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Genetic diversity of high-molecular-weight glutenin subunit compositions in bread wheat landraces originated from Turkey

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Abstract

Focusing on 116 bread wheat landraces, this study investigated high molecular weight glutenin allele polymorphism, gene diversity, genetic variation and linkage disequilibrium (LD) in *Glu-1* loci. To identify gluten alleles, sodium dodesyl sulphate-polyacrylamide, gel electrophoresis was used and for statistical analyses POPGENE software was employed. The results indicated that average genetic variation (h) was the highest in *Glu-B1* (0.6421) and the lowest in *Glu-A1* locus (0.4548); genetic similarity ratio (I) was the highest in *Glu-B1* (1.4170); the highest average genetic diversity (Ht) was observed in *Glu-B1* (0.6575) and the lowest diversity was observed in *Glu-A1* (0.4558). It was also observed that genetic diversity in *Glu-1* locus was largely due to intra-population variations. Inter-population gene flow was also calculated as 4.0051. Marmara and Southeastern Anatolia regions, the results further indicated, had the highest (2.8691) and lowest (0.1694) heterozygosity. Genetic erosion risk for Turkish bread wheat landraces was also seen to be high. Considering the mutual analyses of subunits of nationwide wheat landraces, it is possible to speculate about a limited migration between the landraces. LD of the landraces was largely because of this limited migration and/or epistatic natural selection. Since Turkey is known as the gene centre for major cereals including wheat, barley, rye and oat, where they diversified and spread throughout the world, studying the gluten allele diversity of Turkish bread wheat landraces is important. In addition, this study has revealed the applicability of LD, and neutrality tests to gluten protein diversity for the first time.

Keywords: allele frequency, genetic diversity, genetic similarity, HMW gluten, linkage disequilibrium

Introduction

There are eight regions throughout the world where agricultural practices have been carried out for centuries and which can be classified as gene centres of various species (Vavilov 1951). Turkey is located right at the intersection of two of these centres (Near East and the Mediterranean) and is known as the gene centre for major cereals such as wheat, barley, rye and oat, from which these types diversified and spread throughout the world. These early cultivated forms were diploid (AA) (Einkorn) and tetraploid (AABB) (Emmer) wheat originated in the southeastern parts of Turkey (Heun *et al.*, 1997; Dubcovsky and Dvorak, 2007). Cultivation of these early wheats shifted from there to near east about 9000 years ago and hexaploid bread wheat emerged then for the first time (Feldman, 2001).

High molecular weight glutenin subunits (HMW-GS) are encoded by *Glu*-1 loci present on the long arms of the

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homologous group 1 chromosomes of bread wheat (1A, 1B and 1D) at the *Glu-A1, Glu-B1* and *Glu-D1* loci (Payne *et al.,* 1986). Each locus contains two closely linked genes coding for an *x* and *y* type subunit (Payne *et al.,* 1981), but a variable number of subunits, ranging from three to five, are observed in different bread wheat cultivars. Allelic variation has been reported to be present at each *Glu-1* locus of bread wheat; therefore, a numbering system was used to describe different allelic subunits (Payne *et al.,* 1981; Payne and Lawrence, 1983). HMWGS are part of the glutenin polymers with a number of subunits and allelic variations strongly influencing molecular weight distribution of polymers and, consequently, dough properties (Payne, 1987).

Ewens–Watterson (EW) presented a neutrality test through using allele frequencies to determine the effects of selection (Ewens, 1972). While directional selection supports one or more alleles, balanced selection holds most of the alleles in relatively equal frequencies. EW test employs observed homozygosity statistics (F_{obs} is defined as the sum of squares of allele frequencies) and compares it with the expected value. When the null hypothesis of natural evolution is rejected, this test recommends either balanced selection ($F_{obs} << F_{exp}$) or directional selection ($F_{obs} >> F_{exp}$) impact. EW test was carried out to determine the selection impacts in landraces of bread wheat in Turkey.

Linkage disequilibrium (LD) represents non-random associations causing deviations of alleles on each close-linked locus from the frequencies calculated with Hardy–Weinberg equation (Weir, 1979). Allele or genetic indicator combinations in LD results in deviations from the combination expected from randomized formation of haploids based on the frequencies of these alleles in population. Non-randomized combinations of polymorphisms over certain loci are measured with their LD (Weir, 1979; Ohta, 1982). When the population subunit structuring is prevented and previous subpopulations continue to combine randomly, average homozygosity decreases and heterozygosity increases accordingly. The phenomenon of decreased homozygosity due to combined subpopulations is known as 'Wahlund effect'.

The present study was conducted with the bread wheat landraces of Turkey to determine the genetic variations, diversity, LD, sample heterozygosity and genetic erosion risks.

Material and methods

Wheat materials

In this study, 116 bread wheat landraces originating from different regions of Turkey (Aegean, Central Anatolia, Marmara, Mediterranean, Black Sea, Eastern Anatolia and Southeastern Anatolia) were used (online Supplementary Table S1). They were supplied from 'Ankara Field Crops Central Research Institute' and 'Izmir Aegean Agricultural Research Institute'. Fifteen standard genotypes (Chinese Spring, Drago, Lobeiro, Svevo, Lira, Durambo, Ak 702, Bezostaya 1, Kate-A1, Bayraktar, Mızrak, Yakar, Atay 85, Gerek 79 and Tosunbey) were used as references to identify glutenin alleles.

