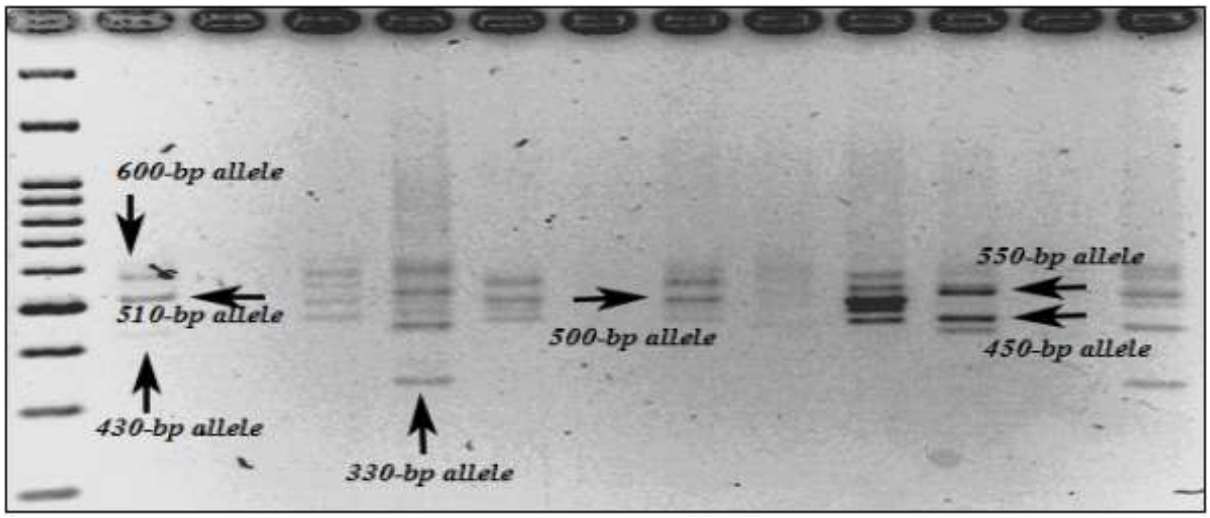

Fig. 2. The amplified products (Alleles) for *T. boeoticum* and *T. urartu* by *Glu-A3.1* specific primer pairs.

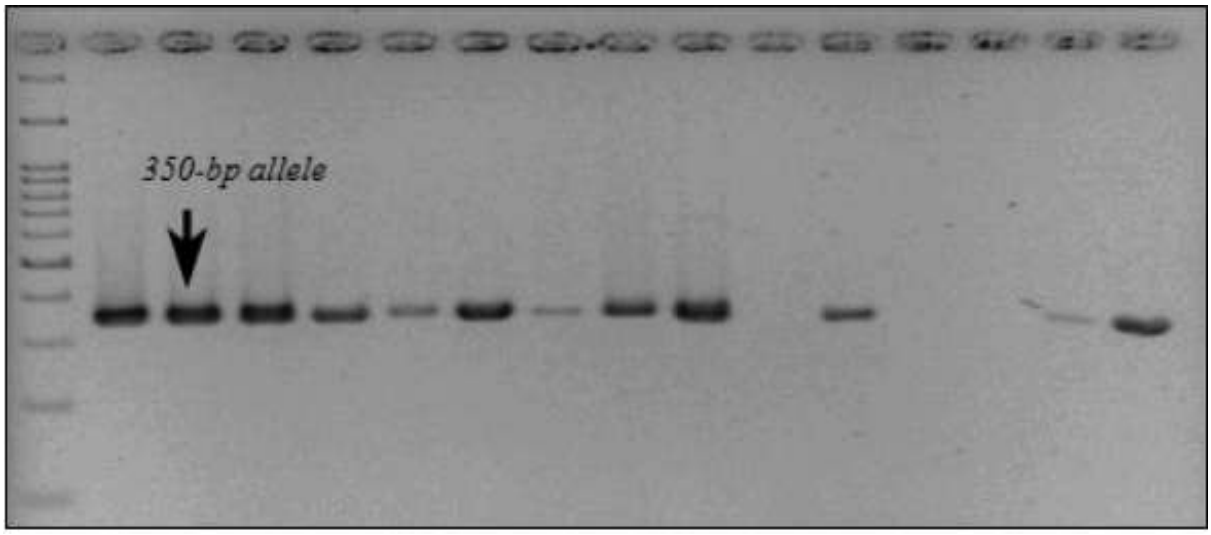

Fig. 3. The amplified products (Alleles) for *T. boeoticum* and *T. urartu* by *Glu-A3.2* specific primer pairs.

The acquisition of the DNA sequence of these important genes now permits their clear monitoring by marker technology. This study screened the mini core-collection of Iranian Einkorn wheat germplasm collected from different parts of Zagros mountains using the locus-specific primers (*Glu-A3.1*, *Glu-A3.2* and *Gli-A2*) developed by Long *et al.* (2005) and Kawaura *et al.* (2005). Results suggest that these specific primer pairs can be used to identify the majority of species reliably, so that they allowed us to confirm that each *Glu-A3* and *Gli-A2* alleles was detected in the core collections. In this collection, 72% of the accessions (29 entries) carried *Glu-A3.2* allele and 92% of the accessions (37 entries) carried *Gli-A2* allele detected by Long *et al.* (2005) and Kawaura *et al.* (2005). At *Glu-A3*, 27% (11 entries) and 37% (15 entries) of accessions respectively carried 450 and 600 bp alleles detected by Long *et al.* (2005). However, in this study, at *Glu-A3* and *Gli-A2* loci, 9 and 2 new alleles were detected using *Glu-A3.1* and *Gli-A2*-s primer pairs, respectively. Previously, Zhang *et al.* (2004) detected one gene, including seven allelic forms cloned from different varieties of wheat, and then developed seven PCR markers to discriminate the protein alleles *Glu-A3a*, *b*, *c*, *d*, *e*, *f* and *g*. Wang *et al.* (2010) characterized one *Glu-3A* gene with seven allelic variants, and developed a number of STS markers for the discrimination of the *Glu-3A* alleles. In the present study we also characterized nine new alleles for the *Glu-3A* which

