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Molecular Detection of Glutenin and Gliadin Genes in the Domesticated and Wild Relatives of Wheat using Allele-specific Markers

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Glutenin and gliadin subunits play a key role in flour processing quality by network formation in dough. Wild relatives of crops have served as a pool of genetic variation for decades. In this study, 180 accessions from 12 domesticated and wild relatives of wheat were characterized for the glutenin and gliadin genes with allele-specific molecular markers. A total of 24 alleles were detected for the *Glu-A3* and *Gli-2A* loci, which out of 19 amplified products identified as new alleles. Analysis of molecular variance (AMOVA) indicated that 90 and 65% of the genetic diversity were partitioned within two *Aegilops* and *Triticum* genera and their species, respectively. Furthermore, all glutenin and gliadin analyzed loci were polymorphic, indicating large genetic diversity within and between the wild species. Our results revealed that allelic variation of *Glu-3A* and *Gli-As.2* is linked to genomic constitutions so that, *Ae. caudata* (C genome), *Ae. neglecta* (UM genome), *Ae. umbellulata* (U genome) and *T. urartu* (A^u genome) harbor wide variation in the studied subunits. Hence, these species can be used in wheat quality breeding programs.

Keywords: *Aegilops*, *Triticum*, glutenin, gliadin, allelic diversity

Introduction

The presence of gliadins and glutenins are important factors in determining the viscoelastic and dough strength properties, which are responsible for bread-making qualities of wheat (Jin et al. 2011). Storage proteins in cereal grain comprise of two major types. In the first type, glutenin a polymer consisting of high-molecular-weight (HMW-GS) and low-molecular-weight subunits (LMW-GS), accounting for 60% of the storage protein in wheat endosperm. HMW-GS are coded by loci, *Glu-A1*, *Glu-B1*, and *Glu-D1*, on the long arms of chromosomes *1A*, *1B*, and *1D*, respectively, while LMW-GS are encoded using *Glu-A3*, *Glu-B3*, and *Glu-D3*, on the short arms of the same chromosomes (Jin et al. 2011). In the second type, gliadin made up of monomeric subunits (Payne 1987). The gliadin loci control the synthesis of a group of proteins, which they further were separated into three types: α/β-gliadin, γ-gliadin and ω-gliadin. The α/β-gliadin subunits are located

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on the *Gli-2* loci of the short arm of the homoeologous group 6 chromosomes, while γ-gliadin, and ω-gliadin are located on the *Gli-1/Gli-3* loci of the short arm of the homoeologous group 1 chromosomes (Payne et al. 1984). Allelic variations of these protein compositions can result in functional differences, such as amino-acid substitutions and Indels (Ma et al. 2006), which may have an effect on dough quality.

Additionally to the variation found in improved modern lines, the search of new variation in wild relatives and alien species has great importance in expanding the wheat germplasm. Wild relatives are species that are closely related to crop plants, which can contribute useful traits such as biotic and abiotic resistance, as well as protein quality (Pour-Aboughadareh et al. 2017b, c). These species are of interest to plant breeders, and major efforts have been made to transfer their genetic variation into domesticated genotypes. The genera *Aegilops* and *Triticum* – belonging to the tribe *Triticeae* within the *Pooideae* subfamily of the grass family *Poaceae* – are important in wheat germplasm due to their evolutionary relationship with the major agricultural crop *T. aestivum* L (Pour-Aboughadareh et al. 2017a). Kimber and Feldman (1987) and van Slageren (1994) described 22 *Aegilops* and five *Triticum* species, which have a wide repertoire of key alleles that can be used in wheat improvement programs. Previously, the analysis of storage proteins of the wild einkorn wheats by specific molecular markers and sodium-dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) indicated the high levels of polymorphism (Caballero et al. 2008; Ahmadi and Pour-Aboughadareh 2015), which suggest these species could be sources of new alleles of the glutenin and gliadin genes. Many studies have been performed on the gliadin and glutenin variation of different *Aegilops* and *Triticum* species, e.g., *Ae. cylindrica* (Wan et al. 2002), *Ae. longissima* (Jiang et al. 2008), *Ae. tauschii* (Xu et al. 2010), *T. urartu* (Caballero et al. 2008; Ahmadi and Pour-Aboughadareh 2015; Cuesta et al. 2017), *T. monococcum* (An et al. 2006) and *T. boeoticum* (Ahmadi and Pour-Aboughadareh 2015). Despite prior studies showing that the wild relatives of wheat had the large variation in glutenin and gliadin subunits, no work has been undertaken to evaluate the whole samples of wild wheat species. With this in mind, the main goal of the current work was to charactererise the allelic variation of glutenin and gliadin in 180 accessions of *Aegilops* and *Triticum* collected from different regions of Iran using specific molecular markers. The results will benefit wheat quality improvement through marker-assisted selection.

