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RESEARCH ARTICLE

Molecular identification and population structure of emmer and einkorn wheat lines with different ploidy levels using SSR markers

Serap Demirel · Fatih Demirel

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Abstract Ancient species (*Triticum monococcum* and *Triticum dicoccum*) of wheat represent a valuable genetic resource for breeding and genetic research. In this study, 81 selected genotypes and 7 commercial cultivars were evaluated with 11 simple sequence repeat (SSR) molecular markers. A total of 93 SSR alleles were detected, giving an average of 8.45 alleles per locus. Consequently, a total of 88 genotypes were assessed for their mean expected heterozygosity (He = 0.486), observed heterozygosity (Ho = 0.121), polymorphism information content (PIC = 0.68), and Shannon's information index (I = 0.918). The clustering analysis separated the genotypes into five subclusters based on the genetic similarity coefficient. Analysis of molecular variance (AMOVA) was performed to evaluate five different clusters. The result of AMOVA was defined as genetic deviation from expectation for clusters (Fis = 0.877, Fit = 0.903, and Fst = 0.211). In Nei's pairwise genetic identity, the highest and lowest were observed between P2-P5 populations (0.39) and P1-P2 populations (0.838). The wide variety of wheat lines can be used as a genetic resource in designing a wheat breeding program to develop new cultivars adapted to different geographic and climatic conditions and can also contribute to breeding programs around the world.

Keywords *Triticum* spp. · Breeding · Genetic diversity · AMOVA · Crops

Introduction

The wheat and closely related species are included in the *Poaceae* (*Gramineae*) family, which is the genus Triticum (Matsuoka 2011). Ancient wheats, including einkorn (*T. monoccocum* L.) and emmer wheat (*T. dicoccum* L.), were discovered for the first time in the Cayonu excavation dating back to 6500–7000 B.C. in Türkiye (Harlan 1998). These species are characterized by hard husks remaining with the grain after threshing.

As with all living species, the evolutionary continuity of plant species is dependent on their ability to adapt to changing environmental conditions. Understanding the genetic diversity of the analyzed plant species is crucial for sustainable agriculture (Sevindik and Efe 2021). It can be possible to develop cultivars that are tolerant and resistant to harsh environmental conditions such as drought with biotechnological

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S. Demirel
Serap Demirel, Molecular Biology and Genetic Department of Van, Yuzuncu Yil University, Van, Türkiye

F. Demirel (✉)
Fatih Demirel, Agriculture Department of Igdir University, Iğdır, Türkiye
e-mail: drfdemirel@gmail.com

applications (Munaweera 2022). The genetic diversity of wheat species is also used in today's wheat breeding studies (Zhang et al. 2010; Gurcan et al. 2017). Successful breeding programs are closely related to the extent and diversity of genetic material. The genetic diversity of wheat serves as the foundation for a breeding program designed to increase wheat yield. Wheat breeding through hybridization necessitates the selection of multiple genotypes regardless of whether the crop is a pure or hybrid variety (Zeb et al. 2009; Kumar et al. 2016a, b). Genetic characteristics are considered selection criteria in wheat breeding. Molecular markers are used in many areas, such as the preparation of detailed physical and genetic chromosome maps in plant organisms, selection of the desired characteristics in plants, increasing the success of classical breeding studies, determining the characteristics of gene sources in plants, genetic studies, determination of transgenic plants (Gupta et al. 2002; Collard and Mackill 2008; Sönmezoglu and Terzi 2018). Understanding the genetic diversity contained in a germplasm collection will have a substantial effect on wheat breeding. Characterization of the germplasm is regarded as a precondition for breeding since it provides new knowledge that may be used for future breeding actions (Demirel 2020). Molecular markers may give opportunities to discover exact genetic diversity across various farmed and wild wheat species that have varying amounts of ploidy (Gurcan et al. 2021). These opportunities can be found in different wheat species.

