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Research Article

ALLELE FREQUENCY OF GLUTENIN SUBUNITS AND GLU-1 QUALITY SCORES IN SOME TURKISH BREAD WHEAT LANDRACES

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Abstract: There are eight centers of origin for cultivated plants and Turkey is located in the interception of two of these centers, the Near East and the Mediterranean. Therefore, Turkey is known to be the gene center for diversification and dispersion of such main cereal crops such as wheat, barley, rye and oat. This study was performed to determine glutenin gene allele frequencies and *Glu-1* quality scores of 116 local wheat landraces of Turkish bread wheat. SDS-PAGE and PCR were used to identify glutenin gene alleles. The results showed that the studied Turkish local wheat landraces contained a total of 19 different subunits (3 subunits in *Glu-A1*, 11 in *Glu-B1* and 5 in *Glu-D1*) with 50 different combinations. The highest and the lowest allelic combinations were determined in East Anatolia and the Aegean regions, respectively. *Glu-A1*c (65.11%), *Glu-B1*b (53.60%) and *Glu-D1*a (58.30%) were the most frequent alleles. The *Glu-1* quality score was found to be 6.07 for the studied genotypes. Among the regions, the highest (7.18) and the lowest (4.80) mean *Glu-1* scores were detected in Marmara and Southeastern Anatolia regions, respectively. 4 accessions (TR32846-6, TR36948-1, TR45105 and TR63536) were reported to have the highest *Glu-1* quality score as 10. 6 genotypes (TR45398-4, TR48025-3, TR33264-6, TR393-5, TR52021-3 and TR45094) had the quality score of 9. Including more new landraces may contribute to discover new *Glu-1* alleles.

Özet: Kültür bitkileri için sekiz orijin merkezi vardır ve Türkiye, bu merkezlerden ikisinin, Yakın Doğu ve Akdeniz'in, kesiştiği yerdedir. Türkiye bu nedenle buğday, arpa, çavdar ve yulaf gibi ana tahıl bitkilerinin çeşitlendiği ve dağıldığı gen merkezi olarak bilinir. Bu çalışmanın amacı, Türk ekmeklik buğdaylarından 116 yerel buğday ırkının glüten allel sıklığı ve *Glu-1* kalite skorunu belirlemektir. SDS-PAGE ve PCR, glüten allellerini tanımlamak için kullanılmıştır. İstatistiksel analizler için POPGENE 1.31 yazılımı kullanılmıştır. İncelenen Türk yerel buğday ırkları toplam 19 farklı alt birim (*Glu-A1*'de 3 alt birim, *Glu-B1*'de 11 ve *Glu-D1*'de 5 alt birim) ve 50 farklı kombinasyon içermektedir. En yüksek ve en düşük allel kombinasyonları sırasıyla Doğu Anadolu ve Ege bölgelerinde belirlenmiştir. *Glu-A1c* (% 65,11), *Glu-B1b* (% 53,60) ve *Glu-D1a* (% 58,30) en sık görülen alleller olarak tespit edilmiştir. *Glu-1* kalite skoru, Türkiye genelinde incelenen genotipler için 6,07 olarak bulunmuştur. Bölgeler arasında ortalama en yüksek (7,18) ve en düşük (4,80) *Glu-1* skorları sırasıyla Marmara ve Güneydoğu Anadolu bölgelerinde tespit edilmiştir. Çalışılan 116 aksesyondan 4 tanesi (TR32846-6, TR36948-1, TR45105 ve TR63536) kalite skoru 10 olup en yüksek Glu-*1* kalite skoruna sahipken 6 tanesinin ise (TR45398-4, TR48025-3, TR33264-6, TR393-5, TR52021-3 ve TR45094) kalite skoru 9 olarak hesaplanmıştır. Daha fazla yerel çeşitlerin çalışmalara eklenmesi yeni *Glu-1* allelerinin keşfedilmesine katkıda bulunabilir.

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Introduction

Vavilov (1951) identified eight centers with gene center status that have long been used for agriculture and Turkey is located in the interception of two of these centers (Near East and the Mediterranean), making the country one of the richest in terms of plant biodiversity. Turkey is also known to be one of the gene centers for diversification and dispersion of main cereal crops such as wheat, barley, rye, and oat. This special future, in addition to being the first place where wheat was cultivated, makes the country for several wild species and landraces of wheat to be available. For instance, the first cultivated forms of diploid and tetraploid wheat originated from Karacadağ (Diyarbakır) in the Southeastern region of Turkey.

These first diploid (AA) and tetraploid genomes (AABB) and their phylogenetic analysis indicate that they

had originated in Southeastern Turkey (Dubcovsky & Dvorak 2007). Cultivation shifted from here to near east about 9.000 years ago and the hexaploid wheat appeared for the first time (Feldman 2001). Cereals occupy 55% of total agricultural and 57% of cultivated areas (21.4 million hectares) in Turkey (Grain sector report 2013). Among the cereals in Turkey, wheat is the pioneering crop with 67% share and the country supplies 3% of world wheat production every year. However, because of quality-related issues, it is still a wheat importing country (TMO 2017). Among the regions, the leading wheat producing region is Central Anatolia (32.4%), followed by Marmara (17.1%), Southeastern Anatolia (13.3%), the Mediterranean (12.8%), Black Sea (10.1%), Aegean (7.4%) and Eastern Anatolia (6.9%) regions.

The quantity and composition of high molecular weight gluten subunits (HMW-GS) are important factors in determining wheat baking properties. Localization of HMW subunit genes on long arms of homologous group 1 was reported by Orth & Bushuk (1974) and Bietzh *et al*. (1975). Each locus contains two linked genes called x and y type which are distinguished by their characteristics and molecular weights (Payne *et al.* 1981). However, as some of those genes are silent, the common wheat possesses 3 to 5 HMW subunits encoded at the Glu-1 loci on the long arms of group 1 chromosomes (1A, 1B, and 1D). The contribution of D genome, followed by B genome considered to have a significant influence on good baking quality (Uthayakumaran *et al.* 2002). Especially, two subunits are expressed always by *Glu-D1*, one or two by *Glu-B1*, one or none (null allele) is expressed by *Glu-A1* loci. If one subunit is expressed by *Glu-A1* or *Glu-B1*, this is always considered as x-type. Rheological properties of the gluten complex are related to the presence or absence of specific subunits of these proteins. The presence of certain HMW subunits is positively correlated with good bread-making quality to determine gluten elasticity (Nakamura 2000, Shewry *et al.* 2003). The relationships between HMW subunits and dough elasticity were determined 40 years ago (Payne *et al.* 1979). Significant differences were found among protein components of wheat grain depending on cultivars, environments and their interactions (Horvat *et al.* 2015, Tok *et al.* 2011). Allelic variations in each *Glu-1* loci were reported in bread wheat genotypes (Lawrence & Shepherd 1980, Payne & Lawrence 1983) and an enumeration system was developed to define different allelic subunits. The definition of HMW subunits coded by *Glu-D1* and *Glu-A1* was described by Payne *et al.* (1983) and Lafiandra *et al.* (1997).