Materials and Methods

Plant Materials

In total, 180 *Aegilops* and *Triticum* accessions from 12 domesticated and wild species were used for the characterization of glutenin and gliadin genes. They belonged to *Ae. cylindrical* Host., *Ae. neglecta* L., *Ae. crassa* Boiss., *Ae. caudata* L., *Ae. speltoides* Tausch., *Ae. triuncialis*, *Ae. tauschii* Coss. and *Ae. umbellulata* Zhuk.,*T. boeoticum* Boiss., *T. urartu* Gandilyan., *T. durum* Desf. and *T. aestivum* L., which collected from natural habitats, deserts, valleys and parts from the Zagros and central Elburz mountains

located in the wide range from the north, northeast, northwest to southwest and central area of Iran. Detailed information about species' genomic constitution and eco-geographical distribution of these materials is listed in Table S1*.

DNA Extraction and PCR Amplification

From each accession, the total genomic DNA was isolated according to the CTAB protocol (Doyle and Doyle 1987). The glutenin and gliadin specific primers used in this study were synthesized according to Long et al. (2005) and Kawaura et al. (2005), and their details are shown in Table S2. The PCR reaction was carried out in 15 µL reaction mixtures contained 7.5 µL master mix (2X), 4.5 µL ddH_2O, 2 µL template DNA and 0.5 µL of each forward/reverse primer pairs. Amplification was run at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 40 seconds, annealing at 53–58.3 °C (varied for each primer) for 45 seconds and elongation at 72 °C for 90 seconds. The final extension was 7 min at 72 °C. Amplified products were visualized on a 2% agarose gel, stained with safe view II and photographed under UV light.

Data Analysis

The alleles scored as 1 and 0 on the basis of presence and absence of each allele, respectively. The distribution of genetic variation within and among accessions and species (AMOVA),the percentage of polymorphic loci (*PPL*), Nei's genetic diversity (*H*) and Shannon's information (*I*) indices was estimated using GenAlEX software version 6.5 (Peakall and Smouse 2006).

Results

Frequency distributions of allelic variants in tested individuals and allelic diversity analysis

Large differences in frequencies of detected alleles for glutenin and gliadin genes were observed in the tested accessions (Table 1). A total of 24 alleles were detected for the *Glu-A3* and *Gli-2A,* which out of 19 amplified products identified as new alleles (Fig. 1). At *Glu-3A* locus, using the specific-locus marker *Glu-3A.1*, 43 (23.9%), 24 (13.3%), 21 (11.7%) and 20 (11.1%) accessions produced 400, 450, 500 and 600-bp alleles, respectively. Based on *Glu-3A.2* marker, 9 (5%), 2 (1.1%), 3 (1.7%), 1 (0.6%), 128 (71.1%), 2 (1.1%), 2 (1.1%) and 1 (0.6%) accessions carried 150, 200, 250, 300, 350, 450, 500 and 800-bp alleles, respectively. Using *Glu-3A.3*, 3 (1.7%), 1 (0.6%), 20 (11.1%), 10 (5.6%), 111 (61.7%) and 3 (1.7%) individuals generated six alleles with the fragment size of 150, 250, 400, 600 and 680-bp, respectively. On the other hand, the frequency of the new alleles for *Gli-As.2* locus was large. In the 180 tested accessions, 148 (82.2%) individuals expressed an allele with size of 210-bp. The frequency of other amplified fragments by

*Further details about the Electronic Supplementary Material (ESM) can be found at the end of the article.

