

GENETIC DIVERSITY OF HIGH AND LOW MOLECULAR WEIGHT GLUTENIN SUBUNITS IN BULGARIAN ACCESSIONS FROM *Aegilops triuncialis* SPECIES

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Abstract

Thirty-eight samples of the species *Aegilops triuncialis* L. were analyzed for HMW and LMW glutenin subunits. A total of 18 alleles were documented at the Glu-U1 locus, with allele frequencies ranging from 2.63% to 15.79%. A total of ten alleles were identified at the Glu-C1 locus, with the alleles Glu-C1h, Glu-C1i, and Glu-C1j classified as rare. The genotypes under scrutiny exhibited substantial allelic variation with respect to both B-LMW-GS and C-LMW-GS. A total of 41 alleles were identified within the B zone, 20 within the Glu-U3 locus, and 21 within the Glu-C3 locus. A total of 35 alleles were identified in the C zone, 21 in the Glu-U3 locus and 14 in the Glu-C3 locus. The presence of a diverse array of glutenin subunits, encompassing both high and low molecular weight variants, within this species, signifies a valuable genetic resource that has the potential to enhance the quality of wheat.

Key words: *Aegilops triuncialis* L., alleles, glutenins, polymorphism, SDS-PAGE.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is one of the most widely cultivated cereal crops globally, serving as a primary source of calories and protein for over one-third of the world's population (Bayisa et al., 2025; Sharma and Sharma, 2025). Its end-use quality, particularly dough elasticity and baking performance, is largely determined by gluten proteins, which form a viscoelastic network in the presence of water (Ortolan and Steel, 2017; Ye et al., 2023). Gluten is composed of two main protein classes: gliadins and glutenins. Glutenins, in turn, are further classified into high molecular weight (HMW-GS) and low molecular weight (LMW-GS) subunits, encoded by the Glu-1 and Glu-3 loci, respectively, located on the homoeologous group 1 and 6 chromosomes (Sharma et al., 2020; Guzmán et al., 2022; Al-Khayri et al., 2023; Spetsov and Daskalova, 2024).

Among glutenin subunits, HMW-GS are particularly critical for dough strength due to their role in forming intermolecular disulfide bonds that contribute to polymer formation (Nucia et al., 2019; Shewry et al., 2022; Wang

et al., 2022, 2023, 2024). Variability in HMW-GS composition is strongly correlated with differences in bread-making quality, with certain allelic variants (e.g., Glu-D1d) being associated with superior performance (Bouabdellah et al., 2025). LMW-GS, although individually smaller, are more numerous and contribute significantly to gluten polymer size and viscoelasticity (Guzmán et al., 2022; Chen et al., 2025). Thus, understanding the genetic diversity of both HMW-GS and LMW-GS is crucial for wheat improvement.

Wild relatives of wheat, such as those in the genus *Aegilops*, are valuable reservoirs of genetic variation not only for disease resistance and for abiotic stress tolerance but also potentially for grain quality traits (Kishii et al., 2019; Adhikari et al., 2023). *Aegilops triuncialis* L., an allotetraploid species with the CCUU genome constitution ($2n = 28$), is naturally distributed across the Mediterranean Basin, including Bulgaria, where it grows in ruderal and disturbed habitats (Feldman and Levy, 2023). It diverged from the wheat lineage approximately 3-4 million years ago and shares genome homology with the U genome, which is

related to the A genome of wheat (Badaeva et al., 2025). This phylogenetic proximity suggests potential for introgressing U-genome-encoded glutenin subunits into wheat via wide hybridization.

Despite its agronomic potential, *Aegilops triuncialis* L. has been poorly characterized for glutenin variation, especially in Eastern Europe. Bulgarian accessions, in particular, have not been systematically analyzed for HMW-GS and LMW-GS composition. Bulgaria's diverse climate and topography support a wide range of genetic variation in wild cereals, making it an ideal region for exploring underutilized genetic resources (Desheva et al., 2013; Kyosev et al., 2025). Understanding the extent of HMW-GS and LMW-GS diversity is essential to identify superior alleles for introgression into commercial wheat cultivars. Therefore, this study aims to assess the allelic diversity of HMW-GS and LMW-GS in Bulgarian *Aegilops triuncialis* L. accessions using electrophoretic profiling, and to evaluate the implications of this diversity for wheat quality breeding.