Subunits of gluten proteins can be identified by Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis. Considering the fact that chains of gluten polymers are stabilized by disulfide bonds, using reducing agents such as DTT and mercaptoethanol in SDS-PAGE makes the identification of gluten subunits easier.

Although Turkey comes third in the production of wheat in the world, quality problems still persist. This study was performed to determine the composition and occurrence frequency of HMW glutenin subunits and the potential of the end-use quality in Turkish wheat landraces to be used in future breeding programs. Analysis of broad collections of landraces enabled the definition of rare alleles on *Glu-1* loci.

Table 1. The wheat specimens studied with their respective distribution regions. All specimens were given using their gen bank accession numbers.

Regions	Wheats (with gen bank accession numbers)	Total Number
Central Anatolia	TR45324-2, TR48061-4, TR53299-1, TR53343-5, TR55002-3, TR46889-2, TR45094, TR47944, TR63316-4, TR35409-1, TR52021-6, TR52021-3, TR35408-4, TR35147-4, TR45303-4, TR45306-2, TR45306-5, TR45308-2, TR53862-5, TR55153-4, TR55164-1, TR55180-1, TR57999-2, TR57999-6, TR32034, TR63536, TR63538	27
Aegean	TR52860-3, TR52860-6, TR52865-1, TR55127-6, TR55140-1, TR55144-1, TR55174-1, TR55201-4, TR52784, TR52873, TR56099	11
Marmara	TR33264-6, TR51937-1, TR51937-2, TR33500-4, TR33500-6, TR38316-4, TR52645, TR52669, TR51937, TR44365, TR45080, TR26746	12
Mediterranean	TR46804, TR52824, TR55316, TR55110, TR37492-1, TR37492-2, TR37492-4, TR32009-5, 393-5, TR26233, TR62808	11
Black Sea	TR14861-5, TR44487-4, TR46873-4, TR44365-4, TR44365-5, TR44984-5, TR32125-3, TR37234-1, TR45105, TR36948-1, TR46861-3, TR44388-6, TR44433-5, TR48373-1, TR54988-1, TR54989-4, TR37383	17
Eastern Anatolia	TR32650-5, TR32668-2, TR32780-2, TR32846-6, TR45370-2, TR48034-6, TR32231-5, TR32231-6, TR45420-5, TR45422-2, TR32273-4, TR39676-4, TR45402-3, TR45398-4, TR47993-1, TR32761-6, TR32881-2, TR39660-1, TR45105-6, TR48025-3, TR47961, TR48050, TR31894-3, TR31894-6, TR32014-5, TR32218, TR63329, TR63322	28
Southeastern Anatolia	TR32218-1, TR32218-5, TR32218-6, TR50443, TR46810-5, TR46822-2, TR38888-6, TR50455-4, TR49018-1, TR31678	10

Table 2. The sequence data for the primers used and expected fragment sizes.

	Alleles	Primer sequences	Expected fragment sizes (bp)	References
P1	Dx2, Dx5	F: GCCTAGCAACCTTCACAATC R: GAAACCTGCTGCGGACAAG	450	Ahmad (2000)
P2	Dy10, Dy12	F: GTTGGCCGGTCGGCTGCCATG R: TGGAGAAGTTGGATAGTACC	576 612	Ahmad (2000)
P3	By18*,By20*,By8, By8*,By9,By16	F:GCAGTACCCAGCTTCTCAA R:CCTTGTCTTGTTTGTTGCC	290-400	Salmanowicz&Dylewicz (2007)
P4	*Axnull*	F: ACGTTCCCCTACAGGTACTA R:TATCACTGGCTAGCCGACAA	920	Salmanowicz&Dylewicz (2007)
P5	*Ax1 + Ax2**	F:CCATCGAAATGGCTAAGCGG R:GTCCAGAAGTTGGGAAGTGC	1500	Salmanowicz&Dylewicz (2007)
P6	*Ax2**	F:CCGATTTTGTTCTTCTCACAC R:CACCAAGCGAGCTGCAGAT	2652	Salmanowicz & Dylewicz (2007)
P7	By20*,By8,By8* By18*, By9	F:TTCTCTGCATCAGTCAGGA R:AGAGAAGCTGTGTAATGCC	750, 710, 660	Salmanowicz & Dylewicz (2007)
P8	*Bx7*	F:ATGGCTAAGCGCCTGGTCCT R:TGCCTGGTCGACAATGCGTCGCTG	2373	Ahmad (2000)
P9	By8	F:TTAGCGCTAAGTGCCGTCT R:TTGTCCTATTTGCTGCCCTT	527	Salmanowicz & Dylewicz (2007)
P10	*Bx14* and *Bx17* cauBx752	F:AGGGGCAGGGAAGAAACACT R:CCAGGCAACACAAATCCATG	642 and 534	Xu *et al.* (2008)
P11	*Bx14* and *Bx17* CauBx642	F:GGGCAATCGGGGTACTTCC R:CCCTTGTCTTGGCTGTTGTC	642 and 534	Xu *et al.* (2008)
P12	Ax2*	F: ATGACTAAGCGGTTGGTTCTT R: ACCTTGCTCCCCTTGTCTTT	1400	Ma *et al.* (2003)
P13	Bx	F: CGCAACAGCCAGGACAATT R: AGAGTTCTATCACTGCCTGGT	650-750	Ma *et al.* (2003)
P14	Bx6, Bx7, Bx7*	F:CAAGGGCAACCAGGGTAC R:AGAGTTCTATCACTGCCTGGT	(850-920), (420-640), (180-280)	Salmanowicz & Dylewicz (2007)
P15	GluD1y10	F:5'-CAACCAATCTCCACAATC-3' R:5'-CTGCAGAGAGTTCTATCA-3'	2210	De Bustos & Jouve (2003)
P16	GluA1-1, 2* Co-dominant Ax2*	F:5'- AAGACAAGGGGAGCAAGGT-3' R:5'- GTGCTCCGCGCTAACATG-3'	1090 1063	Radovanovic & Cloutier (2003)
P17	GluB1 7*, H7 Dominant Bx7	F:5'- CAACAACTTGTGGGGGCCTT-3' R:5'-GCGCTTAGCCATCTCAGTGAAC-3'	1116	Radovanovic & Cloutier (2003)
P18	Co-dominant Bx7	F:5'-ACCTCAGCATGCAAACATG-3' R:5'- GCGCTTAGCCATCTCAGTGAAC-3'	530, 1259, 1302, 3200	Radovanovic & Cloutier (2003)
P19	GluD1- 2, 5 Dx5 Dominant	F:5'-CGTCCCTATAAAAGCCTAGCC-3' R:5'-GGCTAATGTCTCGGAGCTGT-3'	272	Radovanovic & Cloutier (2003)
P20	AX Ax2*, Ax1	F:5'-ATGACTAAGCGGTTGGTTCTT-3' R:5'-GACCTTGCTCCCCTTGTCTTT-3'	1319	Ma *et al.* (2003)
P21	AX Ax2*, Ax1	F:5'-ATGACTAAGCGGTTGGTTCTT-3' R:5'-GACCTTGCTCCCCTTGTCCTG-3'	1500	Ma *et al.* (2003)
P22	Dy10	F:5'-GACAGTCCACCGAGATGG-3' R:5'-GCAAGCTGCAGAGAGTTC-3'	1400, 2000	Mishra *et al.* (2009)
P23	Dx5	F:5'-CATGGTCCTGAACCTTCACC-3' R:5'-CAGAGAGTTCTATCACTGGC-3'	2000	Mishra *et al.* (2009)
P24	1Ax1 (Ax2)	F:5'-CCGAGATGACTAAGCGG-3' R:5'-GCTAACATGGTATGGGCT-3'	1800, 2500	Mishra *et al.* (2009)
P25	Dx5 Dx2	F:5'-CGTCCCTATAAAAGCCTAG-C-3' R:5'-AGTATGAAACCTGCTGCGGAC-3'	478	Ma *et al.* (2003)
P26	Dx5 Dx2	F:5'-CGTCCCTATAAAAGCCTAG-C-3' R:5'-AGTATGAAACCTGCTGCGGAG-3'	450	Ma *et al.* (2003)
P27	Dx5 Dx2	F:5'CGTCCCTATAAAAGCCTAGTT-3' R:5'-AGTATGAAACCTGCTGCGGAC-3'	450	Ma *et al.* (2003)
P28	Dx5 Dx2	F:5'-CGTCCCTATAAAAGCCTAGTT-3' R:5'-AGTATGAAACCTGCTGCGGAG-3'	450	Ma *et al.* (2003)
P29	1Dy10.1	F:5'-ATGGCTAAGCGGC/TTA/GGTCCTCTTTG-3' R:5'-CTATCACTGGCTG/AGCCGACAATGCG-3'	372	Jiang *et al. (*2006)
P30	degenere primer	F:ATCACCCACAACACCGAGCA-3'G R:CTATCACTGGCTA/GGCCGACAATGCG	1800, 2000	Ma *et al.* (2003)
P31	degenere primer	F:AGGGAAAGACAATGGACATG R:TAGTTG/TCCC/TAGAGGCCTCACCTTC	1800, 2000, 2100, 2500	Jiang *et al.* (2006)