The molecular markers are useful for the assessment of genetic diversity such as DAMD (Directed Amplification of Minisatellites DNA), RAPD (Randomly Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeats), SSR (Simple Sequence Repeats), IPBS (Inter Primer Binding Site) (Gurcan et al. 2017; Pinar et al. 2019; Demirel 2020; Yildiz et al. 2021a; Karakaya et al. 2023). Molecular markers provide various advantages, including greater information acquisition, independence from the environment, and stability (Bulunuz Palaz et al. 2023). Simple sequence repeats (SSRs) are extensively utilized in wheat due to their high level of polymorphism, uniform dispersion, and codominant inheritance in the wheat genome (Salem et al. 2015; Uzun et al. 2022). In higher organisms, there are randomly repeated DNA regions whose functions are not yet known but that are thought to have regulatory roles (Rafalski and Tingey 1993). These repeats are called microsatellite and minisatellite according to the number of nucleotides. Microsatellites are referred to as SSR (simple sequence repeats) or STR (short tandem repeats) (Liu 1997). The most common among microsatellites are dinucleotide repeats (AT)n consisting of repeats of 1–6 bp. Repeats such as (AAG)n and (AAT)n are very common in plants (Ellegren et al. 1997). SSRs are more plenty, ubiquitous in presence, inherently hypervariable, and have a high polymorphic information content (PIC) (Gupta et al. 2010).

Using SSR markers, the purpose of this study was to identify the diversity and population structure among wheat lines that may be employed as parents in breeding and hybridization research.

Materials and methods

Plant materials

The investigation utilized 36 Einkorn (*Triticum monococcum* L.) wheat lines, 45 Emmer (*Triticum dicoccum* L.) wheat lines, and seven registered wheat cultivars (Table 1). Plantings of wheat populations that had been gathered from Türkiye's Kars, Kayseri, and Kastamonu regions were carried out in the Iğdır region. For the purposes of breeding, wheat lines were developed from individual spikes that were chosen from populations of wheat that exhibited a variety of traits. Within the parameters of this research, the wheat lines that were obtained were analyzed to assess the genetic variation. In addition, some Einkorn and Emmer wheat spikes are presented in Suppl. Fig. S1.

DNA extraction and SSR marker analysis

Under the circumstances of a greenhouse, the wheat seeds of each line were planted into individual pots. The newly acquired young leaves were used in an isolation procedure for DNA. The extraction of genomic DNA was accomplished by adhering to the CTAB procedure (Doyle and Doyle 1990) with a few adjustments (Aydin et al. 2018). For determining amplification and polymorphism, the PCR methodology that was established by Celik and Aydin (2023) was used. In this investigation, a total of 11 SSR (Table 2) primer pairs were utilized to determine the level of

Table 1 Information on the genotypes used in the study

Code	Species	Code	Species	Code	Species	Code	Species
G1	Emmer	G23	Einkorn	G45	Emmer	G67	Einkorn
G2	Emmer	G24	Einkorn	G46	Emmer	G68	Einkorn
G3	Emmer	G25	Einkorn	G47	Emmer	G69	Einkorn
G4	Emmer	G26	Emmer	G48	Emmer	G70	Einkorn
G5	Emmer	G27	Einkorn	G49	Emmer	G71	Einkorn
G6	Emmer	G28	Emmer	G50	Emmer	G72	Einkorn
G7	Emmer	G29	Emmer	G51	Emmer	G73	Einkorn
G8	Emmer	G30	Einkorn	G52	Emmer	G74	Einkorn
G9	Emmer	G31	Einkorn	G53	Emmer	G75	Einkorn
G10	Emmer	G32	Einkorn	G54	Emmer	G76	Einkorn
G11	Emmer	G33	Einkorn	G55	Emmer	G77	Einkorn
G12	Emmer	G34	Einkorn	G56	Emmer	G78	Einkorn
G13	Emmer	G35	Einkorn	G57	Emmer	G79	Einkorn
G14	Emmer	G36	Einkorn	G58	Emmer	G80	Einkorn
G15	Emmer	G37	Emmer	G59	Emmer	G81	Einkorn
G16	Emmer	G38	Emmer	G60	Einkorn	G82	*T. durum* L. (Doğankent)
G17	Emmer	G39	Einkorn	G61	Einkorn	**G83**	*T. durum* L. (Kızıltan-91)
G18	Emmer	G40	Emmer	G62	Einkorn	G84	*T. durum* L. (Diyarbakır-81)
G19	Emmer	G41	Emmer	G63	Einkorn	G85	*T. durum* L. (Sarıçanak-98)
G20	Emmer	G42	Einkorn	G64	Einkorn	G86	*T. durum* L. (Çeşit1252)
G21	Emmer	G43	Einkorn	G65	Einkorn	G87	*T. aestivum* L. (Bezostaja-1)
G22	Emmer	G44	Emmer	G66	Einkorn	G88	*T. aestivum* L. (Gerek-79)