MATERIALS AND METHODS

The seeds from thirty-eight accessions of the *Aegilops triuncialis* L. collected from the territory of Bulgaria during 2024 (Table 1) were used to analyse HMW and LMW glutenin subunits. Extraction of HMW-GS and LMW-GS was performed using the method of Wang et al. (2022). SDS-PAGE was conducted with 12% (w/v) running gels and 4% (w/v) stacking gels. The gels were run at 30 mA for 1.50 h (Cytiva SE 260 mini-cell apparatus). The gels were stained with a 1% solution of Coomassie Brilliant Blue R-250 in acetic acid, methanol, and water in a ratio of (1:5:4) overnight. The gel plates were decolorized with a solution of acetic acid, methanol, and distilled water (1:2:7). In order to assign the components of HMW-GS, B-LMW-GS, and C-LMW-GS patterns of the tetraploid species *Aegilops triuncialis* L. to U or C genomes, a comparison of glutenin electrophoretic patterns between *Aegilops triuncialis* L. and the diploid species *Aegilops umbellulata* Zhuk. and *Aegilops caudata* L. was undertaken. The nomenclature of the subunits and alleles of HMW-GS (locus Glu-1) corresponds to the terminology of Rodriguez-

Quijano et al. (2001), with the letter C denoting bands from *Aegilops caudata* L. and the letter U denoting bands from *Aegilops umbellulata* Zhuk. For LMW-GS (B- and C-subunits), the different bands were assigned numerical identifiers according to their order of mobility in SDS-PAGE.

The genetic diversity at each locus was calculated using the formula developed by Nei (1973). The level of variation was estimated by dividing the number of patterns found by the number of accessions. A dendrogram was constructed via the unweighted pair group method with arithmetic mean (UPGMA). The implementation of cluster analysis was conducted utilizing the Past4.03 software.

Table 1. Geographic coordinates of collected accessions from *Aegilops triuncialis* L. included in the study