Materials and Methods

Plant materials

116 bread wheat landraces of Turkey obtained from Ankara Field Crops Central Research Institute and Izmir Aegean Agricultural Research Institute were included in the study (Table 1). The specimens were so selected to provide representation of all geographical regions (Aegean, Central Anatolia, Marmara, the Mediterranean, Black Sea, Eastern Anatolia and Southeastern Anatolia) in the country. These were supplied from the "Field Crops Central Research Institute, Ankara" and "Aegean Agricultural Research Institute, İzmir. 15 standard genotypes (Chinese Spring, Drago, Lobeiro, Svevo, Lira, Durambo, Ak 702, Bezostaya 1, KateA1, Bayraktar, Mizrak, Yakar, Atay 85, Gerek 79 and Tosunbey) were used as references to define gluten alleles The wheat specimens studied with their respective distribution regions is provided in Table 1.

DNA extraction and PCR analysis

DNA was isolated from the seeds according to the method described by McCarthy *et al.* (2002) using 31 primer pairs (Table 2). PCR reaction was realized in 15 µl reaction mixture containing 50 nmol of each primer, 0.3 nmol dNTP, 1-2 unit Taq polymerase, 1.5-2.5 mM $MgCl_2$, 10 µg/µl BSA and 30 ng template DNA. Following the initial denaturation at 94°C for 5 minutes, PCR reaction was carried out in 35 cycles of 94°C for 1 minute, 54-65°C for 1-2 minutes and 72°C for 2 minutes. The last extension step was realized at 72°C for 10 minutes. PCR products were run in 1-1.5% agarose-containing ethidium bromide and imaged with a gel documentation system (BioRad MP5 gel documentation system).

Gluten extraction and SDS-PAGE analyses

Gluten proteins were extracted in accordance with the methods described by Gao *et al.* (2012) and Temizgul *et al.* (2018). The methods of Li *et al.* (2012) with slight modification were used for the electrophoresis of gluten proteins. 30-60 mA current was applied per gel during electrophoresis. Gels were stained (dissolved in 187.5 ml of methanol, 225 mg of CBB-R 250; 750 ml 10% TCA and 62.5 ml glacial acetic acid) for 12-24 hours and kept in washing solution (333 ml methanol, 100 ml 10% TCA and 567 ml distilled water) for 5 hours and imaged under the gel documentation system.

Definition of gluten alleles

Gluten alleles of the wheat landraces were defined based on individual HMW subunit distributions in SDS-PAGE in accordance with the methods specified by Payne & Lawrence (1983) and McIntosh *et al.* (1994). The verification of alleles defined by allele-specific primers was also performed.

Calculation of Glu-1 quality scores

Based on the SDS-PAGE profile, relationships between individual HMW subunits and quality was determined by using the method described by Payne (1987a) and scored by using subunit scores of Payne (1987a) and Lukow *et al.* (1989).

POPGENE version 1.31 software was used to draw dendrogram based on Nei's original measurement showing the relationships among the genotypes based on individual subunits (Nei 1972).

Statistical analysis

Data analysis was carried out based on the frequencies of HMW gluten gene alleles. Statistical analyses were performed separately in individual variety and populations for individual alleles, individual sub-units, loci (*Glu-A1*, *Glu-B1*, and *Glu-D1*) and geographical origin (7 regions). POPGENE version 1.31 software was used for statistical analysis. Polymorphism percentages were calculated using Equation 1;

Eq. 1: Polymorphism% = (number of polymorphic allele/number of total alleles) × 100.