based on their size product were dissimilar to alleles identified by Wang *et al.* (2010). As shown in Fig. 4, at the *Gli-A2* locus, we also identified two new alleles with size of 700 and 490 bp. In accordance to the present study, Gu *et al.* (2004) and Kawaura *et al.* (2005) reported a high allelic variation for gliadin genes in wheat germplasm. Zhao-cai *et al.* (2006) indicated that, among the Einkorn wheat, *T. urartu* and *T. boeoticum* species have a high level of allelic variation in *Gli-A1* and *Gli-A2* loci. Furthermore, there still existed several gliadin genes couldn't be detected in A-genome ancestors wheat.

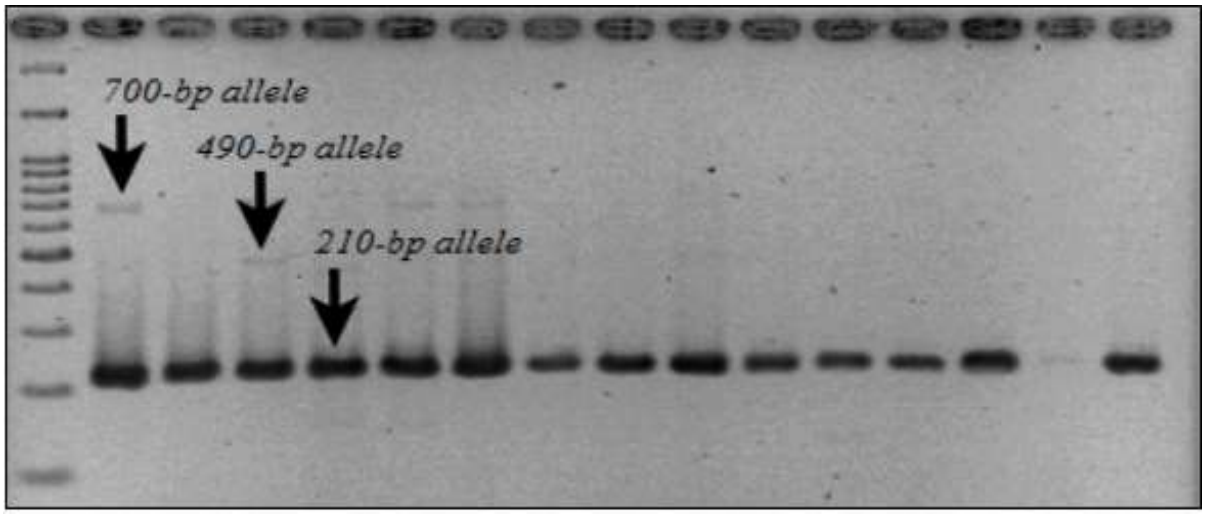

Fig. 4. The amplified products (Alleles) for *T. boeoticum* and *T. urartu* by *Gli-2A* specific primer pairs.

Thus it seems that, there might exist some new gliadin alleles in these accessions and this need to be further study (Metakovsky *et al.,* 1991; Zhao-cai *et al.,* 2006). Allelic status comparison showed that, unlike accessions of *T. urartu*, some accessions of *T. boeoticum* carried many types of alleles. Interestingly, many accessions of *T. urartu* only carried some of alleles. On the other hand, the results of correlation coefficients between geographical parameters with banding patterns showed that, the 350bp allele (detected by *Glu-A3.2* primers pair) was associated with altitude. Regarding to this result and allelic status comparisons, it seems that accessions of *T. urartu* more than *T. boeoticum* are affected by this relation. We do not know the reason (s) for allelic status found within examined accessions of *T. urartu* compared with those of other accessions in this study. Such variation might be due to higher levels of adaptation in the collection sites of these accessions or to higher rates of mutation. The genetic diversity in Einkorn wheat has been estimated and very high level of genetic diversity within populations of *T. boeoticum* and *T. urartu* collected from different areas of Iran had been described by protein, isozymes and DNA markers (Moghaddam *et al.,* 2000; Naghavi *et al.,* 2008, Sofalian *et al.,* 2009, Naghavi *et al.,* 2009, Ahmadi and Pour-Aboughadareh, 2015). Allelic status in breeding materials might be monitored by means of DNA markers. Therefore, the glutenin and gliadin patterns of Einkorn wheat could be used as helpful markers in selecting breeding parents from Einkorn wheat germplasm collection. In the present study, we characterized nine new alleles for *Glu-A3* as well as two new alleles for *Gli-A2*, which it indicated that the higher genetic variations exist in the species of Einkorn wheat from Iran. Moreover, the results revealed a remarkable potential in accessions collected from west and central of Zagros Mountains especially, parts of Kurdistan and Kermanshah provinces as well as capability accessions of *T. urartu* collected from regions of Chaharmahal & Bakhtiari and Kohgiluyeh & Buyer Ahmad provinces, which carried different alleles types. Therefore, these germplasm could be used as good gene pool to broaden the genetic base of bread wheat.

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