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Genetic Diversity of Turkish Cultivated Emmer (*Triticum dicoccum* Schrank) Revealed by Microsatellite Markers

Hatice DEMIR[1], Dogan ILHAN[2*]

ABSTRACT: The aim of this study is to evaluate the genetic diversity of twelve populations for cultivated emmer (*Triticum dicoccum* Schrank ex Schübl) and bread wheat (*Triticum aestivum* L.) species, which are important wheat gene resources and grown in different villages in Kars province (Turkey), using eleven microsatellite markers. SSR primers produced a total of 41 alleles and the average polymorphism percentage was 86.2%. The average number of alleles obtained from primers was 3.72. Polymorphic Information Content (PIC) values varied between 0.14 and 0.37 with the means of 0.26 value. The primers of Xgwm-46 (0.287), Xgwm-154 (0.304) and Xgwmn-361 (0.325) were identified as the most effective primers in understanding the genetic diversity of emmer genotypes. Local emmer wheat had a little higher allelic richness and gene diversity than cultivated wheat. Due to geographic and climatic variations, genetic differentiation was detected in these wheat populations.

Keywords: *Triticum dicoccum*, Genetic diversity, Microsatellite marker, Kars

[1]Hatice DEMIR (**Orcid ID:** 0000-0003-4790-7729), Kafkas University, Graduate School of Natural and Applied Sciences, Program of Biology, Kars, Turkey
[2]Dogan ILHAN (**Orcid ID:** 0000-0003-2805-1638), Kafkas University, Faculty of Science and Letters, Department of Molecular Biology and Genetics, Kars, Turkey

***Sorumlu Yazar/Corresponding Author:** Dogan ILHAN, e-mail: doganilhan@kafkas.edu.tr

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INTRODUCTION

Wheat is one of the most important grains worldwide. In addition, it is the most cultivated and consumed grain in the world. Wheat, which spreads around the world from the region called the 'Bereketli Hilal', which covers the southeast of Turkey, is the main food on all continents today. Because Turkey is in a position where two different gene centers (Akdeniz and Verimli Hilal) overlap, it is the gene center of wheat and many field crops (Özkan et al., 2010). Among the wheat varieties, emmer wheat (*T. dicoccum*), has several advantages (resistance to wheat diseases and drought). Emmer wheat is a potential grain due to its adaptability to the soil, its ability to compete against stress factors, its resistance to environmental conditions, and its high crude protein content (Konvolina et al., 2012a; Konvolina et al., 2012b). Research shows that emmer wheat is also rich in microelements compared to einkorn and durum wheat (Tekin et al., 2018). Molecular markers are used to identify genotypes in many plant species. Microsatellites have been widely used in recent years due to their high level of polymorphism (İlhan et al., 2016). SSRs are one of the most appropriate markers used in many fields such as phylogenetic relationship, genetic diversity, population genetics and genetic mapping (Li-xin et al., 2015; Salehi et al., 2018; Kara et al., 2020; Belete et al., 2020). Information of genetic diversity is important for understanding the extent of genetic variability in existing plant material (Chen et al., 2012). Genetic diversity is the primary requirement to initiate a successful breeding program for the betterment of wheat productivity. The selection of diverse genotypes is the preliminary essential for molecular breeding of wheat (Raj et al., 2017). Genetic variations among emmer populations have been assessed in many countries with diversity analyses; Iran (Tahır and Abdul, 2010), Italy (Pagnotta et al., 2009), Turkey (Teklu et al., 2007; Kaymaz and İzbırak, 2010), Israel (Li et al., 2000; Dong et al., 2009), India (Salunkhe et al., 2013), Ethiopia (Teklu et al., 2006), Europe (Mondini et al., 2014). Research on the emmer has also been carried out in Turkey. However, there are limited studies on the genetic diversity of emmer, local emmer native to Turkey (Arystanbekkyzy et al., 2018; Demirel, 2020). In the current study, the genetic diversity of emmer (*Triticum dicoccum* Schrank) belonging to Kars province (Turkey) was investigated using SSR markers.