Allele frequency of *Glu-1* loci (*Glu-A1*, *Glu-B1*, and *Glu-D1*) was calculated according to Gupta *et al.* (1991). Percentage allele frequencies were calculated by using Equation 2;

Eq. 2: Allele frequency = (number of individual alleles/number of total wheat samples) × 100.

Allelic combination frequencies were calculated using Equation 3;

Eq. 3: Allelic combination frequency = (observed total number of each allelic combinations/number of total wheat samples) × 100.

Results

In A genome of studied genotypes, the frequencies of *Glu-A1*c, *Glu-A1*b, and *Glu-A1*a were calculated to be 65.1%, 26.4%, and 8.5%, respectively (Table 3). In B genome, the frequencies of *Glu-B1*b, *Glu-B1*e, *Glu-B1*d, and *Glu-B1*c were determined to be 44.6%, 16.6%, 10.0%, and 10.7%, respectively. In D genome, the frequencies of *Glu-D1*a, *Glu-D1*d, and *Glu-D1*b were determined to be 59.7%, 27.9%, and 7.7%, respectively. Since quality scores were not determined for these subunits, the contributions of the alleles *Glu-B1*h, *Glu-B1*z, and *Glu-B1*aj to quality score could not be calculated for 7 of the 116 genotypes studied. After determination of rheological characteristics of individual subunits, the contribution of these subunits to the quality can also be determined. The average quality score in the genotypes was calculated to be 6.07. The Marmara and the Southeastern Anatolia regions had 7.18 and 4.8 quality scores as the highest and the lowest, respectively.

Subunit frequencies

Total and region-based subunit frequencies were given in Table 3. The highest frequencies were observed as 65.11%, 54.30%, and 58.30% for the subunits *Glu-A1*c, *Glu-B1*b, and *Glu-D1*a, respectively. *Glu-1* genome subunit frequencies are given in Table 3. Based on the

regions, the frequencies of *Glu-A1a* (18.2%), *Glu-A1b* (50.0%), *Glu-A1c (*80.0%), *Glu-B1b* (58.0%), *Glu-B1e* (46.0%), *Glu-D1a* (86.7%), *Glu-D1d* (50.0%), were the highest in Mediterranean, Marmara, Aegean, Black Sea, Southeastern Anatolia, Aegean and Marmara, respectively.

Table 3. Total and region-based subunit frequencies (%).

Subunits \ Regions		Marmara	Aegean	Mediterranean	Central Anatolia	Black Sea	Eastern Anatolia	South Eastern Anatolia	Total Frequency
	Glu-A1								
a	1	14.2	13.3	18.2	-	15.8	3.3	8.3	8.5
b	2*	50.0	6.7	18.2	32.1	31.6	23.3	16.7	26.4
c	Null	35.8	80.0	63.6	67.9	52.6	73.3	75.0	65.1
	Glu-B1								
a	7	7.0	5.0	-	-	-	-	-	1.7
b	7+8	27.5	56.0	17.0	52.0	58.0	56.0	46.0	44.6
c	7+9	32.0	-	8.0	14.0	9.0	3.3	8.0	10.7
d	6+8	-	24.0	8.0	31.0	4.0	3.0	-	10.0
e	20	13.0	5.0	17.0	3.0	20.0	12.3	46.0	16.6
f	13+16	-	-	-	-	-	6.0	-	0.9
h	14+15	-	-	50.0	-	-	3.3	-	7.6
I	17+18	-	5.0	-	-	-	-	-	0.7
u	7*+8	-	-	-		-	6.7	-	1.0
aj	8	7.0	5.0	-		9.0	-	-	3.0
z	7+15	13.5	-	-		-	9.4	-	3.2
	Glu-D1								
a	2+12	50.0	86.7	45.5	67.9	63.2	40.0	58.3	58.3
b	3+12	-	6.7	22.0	10.7	-	10.0	8.3	8.2
c	4+12	-	-	10.5	3.6	5.3	3.3	8.3	4.3
d	5+10	50.0	6.7	22.0	17.9	31.6	40.0	25.0	27.6
h	2+12*	-	-	-	-	-	6.7	-	1.6

Table 4. Allelic combinations and the frequencies.

Allelic combinations	Subunit combinations	Frequency (%)	Allelic combinations	Subunit combinations	Frequency (%)
*Glu-B1*b/*Glu-D1*a	(7+8, 2+12)	22.48	*Glu-A1*b/*Glu-B1*d/*Glu-D1*c	(2*, 6+8, 4+12)	0.78
*Glu-A1*c/*Glu-B1*b/*Glu-D1*a	(Null, 7+8, 2+12)	11.70	*Glu-A1*a/*Glu-B1*c/*Glu-D1*a	(1, 7+9, 2+12)	0.78
*Glu-A1*b/*Glu-B1*c/*Glu-D1*d	(2*, 7+9, 5+10)	9.35	*Glu-A1*b/*Glu-B1*c/*Glu-D1*b	(2*, 7+9, 3+12)	0.78
*Glu-B1*d/*Glu-D1*a	(6+8, 2+12)	5.45	*Glu-B1*z/*Glu-D1*d	(7+15, 5+10)	0.78
*Glu-A1*b/*Glu-B1*b/*Glu-D1*d	(2*, 7+8, 5+10)	4.70	*Glu-B1*e/*Glu-D1*d	(20, 5+10)	0.78
*Glu-B1*b/*Glu-D1*d	(7+8, 5+10)	4.70	*Glu-A1*c/*Glu-B1*b/*Glu-D1*d	(Null, 7+8, 5+10)	0.78
*Glu-A1*c/*Glu-B1*d/*Glu-D1*a	(Null, 6+8, 2+12)	3.15	*Glu-B1*e/*Glu-D1*c	(20, 4+12)	0.78
*Glu-B1*e/*Glu-D1*a	(20, 2+12)	3.15	*Glu-B1*b/*Glu-D1*c	(7+8, 4+12)	0.78
*Glu-A1*b/*Glu-B1*d/*Glu-D1*b	(2*, 6+8, 3+12)	2.35	*Glu-A1*b/*Glu-B1*e/*Glu-D1*d	(2*, 20, 5+10)	0.78
*Glu-A1*a/*Glu-B1*e/*Glu-D1*a	(1, 20, 2+12)	2.35	*Glu-A1*b/*Glu-B1*i/*Glu-D1*d	(2*, 17+18, 5+10)	0.78
*Glu-A1*b-c/*Glu-B1*b/*Glu-D1*b	(2*, 7+8, 3+12)	1.56	*Glu-A1*a/*Glu-B1*aj/*Glu-D1*a	(1, 8, 2+12)	0.78
*Glu-A1*c/*Glu-B1*z/*Glu-D1*a	(Null, 7+15, 2+12)	1,56	*Glu-A1*a/*Glu-B1*f/*Glu-D1*a	(1, 13+16, 2+12)	0.78
*Glu-B1*z/*Glu-D1*a	(7+15, 2+12)	1.56	*Glu-A1*a/*Glu-B1*aj-e/*Glu-D1*d	(1, 8, 20, 5+10)	0.78
*Glu-B1*b/*Glu-D1*b	(7+8, 3+12)	1.56	*Glu-A1*a/*Glu-B1*e/*Glu-D1*a	(1, 20, 2+12)	0.78
*Glu-B1*u/*Glu-D1*a	(7*+8, 2+12)	1.56	*Glu-A1*a/*Glu-B1*z/*Glu-D1*a	(1, 7+15, 2+12)	0.78
*Glu-A1*c/*Glu-B1*e/*Glu-D1*d	(Null, 20, 5+10)	1.56	*Glu-A1*b/*Glu-B1*d/*Glu-D1*d	(2*, 6+8, 5+10)	0.78
*Glu-A1*c/*Glu-B1*e/*Glu-D1*a	(Null, 20, 2+12)	1.56	*Glu-A1*b/*Glu-B1h*/*Glu-D1*d	(2*, 14+15, 5+10)	0.78
*Glu-A1*b/*Glu-B1*b/*Glu-D1*c	(2*, 7+8, 4+12)	1.56	*Glu-A1*b/*Glu-B1*f/*Glu-D1*d	(2*, 13+16, 5+10)	0.78
*Glu-A1*c/*Glu-B1*b/*Glu-D1*b	(Null, 7+8, 3+12)	1.56	*Glu-A1*a/*Glu-B1*f	(1, 13+16)	0.78
*Glu-A1*b/*Glu-B1*a/*Glu-D1*d	(2*, 7, 5+10)	0.78			