№	Accession number	Geographical latitude	Geographical longitude	Altitude	Locality
1	C4E0032	42°1'36.462"N	24°42'58.212"E	670	Galabovo, Plovdiv
2	C4E0033	41°39'15.732"N	25°52'16.104"E	199	meandrite na Arda, Haskovo
3	C4E0051	42° 30' 31.8"N	24° 48' 51.0"E	360	Pesnopoy, Plovdiv
4	C4E0052	42°2'443.161"N	27°42'5.513"E	8	Sozopol, Burgas
5	C4E0200	41°47'21.82"N	25°21'55.823"E	402	Pchelarovo, Kardzhali
6	C4E0205	41°25'49.495"N	25°21'40.377"E	285	Samodiva, Kardzhali
7	C4E0207	41°22'51.739"N	25°13'35.430"E	360	Deletz, Kardzhali
8	C4E0209	41°25'26.848"N	25°21'46.488"E	287	Bregovo, Kardzhali
9	C4E0190	43°15'26.323"N	27°49'25.807"E	134	Aksakovo, Varna
10	C4E0061	42°24'39.668"N	24°46'45.351"E	237	Dolino sahrane, Stara Zagora
11	C4E0195	41°52'26.533"N	25°20'36.085"E	249	Susam, Haskovo
12	C4E0044	42° 26' 51.3"N	24° 11' 31.9"E	255	Brestnik, Plovdiv
13	C4E0088	42°44'43.207"N	27°44'31.542"E	100	Kosharitz, Burgas
14	C4E0091	42°46'17.248"N	27°47'35.313"E	805	Banya, Burgas
15	C4E0148	41°57'36.773"N	23°35'52.192"E	805	Daganovo, Blagoevgrad
16	C4E0149	41°57'34.228"N	23°35'45.264"E	805	Daganovo, Blagoevgrad
17	C4E0153	41°52'27.781"N	23°35'30.874"E	725	Eleshtitsa, Blagoevgrad
18	C4E0158	41°39'37.804"N	23°44'4.392"E	551	Gospodintzi, Blagoevgrad
19	C4E0162	41°34'34.388"N	23°44'35.937"E	540	Gotze Delchev, Blagoevgrad
20	C4E0163	41°35'59.904"N	23°40'48.084"E	871	Dobrotino, Blagoevgrad
21	C4E0164	41°31'17.870"N	23°32'36.500"E	773	Pirin, Blagoevgrad
22	C4E0165	41°29'49.373"N	23°29'36.934"E	306	Gorno Spanchevo, Blagoevgrad
23	C4E0168	41°27'13.411"N	23°26'22.104"E	188	Kalimantzi, Blagoevgrad
24	C4E0170	41°27'6.779"N	23°23'37.109"E	248	Harsovo, Blagoevgrad
25	C4E0176	41°27'23.806"N	23°22'28.922"E	230	Kromidovo, Blagoevgrad
26	C4E0178	41°27'31.041"N	23°20'45.984"E	172	Kapatovo, Blagoevgrad
27	C4E0181	41°27'18.600"N	23°19'23.226"E	143	Levanovo, Blagoevgrad
28	C4E0182	41°28'45.584"N	23°15'21.948"E	103	Starchevo, Blagoevgrad
29	C4E0183	41°28'45.584"N	23°15'21.948"E	103	Starchevo, Blagoevgrad
30	C4E0184	41°27'40.311"N	23°15'41.488"E	90	Rupite, Blagoevgrad
31	C4E0185	41°55'31.691"N	23°12'22.29"E	491	Gradevo, Blagoevgrad
32	C4E0197	41°55'45.847"N	25°20'39.434"E	321	Haskovski mineralni bani, Haskovo
33	C4E0218	42°17'31.941"N	22°39'56.116"E	551	Lozno, Kjustendil
34	C4E0014	42°9'54.708"N	24°8'15.162"E	274	Varvara, Pazardzhik
35	C4E0015	42°10'10.956"N	24°6'49.006"E	352	Simenovetz, Pazardzhik
36	C4E0018	42°12'25.344"N	24°30.090"E	313	Belovo, Pazardzhik
37	C4E0023	42°12'1.5328"N	24°42'4.5468"E	784	Tzar Kaloyan, Plovdiv
38	C4E0027	42°2'39.1344"N	24°41'36.355"E	1078	baza Iglika, Plovdiv

RESULTS AND DISCUSSIONS

Glutenin proteins, particularly the high molecular weight (HMW GS) and low molecular weight (LMW GS) subunits, play a decisive role in dough rheology and, consequently, in end use quality. *Aegilops*

species generally exhibit high genetic diversity in both HMW-GS and LMW-GS. This diversity can be harnessed to introduce new alleles into the wheat gene pool, which is often limited in genetic variation (Cuesta et al., 2016; Ahmadi et al., 2018; Tariq et al., 2018; Goldasteh et al., 2019; Mohamed et al., 2022; Jiang et al., 2023). In this study employed sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) to characterize the glutenin subunit composition of 38 *Aegilops triuncialis* L. accessions collected from diverse agro ecological zones across Bulgaria.

A total of 28 distinct alleles were identified across the Glu-1 loci, distributed between the Glu-U1 and Glu-C1 regions. In the Glu-U1 locus, 18 alleles were detected, with frequencies ranging from 2.63% to 15.79%. The most common alleles were Glu-U1c and Glu-U1j, each occurring in 15.79% of samples, followed by Glu-U1b and Glu-U1d, each present in 10.53%. Alleles Glu-U1g and Glu-U1a were found in 7.89% of samples, while Glu-U1f appeared in 5.26%. The remaining 11 alleles were each detected at the lowest observed frequency of 2.63% (Tables 2 and 4).

At the Glu-C1 locus, ten alleles were identified. The most prevalent were Glu-C1d (encoding subunit 4) at 21.05% and Glu-C1f (associated with subunits 2 and 7) at 18.42%. Glu-C1g was observed in six genotypes, and Glu-C1b and Glu-C1c each occurred in 13.16% of samples. Alleles Glu-C1h, Glu-C1i, and Glu-C1j were considered rare, each appearing in only a single genotype (Tables 2 and 4).