molecular diversity and population structure (Gurcan et al. 2017; Yildiz et al. 2021b). Amplified PCR (polymerase chain reaction) products were electrophoresed on a 2.5% (w v^{-1}) agarose gel in a Tris-borate-EDTA (TBE) buffer for 120 min; ethidium bromide was used to stain the gel after electrophoresis; and an Imager Gel Doc XR + system (Bio-Rad, United States) was used to observe and photograph the results (Fig. 1). As a molecular size marker, the GeneRuler 100 bp DNA Ladder was utilized (ThermoFischer Scientific Waltham).

Data analysis

For molecular analysis, a genetic similarity and clustering matrix based on the quantity of shared alleles were generated, and the observed heterozygosity (Ho), expected heterozygosity (He), and polymorphism information content (PIC) were computed by the PowerMarker V3.025 software (Liu and Muse 2005). Shannon information index (I) for 11 SSR markers was calculated using the GENALEX V6.5 program (Peakall and Smouse 2006). Population structure of genotypes and the optimal value of ΔK were generated by STRUCTURE software and Structure Harvester (Pritchard et al. 2000; Earl and vonHoldt 2012). For AMOVA and population information (N: sample number, Na: number of different alleles, Ne: number of effective alleles, I: Shannon's information index, Ho: observed heterozygosity, He: expected heterozygosity, uHe: unbiased expected heterozygosity, and F: fixation index), the GENALEX program was used. In addition, Nei's genetic identity and distance matrices among subpopulations were created. UPGMA cluster analysis was utilized to assess patterns of diversity among the wheat entries based on the genetic similarity matrix generated by MEGA7 software (Kumar et al. 2016a, b).

Result and discussion

Molecular characterization

The results from 93 polymorphic bands scored using 11 SSR primers containing divergent linkage groups (LG) were utilized to carry out molecular