Table 5. Region-based allelic combinations and their frequencies.

Region	Allelic combinations	Subunit combinations	Frequency (%)	Glu-1 Score	Average Glu-1 score
Marmara	*Glu-A1*b/*Glu-B1*c/*Glu-D1*d	(2*, 7+9, 5+10)	35.71	9	7.18 ± 2.34
	*Glu-B1*b/*Glu-D1*a	(7+8, 2+12)	14.29	5	
	*Glu-A1*b/*Glu-B1*a/*Glu-D1*d	(2*, 7, 5+10)	7.14	9	
	*Glu-B1*e/*Glu-D1*a	(20, 2+12)	7.14	3	
	*Glu-A1*a/*Glu-B1*e/*Glu-D1*a	(1, 20, 2+12)	7.14	6	
	*Glu-A1*c/*Glu-B1*b/*Glu-D1*a	(null, 7+8, 2+12)	7.14	6	
	*Glu-B1*z/*Glu-D1*a	(7+15, 2+12)	7.14	?	
Aegean	*Glu-A1*c/*Glu-B1*b/*Glu-D1*a	(null, 7+8, 2+12)	26.67	6	5.10 ± 1.42
	*Glu-B1*b/*Glu-D1*a	(7+8, 2+12)	20.00	5	
	*Glu-A1*c/*Glu-B1*d/*Glu-D1*a	(null, 6+8, 2+12)	13.33	3	
	*Glu-A1*c/*Glu-B1*b/*Glu-D1*b	(null, 7+8, 3+12)	6.67	6	
	*Glu-A1*a/*Glu-B1*aj/*Glu-D1*a	(1, 8, 2+12)	6.67	?	
Mediterranean	*Glu-B1*b/*Glu-D1*a	(7+8, 2+12)	18.18	5	5.70 ± 2.14
	*Glu-B1*b/*Glu-D1*b	(7+8, 3+12)	18.18	5	
	*Glu-A1*b/*Glu-B1*d/*Glu-D1*d	(2*, 6+8, 5+10)	9.09	8	
	*Glu-A1*b/*Glu-B1*c/*Glu-D1*d	(2*, 7+9, 5+10)	9.09	9	
	*Glu-B1*b/*Glu-D1*c	(7+8, 4+12)	9.09	4	
	*Glu-B1*e/*Glu-D1*a	(20, 2+12)	9.09	3	
	*Glu-A1*c/*Glu-B1*b/*Glu-D1*a	(null, 7+8, 2+12)	9.09	6	
Central Anatolia	*Glu-B1*b/*Glu-D1*a	(7+8, 2+12)	39.29	5	5.63 ± 2.38
	*Glu-B1*d/*Glu-D1*a	(6+8, 2+12)	14.29	3	
	*Glu-A1*b/*Glu-B1*c/*Glu-D1*d	(2*, 7+9, 5+10)	14.29	9	
	*Glu-A1*b/*Glu-B1*b/*Glu-D1*b	(2*, 7+8, 3+12)	10.71	6	
	*Glu-A1*c/*Glu-B1*d/*Glu-D1*a	(null, 6+8, 2+12)	7.14	4	
	*Glu-A1*b/*Glu-B1*b/*Glu-D1*d	(2*, 7+8, 5+10)	3.57	10	
	*Glu-A1*b/*Glu-B1*b/*Glu-D1*c	(2*, 7+8, 4+12)	3.57	7	
	*Glu-A1*c/*Glu-B1*b/*Glu-D1*a	(null, 7+8, 2+12)	3.57	6	
Black Sea	*Glu-B1*b/*Glu-D1*a	(7+8, 2+12)	26.32	5	6.65 ± 1.73
	*Glu-A1*c/*Glu-B1*b/*Glu-D1*a	(null, 7+8, 2+12)	21.05	6	
	*Glu-A1*b/*Glu-B1*c/*Glu-D1*d	(2*, 7+9, 5+10)	5.26	9	
	*Glu-B1*b/*Glu-D1*d	(7+8, 5+10)	5.26	7	
	*Glu-A1*b/*Glu-B1*b/*Glu-D1*d	(2*, 7+8, 5+10)	5.26	10	
	*Glu-A1*b/*Glu-B1*d/*Glu-D1*c	(2*, 6+8, 4+12)	5.26	5	
	*Glu-A1*c/*Glu-B1*aj-e/*Glu-D1*a	(null, 8, 20, 2+12)	5.26	?	
	*Glu-A1*a/*Glu-B1*c/*Glu-D1*a	(1, 7+9, 2+12)	5.26	7	
	*Glu-A1*b/*Glu-B1*e/*Glu-D1*d	(2*, 20, 5+10)	5.26	8	
	*Glu-A1*a/*Glu-B1*e/*Glu-D1*a	(1, 20, 2+12)	5.26	6	
Easter Anatolia	*Glu-B1*b/*Glu-D1*d	(7+8, 5+10)	16.67	7	6.57 ± 2.10
	*Glu-B1*b/*Glu-D1*a	(7+8, 2+12)	10.00	5	
	*Glu-B1*u/*Glu-D1*a	(7*+8, 2+12)	6.67	5	
	*Glu-A1*c/*Glu-B1*e/*Glu-D1*a	(null, 20, 2+12)	6.67	4	
	*Glu-A1*c/*Glu-B1*h/*Glu-D1*a	(null, 14+15, 2+12)	3.33	?	
	*Glu-A1*b/*Glu-B1*b/*Glu-D1*d	(2*, 7+8, 5+10)	3.33	10	
	*Glu-A1*b/*Glu-B1*b/*Glu-D1*b	(2*, 7+8, 3+12)	3.33	7	
	*Glu-A1*c/*Glu-B1*d/*Glu-D1*a	(null, 6+8, 2+12)	3.33	4	
	*Glu-A1*c/*Glu-B1*z/*Glu-D1*a	(null, 7+15, 2+12)	3.33	?	
	*Glu-B1*z/*Glu-D1*h	(7+15, 2+12*)	3.33	?	
	*Glu-B1*z/*Glu-D1*d	(null, 7+15, 5+10)	3.33	?	
	*Glu-B1*b/*Glu-D1*h	(7+8, 2+12*)	3.33	5	
	*Glu-A1*b/*Glu-B1*c/*Glu-D1*d	(2*, 7+9, 5+10)	3.33	9	
	*Glu-A1*c/*Glu-B1*b/*Glu-D1*d	(null, 7+8, 5+10)	3.33	8	
	*Glu-A1*b-c/*Glu-B1*f/*Glu-D1*d	(2*, 13+16, 5+10)	3.33	10	
	*Glu-A1*c/*Glu-B1*b/*Glu-D1*b	(null, 7+8, 3+12)	3.33	6	
	*Glu-A1*c/*Glu-B1*e/*Glu-D1*d	(null, 20, 5+10)	3.33	6	
South Eastern Anatolia	*Glu-B1*b/*Glu-D1*a	(7+8, 2+12)	16.67	5	4.80 ± 1.77
	*Glu-B1*e/*Glu-D1*a	(20, 2+12)	16.67	3	
	*Glu-A1*c/*Glu-B1*b/*Glu-D1*a	(null, 7+8, 2+12)	16.67	6	
	*Glu-B1*e/*Glu-D1*c	(20, 4+12)	8.33	2	
	*Glu-A1*b/*Glu-B1*c/*Glu-D1*b	(2*, 7+9, 3+12)	8.33	7	
	*Glu-A1*c/*Glu-B1*e/*Glu-D1*d	(null, 20, 5+10)	8.33	6	
	*Glu-B1*e/*Glu-D1*d	(20, 5+10)	8.33	5	