Gluten extraction and sodium dodecyl sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) analyses

Glutenin proteins were extracted in accordance with the method described by Gao *et al.* (2012). The Li *et al.* (2012) method was used with slight modification for electrophoresis of glutenin subunits. 30–60 mA currents were applied per gel during electrophoresis. Gels were stained with comassie blue for 12–24 h and were kept in washing solution for 5 h. The ingredient of staining (225 mg of CBB-R 250 was dissolved in 187.5 ml of methanol, 750 ml 10% TCA and 62.5 ml glacial acetic acid) and washing solution (333 ml methanol, 100 ml 10% TCA and 567 ml distilled water) was modified and imaged under the gel documentation system.

Identification of glutenin alleles

Glutenin alleles of local wheat populations were numbered as described by Payne and Lawrence (1983) and McIntosh *et al.* (1994). Verification of alleles defined by allele specific primers was also performed.

Statistical analysis

Data analysis was carried out based on frequencies of HMW-GS alleles. Statistical analyses were performed for individual alleles, subunits, loci and geographical regions. Allele frequency of *Glu-1* loci of the wheat landraces obtained from seven different regions of Turkey was calculated according to Gupta *et al.* (1991). Percentage allele frequencies were calculated by using the formula; '(number of individual alleles/number of total wheat samples) × 100'.

POPGENE version 1.31 software was used for statistical analysis. Genetic diversity analyses in each sample were performed by using allele frequencies, the effective number of alleles per locus (Ne), observed number of alleles (Na), Nei's gene diversity and Shannon's Information index (Lewontin, 1972) according to the unbiased estimate method of Nei's (1973). Polymorphism percentages were calculated using the formula; 'number of polymorphic allele/number of total alleles'. EW homozygosity (or neutrality) test in samples and regions was calculated by employing

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sampling distribution of neutral alleles according to the Manly (1985). Observed value of F gives the homozygosity of sample. Genetic diversity analyses in subpopulations were performed according to Nei (1987). Ht, Hs, Gst and Nm indicate average genetic diversity, genetic diversity within populations; genetic diversity between populations, gene flow between populations, respectively. Nm was calculated by the formula '0.5(1-Gst)/Gst' according to the McDermott and McDonald (1993). Genetic identity and genetic distance analyses between populations were performed by Nei's original and unbiased measures according to Nei (1973, 1978). Dendrogram was drawn according to Nei (1973, 1978). Dendogram was constructed by the method of UPGMA (Unweighed Pair Group Method with Arithmetic Mean). This method has been modified from NEIGHBOR according to the PHYLIP 3.5 procedure. LD in unique populations was calculated according to the Weir (1979). Two loci analyses in subpopulations were performed according to the Ohta (1982). Multilocus analyses (single locus effect; average gene diversity (MH), variance of the diversity (VH) and Wahlund's effect (WH), two locus effect, average disequilibrium (MD), Wahlund's effect (WC), the interaction between MD and WC, variance of disequilibrium (VD), co-variance of interaction (CI), total variance and average variance) were then performed according to Brown and Feldman (1981). This analysis gives observed heterozygosity and distribution normality in populations. Comparative analyses of subunits in populations were performed considering the expected and observed values of each subunit in populations.

Results

HMW gluten polymorphism

In this study, 22 different individual alleles and 19 HMW subunits were identified in 39 different combinations of Turkish bread wheat landraces. Gluten subunit frequencies and representative gluten band profiles obtained in this study are presented in Table 1 and Fig. 1.

Genetic variation analyses

Mean genetic variation among the nationwide landraces of wheat was determined to be (h) 0.455 at the *Glu-A1*, 0.642 at the *Glu-B1* and 0.600 at the *Glu-D1* locus. Mean genetic variation at the *Glu-1* locus was (h) 0.566 ± 0.098. Mean Shannon Information Index (genetic similarity ratio) (I) was found to be 0.759 in *Glu-A1*, 1.417 in *Glu-B1* and 1.179 in *Glu-D1* locus. Mean Shannon Information Index (I) was found to be 1.118 ± 0.333 (Table 1).

The highest genetic variation was observed in the Mediterranean areas (0.623 ± 0.091) and the lowest variation was observed in the Aegean regions (0.253 ± 0.153). Considering the genetic similarity with regard to subunits, the highest value was observed in the Mediterranean areas (1.163 ± 0.253) and the lowest value was observed in Aegean regions (0.456 ± 0.263) (Table 2).

Region-based genetic variation analyses of *Glu-1* locus (*Glu-A1, Glu-B1* and *Glu-D1*) are provided in Table 2. Results revealed the highest genetic diversity of *Glu-A1* locus in Marmara (0.569) and the least diversity in Aegean region (0.165). The highest value of *Glu-B1* locus was observed in Marmara (0.722) and the lowest value was observed in the Aegean regions (0.429). The highest and the lowest values of *Glu-D1* locus were 0.711 and 0.165 for Mediterranean and Aegean regions, respectively.

The highest Shannon information index of *Glu-A1* locus was observed in the Black Sea regions (0.924) and the lowest value was observed in the Aegean regions (0.305). The highest index value of *Glu-B1* locus was observed in Marmara (1.424) and the lowest value was observed in the Aegean regions (0.759). The highest and the lowest values of *Glu-D1* locus were 1.414 and 0.305 for the Mediterranean and the Aegean regions, respectively (Table 2).

