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Phylogeographic Structure of Kars Emmer Wheat (*Triticum dicoccum* Schrank ex Schübl) in Turkey Explained by SSR Markers

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ABSTRACT: Turkey has a role in the distribution of many plants across continents and it is the main center of wheat. Due to its climatic characteristics and geostrategic importance, Turkey also has important genetic resources for the cultivation and development for many local wheat varieties. Therefore, it is important to determine local wheat genotypes that can adapt to different ecological conditions in Turkey and define ones having high performance in terms of efficiency and quality characteristics to make them useful. Emmer wheat which is grown in and around Kars City in northeastern Anatolia region is seen as valuable. In the present study, the phylogeographic structure analyses of 10 emmer (*Triticum dicoccum* Schrank) wheat populations and 2 populations of cultivated wheat (*Triticum aestivum*) obtained from Kars region were made by Simple Sequence Repeats (SSR). Within the scope of the study, Principal Coordinates Analysis (PCoA), phylogenetic analysis, and genetic-geographic distance analysis were performed. 3 main groups of differentiation at the populations were supported by PCoA and phylogenetic analyses. The comparison between geographic and genetic distance matrices for all genotypes revealed a statistically negative correlation (R^2=0.04). Emmer wheat is an important local genetic resource and the cultivation area in agriculture should be expanded and used in breeding studies. In addition, it has been determined that SSR markers can give more comprehensive results with higher numbers in wheat genotyping studies.

Keywords: Emmer wheat, Phylogeography, *Triticum dicoccum*, SSR, Kars

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INTRODUCTION

Cereals are among the first plants to be cultured and they belong to the family of wheat (Gramineae/Poaceae) (Verma et al., 2019). In terms of planting area and production quantity, wheat is important grain group in Turkey as in the world (Karagöz, 2014). In ancient times, primitive species were collected directly from nature and consumed as nutrients. *Triticum monococcum* L.(Siyez) and *Triticum dicoccum* S. (emmer) such primitive wheat species were used in this way for many years and they were later cultured by producers (Karagöz, 1996). Emmer group wheat Bronze Age (10000-4000 BC) has maintained its existence throughout. In this process naked wheat has gradually replaced the emmer, especially the tetraploid species. (Harlan, 1981; Perrino and Hammer, 1982). *Triticum urartu* (wild diploid wheat, 2n=14, AuAu) and *Aegilops speltoides* (wild grass plant, 2n=14, BB) hybridized naturally about 30-50 thousand years ago and occurred in *Triticum dicoccoides* (wild emmer, 4n=28, AuAuBB) with their chromosomes folded. *Triticum dicoccum* (culture Gernik wheat 4n=28 and AuAuBB) has been domesticated as a result of natural-artificial selections from the species *Triticum dicoccoides* (Peng et al., 2011; Dhanavath and Prasada, 2017). Today, emmer wheat is grown in Ethiopia, Iran, Turkey, the Caucasia, the Volga Valley, the republics of Former Yugoslavia, Central Europe, Italy, Spain and India (Zaharieva et al., 2010). Molecular marker techniques (RAPD, RFLP, SSR, AFLP etc.) are preferred for the purpose of identifying genotypes in many species, especially cereals (Grover and Sharma, 2015). In many studies, it has been proven that SSRs in plant species have a high level of informative (İlhan et al., 2016; Rafeipour et al., 2016; Abbasov et al., 2018). SSRs are among the most appropriate markers used in many fields such as phylogenetic relationship, genetic diversity, population genetics, genetic mapping (Kacem et al., 2017; Salehi et al., 2018; Kara et al., 2020). A great deal of studies has been done to investigate genetic diversity, phylogenetic relationships using SSR in wheat (Ehtemam et al., 2010; Tahır and Abdul, 2010; Asmamaw et al., 2019;). It is valuable to investigate phylogeographic structure of populations for acceptable evaluation of local genetic plant resources found in Turkey. In addition, it is necessary to evaluate local genetic resources with correct methods (Demirel et al., 2019). Research on the emmer has also been carried out in Turkey. However, there are limited studies on the genetic diversity of Turkish emmer wheat (Arystanbekkyzy et al., 2018; Demirel, 2020). In the current study, phylogeographic analyses of emmer wheat (*Triticum dicoccum* Schrank) belonging to Kars province (Turkey) was carried out using SSR markers.