Table 1. Frequencies of alleles for glutenin and gliadin genes in 180 wild relatives of wheat

Marker	Size	Allele	*Aegilops*									*Triticum*					Total individuals	
			caudata (n = 7)	*crassa* (n = 14)	*cylindrica* (n = 19)	*neglecta* (n = 11)	*speltoides* (n = 6)	*tauschii* (n = 20)	*triuncialis* (n = 15)	*umbellulata* (n = 17)	Total	*aestivum* (n = 19)	*boeoticum* (n = 17)	*durum* (n = 18)	*urartu* (n= 17)	Total	No.	Frequency (%)
Glu-3A.1	400	*a-new*	0.0	0.0	0.0	90.9	0.0	0.0	66.7	41.2	24.8	0.0	41.2	0.0	52.9	22.5	43	23.9
	450	*b*	0.0	0.0	5.3	0.0	0.0	5.0	0.0	0.0	1.8	21.1	0.0	100.0	0.0	31.0	24	13.3
	500	*c-new*	71.4	14.3	0.0	0.0	0.0	0.0	0.0	0.0	6.4	0.0	41.2	0.0	41.2	19.7	21	11.7
	600	*d*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.5	0.0	100.0	0.0	28.2	20	11.1
Glu-3A.2	150	*a-new*	0.0	21.4	0.0	9.1	0.0	5.0	0.0	17.6	8.3	0.0	0.0	0.0	0.0	0.0	9	5.0
	200	*b-new*	0.0	0.0	0.0	0.0	0.0	0.0	13.3	0.0	1.8	0.0	0.0	0.0	0.0	0.0	2	1.1
	250	*c-new*	14.3	0.0	5.3	0.0	0.0	5.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	3	1.7
	300	*d-new*	0.0	0.0	5.3	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	1	0.6
	350	*e*	0.0	50.0	89.5	72.7	50.0	45.0	80.0	64.7	61.5	78.9	100.0	94.4	82.4	85.9	128	71.1
	450	*f-new*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.8	1.8	0.0	0.0	0.0	0.0	0.0	2	1.1
	500	*g-new*	0.0	0.0	0.0	0.0	0.0	10.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	2	1.1
	800	*h-new*	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	1	0.6
Glu-3A.3	150	*a-new*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.9	0.9	0.0	5.9	0.0	5.9	2.8	3	1.7
	250	*b-new*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.9	1.4	1	0.6
	400	*c-new*	71.4	0.0	0.0	0.0	0.0	0.0	0.0	64.7	14.7	0.0	5.9	0.0	17.6	5.6	20	11.1
	600	*d-new*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	58.8	14.1	10	5.6
	680	*e*	0.0	92.9	84.2	27.3	33.3	60.0	73.3	0.0	52.3	84.2	82.4	88.9	58.8	76.1	111	61.7
Gli-As.2	210	*a*	71.4	85.7	84.2	81.8	83.3	80.0	73.3	76.5	79.8	94.7	64.7	94.4	88.2	85.9	148	82.2
	350	*b-new*	14.3	7.1	0.0	9.1	0.0	0.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0	0.0	3	1.7
	450	*c-new*	0.0	0.0	0.0	45.5	33.3	0.0	0.0	5.9	7.3	0.0	0.0	0.0	0.0	0.0	8	4.4
	500	*d-new*	14.3	35.7	0.0	0.0	0.0	5.0	6.7	0.0	7.3	5.3	0.0	0.0	0.0	1.4	9	5.0
	600	*e-new*	14.3	42.9	0.0	54.5	0.0	10.0	0.0	0.0	14.7	0.0	0.0	0.0	0.0	0.0	16	8.9
	700	*f-new*	0.0	0.0	15.8	45.5	50.0	0.0	0.0	0.0	10.1	15.8	0.0	0.0	0.0	4.2	14	7.8

this marker was small, so that 3 (1.7%), 8 (4.4%), 9 (5%), 16 (8.9%), 14 (7.8%) and 4 (2.2%) individuals generated fragments with 350, 450, 500, 600, 700 and 800-bp, respectively. In general, our results revealed that frequencies of new alleles in *Aegilops* are more than *Triticum* genus (Table 1). The result of analysis of molecular variance (AMOVA) showed that more than 90 and 65% of the genetic diversity was partitioned within two genera and different species, while the variation between them were 10 and 35%