The genetic diversity indices calculated for the Glu-U1 locus and Glu-C1 locus were high, respectively 0.907 and 0.843 indicating a high level of allelic polymorphism and variability at HMW-GS loci (Table 2).

In accordance with the findings of our study, a high degree of genetic diversity with regard to HMW-GS has also been identified in other *Aegilops* species. Research conducted on *Aegilops tauschii* Coss. has revealed significant variations in HMW-GS. For instance, 14 HMW-GS combinations have been identified in Iranian specimens, suggesting a high degree of genetic diversity (Tahernezhad et al., 2013). In the Sitopsis section of *Aegilops*, six new HMW-GS alleles have been identified in species of this section, with a large molecular weight due to the

insertion of fragments (Jiang et al., 2012). Medouri et al. (2014) have documented the extensive array of high molecular weight glutenin subunits and low molecular weight glutenin subunits in Algerian *Aegilops geniculata* Roth. As demonstrated by Kozub et al. (2012), significant genetic diversity has been identified, with studies identifying numerous alleles for glutenin subunits (Gli-U1 and Gli-M1) across various populations of *Aegilops biuncialis* Vis.

Table 2. Allele frequency in HMW glutenin subunits and genetic diversity index in the Glu-U1 and Glu-C1 loci in samples from *Aegilops triuncialis* L.

Zone	Locus	Allele	Subunits	Frequency
A	Glu-U1	a	1+5	5.26
		b	1+6	10.53
		c	1+7	15.79
		d	1+8	10.53
		e	2+6	2.63
		f	2+7	5.26
		g	2+8	7.89
		h	3+6	2.63
		i	3+7	2.63
		j	3+8	15.79
		k	3+9	5.26
		l	4+8	2.63
		m	6+9	2.63
		n	7+8	2.63
		o	8+9	2.63
		p	9	2.63
		q	2+6+10	2.63
		r	5+7+9	2.63
	H index		0.907	
	Glu-C1	a	1	5.26
		b	2	13.16
		c	3	13.16
		d	4	23.68
		e	1+9	5.26
		f	2+7	18.42
		g	2+9	15.79
		h	4+8	2.63
		i	4+9	2.63
		j	2+7+9	2.63
	H index		0.843	

The Glu3-loci in wheat and its relatives, including *Aegilops* species, encode LMW-GSs, which are essential components of the gluten protein network. LMW-GSs significantly affect dough elasticity, viscosity, and overall bread-making quality (Pourmohammadi et al., 2023; Zhao et al., 2025). In this study set of genotypes of *Aegilops triuncialis* L. showed significant allele variations for B-LMW-GS and C-LMW-GS.

Forty-one alleles were identified in zone B (Tables 3 and 4), 20 in the Glu-U3 locus and 21 in the Glu-C3 locus. In the Glu-U3 locus, sixteen of the alleles were found with the same frequency (2.63%). It was observed that only Glu-U3d (encoding subunits 1 and 9) exhibited a frequency of 28.95%. In the Glu-C3 locus, thirteen distinct alleles were identified in the

analyzed genotypes. Three alleles (Glu-C3c, Glu-C3j, and Glu-C3k) were found with a frequency above 10%. The most prevalent allele (Glu-C3k, encoding subunits 3+12) was identified in six samples (representing 15.79% of the total).

35 alleles were identified in the C-zone (Table 3), 21 of which were located in the Glu-U3 locus and 14 in the Glu-C3 locus. In the Glu-U3 locus, eleven of the alleles were classified as rare and have a frequency of 2.63%. The analysis revealed the presence of Glu-U3b and Glu-U3c (encoding subunits 2 and 3, respectively) in 5 and 4 samples, respectively, with a frequency of 13.16% and 10.53%, respectively. At the Glu-C3 locus, eight alleles were identified in individual genotypes. One allele (Glu-C3f) was identified in six samples, one allele (Glu-C3c) was recorded in another six samples, and frequencies of 13.16% were found for the alleles Glu-C3b, Glu-C3d, and Glu-C3g (Tables 3 and 4).