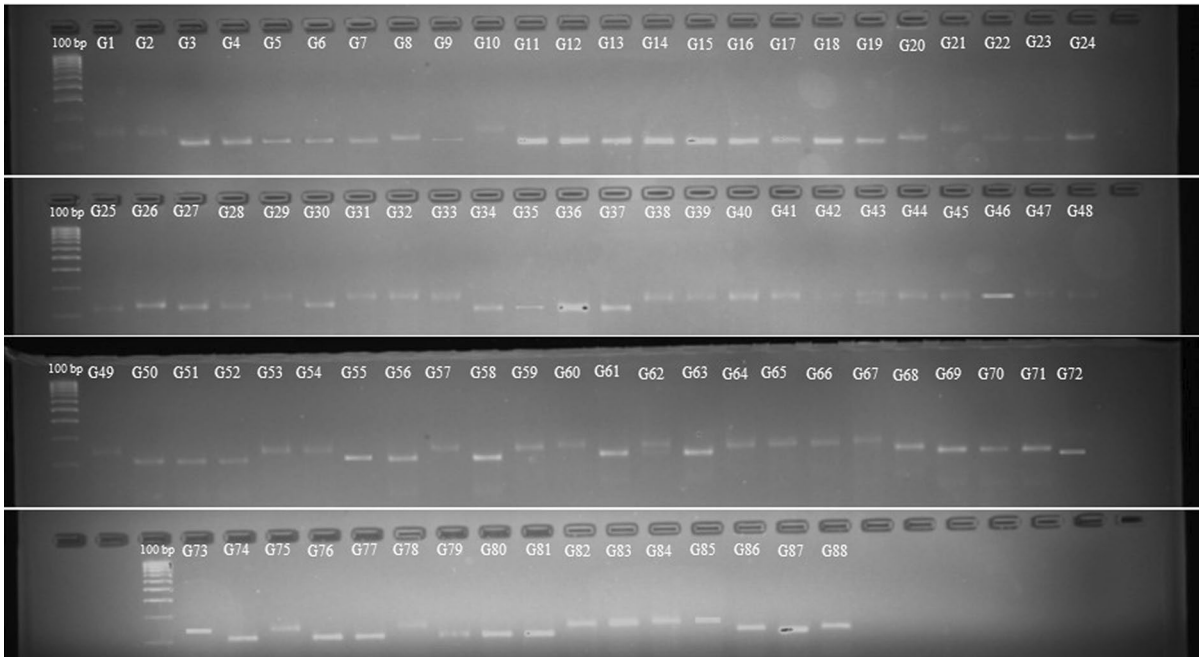


Fig. 1 PCR amplification profile of simple sequence repeat marker Xgwm312 on agarose gel

characterisation and estimate the basics marker characteristics, described in Table 2. The number of alleles found in each marker varied anywhere from 5 to 15, with 8.45 being the mean value. The marker WMC177 provided the greatest number of alleles, which was 15, while the Xgwm135 and Xgwm312 tests produced the fewest number of alleles, each of which was also 5. This finding is much lower than the number that was reported in several earlier research (Teklu et al. 2006; Gurcan et al. 2017),

Table 2 Summary of genetic parameters for 88 wheat genotypes using 11 simple sequence repeat markers

Marker	Forward (5′-3′)	Reverse (5′-3′)	N	LG	Ho	He	I	PIC
Xgwm135	TGTCAACATCGTTTTGAAAAGG	ACACTGTCAACCTGGCAATG	5	1 A	0.412	0.388	0.646	0.555
Xgwm312	ATCGCATGATGCACGTAGAG	ACATGCATGCCTACCTAATGG	5	2 A	0.067	0.288	0.500	0.520
Xgwm136	GACAGCACCTTGCCCTTTG	CATCGGCAACATGCTCATC	7	1 A	0.073	0.524	0.978	0.786
Xgwm71	GGCAGAGCAGCGAGACTC	CAAGTGGAGCATTAGGTACACG	9	2 A	0.103	0.514	0.989	0.642
WMC177	AGGGCTCTCTTTAATTCTTGCT	GGTCTATCGTAATCCACCTGTA	15	2 A	0.046	0.571	1.266	0.862
Xgwm296	AATTCAACCTACCAATCTCTG	GCCTAATAAACTGAAAACGAG	7	2 A	0.309	0.567	1.015	0.734
Xgwm372	AATAGAGCCCTGGGACTGGG	GAAGGACGACATTCCACCTG	8	2 A	0.053	0.454	0.824	0.632
Dupw167	CGGAGCAAGGACGATAGG	CACCACACCAATCAGGAACC	7	6 A	0.063	0.543	1.029	0.725
Xgwm570	TCGCCTTTTACAGTCGGC	ATGGGTAGCTGAGAGCCAAA	9	6 A	0.037	0.362	0.646	0.552
Xgwm427	AAACTTAGAACTGTAATTTCAGA	AGTGTGTTCATTTGACAGTT	12	6 A	0.061	0.644	1.312	0.807
Xgwm635	TTCCTCACTGTAAGGGCGTT	CAGCCTTAGCCTTGGCG	9	7 A	0.107	0.490	0.893	0.665
Mean			8.45	–	0.121	0.486	0.918	0.680
Total			93					

Number of polymorphic bands (N); Linkage group (LG); Observed heterozygosity (Ho); Expected heterozygosity (He); Shannon information index (I); Polymorphism information content (PIC)