MATERIALS AND METHODS

Plant Materials and DNA Extraction

Seeds from 10 different populations of local emmer (*Triticum dicoccum* Schrank) wheat used in the study were obtained from farmers in different villages of Kars (Table 1, Fig. 2). Since this wheat species is an autogam, a sample was taken from each population. In addition, seeds of 2 cultivated wheat (*Triticum aestivum* L.) obtained from farmers in different villages of Kars province to compare genetic diversity were also included in the study (Table 1, Fig. 2). The seeds of wheat populations were germinated and grown on viols in the climate room, which included a 16/8 - hour long day photoperiod and a room temperature of 25 ^{0}C. DNA extraction was conducted using CTAB method from young leaves of wheats grown in plant growing rooms (Doyle and Doyle, 1990). These resulting DNAs were diluted to a concentration of 10 ng/µl and made ready for use in PCR reactions.

Table 1. Wheat materials used in this study and their locations

Population No	Type of Wheat	Wheat Locations	Number of Local Varieties	Number of Individuals
1	*Triticum aestivum* L.	Dağpınar Village (Digor District)	1	1
2	*Triticum aestivum* L.	Aşağı kotanlı Village (Selim District)	1	1
3	*Triticum dicoccum* Schrank ex Schübl.	Benliahmet Village (Selim District)	1	1
4	*Triticum dicoccum* Schrank ex Schübl.	Koçköy Village (Arpaçay District)	1	1
5	*Triticum dicoccum* Schrank ex Schübl.	İncesu Village (Susuz District)	1	1
6	*Triticum dicoccum* Schrank ex Schübl.	Dikme Village (Kars Town)	1	1
7	*Triticum dicoccum* Schrank ex Schübl.	Kürekdere Village (Arpaçay District)	1	1
8	*Triticum dicoccum* Schrank ex Schübl.	Dağpınar Village (Digor District)	1	1
9	*Triticum dicoccum* Schrank ex Schübl.	Geçit Village (Akyaka District)	1	1
10	*Triticum dicoccum* Schrank ex Schübl.	Mezraa Village (Kars Town)	1	1
11	*Triticum dicoccum* Schrank ex Schübl.	Değirmenköprü Village (Arpaçay District	1	1
12	*Triticum dicoccum* Schrank ex Schübl.	Arpaçay Town	1	1

Amplification and Analysis of SSR Markers

The PCR process was performed on Bioneer Global Genomics Partner Thermocycler. For PCR reactions, 9.5 µl ddH_20, 1 µl F primer and 1 µl R primer (10 µM of primers) and 12.5 µl Taq 2x Master Mix (25 mM $MgCl_2$) were used with a final volume of 25 µl containing 1 µl of diluted genomic DNA (10 ng/1 µl). PCR cycles: pre-denaturation 3 min at 94 ^{0}C, 45 cycles of denaturation 1 min at 94 ^{0}C, for 1 min at 56-59 ^{0}C, and for 2 min at 72 ^{0}C, followed by a cycle for 10 min at 72 ^{0}C. The bands provided by PCR products were determined by agarose gel electrophoresis. SSR-PCR products (100-700 bp) were run on prepared 5% agarose gel. Ethidium bromide (3 µl) was added to the gel to display the resulting bands in UV light. The gel images were interpreted and then scored based on the allele size of the bands. In the present study, 11 SSR markers with easy scoring and high amplification ability were selected (Röder et al., 1998) (Table2).