Inter-population neutrality tests

Interpopulation EW neutrality test indicated that dispersion of gluten subunits in Glu-1 loci had balanced selection in Turkish bread wheat landraces (Obs. $F < 0.5$). EW neutrality test for the material analysed revealed that *Glu-A1b, Glu-A1c, Glu-B1b, Glu-B1c, Glu-B1d, Glu-B1e, Glu-D1a* and *Glu-D1d* subunits had balanced selection, while other subunits had directional selection (Table 1).

Genetic diversity analyses

Genetic diversity at the *Glu-A1* locus of nationwide landrace wheat was found to be (Ht) 0.456 genetic diversity within the populations was (Hs) 0.402, genetic diversity among the populations was (Gst) 0.117 (Table 1) and estimated gene flow between the populations (Nm) was calculated as 3.767. A 100% polymorphism was observed among the subunits in *Glu-A1* locus. Genetic diversity at the *Glu-B1* locus of nationwide landrace wheat was found to be (Ht) 0.658, genetic diversity within the populations was (Hs) 0.582, genetic diversity among the populations was (Gst) 0.115 and estimated gene flow between the populations (Nm) was calculated as 3.843. A 100% polymorphism was observed among the subunits in *Glu-B1* locus. Genetic diversity at the *Glu-D1* locus of nationwide landrace wheat was found to be (Ht) 0.586, genetic diversity within the populations was (Hs) 0.527, genetic diversity

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Table 1. Genetic diversity analyses; Ewens–Watterson neutrality test* and allele frequencies for *Glu-1* locus

Locus	NS	Na	Ne	H	I	Ht	Hs	Gst	Nm**
Glu-A1		3	1.834	0.455	0.759	0.456	0.402	0.117	3.767
Glu-B1	116	9	2.794	0.642	1.417	0.658	0.582	0.115	3.843
Glu-D1		7	2.501	0.600	1.179	0.586	0.527	0.102	4.425
Average		6.333	2.377	0.566	1.118	0.567	0.504	0.111	4.005
Std. Dev.		3.055	0.492	0.098	0.333	0.011	0.008	–	–
		NS	Obs. F	Min. F	Max. F	Mean	SE	L95	U95
Glu-A1		3	0.545	0.333	0.966	0.681	0.032	0.374	0.966
Glu-B1	116	9	0.358	0.111	0.872	0.309	0.011	0.166	0.576
Glu-D1		7	0.3998	0.143	0.902	0.384	0.019	0.208	0.748
		Freq. (%)	Obs. F	Min. F	Max. F	Mean	SE	L95	U95
Glu-A1a		5.17	0.844	0.500	0.985	0.807	0.029	0.504	0.985
Glu-A1b	116	25.86	0.612	0.500	0.985	0.814	0.027	0.504	0.985
Glu-A1c		68.97	0.578	0.500	0.985	0.811	0.029	0.502	0.985
Glu-B1a		1.72	0.969	0.500	0.985	0.813	0.029	0.505	0.985
Glu-B1b		56.03	0.504	0.500	0.985	0.812	0.028	0.502	0.985
Glu-B1c		12.07	0.807	0.500	0.985	0.818	0.028	0.502	0.985
Glu-B1d	116	12.93	0.783	0.500	0.985	0.821	0.026	0.502	0.985
Glu-B1e		10.34	0.738	0.500	0.985	0.819	0.029	0.502	0.985
Glu-B1f		3.45	0.969	0.500	0.985	0.817	0.029	0.504	0.985
Glu-B1h		0.86	0.926	0.500	0.985	0.815	0.028	0.502	0.985
Glu-B1i		0.86	0.985	0.500	0.985	0.818	0.029	0.504	0.985
Glu-B1z		1.72	0.969	0.500	0.985	0.817	0.029	0.504	0.985
Glu-D1a		56.03	0.511	0.500	0.985	0.821	0.028	0.504	0.985
Glu-D1b		8.62	0.857	0.500	0.985	0.816	0.028	0.505	0.985
Glu-D1c		4.31	0.926	0.500	0.985	0.819	0.027	0.504	0.985
Glu-D1d	116	27.59	0.598	0.500	0.985	0.817	0.029	0.502	0.985
Glu-D1h		1.72	0.969	0.500	0.985	0.818	0.027	0.504	0.985
Glu-D1l		0.86	0.985	0.500	0.985	0.818	0.027	0.504	0.985
Glu-D1–5		0.86	0.985	0.500	0.985	0.816	0.028	0.502	0.985

NS, number of samples; Na, observed number of alleles; Ne, effective number of alleles; h, Nei's gene diversity; I, Shannon's Information Index; Ht, average genetic diversity; Hs, genetic diversity within populations; Gst, genetic diversity among populations; Nm, gene flow between populations.
Number of polymorphic regions: 3.
Ratio of polymorphic regions (%): 100.00.
*This statistic was calculated by using 1000 representative samples.
**Nm, 0.5 (1 – Gst)/Gst; Freq., frequencies; SE. standard error; L95 and U95, 95% lower and upper confidence interval.

among the populations was (Gst) 0.102 and estimated gene flow between the populations (Nm) was calculated as 4.425. Again 100% polymorphism was observed among the subunits in *Glu-D1* locus.