MATERIALS AND METHODS

Plant Materials and DNA Extraction

The seeds of ancestral wheat populations used in the study were obtained from farmers in the villages of Kars (Table 1). Seed samples were sown in pots in the growth chamber containing 16/8 lighting periods and a room temperature of 25 ^{0}C. Following the germination period, DNA isolation was performed using the CTAB method from the young leaves of the 2-weeks-old plants (Doyle and Doyle, 1990). The isolated DNAs were then 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

PCR reactions were carried out in Bioneer Global Genomics Partner Thermocycler. For these reactions; 25 µl total volume containing 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 $MgCl_2$), 1 µl of diluted genomic DNA (10 ng/1 µl) was used. PCR cycle; pre-denaturation at 94^0C for 3 minutes, 45 cycles of denaturation at 94^0C for 1 minute, 56-59^0C for 1 minute, and 72^0C for 2 minutes and 72^0C for 10 minutes was set as a cycle. PCR products (100-700 bp) were run in 5% agarose gel electrophoresis and visualized at UV. Scoring was done based on the bands obtained. Among the 30 SSR markers used in the study, eleven SSR markers were easily scored and those with high amplification ability were selected (Table 2).

Table 2. Primers of eleven SSR markers used in the study (Röder et al., 1998)

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

Because we used codominant SSR markers in the analysis, we scored the bands as 1-0. We used the GenAlEx 6.1 (Peakall and Smouse, 2001) program to determine genetic distances among populations. We made calculations using POWERMARKER v3.25 (Liu and Muse, 2005) software for

basic criteria such as the number of alleles, major allele frequencies, gene diversity and the polymorphic information content (PIC), which are important parameters of genetic diversity. We calculated the number of polymorphic bands and percentage of polymorphism for all primers using GenAlEx 6.1 (Peakall and Smouse, 2001) and POWERMARKER v3.25 (Liu and Muse, 2005) programs.

RESULTS AND DISCUSSION

Genetic Diversity Analysis

To assess the genetic diversity of twelve wheat genotypes, parameters such as the number of alleles, major allele frequency, gene diversity and the PIC values were analyzed. 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 et al., (1983). When we took into consideration 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	-

In this study, eleven SSR markers were produced total of 41 polymorphic bands and the average polymorphism percentage was 86.2%. We calculated the PIC value to determine the effectiveness of SSR markers. PIC value was observed in the Xgwm-340 primer pair with the highest value of 0.375, while the lowest value was obtained from the Xgwm-95 primary pair with 0.190 in emmer genotypes. In terms of PIC values, the primers Xgwm-361 with 0.325, Xgwm-154 with 0.304 and Xgwm-46 with 0.287 had high values. In emmer genotypes Xgwm-46, Xgwm-154 and Xgwmn-361 primers were understood to be the most effective primers in assessing their genetic diversity. The mean PIC values for emmer genotypes was 0.264 (Table 4).

Table 4. Total band numbers and the PIC values revealed by amplifications of two species (*T. dicoccum, T. aestivum*) with eleven SSR markers

No	Primer	Monomorphic Bands	Polymorphic Bands	Total Bands	The Percentage of Polymorphism (%)	PIC Value
1	Xgwm-46	-	6	6	100	0.287
2	Xgwm-95	1	2	3	66.6	0.190
3	Xgwm-120	1	4	5	80	0.280
4	Xgwm-154	1	2	3	66.6	0.304
5	Xgwm-155	1	3	4	75	0.241
6	Xgwm-340	1	4	5	80	0.375
7	Xgwm-361	-	2	2	100	0.325
8	Xgwm-389	-	9	9	100	0.233
9	Xgwm-540	-	2	2	100	0.243
10	Xgwm-558	1	4	5	80	0.239
11	Xgwm-601	-	3	3	100	0.195
Total		6	41	47		
Means			3.72		86.2	0.26

When the emmer wheat compared to cultivated wheat, the mean number of alleles (3.45), gene diversity (0.30) and polymorphic information content (0.24) values in emmer prove that genetic diversity was higher than cultivated wheat (Table 5). In fact, this difference emerged as a result of the analysis shows that the genetic diversity of *T. dicoccum* species is maintained at a certain level. Because genetic homogeneity can be seen due to artificial selection in many forms of culture.