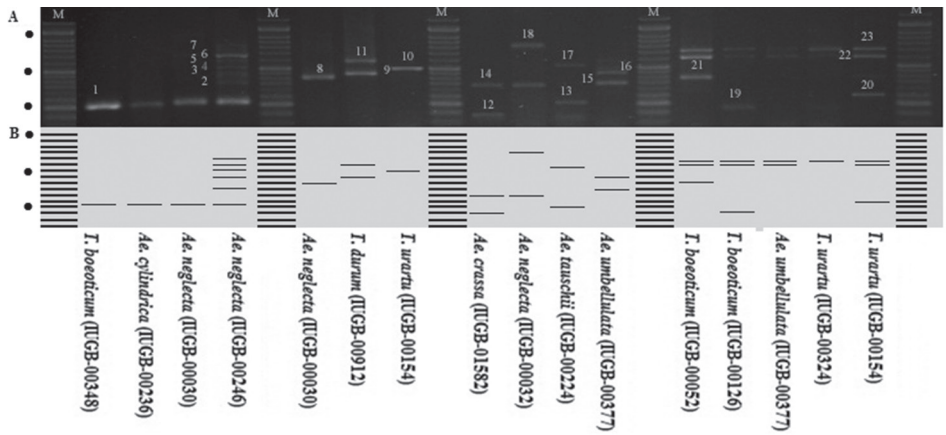

Figure 1. Panel A: image of agarose gel electrophoretic separation of the PCR products. Panel B: simulated electrophoretic image of the PCR products after normalization and thresholding. Each lane corresponds to different new alleles found. The molecular marker is a 50 bp Plus DNA ladder. In Panel B, dots label the 200, 500 and 1200 bp bands with stronger appearance in Panel A. M, ladder; 1, *Gli-As.2a*; 2, *Gli-As.2b*; 3, *Gli-As.2c*; 4, *Gli-As.2d*; 5, *Gli-As.2e*; 6, *Gli-As.2f*; 7, *Gli-As.2g*; 8, *Glu-3A.1a*; 9, *Glu-3A.1b*; 10, *Glu-3A.1c*; 11, *Glu-3A.1d*; 12, *Glu-3A.2a*; 13, *Glu-3A.1b*; 14, *Glu-3A.1d*; 15,*Glu-3A.1e*; 16,*Glu-3A.1f*; 17, *Glu-3A.1g*; 18, *Glu-3A.1h*; 19, *Glu-3A.2a*; 20,*Glu-3A.2b*; 21, *Glu-3A.2c*; 22, *Glu-3A.2d*; 23, *Glu-3A.2e*. For explanation of IUGB codes, see Table S1

Table 2. Analysis of molecular variance (AMOVA) in the wild relatives of wheat based on glutenin and gliadin markers

Source	*df*	Sum of squares	Mean squares	Estimated variance components	Total variance (%)
Genera					
Among populations	1	16.10	16.10	0.17	10
Within populations	178	280.72	1.57	1.57	90
PhiPT = 0.097, *P* = 0.010					
Species					
Among populations	11	110.95	10.08	0.60	35
Within populations	168	185.87	1.11	1.11	65
PhiPT = 0.353, *P* = 0.010					

(Table 2). Also, the highest level of diversity and percentage of polymorphism loci (*PPL*) was observed in *Aegilops* than *Triticum* genus (87.50 *vs.* 58.33%) (Table S3). Of the 12 different species, the highest values of *PPL*, *H* and *I* indices was estimated for *Ae. neglecta* followed by *T. urartu*, *Ae. crassa*, *Ae. caudata* and *Ae. umbellulata*.

Distributions of detected alleles in the wild relatives of wheat

Table 1 also presents the frequency of discovered alleles at loci analyzed in the domesticated and wild species of wheat. Additionally, allelic patterns observed by each accession are given in Table S4. As shown in Table 1, among the different species, only *Ae. neglecta* (90.9%), *Ae. triuncialis* (66.7%), *T. urartu* (52.9%), *T. boeoticum* (41.2%) and *Ae. triuncialis* (41.2%) amplified the *Glu-3A.1a* allele. All accessions of *T. durum* (100%) along with some individuals of *T. aestivum* (21.1%), *Ae. cylindrica* (5.3%) and *Ae. tauschii* (5%) possessed the *Glu-3A.1b* allele. *T. boeoticum* (41.2%), *T. urartu* (41.2%) *Ae. caudata* (71.4%) and *Ae. crassa* (14.3) amplified the *Glu-3A.1c*. Moreover, only *T. durum* and some of individuals of *T. aestivum* presented amplification for *Glu-3A.1d* allele.