Genetic identity and genetic distances

Region-based genetic similarities and distances among the wheat landraces are provided in Table 3. Results revealed that the highest similarity in *Glu-1* locus was observed between the Mediterranean and the Black Sea regions (0.952), the Black Sea and Eastern Anatolia (0.941) and Central Anatolia and the Mediterranean regions (0.939), respectively.

On the other hand, the highest distance in *Glu-A1, Glu-B1* and *Glu-D1* loci were observed between Marmara and the Aegean (0.468), Southeastern and Marmara (0.291) and the Mediterranean and Marmara regions (0.269), respectively (Table 3).

The dendrogram drawn according to original measurements of Nei (1972) revealed that the Mediterranean, the

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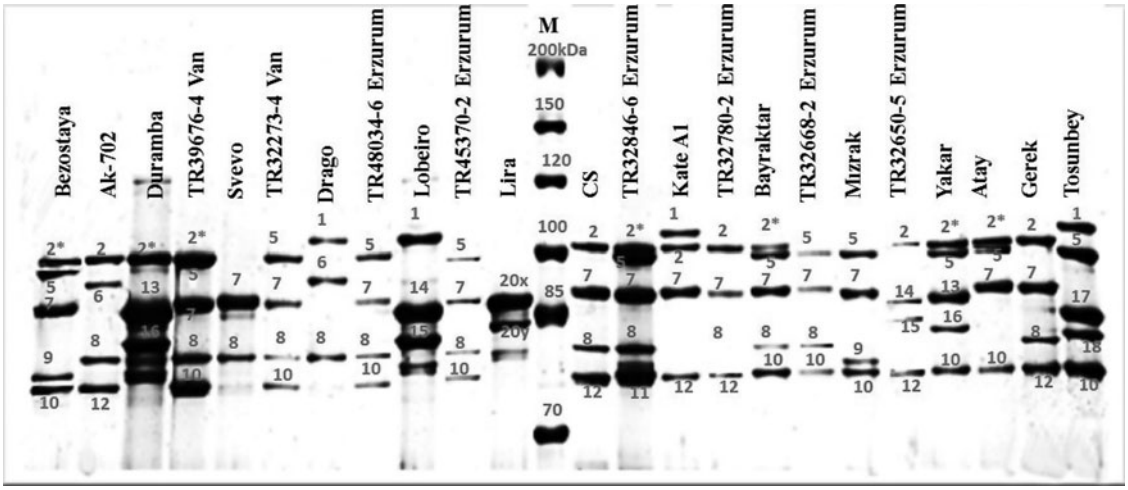

Fig. 1. HMW gluten band profiles as a result of SDS-PAGE separation of *Glu-1* locus of Turkish bread wheat landraces.

Black Sea and Central Anatolia regions were clustered together (Fig. 1). Other regions are clustered separately.

LD, single and/or multiple loci and heterozygosity analyses

Considering the LD, significant LD was detected among individual subunits ($P < 0.05$). In general, it can be inferred that there was a limited migration between wheat landraces. LD occurs because of a limited migration. However, the LD between *Glu-A1b* and *Glu-D1a* and between *Glu-D1d, Glu-D1a* subunit and *Glu-D1d* suggest an epistatic natural selection. The highest heterozygosity (2.8691) was observed in Marmara region. The heterozygosity was the lowest in Southeastern Anatolia (0.1694) and Eastern Anatolia region (0.1859) (Table 4). In terms of single locus effect, average gene diversity was calculated to be 0.6895 (with a variance of diversity of 0.0454 and Wahlund effect of −0.0172). Considering the comparative analysis of subunits carried out on subunits of wheat landraces, dispersion of *Glu-A1c, Glu-B1c, Glu-B1d, Glu-B1e* and *Glu-D1a* were significantly different ($P < 0.05$). For the dispersion of other subunits, significant difference was not observed between expected and observed values ($P > 0.05$).

Multiple locus analyses on bread wheat landraces revealed that the values observed in regions, except for the Aegean and Eastern Anatolia, were higher than upper confidence interval of 95% (U2-U4) (Table 4). Therefore, it was assumed that the samples did not exhibit normal distribution in these landraces. However, samples exhibited normal distribution in the Aegean and Eastern Anatolia regions.

Population subunits were subjected to Brown and Feldman (1981) multiple locus analysis. With regard to single locus effect, average gene diversity (MH) was calculated as 0.6895; variance of the diversity (VH) was 0.0454 and Wahlund's effect (WH) was −0.0172. With regard to two locus effect, average disequilibrium (MD) was 0.3535; Wahlund's effect (WC) was 0.0108; the interaction between MD and WC (AI) was 0.0307; variance of disequilibrium (VD) was 0.1622; co-variance of interaction (CI) was −0.0592; total variance was (MH + VH + WH + MD + WC + AI) 1.1128 and average variance was (MH + MD + AI + VD + CI) 1.1767. Considering the mutual analyses of subunits of nationwide wheat landraces, one may speculate on the possibility of a limited migration between the landraces. LD of the landraces was largely due to this limited migration and/or epistatic natural selection [$(D'IS)^2 > (D'ST)^2$ and $(DST)^2 > (DIS)^2$; $(D'IS)^2 < (D'ST)^2$ and $(DST)^2 < (DIS)^2$ respectively].