Table 2. Primers and references of 11 SSR markers used in the study

Marker	Forward Primer	Reverse Primer	Primer References
Xgwm46	GCA CGT GAA TGG ATT GGA C	TGA CCC AAT AGT GGT GGT CA	Röder et al., 1998
Xgwm95	GAT CAA ACA CAC ACC CCT CC	AAT GCA AAG TGA AAA ACC CG	Röder et al., 1998
Xgwm120	GAT CCA CCT TCC TCT CTC TC	GAT TAT ACT GGT GCC GAA AC	Röder et al., 1998
Xgwm154	TCA CAG AGA GAG AGG GAG GG	ATG TGT ACA TGT TGC CTG CA	Röder et al., 1998
Xgwm155	CAA TCA TTT CCC CCT CCC	AAT CAT TGG AAA TCC ATA TGC C	Röder et al., 1998
Xgwm340	GCA ATC TTT TTT CTG ACC ACG	ACG AGG CAA GAA CAC ACA TG	Röder et al., 1998
Xgwm361	GTA ACT TGT TGC CAA AGG GG	ACA AAG TGG CAA AAG GAG ACA	Röder et al., 1998
Xgwm389	ATC ATG TCG ATC TCC TTG ACG	TGC CAT GCA CAT TAG CAG AT	Röder et al., 1998
Xgwm540	TCT CGC TGT GAA ATC CTA TTT C	AGG CAT GGA TAG AGG GGC	Röder et al., 1998
Xgwm558	GGG ATT GCA TAT GAG ACA ACG	TGC CAT GGT TGT AGT AGC CA	Röder et al., 1998
Xgwm601	ATC GAG GAC GAC ATG AAG GT	TTA AGT TGC TGC CAA TGT TCC	Röder et al., 1998

Data Analysis

The scoring process was carried out using 1–0 technique due to the codominant marker system and data analyses were performed. The software Genalex 6.1 (Peakall and Smouse, 2001) was prefered to determine the genetic distances between populations using by the scoring data. Analyses were managed to these criteria using basic parameters such as allele frequencies, major allele frequencies, gene diversity and polymorphism information content (PIC) in determining genetic diversity. The powermarker v3.23 (Liu and Muse, 2005) software program was used for the calculations. PCoA (Principal Coordinate Analysis) was performed using the GenAlEx (Peakall and Smouse, 2001) software to identify genetic variations of populations. The neighbour-joining dendrogram was created using the POWERMARKER v3.25 (Liu and Muse, 2005) program. The neighbour-joining tree was visualized

using the DENDROSCOPE program for the molecular relationship of 12 individuals at the population level using the data obtained from here (Huson et al., 2010).

RESULTS AND DISCUSSION

Principal Coordinate Analysis (PCoA) and Molecular Phylogenetic Analysis

Principal coordinate analysis (PCoA) was performed for 10 tetraploid and 2 hexaploid wheat genotypes for the aim of explaining the variations between populations at a minimum level *T. dicoccum* and *T. aestivum* species and wheat species are encoded with different colors. PCoA analysis examined *T. dicoccum* and *T. aestivum* wheat populations are grouped similarly to NJ dendrogram results (Fig. 1). The results show that *Triticum aestivum* forms a clearly distinct group from *Triticum dicoccum*. The cultivated wheat (*Triticum aestivum*) that we used for comparison in the study is expected to exhibit close relationship. According to the genetic distance values, 6 (Dikme) and 8 (Dağpınar) numbered populations were the closest to each other, while 4 (Koçköyü) and 11 (Değirmenköprü) numbered populations were the most distant to each other (Fig. 1). The distribution of *T. dicoccum* populations of the same species into different groups points to the level of genetic variation among genotypes.

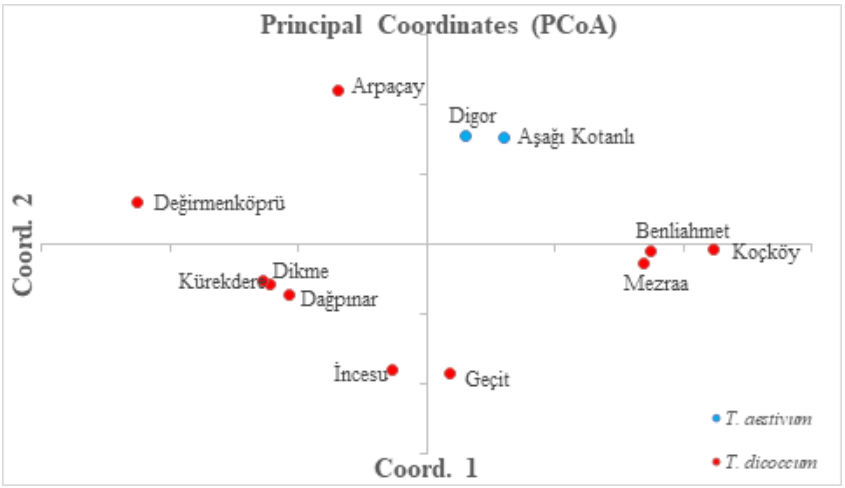

Figure 1. Clusters based on the two main components obtained from the analysis using the 11 SSR marker, emmer wheat populations belonging to different locations (*T. dicoccum*) separation from each other and separation by *T. aestivum* population