Ae. umbellulata, *T. urartu*, *Ae. cylindrica*, *T. boeoticum* and *Ae. crassa* carried the *Glu-3A.1c* allele by frequencies of 64.7, 35.3, 21.1, 11.8 and 7.1%, respectively.

The frequency of *Glu-3A.1d* was high in *Ae. triuncialis* (100%) and among other species only *Ae. neglecta* (18.2%), *Ae. crassa* (14.3%), *Ae. tauschii* (10%) and *T. boeoticum* (5.9%) presented amplification for this allele. Although the *Glu3A-1e* had the high frequency among some species, *Ae. caudata*, *Ae. crassa*, *Ae. speltoides* and *T. boeoticum* did not any amplify fragment for this allele. However, *Ae. crassa* and *T. boeoticum* along with *Ae. triuncialis* and *Ae. tauschii* produced the *Glu-3A.1h* allele (7.1, 11.8, 100 and 10%, respectively).

According to allelic patterns revealed by *Glu-3A.2* marker, some accessions belong to *Ae. crassa* (21.4%), *Ae. umbellulata* (17.6%), *Ae. neglecta* (9.1%) and *Ae. tauschii* (5%) amplified the *Glu-3A.2a* allele with 150-bp. *Glu-3A.2b* allele only presented in some accessions of *Ae. triuncialis* (13.3%). The *Glu-3A.2c* allele also presented in some of *Aegilops* species such as *Ae. caudata*, *Ae. cylindrica* and *Ae. tauschii* with frequencies of 14.3, 5.3 and 5%, respectively. The *Glu-3A.2d*, as a new allele, only was found in some of individuals of *Ae. cylindrica* (5.3%). On the other hand, all species except *Ae. caudata* possessed the *Glu-3A.2e* allele and the highest frequency of it observed in *T. boeoticum*, *T. durum*, *T. urartu* and *T. aestivum* (100, 94.4, 82.4 and 78.9%, respectively). Frequencies of *Glu-3A.2f*, *Glu-3A.2g* and *Glu-3A.2h* alleles were low and only *Ae. umbellulata* (11.8%), *Ae. tauschii* (10%) and *Ae. neglecta* (0.9%) carried these alleles, respectively. Allelic frequencies revealed by *Glu-3A.3* marker were similar to other glutenin markers. The highest frequency of *Glu-3A.3a* allele was found for *Ae. tauschii*, *Ae. speltoides*, *Ae. neglecta* and *Ae. cylindrica* (65, 16.7, 9.1 and 5.3%, respectively). This allele was not observed in other species. The *Glu-3A.3a* allele was observed in *T. urartu*, *T. boeoticum* and *Ae. umbellulata* with frequencies of 5.9% for each species. The *Glu-3A.3b* allele only observed in some *T. urartu* accessions (5.9%). Among different species, *Ae. caudata* fol-

lowed by *Ae. umbellulata*, *T. urartu* and *T. boeoticum* carried the *Glu-3A.3c* with frequencies of 71.4, 64.7, 17.6 and 5.9%, respectively. Moreover, the *Glu-3A.3d* allele only was found in *T. urartu* with frequencies of 58.8%. The *Glu-3A.3e* allele widely presented in all of the species (except *Ae. umbellulata*), and the highest frequencies was detected for *Ae. crassa* (92.9%), *T. durum* (88.9%), *Ae. cylindrica* (84.2%), *T. aestivum* (84.2%), respectively.

At the *Gli-As* locus, *Gli-As.2a* allele presented in all of species with high frequencies, while the *Gli-As.2b* allele only presented in *Ae. caudata*, *Ae. crassa* and *Ae. neglecta*. The *Gli-As.2c* allele mainly detected in some accessions of *Ae. neglecta*, *Ae. speltoides* (as the putative donor of B genome) and *Ae. umbellulata* with frequencies of 45.5, 33.3 and 5.9%, respectively. Also, *Ae. caudata* (14.3%), *Ae. crassa* (35.7%), *Ae. tauschii* (5%), *Ae. triuncialis* (6.7%) and *T. aestivum* (5.3%) possessed the *Gli-As.2d*. The *Gli-As.2e* only detected in *Ae. neglecta*, *Ae. crassa*, *Ae. caudata* and *Ae. tauschii* (with frequencies of 54.5, 42.9, 14.3 and 10%, respectively). The *Gli-As.2f* allele mainly amplified in some of accessions of *Ae. speltoides* (50%), *Ae. neglecta* (45.5%), *Ae. cylindrica* (15.8%) and *T. aestivum* (15.8%). Furthermore, *Gli.As.2g* as the rare allele was only found in few accessions of *Ae. caudata* (14.3%), *T. aestivum* (10.5%) and *Ae. neglecta* (9.1%).