Discussion

Considering the fact that Turkey is among the gene centres for wheat (Vavilov, 1951), we expected high genetic diversity in *Glu-1* loci of local wheat landraces in this country and studied 116 wheat genotypes for gluten allele polymorphism to determine gluten allele profile of landraces and high quality score wheat genotypes as candidates for breeding. Landraces are great resources for allele diversity of different agronomic characters and in this study local wheat genotypes are investigated for gluten allele diversity. Although, there are several articles reporting the diversity of gluten alleles for different regions of the world, Turkish wheat landraces were not screened thoroughly for gluten allele diversity. Fourteen different alleles were defined in Turkish durum wheat in a study carried out by Turchetta *et al.* (1995) and 27 in Asian wheat (Terasawa *et al.*, 2010). In this study, 22 different individual alleles and 19 HMW subunits were identified in 39 different combinations in Turkish bread wheat landraces (Fig. 2).

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Table 2. Region-based genetic variation analyses of *Glu-1* locus

Regions	NS	Locus	Na	Ne	h	I
		Glu-A1	3.000	2.323	0.569	0.918
		Glu-B1	5.000	3.600	0.722	1.424
Marmara	12	*Glu-D1*	2.000	2.000	0.500	0.693
Region		Mean	3.333	2.641	0.597	1.012
		Std. Dev.	1.528	0.846	0.114	0.374
		Glu-A1	2.000	1.198	0.165	0.305
		Glu-B1	3.000	1.754	0.429	0.759
Aegean	11	*Glu-D1*	2.000	1.198	0.165	0.305
Region		Mean	2.333	1.383	0.253	0.456
		Std. Dev.	0.577	0.321	0.153	0.263
		Glu-A1	3.000	2.123	0.529	0.908
		Glu-B1	4.000	2.689	0.628	1.169
Mediterranean	11	*Glu-D1*	5.000	3.457	0.711	1.414
Region		Mean	4.000	2.756	0.623	1.163
		Std. Dev.	1.000	0.669	0.091	0.253
		Glu-A1	2.000	1.800	0.444	0.637
		Glu-B1	3.000	2.488	0.598	0.989
Central Anatolia	27	*Glu-D1*	4.000	2.031	0.508	0.949
Region		Mean	3.000	2.106	0.517	0.858
		Std. Dev.	1.000	0.350	0.077	0.193
		Glu-A1	3.000	2.240	0.554	0.924
		Glu-B1	4.000	1.889	0.471	0.917
Black Sea	17	*Glu-D1*	3.000	2.240	0.554	0.924
Region		Mean	3.333	2.123	0.526	0.921
		Std. Dev.	0.577	0.203	0.048	0.005
		Glu-A1	2.000	1.600	0.375	0.562
		Glu-B1	8.000	2.292	0.564	1.307
Eastern Anatolia	28	*Glu-D1*	5.000	3.039	0.671	1.278
Region		Mean	5.000	2.310	0.537	1.049
		Std. Dev.	3.000	0.719	0.149	0.422
		Glu-A1	2.000	1.219	0.180	0.325
		Glu-B1	4.000	2.941	0.660	1.194
Southeastern	10	*Glu-D1*	4.000	2.381	0.580	1.089
Anatolia		Mean	3.333	2.181	0.473	0.869
Region		Std. Dev.	1.155	0.878	0.257	0.474
		Glu-A1	3.000	1.834	0.455	0.759
		Glu-B1	9.000	2.794	0.642	1.417
Overall Turkey	116	*Glu-D1*	7.000	2.501	0.600	1.179
		Mean	6.333	2.377	0.566	1.118
		Std. Dev.	3.055	0.492	0.098	0.333

Na, observed number of alleles; Ne, effective number of alleles; h, Nei's gene diversity; I, Shannon's Information index; NS, number of samples.

In *Glu-A1* locus, three alleles (*Glu-A1a, Glu-A1b* and *Glu-A1c*) were defined in Turkish durum wheat in a study carried out by Branlard *et al.* (1989). Correspondingly, three alleles (*Glu-A1a, Glu-A1b* and *GluA1c*) were defined in *Glu-A1* locus of Turkish bread wheat in this study. In *Glu-B1* locus, seven alleles were defined in Turkish durum wheat. However, nine alleles (*Glu-B1a, Glu-B1b, Glu-B1c, Glu-B1d, Glu-B1e, Glu-B1f, Glu-B1h, Glu-B1i* and

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Table 3. Genetic similarity (upper diagonal) and genetic distances (lower diagonal) in *Glu-1* locus

Regions	Central Anat.	Aegean	Marmara	Mediterr.	Black Sea	Eastern Anat.	Southeastern Anat.
Central Anatolia	****	0.921	0.802	0.938	0.936	0.891	0.861
Aegean	0.082	****	0.626	0.913	0.899	0.845	0.884
Marmara	0.221	0.468	****	0.764	0.830	0.792	0.748
Mediterranean	0.064	0.091	0.269	****	0.952	0.924	0.911
Black Sea	0.066	0.107	0.186	0.049	****	0.941	0.875
Eastern Anatolia	0.116	0.169	0.233	0.079	0.061	****	0.879
Southeastern Anatolia	0.149	0.123	0.291	0.093	0.133	0.128	****

Glu-B1z) were defined in *Glu-B1* locus of Turkish bread wheat landraces in this study. In *Glu-D1* locus, five alleles had so far been defined in Turkish durum wheat (Branlard *et al.,* 1989): notwithstanding, seven alleles (*Glu-D1a, Glu-D1b, Glu-D1c, Glu-D1d, Glu-D1h, GluD1l* and Glu-D15) were defined in *Glu-D1* locus of Turkish bread wheat landraces in this study.