Table 5. Diversity statistics of two species (*T. dicoccum* and *T. aestivum*) for twelve genotypes based on eleven SSR loci (in parentheses)

Parameters	Overall	*T. aestivum*	*T. dicoccum*
The Number of Individuals	12	2	10
Number of Alleles	3.72 (2.00-9.00)	2 (0.00-2.00)	3.45 (0.00-9.00)
Major Allele Frequency	0.76 (0.50-0.91)	0.78 (0.50-1.00)	0.78 (0.50-1.00)
Gene Diversity	0.32 (0.15-0.48)	0.21 (0.00-0.50)	0.30 (0.00-0.50)
Polymorphic Information Content	0.26 (0.14-0.37)	0.16 (0.00-0.37)	0.24 (0.00-0.37)

Microsatellites used in this study are used in many areas such as phylogenetic relationship, genetic diversity, genetic mapping, population genetics, evolutionary genetics (Zarei et al., 2016; Kumar et al., 2016; İlhan et al., 2016; Abbasov et al., 2018; Kara et al., 2020). SSR markers have been extensively used to detect variability in wheat genotypes and to evaluate their genetic diversity (Chen et al., 2012). As a result of the study conducted with eleven SSR primers, the primers used in emmer populations produced fragments in the ranges suitable for fragment ranges in the literature. In the current study, eleven SSR primer pairs produced a total of 41 alleles with averages of polymorphism percentage of 86.2%. The average number of alleles obtained from SSR primers was 3.72 (Table 4). Salunkhe et al., (2013) detected the average allele number in their study as 3.87, Akfirat and Uncuoglu, (2013) found 3.09 alleles per locus the average allele number, Salem et al., (2008) found the average number of alleles to be 3.2. It is observed that these obtained values are highly compatible with this study. The PIC values and genetic diversity are very helpful parameters to measure the polymorphism between the genotypes used in breeding programs (Robbana et al., 2019; Mourad et al., 2020). In the present study, the PIC values ranged between 0.14 and 0.37. The average PIC value was 0.26. The primer Xgwm-340 had the highest PIC value, and the primer Xgwm-95 gave the lowest PIC value. In terms of the PIC values, the primer pairs of Xgwm-46 (0.287), Xgwm-154 (0.304) and Xgwmn-361 (0.325) were identified as the most effective primers in understanding the genetic diversity of emmer genotypes (Table 4). The PIC values obtained in this study were determined in the literature samples (Emebiri et al., 2008; Ren et al., 2013; Rafeipour et al., 2016; Zatybekov et al., 2020; Mourad et al., 2020) but some of them are high or

low. This difference may be caused by the samples of emmer used in the study and the presence of SSR markers used in the study in different numbers and localities. In this study, gene diversity values ranged between 0.15 and 0.48. The average gene diversity value was 0.32. Arystanbekkyzy et al., (2018) found the average gene diversity value to be 0.48, Mourad et al., (2020) detected the average gene diversity in their study as 0.29. Genetic distance is the distance between populations. The high genetic distance value indicates that the difference between locations is excessive. In the present study, we saw that the most genetic distance was between the populations of Koçköy and Değirmenköprü in *T. dicoccum* populations, while the minimum genetic distance was between Dikme and Dağpınar in *T. dicoccum* populations (Table 3). Although they are in the same geography, the main reason for the differences in these wheat populations is due to altitude and climatic differences rather than genetic distance. There are studies in the literature with similar results supporting these findings. Fahima et al., (2002) stated 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 reported that a clear genetic differentiation was observed even though the distance between the populations was not much.

CONCLUSION

In this study, we investigated genetic diversity for Turkish cultivated emmer with microsatellite marker SSR native to Kars region. We found that this local wheat species had a moderate genetic diversity and allelic richness. Due to the different geographical and climatic variations, differentiation was detected in wheat populations. Local emmer wheat had a little higher allelic richness and gene diversity than cultivated wheat. Emmer wheat, whose planting area is gradually decreasing, is quite resistant to biotic and abiotic environmental conditions, planting areas should be increased and it should be explained that it is suitable for organic agriculture. It is thought that this study will be a reference for the recognition of kavilca wheat (emmer), which is also very rich in terms of nutrient content and to expand the cultivation area in agriculture. In addition, genotypes showing superior characteristics of this wheat population showing variation at the 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 genetic diversity analyses.

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Conflict of Interest

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

Authors Contributions

The authors declare that they contribute equally to the article.

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