but it is significantly higher than the number that was recorded in a number of earlier studies (Eujay et al. 2002; Salunkhe et al. 2013; Asmamaw et al. 2019). The value of observed heterozygosity (Ho) ranged from 0.037 for marker Xgwm570 to 0.412 for Xgwm135, with a mean of 0.121. The gene diversity (He) of markers ranged from 0.288 to 0.644, with marker Xgwm312 having the lowest score and marker Xgwm427 having the highest score, with the mean He score being 0.486. The Shannon information index (I) of markers ranged from 0.5 to 1.312, with marker Xgwm312 having the lowest score and marker Xgwm427 having the highest score, with the mean I score being 0.918. The average I value determined in this study was lower than the previous study conducted using SSR marker systems by Peng et al. (2009); however, it was higher than the results of Bhandawat et al. (2020). According to the standards established by Vaiman et al. (1994), PIC values were categorized as follows: those with a value of more than 0.5 were considered to have a high value, those with a value ranging from 0.5 to 0.25 were considered to have a medium value, and those with a value of less than 0.25 were considered to have a low value. The PIC values of the loci varied from 0.52 (Xgwm312) all the way up to 0.862 (WMC177), with a mean value of 0.68. The current investigation found that the PIC values for all 11 SSR markers were quite high. In a prior investigation of wheat genotypes using SSR markers, the researchers (Gurcan et al. 2017; Asmamaw et al. 2019) found the same conclusions related to PIC. Previous research has shown that when the PIC is more than 0.5, the marker has the greatest variety, which indicates a significant allelic diversity among germplasms. On the other hand, when PIC is less than 0.25, the marker has the least amount of variety (Kamara et al. 2020; Yildiz et al. 2021b). Considering these findings, which have an average PIC value of 0.68, it may be deduced that there is an adequate level of genetic heterogeneity and variation among the genotypes.

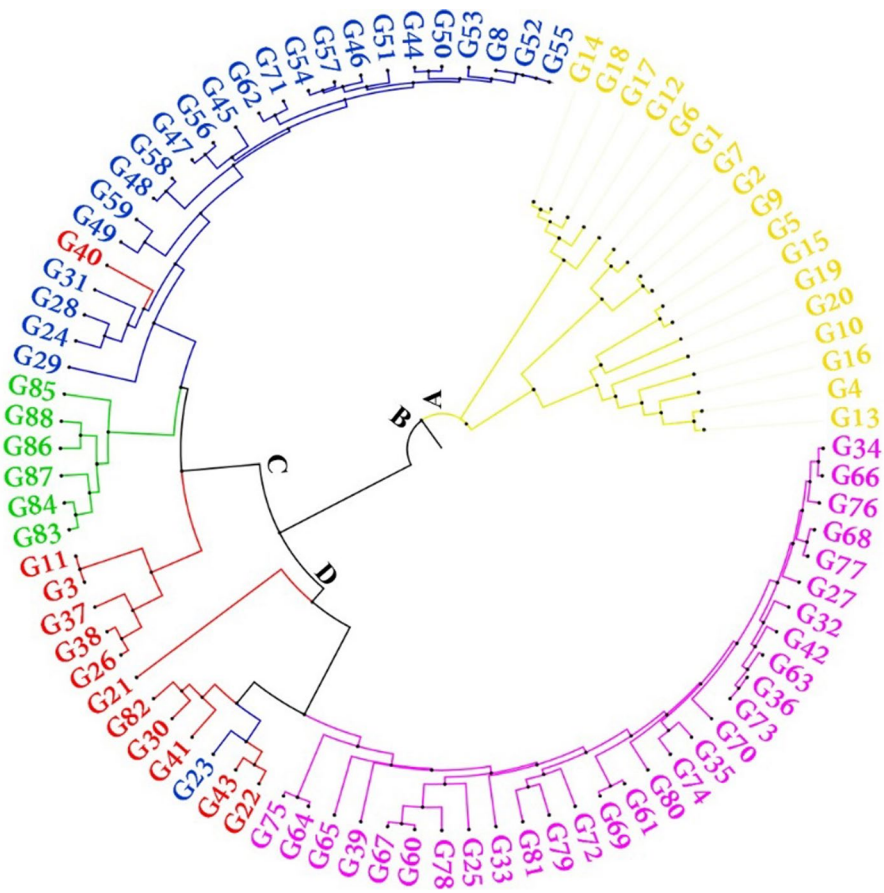
Because of this, the WMC177 marker located in linkage group (LG) 2 A was discovered to be the most polymorphic marker. This means that it has the potential to achieve discriminating ability for more marker-assisted selection studies as well as genetic diversity research in wheat breeding.