An alternative approach to summarizing and forecasting genetic diversity can be based on Shannon's diversity index (Sherwin, 2010). Shannon diversity index offers a possible route for integrating diversity across all levels of biological organization (Smouse and Ward, 1978). Thus, this index could also be used to forecast and summarize allele diversity in different populations of the same species. Shannon's index accounts for both abundance and evenness of the alleles present. Shannon's equitability (EH) can be calculated by dividing H by Hmax. Equitability assumes a value between 0 and 1 with 1 being complete evenness. Shannon's equitability information indices for Turkish bread wheat landraces were 0.691, 0.645 and 0, 606 for Glu-A1, Glu-B1 and Glu-D1, respectively. Considering these results, Turkish bread wheat landraces were more homogeneous in Glu-A1 locus and more heterogeneous in Glu-D1 locus. These results indicate that there is a considerable amount of diversity in Turkish bread wheat landraces.

Gene flow refers to the movement of genes between populations, and takes place in plants through the dispersion of seed or pollens. Gene flow through cross-pollination occurs at such a level in all crops, even in an autogamous plant like wheat (Ellstrand *et al.*, 1999). Generally, if gene flow (Nm) is higher than 1, the differentiation in population is slow. On the contrary, when gene flow (Nm) is smaller than 1, local differentiation of population occurs. In the current study average gene flow is higher than 1 (4.00), which is indicative of a minor differentiation of populations. Although we do not expect that much scale of gene flow in an autogamous species, seed-mediated gene flow may occur through exchange of seed materials among growers.

Unpredictably, although there is geographical barrier between the Mediterranean and the Black sea region, they were genetically identical in UPGMA. Marmara region is the most distant to other regions. This result is expected since this is an industrial region and wheat cultivation is quite low there.

Van Hintum and Elings (1991) assessed the phenotypic and gluten-based relationships in Syrian local durum wheat cultivars with different geographical origins. Researchers identified 19 HMW subunits in 48 different combinations. In this study, 21 individual alleles and 19 HMW subunits were identified in 39 different combinations. Gianibelli *et al.* (2002) studied biochemical and molecular characterization of *Glu-1* locus of Argentinean wheat cultivars. Researchers analysed 107 wheat collections of Argentina through SDS-PAGE and identified 11 alleles of which three were coded on *Glu-A1*, six on *Glu-B1* and two on *Glu-D1*. The researchers observed the highest frequency in *Glu-D1d* allele. Lerner *et al.* (2009) also studied endosperm storage protein diversity in Argentinean bread wheat cultivars and they observed three, six and two alleles in 17 allelic combinations at the Glu-A1, Glu-B1 and Glu-D1 loci, respectively. The mean genetic variation index (H) observed for the glutenin loci was 0.589 for the studied cultivars. The highest frequencies for the observed alleles were Glu-A1b (63.9%), Glu-B1c (41.2%), and Glu-D1d (94.1%).

In Turkish cultivars, *Glu-A1*c subunit had the highest frequency with 68.97%. Argentinean wheat types were distributed in 18 different groups based on HMW-GS contents and *Glu-A1a/Glu-B1al/Glu-D1d* had the highest frequencies (22%) (Gianibelli *et al.,* 2002). In Turkish bread wheat landraces, *Glu-B1b/Glu-D1a* allelic combination had the highest ratio (22.48%).

Primitive and less cultivated genotypes are generally considered as significant source of variation with regard to disease resistance, kernel protein quality and resistance against various ecological stresses (Porceddu *et al.,* 1988; Kaplan *et al.,* 2014). In previous studies, higher variations were reported in HMW subunits of Turkish wheat compared with the Italian, Australian, American, Canadian, Spanish and French wheat cultivars (Autran and Feillet, 1985; Margiotta *et al.,* 1987; Carrillo *et al.*, 1990; Liu and Shepherd, 1991). These were expected results since

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Table 4. Multiple locus analysis in single populations and Hypothesis tests for observed values of K