Allelic combinations and frequencies

Allelic combinations and their frequencies are given in Table 4. The results indicated the presence of 39 different allelic combinations. Among these combinations, *Glu-B1*b/*Glu-D1*a was the most frequent combination with a 22.48% frequency value, followed by *Glu-A1*c/*Glu-B1*b/*Glu-D1*a, *Glu-A1*b/*Glu-B1*c/*Glu-D1*d, and *Glu-B1*d/*Glu-D1*a allelic combinations with 11.7%, 9.35%, and 5.45% frequencies, respectively. The rest of the alleles were observed with a frequency less than 5%.

Region-based allelic combinations and Glu-1 scores

The frequencies of HMW gluten subunits and their *Glu-1* scores are given in Table 5. In some of the genotypes, the effect of some subunits on quality score could not be calculated since the effect of these subunits on quality score was not determined. The quality score was 10 in four accessions (TR32846-6, TR36948-1, TR45105 and TR63536), and 9 in 6 accessions (TR45398-4, TR48025-3, TR33264-6, 393-5, TR52021-3, TR45094). The highest (17) and the lowest (5) allelic combinations were observed in Eastern Anatolia and Aegean regions, respectively (Table 5).

Development of dendrogram based on subunits of Glu-1 loci

When the dendrograms (Nei 1972) drawn based on individual subunits were investigated, the Mediterranean region clustered with Black sea (genetic distance 0.32) and Aegean region clustered with Central Anatolia (genetic distance 0.52) (Fig. 1). Marmara (genetic distance 1.35), Eastern Anatolia (genetic distance 0.37), and Southeastern Anatolia (genetic distance 0.52) regions were separately clustered.

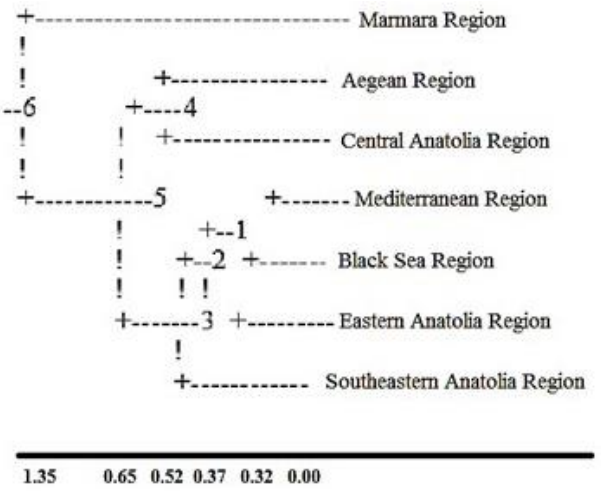

Fig. 1. Dendrogram showing the relationships of the genotypes based on individual subunits of *Glu-1* loci.

Discussion

The determination of HMW-GS composition in wheat cultivar collections from different countries has been studied (Nucia *et al.* 2019). The contribution of individual subunits on dough quality is quite different (Payne *et al.* 1987, Lukow *et al.* 1989). While quality score of *Glu-D1*d is the highest (quality score 4), the contribution of *Glu-A1*c, *Glu-B1*a, *Glu-B1*d, *Glu-B1*e, and *Glu-D1*c is considered to be the lowest (quality score 1). In the present study, the highest *Glu-D1*d frequency (50%) was observed in Marmara and the lowest was observed in Aegean regions (6.67%). The frequency of this subunit was 27.6% throughout Turkey (Table 2). The highest *Glu-1* score with 7.18 was observed in Marmara followed by Black Sea with 6.65, Eastern Anatolia with 6.57, Mediterranean with 5.70, Central Anatolia with 5.63, Aegean with 5.10, and Southeastern Anatolia regions with 4.8 (Table 5).