The Nei diversity index for B-LMW-GS was 0.961 and for C-LMW-GS respectively 0.967 (Table 4). The provided values, represent high genetic diversity for Low Molecular Weight Glutenin subunits in *Aegilops triuncialis* L., with the C-type locus showing slightly greater diversity than the B-type locus, indicating rich allelic variation important for baking quality. These results suggest a complex genetic makeup for glutenins, crucial for dough strength and elasticity in wheat breeding (Wang et al., 2024; Zhao et al., 2025, 2025a). Wang et al. (2018) identify specific LMW-GS from *Aegilops umbellulata* Zhuk., that enhance gluten networks, benefiting traits like dough development time, stability, gluten index, and loaf structure in improved wheat varieties. In the species *Aegilops comosa* Sm. novel LMW-GS genes were isolated, showing significant polymorphisms and evolutionary divergence (Wang et al., 2011; Huang et al., 2018), while in *Aegilops tauschii* Coss. three LMW-GS genes were characterized, showing high homology with bread wheat and related species (Pei et al., 2007).

Based on the HMW- and LMW-glutenin subunit profiles, a UPGMA dendrogram (Figure 1) was constructed to assess genetic relatedness among

the studied genotypes, revealing four major clusters with distinct molecular and geographic patterns.

The first cluster includes four genotypes - 24, 25, 29, and 12 exhibiting high variability in the B and C zones of the LMW-GS at the Glu-C3 locus. Genotypes 24, 25, and 29 originate from the Blagoevgrad region, while genotype 12 was collected in Plovdiv, suggesting possible regional genetic divergence. Cluster II consists of two genotypes - 21 and 26 - both from Blagoevgrad, which share rare alleles in the B-LMW-GS region, highlighting unique genetic features within this group. The largest cluster (Cluster III) comprises 23 accessions, further subdivided into four well-defined subgroups. Subgroup 1 includes genotypes 36 (from Plovdiv region), 32 (from Haskovo region), 33 (from Kardzhali region), and 27 and 30 (from Blagoevgrad region), and is characterized by a strong presence of the Glu-U1d and Glu-C1c alleles. Subgroup 2 features genotypes 1 (from Plovdiv region), 31 (from Blagoevgrad region), 14 (from Burgas region), and 10 (from Stara Zagora region), dominated by the Glu-U3c allele in B-LMW-GS. Subgroup 3 is defined by the prevalent Glu-U3d allele in B-LMW-GS and includes primarily genotypes from Blagoevgrad region (4, 15-20, 22), with only two exceptions: genotype 2 (from Haskovo region) and 13 (from Burgas region). Subgroup 4 contains genotypes 23 and 28 (from Pazardzhik region) and 34 and 35 (from Blagoevgrad region), all carrying rare alleles in the C-LMW-GS region. The fourth cluster groups nine genotypes 3, 37, 38 (from Plovdiv region), 5-8 (from Kardzhali region), 9 (from Varna region), and 11 (from Haskovo region) - distributed across three subgroups and marked by a shared predominance of the Glu-C1d (A-HMW-GS) and Glu-C3f (C-LMW-GS) alleles, suggesting a common genetic background despite geographic dispersion. The dendrogram identifies several genotype pairs with exceptionally high genetic similarity, including 17-18, 13-22, 16-18, 34-35, and 3-7, all exhibiting Dice similarity indices between 0.737 and 0.870 (Figure 1). These close relationships underscore potential duplication, shared ancestry, or limited genetic differentiation within these pairs.

Table 3. Frequency of alleles in LMW glutenin subunits and genetic diversity index in the Glu-U3 and Glu-C3 loci in samples from *Aegilops triuncialis* L.