The UPGMA algorithm was used so that the dendrogram could be constructed. In Fig. 2, we presented an optimal tree that had the sum. With the use of the Maximum Composite Likelihood approach, the distances between genotypes were calculated (Tamura et al. 2004). Population genetic structure was also examined among 88 genotypes. The K value of this analysis was determined to be $K=5$, implying the existence of five populations (P1, P2, P3, P4, and P5) among the wheat gene pool. The design of populations was visualized in a graph (Fig. 3). The genotypes for which population structure was determined in Fig. 3 were shown with the same colors in the dendrogram (Fig. 2).

The 88 genotypes were divided into two main groups (A and B). Individually from other genotypes, the P4 population's genotypes were clustered into a singular group (A). The A group consisted exclusively of *T. dicoccum* L. species. The B group split into two subgroups, C and D. Registered cultivars (except G82) were clustered into the C group in the dendrogram. In addition, they were within P2 in Fig. 3. All the P5 (Fig. 3) genotypes in group D (Fig. 2) were *T. monococcum* L. species. In general, 11 SSR markers divided different species of wheat into separate clusters. The present results showed similarity with the reports of Gurcan et al. (2017). Therefore, the classification of wheats into various clusters according to their species suggested that there may be evidence of the evolution of diverse progenitors from ancient wheats to cultivated wheats.

Analysis of molecular variance

Analysis of molecular variance (AMOVA) was executed using data from wheat genotypes and STRU CTURE results. AMOVA demonstrated a lower proportion of variety among populations (21%) and within individuals (10%) as compared to variation among individuals within populations (69%) (Table 3). These results agreed with reports from previous studies. They also noted that in their research they employed wheat genotypes that had a large amount of diversity (Ramya et al. 2015; Soriano et al. 2016; Kabbaj et al. 2017). The values of Fit (Individual within the total population), Fis (Individual within the subpopulation) and Fst (Subpopulation within the total population) were 0.903, 0.877, and 0.211 for significance at $P<0.001$, respectively. These results were higher than the reported values of Asmamaw et al. (2019). Wright's F-statistics provide a unified



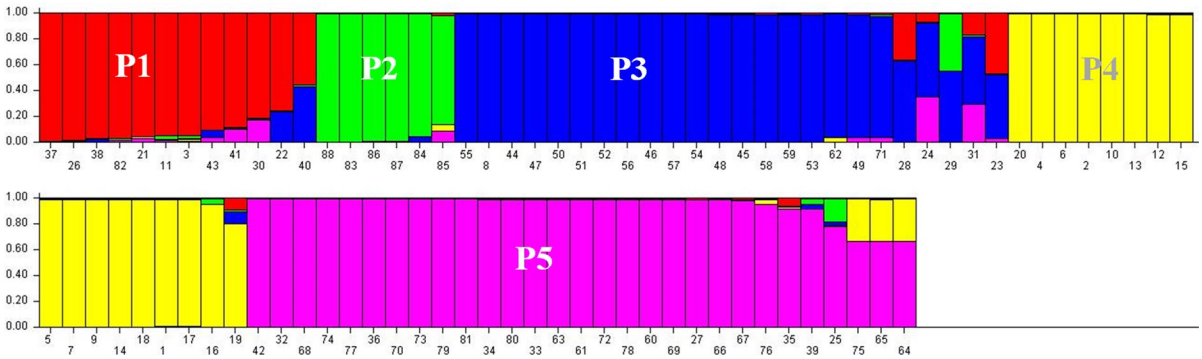


Fig. 2 Dendrogram of 88 wheat genotypes. The colors on the dendrogram represent the populations in the structure result

Fig. 3 Population structure analysis of 88 wheat genotypes

perspective of genetic variation at three hierarchical levels of population structure (Conner and Hartl 2004). As a result, all loci exhibited a high level of inbreeding (0.877), which caused heterozygosity. Fst, a measure of population differentiation due to genetic differentiation, was moderately high (0.211). It could be due to high levels of variation among individuals (Chao et al. 2007).