Observing the highest quality score in Marmara and the lowest in the Southeastern region is an expected outcome. Southeastern Turkey is on the interception of gene centers for wheat and its wheat is intensively studied and high-quality score wheat genotypes are already selected for breeding. Marmara region is the most industrialized region and agricultural activity is quite poor in this region, therefore, the landraces might not be used to select for high-quality score cultivar breeding. Throughout Turkey, 19 different subunits (3 *Glu-A1*, 11 *Glu-B1* and 5 *Glu-D1*) were observed in 39 different combinations (Tables 3 and 4). Among the allelic combinations *Glu-B1*b/*Glu-D1*a was the highest with 22.48% frequency, followed by *Glu-A1*c/*Glu-B1*b/*Glu-D1*a with 11.70%, *Glu-A1*b/*Glu-B1*c/*Glu-D1*d with 9.35% and *Glu-B1*d/*Glu-D1*a with 5.45%. Other alleles were observed with less than 5% frequency. The highest allelic variation with 16 subunits and 17 different combinations was observed in Southeastern Anatolia. *Glu-B1*b/*Glu-D1*d allelic combinations with 16.67% frequency were the most frequent allelic combinations in this region (Table 5). Based on Nei's original measurement (Nei 1972), while the highest similarity among regions for individual subunits of *Glu-1* loci was observed between Black Sea and the Mediterranean regions (0.9935), the lowest similarity was observed in Marmara and the Aegean regions (0.953). When the dendrogram was drawn based on Nei's (1972) original measurements, the Mediterranean clustered with the Black Sea and the Aegean with the Central Anatolia accessions. Other regions were separately clustered (Fig. 1).

Nakamura *et al.* (1999) studied variations in the HMW subunits of *Glu-1* loci of Kapon wheats and identified 14 different alleles, 3 of which were on *Glu-A1*, 6 on *Glu-B1* and 5 on *Glu-D1* loci. In the present study, 19 different subunits were identified and 3 of them were on *Glu-A1*, 11 of them on *Glu-B1* and 5 of them on *Glu-D1* loci. The frequency of null alleles located on chromosome 1A was reported to be high (74%) on Japanese wheat especially on Norin variety. The frequency of this allele was also found to be high in landraces of Turkish wheat (65.11 %). While *Glu-B1*a, *Glu-B1*f, *Glu-B1*h, *Glu-B1*j, and *Glu-B1*k subunits were not observed in Japanese bread wheat (Nakamura *et al.* 1999), only *Glu-B1*j and *Glu-B1*k

subunits were not observed in Turkish wheat genotypes used in this study. While the frequency of 2+12 subunit coded by *Glu-D1*a allele was 55% in Japanese wheat, it was only 1.5% in 5+10 subunit coded by *Glu-D1*d. The average frequencies of these alleles were found to be 58.3% and 27.6% in Turkish genotypes, respectively. The frequencies of *Glu-D1*a and *Glu-D1*d subunits were 86.7% and 50% in Aegean and Marmara regions, respectively. The frequency of *Glu-D1d* subunit was found to be high in European wheat varieties (Payne *et al.* 1984). The frequency of this subunit was also found to be high in Turkish wheat landraces, especially in Marmara region (average 27.6%, Marmara region 50.0%). Cabellero *et al.* (2009) investigated the seed storage protein diversity of the wild diploid wheat genotypes obtained from Lebanon and Turkey. They determined 10 alleles at *Glu-A1*, 16 alleles at *Glu-A3*, 15 alleles at *Gli-A1*, 18 alleles at *Gli-A2* and detected 4 frequent, 2 infrequent, and 3 rare alleles. In the present study, 11 frequent, 7 infrequent and 4 rare alleles were determined.

Van Hintum & Elings (1991) evaluated the Syrian durum wheat genotypes based on phenotype and gluten content. They observed 19 HMW subunits in 48 different combinations. In the present study, 22 individual alleles and 19 HMW subunits were detected in 39 different combinations. Gianibelli *et al.* (2002) studied molecular and biochemical characterizations of Argentinian wheat cultivars, identified the allelic variations, and calculated the allele frequencies. Of the 11 alleles, 3 were coded by *Glu-A1*, 6 were coded by *Glu-B1* and 2 were coded by *Glu-D1* loci. Null allele frequency was found to be quite low (1.1%) and *Glu-D1*d was the highest in frequency. However, *Glu-A1*c subunit was the highest with the frequency of 65.1% among the alleles in Turkish landraces. Gianibelli *et al.* (2002) also calculated the quality score taking the *Glu-A1* into account and separated the Argentinian wheat into 18 groups. *Glu-A1*a/*Glu-B1*bq/*Glu-D1*d was observed in highest frequency (22%). In Turkish landraces, *Glu-B1*b/*Glu-D1*a was observed in highest frequency.

Payne (1987b) found the quality score of world wheat collection as 9.5. The quality score of Turkish wheat landraces was found to be significantly lower (6.07). Notwithstanding, quality score is not determined by only HMW-GS, the contribution of LMW-GS and Gliadins should also be taken into account (MacRitchie *et al.* 1990).

Payne & Lawrence (1983) published the catalogue of *Glu-1* alleles. They determined 3, 11, and 7 alleles in *Glu-A1, Glu-B1*, and *Glu-D1,* respectively. Additional alleles were also determined but most of those alleles were found to be in *Glu-B1* loci (Pogna *et al.* 1990). In the present study, a possible new allele (Dy12*) was determined in *Glu-D1* locus. This new subunit was differentiated considering its faster movement in SDS-PAGE.

Branlard *et al.* (1989) observed 3 allelic variations in *Glu-A1* of 165 Turkish durum wheat cultivars. *Glu-A1*-1 allele was coding an x subunit that had bigger electrophoretic mobility than 2*. The researchers suggested that this allele was similar to previously discovered two alleles (*Glu-A1*V, *Glu-A1*VI). The null allele with 68.9% frequency was the most frequent allele followed by *Glu-A1*b (28.3%) and *Glu-A1*-1 (3.8%). Seven different *Glu-B1* allele variants were identified in previous studies subjecting the Turkish wheats (Branlard et al. 1989) . These consisted of 5 different x and y type subunit combinations. Of the 7 *Glu-B1*, 5 (*Glu-B1*b, *Glu-B1*d, *Glu-B1*e, *Glu-B1*h, and *Glu-B1*z) were observed among Turkish wheat samples (Branlard *et al.* 1989, Payne *et al.* 1981). In the present study, 11 subunits were determined in *Glu-B1* loci (Table 3).