Populations	Obs. value		Exp. value		Heterozygosity		L2	U2	SEM2	L3	U3	SEM3	L4	U4	SEM4
Central Anatolia region	M1	1.550	EM1	1.550	X2	0.999	0.413	1.061	0.165	−0.347	0.299	0.165	0.467	2.094	0.415
	M2	1.473	EM2	0.737	X3	2.116									
	M3	−0.073	EM3	−0.024	X4	1.438									
	M4	3.121	EM4	1.281	Aver.	1.518									
Aegean region	M1	0.760	EM1	0.760	X2	0.238	0.114	0.928	0.208	−0.353	0.791	0.292	−0.748	2.241	0.763
	M2	0.645	EM2	0.521	X3	0.538									
	M3	0.337	EM3	0.219	X4	0.337									
	M4	0.998	EM4	0.747	Aver.	0.371									
Marmara region	M1	1.792	EM1	1.792	X2	1.433	0.227	1.165	0.239	−0.635	0.389	0.261	−0.152	2.495	0.675
	M2	1.693	EM2	0.696	X3	4.881									
	M3	−0.725	EM3	−0.123	X4	2.293									
	M4	3.856	EM4	1.171	Aver.	2.869									
Mediterrranean region	M1	1.868	EM1	1.868	X2	0.919	0.200	1.177	0.249	−0.721	0.399	0.286	−0.302	2.615	0.744
	M2	1.321	EM2	0.689	X3	2.405									
	M3	−0.548	EM3	−0.161	X4	1.417									
	M4	2.794	EM4	1.156	Aver.	1.580									
Black Sea region	M1	1.578	EM1	1.578	X2	0.753	0.334	1.153	0.209	−0.451	0.374	0.210	0.261	2.331	0.528
	M2	1.303	EM2	0.743	X3	5.625									
	M3	−0.254	EM3	−0.038	X4	1.115									
	M4	2.741	EM4	1.296	Aver.	2.498									
Eastern Anatolia region	M1	1.610	EM1	1.610	X2	0.089	0.391	1.011	0.158	−0.357	0.261	0.157	0.402	1.980	0.403
	M2	0.764	EM2	0.701	X3	0.335									
	M3	−0.064	EM3	−0.048	X4	0.133									
	M4	1.349	EM4	1.191	Aver.	0.186									
Southeastern Anatolia region	M1	1.420	EM1	1.420	X2	0.825	0.142	1.089	0.242	−0.492	0.459	0.243	−0.228	2.155	0.608
	M2	1.124	EM2	0.616	X3	−1.624									
	M3	0.010	EM3	−0.016	X4	1.307									
	M4	2.223	EM4	0.964	Aver.	0.169									

M1–M4 are the first four observed values, EM1–EM4 are respectively the expected values of these four values, X2–X4 are heterozygosity ratios (Xi = Mi/EMi-1), SEM2-SEM4, standard error of M2-M4; L2-L4, 95% lower confidence interval of M2–M4; U2–U4, 95% upper confidence interval of M2–M4; Aver, average [According to the Brown *et al.*, 1980; Brown and Feldman, 1981].

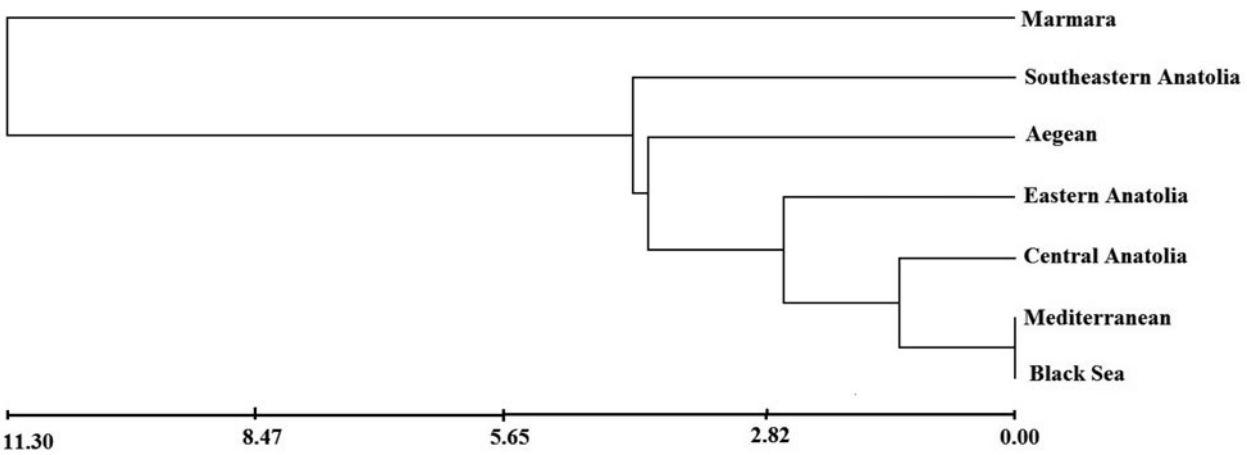

Fig. 2. Regional dendogram for *Glu-1* locus.

Turkish lines are highly old and local races with centuries of history. Intensive breeding probably decreased the genetic variations among western wheat (Porceddu *et al.,* 1988). Notwithstanding, a study carried out by Manifesto *et al.* (2001) demonstrated that there was no significant decrease in genetic diversity of Argentinian bread wheat cultivars released in the last half century.

Terasawa *et al.* (2010) studied genetic variation in high molecular weight gluten subunits of Asian wheat and identified three alleles in *Glu-A1* locus and nine alleles in *Glu-B1* locus. *Glu-B1*b was observed in 76.5% of accessions. In the present study, frequency of this allele was observed as 56.03%. Terasawa *et al.* (2010) reported similar characteristics for Western Asian, Afghan and Eastern Asian wheat but indicated different characteristics for Caucasian and Central Asian wheats. As previously indicated by Terasawa *et al.* (2009) and Lagudah *et al.* (1987), *Glu-A1*c, *Glu-B1*b and *Glu-D1*a were the most common genotypes in Western and Eastern Asia. These alleles had the highest frequencies also in the present study. These alleles were also reported to be common in Japan and Xinjiang border lines (Nakamura, 2000; Cong *et al.,* 2005). All these outcomes indicated that *Glu-A1*c, *Glu-B1*b and *Glu-D1*a genotypes were dominant from Afghanistan to Far East, from Mesopotamia to Central Asia. This genotype is not common in European endemic wheat (Gregova *et al.,* 1999, 2006). Genetic diversity level is relatively low in central parts of Asia (Western Asia, Afghanistan, Eastern Asia) and relatively high in border lines (Caucasia, Central and Southern Asia). Such results were due to differences in frequencies of typical Asian genotypes (Terasawa *et al.,* 2010).