Molecular phylogenetic analysis was performed to confirm the results from PCoA and to determine the molecular relationships of 12 wheat genotypes. POWERMARKER v3.25 software was used to perform this analysis. Data from the program was visualized with the DENDROSCOPE program, which was created to determine the molecular relationship of 12 individuals at the population level (Fig. 2). Based on the dendrogram, the 12 populations were divided into three main groups. Of these groups, the first main group was divided into two subgroups. In one of these subgroups, the İncesu population, and in the other subgroup, the Kürekdere, Dağpınar, Değirmenköprü and Dikme populations were clustered. In its second main group, it was seen to be seperated into two subgroups. Both subgroups formed subgroups again. The populations of Mezraa, Benliahmet and Koçköy villages were grouped in the first subgroup of the second main group. The Aşağı Kotanlı, Digor and Arpaçay populations were clustered in the other subgroup of the second main group. In the third main group, only the population is present.

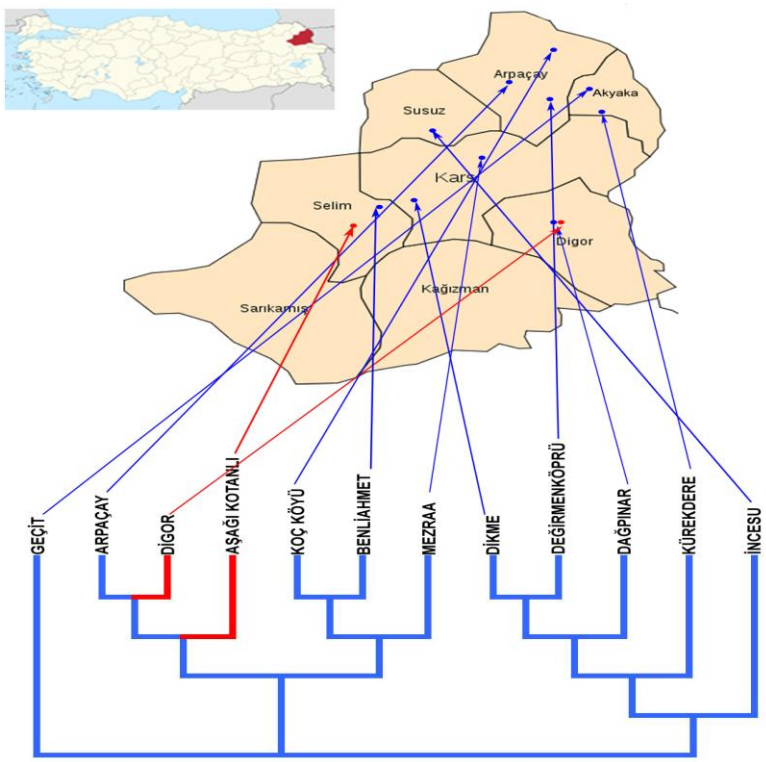

Figure 2. Dendrogram shows geographic origins of 12 wheat populations together with Neighbour Joining tree based on genetic distances

Genetic Distance Analysis

Genetic distance is an expression of divergence between populations. If the distance value is small, it can be understood that there is a close genetic relationship; if the distance value is large, the genetic relationship is more distant. In the present study, the genetic distances between populations were computed with respect to the genetic distance calculation method of Nei (Nei, 1983). When we look at the results of the genetic distance for the populations, while the least genetic distance was between Dikme and Dağpınar villages in *T. dicoccum* populations, the maximum genetic distance was between Koçköy and Değirmenköprü villages in *T. dicoccum* populations (Table 3).

Table 3. Genetic distance matrix of *T. aestivum* and *T. dicoccum* populations based on SSR analysis calculated for all loci according to Nei et al., (1983)

Pop	1	2	3	4	5	6	7	8	9	10	11	12
1	-											
2	0.18	-										
3	0.16	0.18	-									
4	0.19	0.15	0.09	-								
5	0.19	0.19	0.19	0.18	-							
6	0.16	0.16	0.12	0.15	0.13	-						
7	0.16	0.14	0.14	0.19	0.13	0.06	-					
8	0.18	0.14	0.16	0.13	0.11	**0.04**	0.08	-				
9	0.18	0.20	0.12	0.17	0.15	0.10	0.10	0.12	-			
10	0.14	0.16	0.10	0.13	0.17	0.14	0.14	0.14	0.12	-		
11	0.18	0.18	0.18	**0.23**	0.19	0.08	0.10	0.10	0.16	0.20	-	
12	0.12	0.12	0.14	0.17	0.21	0.10	0.12	0.12	0.20	0.16	0.10	-