Zone	Locus	Allele	Subunits	Frequency	Zone	Locus	Allele	Subunits	Frequency
B	Glu-U3	a	1+6	2.63	C	Glu-U3	a	1	5.26
		b	1+7	2.63			b	2	13.16
		c	1+8	13.16			c	3	10.53
		d	1+9	28.95			d	4	7.89
		e	2+10	5.26			e	5	2.63
		f	2+8	2.63			f	6	5.26
		g	2+9	13.16			g	1+11	2.63
		h	3+13	2.63			h	1+8	7.89
		i	4+11	2.63			i	1+9	5.26
		j	4+13	2.63			j	2+10	2.63
		k	4+14	2.63			k	2+12	7.89
		l	1+10+14	2.63			l	2+13	5.26
		m	1+5+14	2.63			m	2+9	5.26
		n	1+7+16	2.63			n	3+10	2.63
		o	1+8+14	2.63			o	3+11	2.63
		p	2+5+15	2.63			p	3+12	2.63
		q	2+6+16	2.63			q	3+13	2.63
		r	2+7+14	2.63			r	3+9	2.63
		s	2+8+15	2.63			s	4+12	2.63
		t	3+7+16	2.63			t	4+13	2.63
	H index	0.868					u	6+13	2.63
	Glu-C3	a	1+10	2.63		Glu-C3	a	3	2.63
		b	1+8	2.63			b	4	13.16
		c	2+10	10.53			c	5	15.79
		d	2+11	2.63			d	6	13.16
		e	2+12	2.63			e	7	10.53
		f	2+13	5.26			f	8	15.79
		g	2+6	2.63			g	9	13.16
		h	2+7	7.89			h	1+8	2.63
		i	3+10	2.63			i	3+11	2.63
		j	3+11	13.16			j	4+11	2.63
		k	3+12	15.79			k	5+11	2.63
		l	3+13	5.26			l	5+9	2.63
		m	3+7	2.63			m	6+10	2.63
		n	3+9	2.63			n	8+11	2.63
		o	4	2.63			H index		0.882
		r	5	2.63					
		s	6	2.63					
		t	8	2.63					
		p	4+10	5.26					
		q	4+12	5.26					
		u	2+9+13	2.63					
	H index	0.920							

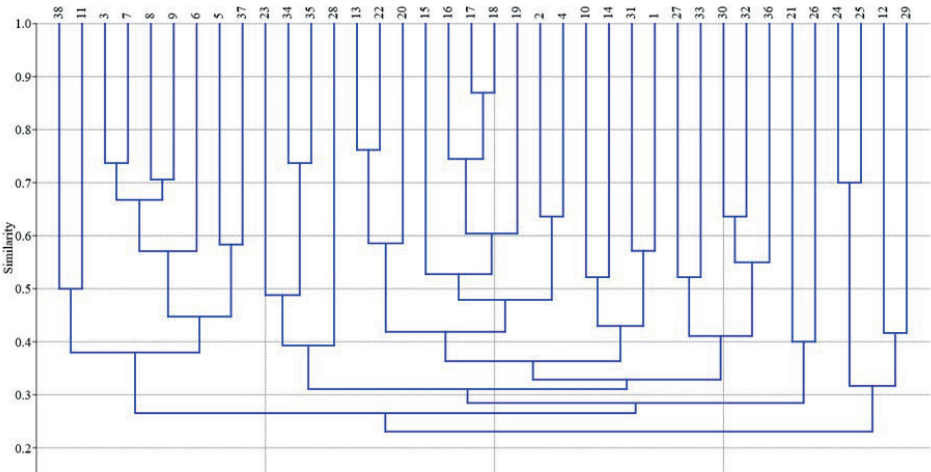


Figure 1. Grouping of 38 genotypes of *Aegilops triuncialis* L. using the UPGMA method (Similarity index-Dice) (The numbers of the genotypes in the dendrogram correspond to the numbers in Table 1)

Table 4. Allele composition of HMW-GS, B-LMW-GS and C-LMW-GS subunits in accessions of the species *Aegilops triuncialis* L