Caballero *et al.* (2009) studied genetic diversity in seed storage proteins of diploid wheat in Turkey and Lebanon and reported higher genetic diversity for Turkish populations (Ht = 0.740) than for Lebanese populations (Ht = 0.685). In the present study, genetic diversity (Ht) in Turkish bread wheat landraces was observed as 0.5666. Marshall and Brown (1975) method are commonly employed to find out the distribution pattern of alleles in different species. According to this method, alleles are defined as frequent if the allele frequency is over 5%, seldom if the frequency is below 5% and rare if the frequency is below 1%. Caballero *et al.* (2009) reported four as frequent, two as seldom and three as rare alleles in *Glu-1* locus. The researchers identified 22 alleles in Turkish local bread wheat cultivars as of 11 frequent, seven seldom and four rare. According to intra-population and inter-population gene diversity analysis, Turkish populations ($A = 10.75$) exhibited higher diversity than the Lebanese populations ($A = 5.75$) (A = number of alleles in each locus). In the present study, intra- and inter-population gene diversity ratio was observed as 6.3333 ± 3.0551. Besides, higher genetic erosion risk was reported for Turkish populations than for Lebanese populations (Caballero *et al.,* 2009) so much so that while Ne value was representing 51% of alleles in Lebanese populations, this ratio was 39% in Turkish populations (Ne = impact values of alleles). Similarly, Ne value representing 37.52% of alleles of Turkish bread wheat landraces indicates the higher genetic erosion in the current study. Although the researchers reported genetic similarity (I) as 0.357 (Caballero *et al.,* 2009), the value was observed as 1.1183 in the present study. In a previous study, HMW-GS-based analyses revealed the genetic diversity as 0.45 for Argentinean, 0.68 in Greek, 0.67 in Spanish, 0.60 in Chinese, 0.59 in American, 0.58 in Australian and 0.46 in Russian wheat (Cooke, 1995). In the present study, genetic diversity was observed as 0.5657. Genetic erosion risk was high in Turkish local bread wheat lines (Ne value represented 37.52% of alleles).

There are insufficient studies about homozygosity (or neutrality) and LD of wheat landraces and cultivars. However, in the present study, possible use of these tests was assessed to determine selection effects in local populations. Neutrality test allows the researchers to gain information about

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balanced or directional selection of populations under the null hypothesis of evolution. Therefore, in this study, the data obtained from SDS-PAGE analysis were analysed in POPGENE statistics software and ultimately EW neutrality tests and LD were applied in Turkish bread wheat landraces. EW neutrality test revealed that *Glu-A1b, Glu-A1c, Glu-B1b, Glu-B1c, Glu-B1d, Glu-B1e, Glu-D1a* and *Glu-D1d* subunits had balanced and other subunits had directional selection. Significant LD between individual subunits was determined ($P < 0.05$). LD in varieties were largely because of limited migration [$(D'IS)^2 > (D'ST)^2$and $(DST)^2 > (DIS)^2$]. However, an epistatic natural selection can be mentioned for the LD between *Glu-A1b* subunit and *Glu-D1a* and *Glu-D1d* subunits and between *Glu-D1a* and *Glu-D1d* subunits [$(D'IS)^2 < (D'ST)^2$ and $(DST)^2 < (DIS)^2$].

Single and multiple locus analyses were also performed for Turkish bread wheat landraces in the present study. Average gene diversity with regard to single locus effect was calculated as 0.6895. Considering the comparisons made for intra- and inter-populations of Turkish bread wheat landraces, significant differences were observed in distribution of *Glu-A1c, Glu-B1c, Glu-B1d, Glu-B1e* and *Glu-D1a* ($P < 0.05$).

Wahlund effect is defined as the increased frequency of homozygotes in subdivided populations. It is the reduction in the overall heterozygosity of a population as a result of subpopulation structures. Essentially, if two or more subpopulations have independent allele frequencies, then the overall heterozygosity is reduced, irrespective of whether those subpopulations are in Hardy–Weinberg equilibrium. We might then suspect that selection, or some other factors, was favouring homozygotes. Multiple locus analyses on present landraces revealed that samples did not exhibit normal distribution in regions except for the Aegean and Eastern Anatolia regions. Multiple locus analyses for subunits in populations revealed that average gene diversity with regard to single locus effect was 0.6895 and average gene imbalance with regard to two locus effect was 0.3535 and Wahlund's effect was calculated as 0.0108.

This result indicates higher gene flow and lower heterozygosity in the studied Turkish bread wheat landraces. As a conclusion, studying the gluten allele diversity of Turkish bread wheat landraces is important for breeders, since Turkey is known as one of the gene centres for major cereals including wheat, barley, rye and oat, where they diversified and spread throughout the world. In addition, population structure, LD and neutrality tests were applied for the first time to show the selection effect and genetic erosion risk based on gluten protein diversity of landraces representing the whole country. Considering overall results, there is significant amount of genetic variation in HM weight gluten subunits existing in Turkish bread wheat landraces and they still could be used as a genetic source for breeding purposes.

Supplementary Material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262116000356

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Conflict of Interest

The authors declare that they have no conflict of interest.

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