Correlation of Geographic and Genetic Distances

To elucidate the relationship between geographic distance and genetic distance, we computed the genetic distance matrix for populations using GenAlEx 6.1 (Peakall and Smouse, 2001) software. The latitude and longitude values of the populations are given in Table 4. Then, we determined the

geographic distance matrix of the populations (Table 5). A statistically weak negative correlation was found in the comparison between geographic distance and genetic distance for all genotypes (R^2=0.04) (Fig. 3).

Table 4. Latitude and longitude data of *T. dicoccum* and *T. aestivum* wheat populations

Pop. Number	Wheat Locations	Latitude	Longitude
1	Dağpınar Village (Digor District)	40.46344900	43.32416601
2	Aşağı kotanlı Village (SelimDistrict)	40.46218999	42.90562599
3	Benliahmet Village (SelimDistrict)	40.48790500	42.90257291
4	Koçköy Village (ArpaçayDistrict)	40.86334199	43.51571701
5	İncesu Village (SusuzDistrict)	40.74201600	43.12925090
6	Dikme Village (Kars Town)	40.50762901	42.98111494
7	Kürekdere Village (ArpaçayDistrict)	40.71816590	43.49843090
8	Dağpınar Village (Digor District)	40.46344900	43.32416601
9	Geçit Village (AkyakaDistrict)	40.77485460	43.51654368
10	MezraaVillage (Kars Town)	40.69680501	43.17520889
11	Değirmenköprü Village (ArpaçayDistrict)	40.81860901	43.42140101
12	Arpaçay Town	40.84674701	43.33209501

Table 5. Geographic distance matrix (km) was calculated for all genotypes of *T. dicoccum* and *T. aestivum* populations

Pop	1	2	3	4	5	6	7	8	9	10	11	12
1	0											
2	35	0										
3	36	3	0									
4	47	68	66	0								
5	35	36	34	35	0							
6	29	8	7	60	29	0						
7	32	58	56	16	31	50	0					
8	0	35	36	47	35	29	32	0				
9	38	62	61	10	33	54	6	38	0			
10	29	35	33	34	6	27	27	29	30	0		
11	40	59	57	9	26	51	13	40	9	25	0	
12	43	56	54	16	21	48	20	43	17	21	8	0

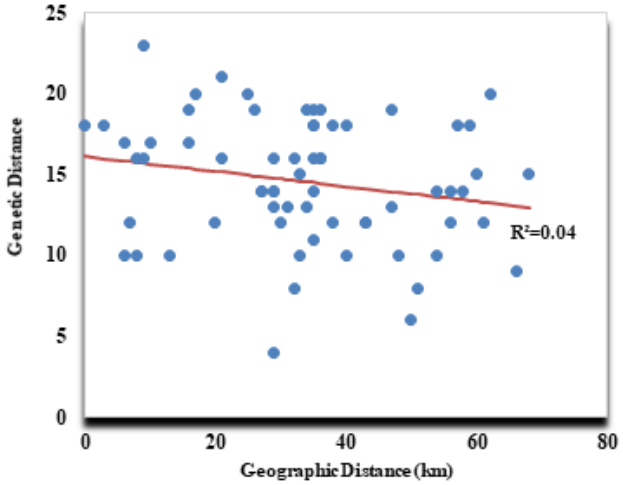

Figure 3. Mantel test regression between geographic and genetic distance matrices of 12 populations