Discussion

Glutenin and gliadin subunits play a crucial role in flour processing quality by network formation in dough. Due to this character, they allow wheat flour to be processed into bread, paste, noodles and other food products (Ma et al. 2006). Wild relatives and progenitors of wheat are of interest to plant breeders, and major efforts have been made to transfer their genetic variation into domesticated genotypes. These species have a wide repertoire of key alleles that can be used in wheat improvement programs (Pour-Aboughadareh et al. 2017a). In regard to storage proteins, extensive allelic diversity of glutenin and gliadin subunits have been discovered in several of wild relatives of wheat and previous studies have indicated that HMW glutenin subunits from different species of *Aegilop*s such as *Ae. tauschii* have a significant influence on the processing quality of the synthetic hexaploid wheat (Zhang et al. 2013). As the major components of wheat storage proteins, LMW-GSs are significantly correlated with dough extensibility and dough strength (Zhang et al. 2012). In many studies, the correlations between allelic variations of *Glu-3* and bread-making quality in common wheat are well established. Also, the linkage of specific LMW-GS proteins with all *Glu-3* alleles makes it possible to discern the roles of individual LMW-GS in wheat flour functionality and develop new lines with improved quality (Lee et al. 2016). In the present study, we dissected the allelic variability of subunits of glutenin and gliadin in 180 accessions of wheat germplasm collected from different regions of Iran. According to results, all glutenin and gliadin loci analyzed were polymorphic, indicating large genetic diversity within and between species. Similarly, Ahmadpoor et al. (2014) and Ahmadi and Pour-Aboughadareh (2015) indicated that some of species of *Aegilops* and *Triticum* such as *Ae. triuncialis*, *Ae. columnaris*, *Ae. biuncialis*, *Ae. crassa*, *Ae. cylindrica*, *Ae. ovate*, *T. boeoticum* and *T. urartu* had high levels of

allelic diversity in HMW and LMW subunits. Our results indicated that, the accessions from *Aegilops* have more allelic diversity than *Triticum*. Also, the results from AMOVA revealed a higher distribution of genetic variation within two genera and different species as compared to between them (Table 2). In addition, the highest percentage of polymorphism loci was detected in *Aegilops* than *Triticum* accessions. However, the highest values of Nie's genetic diversity (*H*) and Shannon's information (*I*) indices observed for *Triticum* accessions. Hence, these information could be useful to select material from these wild relatives for breeding purposes, because other interesting genes from different *Aegilops* and *Triticum* species could be also inherited and be present in various advanced wheat genotypes (Aguiriano et al. 2006; Ahmadi and Pour-Aboughadareh 2015).

More importantly, as shown in Table 1 and Fig. 1, we discovered 19 new allelic variants for *Glu-3A* and *Gli-2A* loci using four locus-specific primer pairs in the tested wheat germplasm. From species viewpoint, frequencies of new alleles in the various species were different. Regard to allelic status comparison, we surmise that present of new alleles in some of species linked to genomic constitution. For instance, *Glu-3A.1a* allele only presented in species that possessing U-genome, *Ae. umbellulata* (U genome), *Ae. triuncialis* (UC genome) and *Ae. neglecta* (UM genome), and *Glu3A.1c* linked to present of M genome in *Ae. neglecta* (UM) and Ae. crassa (MD). The *Glu-3A.2c* was associated with D and C genomes, so that *Ae. cylindrica* and its parental species, *Ae. caudata* (C genome) and *Ae. tauschii* (D genome), possessed this new allele. Furthermore, *Glu-3Ac* allele only exist in neglected diploid wheat; *Ae. caudata* (C genome), *Ae. umbellulata* (U genome), *T. urartu* (A^u genome) and *T. boeoticum* (A^b genome). Also, *T. urartu*, as A-genome donor of common wheat, carried two new alleles *Glu-3A.3c* and *Glu-3A.3d*. In the present study, the number of allelic variants exceeded that found by Zhang et al. (2013) in common wheat, or by Wang et al. (2010), Ahmadpoor et al. (2014), Ahmadi and Pour-Aboughadareh (2015), Luo et al. (2015) and Cuesta et al. (2017) in wild relatives of wheat. The development of allele-specific markers is based on the polymorphisms of nucleotide sequences among different alleles, and the relationship between markers and phenotypes needs to be established. In this regard, Wang et al. (2009) discovered several new alleles in different wheat genotypes and designed seven PCR markers to discriminate the protein alleles *Glu-A3a*, *b*, *c*, *d*, *e*, *f* and *g*. In another study, Wang et al. (2010) characterized one *Glu-3A* gene including four allelic variants, and developed a set of STS markers for the discrimination of the *Glu-3A* alleles.