Primitive cultivars and locally grown landraces were considered to be the sources of variation for grain protein quality, disease resistance, and resistance to abiotic stress conditions (Porceddu *et al.* 1988, Kaplan *et al.* 2014). HMW subunit variations in Turkish bread wheat landraces were found to be higher compared to Australian, Italian, American, Canadian, French and Spanish wheat samples (Autran & Feillet 1985, Margiotta *et al.* 1987, Carrillo *et al.* 1990). This outcome is somewhat expected since intensive breeding works have decreased the variation in western wheat genotypes (Porceddu *et al.* 1988).

Morgunov *et al.* (1993) and Sultana *et al.* (2007) stated that the increase in HMW score was related to a decrease in diversity in gluten alleles. A similar phenomenon was also observed in wheats grown in Dobrudzha Agricultural Institute (Atanasova *et al.* 2009). That is why it is crucial to use *Glu-B1*f, *Glu-B1*h and *Glu-B1*i subunits to increase quality score. This situation may decrease genetic diversity and increase end-product quality (Liu *et al.* 2007). In the present study, the observation of these alleles was also quite low in 116 Turkish wheat landraces (approximately 7%). The frequency of *Glu-B1*h (14+15) was observed to be 55% in the Mediterranean region. Maintenance of *Glu-A1*b (2*) and *Glu-D1*d (5+10) alleles is important. These alleles contribute to quality supported with *Glu-B1*i (17+18), *Glu-B1*f (13+16) and *Glu-B1*h (14+15) (Tsenov *et al.* 2009).

Terasawa *et al.* (2010) studied the genetic variation of high molecular weight gluten subunits and identified 3, 9 and 15 alleles in *Glu-A1*, *Glu-B1* and *Glu-D1*, respectively. *Glu-A1*c (74.4%), *Glu-B1*b (76.5%) and *Glu-D1*a (81.5%) were the most frequently observed alleles. Although *Glu-D1*a (46.9%) was the most frequently observed allele in Central Asia, it was lower in all the other regions except Caucasian region. A total of 83 allelic combinations were determined on *Glu-1* loci in their studies. Among the allelic combinations, *Glu-A1*c/*Glu-B1*b/*Glu-D1*a was the most frequently observed genotype. The frequency of this allelic combination was found to be 11.70% for Turkish cultivars. The most frequently observed allelic combination was *Glu-B1*b/*Glu-D1*a (22.48%). Although western Asian, Afghanistan, and Eastern Asian wheats were exhibiting

similar characteristics, Caucasian and Central Asian wheats differed from these three regions. As it is reported by Terasawa *et al.* (2009) and Lagudah *et al.* (1987), the most common genotypes were determined to be *Glu-A1*c (null), *Glu-B1*b (7+8), and *Glu-D1*a (2+12) among Western and Eastern Asian genotypes. These alleles were also found to be the highest in frequency in the present study. Those results, the results of Terasawa *et al*. (2009) and Lagudah *et al.* (1987) indicated that *Glu-A1*c, *Glu-B1*b, and *Glu-D1*a genotypes were dominant in regions extending from Mesopotamia, Afghanistan, and Far East to Central Asia. In Southern Asia, *Glu-A1*c, *Glu-B1*i, and *Glu-D1*a were the most frequently observed genotypes in a study reported by Terasawa *et al.* (2010). This genotype was considered to be a modified version of the typical Asian genotype in the sense that only *Glu-B1*i allele was replacing *Glu-B1*b allele. *Glu-B1*i allele was observed rarely in other regions of Southern Asia. This allele was also found to be very rare in the present study (0.78%). Similarly, this allele was also rare among European endemic wheat genotypes (Gregova *et al.* 1999, 2006, Juha'sz *et al.* 2003). This is why researchers considered that *Glu-B1*i had appeared in Southern Asia (Terasawa *et al.* 2010). *Glu-B1*i allele was providing more firmness to dough compared to *Glu-B1*b allele (Payne & Lawrence 1983, Mondal *et al.* 2008). *Glu-D1*d allele is common in Caucasian and Central Asian accessions. The high frequency of this allele in Caucasian and Central Asia is remarkable. In the same region *Glu-A1*b and *Glu-B1*a alleles have also been observed in high frequency (Terasawa *et al.* 2010). *Glu-D1*d allele is known to contribute to bread-making quality. This allele is introduced to modern wheat genotypes to increase bread-making quality (Wrigley *et al.* 2015, online). The frequency of this allele was calculated to be 27.91% in the present study and the allele was observed in 50% of the wheat genotypes. This allele was also in high frequency in European wheat (Gregova *et al.* 1999, 2006, Juha'sz *et al.* 2003). However, researchers suggested the Caucasian region as the center of origin for this allele and its dispersion to other regions (Dvorak *et al.* 1998).

In conclusion, the quality score was found to be low in the studied genotypes (6.07). High quality score genotypes might have already been selected for breeding purposes by agricultural institutes. Relatively high quality scores in Black Sea region, where wheat breeding studies is relatively low, and in highly industrialized Marmara region support that claims. Considering the individual alleles in *Glu-1* loci, the highest similarity was observed between Black Sea and the Mediterranean regions (0.9953) and the lowest similarity was between Marmara and Aegean regions (0.9472). When individual alleles and subunits are used for cluster analysis in *Glu-1* loci, the Mediterranean region clustered with Black Sea and Aegean region clustered with Central Anatolia. Other regions are individually clustered and separated from these regions.

Among the studied genotypes, 4 accessions (TR32846-6, TR36948-1, TR45105 and TR63536) were determined to be reaching to the highest score (quality score 10). Of the 116 studied accessions, 6 genotypes (TR45398-4, TR48025-3, TR33264-6, TR393-5, TR52021-3 and TR45094) had the quality score of 9. To investigate new *Glu-1* alleles, more landraces need to be studied. To verify new putative alleles, 2D gel electrophoresis and peptide sequencing could also be applied in addition to PCR and SDS-PAGE.

Although we detected 50 different allelic combinations among the studied accessions, we were able to calculate the quality score of 39 accessions. Glu-B1b/Glu-D1a with 22.48% frequency was the most frequent combination. This was followed by Glu-A1c/Glu-B1b/Glu-D1a, Glu-A1b/Glu-B1c/Glu-D1d and Glu-B1d/Glu-D1a allelic combinations with 11.7%, 9.35% and 5.45% frequencies, respectively. The highest quality scores were observed in Glu-A1b/Glu-B1c/Glu-D1d (quality score 10 with 4.70% frequency) and in Glu-A1b/Glu-B1b/Glu-D1d (quality score 9 with 9.35% frequency) allelic combinations, respectively.

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