Studies such as gene tagging, genetic mapping, genetic diversity, phylogenetic analysis using DNA markers have become particularly easy after the discovery of polymerase chain reaction (Joshi et al., 2000). In wheat, the most common cereal group, molecular markers are used to determine differences in specific loci at species or population level. Molecular markers are the tools involved in the explaining of variations by utilizing the knowledge of polymorphism among populations or genotypes (Madhumati, 2014; Mandal et al., 2018) The SSRs used in the present study are among the molecular marker techniques used in many fields such as phylogenetic relationship, genetic diversity, genetic mapping, population genetics, evolutionary genetics (Pagnotta et al., 2009; Khan et al., 2015; Kumar et al., 2016; Zarei et al., 2016). In the current study, Principal Coordinate Analysis (PCoA) was performed to identify genetic variations. PCoA results showed *T. aestivum* populations located a separate group (Digor and Aşağı Kotanlı populations) as expected. All of emmer wheat populations were originated from Kars region, i.e. from similar eco-geographic region. However, the PCoA analysis revealed that different clusters are observed. The populations of Benliahmet, Koçköy and Mezraa and the populations of Incesu, Dikme, Kürekdere and Dağpınar are clustered close together. The Geçit population formed a separate cluster. Arpaçay population was also in the same group as *T. aestivum* in dendrogram, while it was in different regions in PCoA analysis (Fig. 1). There are similar studies supporting these results. Fahima et al., (2002) reported that the genetic distances determined in their studies with wild emmer wheat from Turkey and Israel and the geographical distances between the populations were not related. Shizuka et al., (2015) investigated the genetic variation in *Triticum turgidum* ssp. *dicoccoides* populations and stated that a clear genetic differentiation was observed even though the distance between the populations was not much. These results can be explained by the differences of other geographical factors such as climate, latitude and longitude. In emmer wheat populations, there is no correlation between the locations of all genotypes and their genetic distances. A distance-based Neighbour Joining (NJ) tree was created to determine the molecular relationships of 12 populations using SSR markers in this study. When the dendrogram is analysed, emmer wheat was divided into three main groups. One of these main groups was only the Geçit population. The other main groups were the seperated subgroups within themselves. Also *T. aestivum* populations showed clustering together. Arpaçay *T. dicoccum* population was also in the same group as *T. aestivum* populations (Fig. 2). Similar results were obtained in other studies with *T. dicoccum* and *T. aestivum* wheat. Salunkhe et al., (2013) dendrograms using UPGMA in their molecular relationship analysis with SSR markers in *T. dicoccon* wheat and reported that 3 clusters were formed for 9 populations. Sarkar et al., (2014) examined 35 wheat varieties with 30 SSR markers and dendograms with UPGMA method and reported that all wheat genotypes were clustered in 3 main groups. Genetic distance is distance between populations. The high genetic distance value indicates that the difference between locations is excessive. In the present study, at the genetic distance matrix of 12 populations, we found that the most genetic distance was between the populations of Koçköy and Değirmenköprü in *T. dicoccum* populations, while the minimum genetic distance was between the Dikme and Dağpınar in *T. dicoccum* populations (Table 3). For all genotypes, our comparison between geographic distance and genetic distance revealed a statistically weak negative correlation (R^2=0.04) (Table 5). There are numerous studies supporting the results of this study. Dong et al., (2009) analyzed 15 wild emmer wheat populations with 25 EST-SSR markers. In their study, they found the value of genetic distance between populations to be 0.4 on average and they stated that populations that were geographically distant from each other have lower genetic distance and that genetic distance was independent of the geographic region. Oliveria et al., (2012) examined 53 wheat populations of tetraploids and reported that they found a weak correlation between geographical distance and genetic

distance (r = 0.21). Ren et al., (2013) in their study of genetic diversity in wild emmer wheats, they found r = 0.05 according to the results of the mantel test they performed to determine the relationship between genetic and geographical distance and stated that the genetic differentiation between populations was independent of geographical distance. Based on these studies, genetic differentiation among populations is not due to geographical distance in our study.

CONCLUSION

In the current study, we performed phylogeographic analyses for Kars's local emmer wheat together with cultivated *T. aestivum* using SSR markers. Due to the different geographical and climatic variations within Kars province, the phylogeographic structure of emmer wheat species in 3 main groups has also been supported by PCoA and phylogenetic studies. It is thought that this study will be a reference for the recognition of kavilca wheat (emmer), which is also rich in terms of nutrient content, and to expand the cultivation area in agriculture. In addition, genotypes showing superior characteristics of this wheat populations showing variation at molecular level are determined and it is thought that durum and bread wheat can be used successfully in breeding studies. It is also understood in parallel with literature studies that SSR markers are suitable molecular tools for molecular relationship, and genetic-geographically based distance analyses.

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Conflict of Interest

I declare that there is no conflict interest for writers of this article.

Authors Contributions

The authors declare that they contribute equally to the article.

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