№	Genotype	A subunits				B subunits				C subunits			
		Glu-U1	Allele	Glu-C1	Allele	Glu-U3	Allele	Glu-C3	Allele	Glu-U3	Allele	Glu-C3	Allele
1	CE40032	1+7	c	2	b	1+8	c	2+10	c	1+8	h	5	c
2	CE40033	1+7	c	2+7	f	1+9	d	3+12	k	3+10	n	4+11	j
3	CE40051	3+8	k	2	b	2+9	g	4+12	q	2+10	j	4	b
4	CE40052	4+8	l	2	b	1+9	d	2+12	e	3	c	8	f
5	CE40200	5+7+9	r	2+7+9	j	2+8+15	s	2+7	h	2+12	k	6	f
6	CE40205	6+9	m	4	d	2+6+16	q	2+7	h	6+13	u	8	f
7	CE40207	7+8	n	4	d	2+9	g	4+10	p	2	b	8	f
8	CE40209	8+9	o	4	d	2+9	g	4+12	p	3	c	8	f
9	CE40190	9	p	4	d	2+9	g	3+12	k	2	b	8	f
10	CE40061	2+7	f	2+7	f	1+8	c	3+13	l	6	f	8	f
11	CE40195	3+6	h	2+7	f	2+5+15	p	2+6	g	1	a	5	c
12	CE40044	1+7	c	1	a	1+6	a	1+10	a	4+12	s	8+11	n
13	CE40088	1+6	b	2+7	f	1+5+14	m	1+8	b	4	d	6	d
14	CE40091	2+7	f	2	b	1+8+14	o	2+9+13	u	3+13	q	6	d
15	CE40148	2+8	g	2+9	h	2+9	g	3+12	k	1+8	h	3+11	i
16	CE40149	2+8	g	2+9	g	1+9	d	3+12	k	6	f	7	e
17	CE40153	2+6+10	q	2+7	f	1+9	d	2+13	f	1+9	i	4	b
18	CE40158	2+8	g	2+9	g	1+9	d	2+11	d	1+9	i	4	b
19	CE40162	3+8	j	2+9	g	1+9	d	3+11	j	1	a	9	g
20	CE40163	2+6	e	2+7	f	1+9	d	3+10	i	4	d	9	g
21	CE40164	1+5	a	2+7	f	2+10	e	2+13	f	3	c	9	g
22	CE40165	1+8	d	2	b	1+10+14	l	3+9	n	4	d	9	g
23	CE40168	1+7	c	1	a	1+7+16	n	3+7	m	3+11	o	3	a
24	CE40170	1+6	b	1+9	e	3+7+16	t	4+10	p	2	b	9	g
25	CE40176	1+5	a	1+9	e	3+13	h	5	r	2	b	5	c
26	CE40178	1+6	b	2+9	g	4+11	i	4	o	1+11	g	4	b
27	CE40181	1+8	d	2+9	g	4+14	k	6	s	2+9	m	5+9	l
28	CE40182	1+7	c	3	c	2+7+14	r	2+7	h	3+9	r	6+10	m
29	CE40183	1+6	b	4+8	h	1+7	b	2+10	c	4+13	t	6	d
30	CE40184	1+8	d	3	c	1+8	c	2+10	c	2+13	l	4	b
31	CE40185	3+8	j	4	d	1+8	c	2+10	c	1+8	h	1+8	h
32	CE40197	3+8	j	3	c	1+8	c	3+12	k	2+13	l	7	e
33	CE40218	1+7	c	4+9	i	4+13	j	8	t	2+9	m	5+11	k
34	CE40014	3+8	j	3	c	1+9	d	3+11	j	3	c	5	c
35	CE40015	3+9	k	4	d	1+9	d	3+11	j	3+12	p	5	c
36	CE40018	1+8	d	3	c	1+9	d	3+13	l	2	b	7	e
37	CE40023	3+7	i	4	d	2+10	e	3+12	k	2+12	k	6	d
38	CE40027	3+8	j	4	d	2+8	f	3+11	j	5	e	7	e
H		0.956				0.961				0.967			

CONCLUSIONS

This study presents the first comprehensive SDS-PAGE analysis of high- and low-molecular-weight glutenin subunit (HMW-GS and LMW-GS) diversity in naturally occurring *Aegilops triuncialis* populations from Bulgaria. The findings reveal remarkable diversity in both HMW-GS and LMW-GS profiles, including the detection of several rare subunit variants, underscoring the species' role as an untapped genetic reservoir for enhancing wheat end-use quality. SDS-PAGE emerges as a robust tool for early-generation screening in breeding programs, enabling the rapid identification of desirable subunit combinations. Future research should synergize protein electrophoresis with high-resolution genomic approaches to precisely map functional loci and enable targeted introgression of beneficial alleles into cultivated wheat, thereby accelerating the development of superior grain quality traits.

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