Up to now, gliadin genes, not subjected to direct selection by wheat breeders, could be linked to genes directly selected from different wild relatives and ancestral species. Therefore, these new gliadin alleles might be associated with specific genomic constitution. The large number of new alleles indicates that some of the *Aegilops* and *Triticum* species are rather unique. This allelic diversity is conserved nowadays because wheat breeders did an important effort to collect and maintain this germplasm in *ex-situ* collections before being affected by the genetic erosion in the field (Aguiriano et al. 2006). Extensive researches have been performed to characterize novel gliadin genes among species of *Aegilops* and *Triticum* genera (Li et al. 2013; Zhang et al. 2015; Huang et al. 2016). In the present study, the *Gli-As.2a* allele was observed in all of *Aegilops* and *Triti-*

cum species. Of the new alleles detected in *Gli-As.2* locus, *Gli-As.2d*, *e*, *f* and *g* only discovered in *T. aestivum*, while these alleles were absent in other *Triticum* species. In contrast, allelic variability in *Aegilops* species was very high and some species had more than one new allele. The *Gli-As.2b*, *d* and *e* new alleles mainly were detected in *Ae. caudata*, *Ae. crassa* and *Ae. neglecta*. Also, *Ae. neglecta* possessed two new alleles *Gli-As.2e* and *f*. These results reveal that these species could be considered as valuable gene resources for seed storage proteins (Huang et al. 2016).

Wild relatives and progenitors of wheat are of interest to plant breeders, and major efforts have been made to transfer their genetic variation into domesticated genotypes. These species have a wide repertoire of key alleles that can be used in wheat improvement programs (Pour-Aboughadareh et al. 2017a). Of the wild relatives of common wheat, only some species such as *Ae. tauschii, T. urartu*, *T. monococcum* and *T. dicocoides*, have been explored and exploited for HMW and LMW characteristics. In conclusion, we reported an allelic diversity of glutenin and gliadin genes in different *Aegilops–Triticum* species that found a wide range of variability among various wild relatives of wheat using several locus-specific markers. These results can be used as a source of quality protein genes for wheat breeding and for studying the genetics of storage proteins. We also revealed that allelic status of *Glu-3A* and *Gli-As.2* is linked to genomic constitutions, so that the di- and tetraploid wild relatives possessing U, C, D and A genomes harbor wide variation in the glutenin and gliadin subunits. Hence, these results may open up new avenues for rethinking the connections between other progenitors and wild relatives with improving the dough quality. Consequently, it is suggested that the discovery of this highly diverse gene pool should encourage researchers to explore valuable and new alleles for the improvement of new varieties that are adapted to new uses.

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Electronic Supplementary Material (ESM)

Electronic Supplementary Material (ESM) associated with this article can be found at the website of CRC at https://akademiai.com/loi/0806

Electronic Supplementary *Table S1*. List of the *Aegilops – Triticum* accessions analyzed, their provenance and genome classification

Electronic Supplementary *Table S2*. PCR-primers of molecular markers used for the detection of allelic diversity of glutenin and gliadin genes

Electronic Supplementary *Table S3*. Estimated genetic diversity parameters in *Aegilops* and *Triticum* species

Electronic Supplementary *Table S4*. Allelic patterns for gluten and gliadin genes in 180 *Aegilops* and *Triticum* accessions