Table 4 presents the results of the reporting of genetic parameters of populations obtained by STRU

Table 3 Analysis of molecular variance for five wheat populations

Source	Degrees of freedom	Sum of squares	Mean squares	Estimated variation	Percentage
Among populations (P)	4	143.565	35.891	0.890	21
Individuals within P	83	519.617	6.260	2.926	69
Individuals	88	36.000	0.409	0.409	10
Total	175	699.182		4.225	100

CTURE analysis. The number of different alleles (Na) was most observed in the P3 population with 5.364 and least observed in the P2 population with 2.455. The population with the highest observed number of effective alleles (Ne) was P3, which had 3.246, whereas the population with the lowest recorded number of effective alleles was P4, which had 1.32. The highest value and the lowest value of Shannon's information index (I) were 1.246 for the P3 population and 0.37 for the P4 population, respectively. The mean of Shannon's information index was 0.918 for all the populations. The population with the greatest value of observed heterozygosity (Ho) was the P1 population, which had a value of 0.217. The population with the lowest value was the P4 population, which had a value of 0.048. The mean of observed heterozygosity values was 0.121 for all the populations. The highest value and the lowest value of expected heterozygosity (He) were 0.609 for the P3 population and 0.183 for the P4 population, respectively. The mean expected heterozygosity value was 0.486 for all the populations. The highest and lowest values of unbiased expected heterozygosity (uHe) for the P3 and P4 populations, respectively, were 0.631 and 0.19. The mean of unbiased expected heterozygosity values was 0.513 for all the populations. The highest value and the lowest value of fixation index (F) were 0.877 for the P3 population and 0.571 for the P4 population, respectively. The mean unbiased fixation index was 0.713 for all the populations.

In Nei's pairwise genetic identity, the lowest genetic identity was observed between P2 and P5 populations (0.39). Crossing genotypes from distant clusters may result in wider genetic variability and more heterosis in wheat lines. On the other hand, the highest genetic identity was observed between P1 and P2 populations (0.838). This might be due to the low criteria for wheat lines in this study or the high variance exchange between selecting wheat genotypes. Also, the differences observed in the wheat genotypes could result at least partly from the combined impacts of mutation, genetic drift, selection, and migration (Asmamaw et al. 2019) (Table 5).

In wheat, molecular markers based on simple sequence repeat (SSR) have been the most widely used in recent years due to their wide distribution in the genome, codominance, high polymorphism and reproducibility, and ease of analysis (Gurcan et al. 2017). Several studies have shown that SSR markers are an effective tool for analyzing wheat germplasm collections' genetic diversity and subpopulation organization (Salem et al. 2015; Soriano et al. 2016; Yildiz et al. 2021b).

Researching wheat's genetic variety is very important if one wants to comprehend the genetic makeup of wheat (Gurcan et al. 2017). The results of this research may be useful for wheat breeders and anyone who are interested in the improvement of wheat's

Table 4 Summary of genetic parameters for 5 populations

Population	N	Na	Ne	I	Ho	He	uHe	F
P1	9.909	3.636	2.581	1.041	0.217	0.579	0.611	0.623
P2	5.273	2.455	2.093	0.766	0.189	0.486	0.538	0.582
P3	15.091	5.364	3.246	1.246	0.081	0.609	0.631	0.877
P4	13.909	2.545	1.320	0.370	0.048	0.183	0.190	0.571
P5	17.727	5.273	2.897	1.166	0.069	0.573	0.594	0.865
Total	12.382	3.855	2.428	0.918	0.121	0.486	0.513	0.713

Sample number (N); Number of different alleles (Na); Number of effective alleles (Ne); Shannon's information index (I); Observed heterozygosity (Ho); Expected heterozygosity (He); Unbiased expected heterozygosity (uHe); Fixation index (F)

Table 5 Pairwise population matrix of genetic identity (down) and genetic distance (up)

	P1	P2	P3	P4	P5
P1	–	0.176	0.583	0.599	0.845
P2	0.838	–	0.812	0.796	0.943
P3	0.558	0.444	–	0.450	0.284
P4	0.550	0.451	0.637	–	0.817
P5	0.430	0.390	0.753	0.442	–

genetic resources. As a result, the agricultural gene pool must be appraised according to the degree of genetic diversity (Yildiz et al. 2021b), and the varied genotypes ought to be exploited in a variety of breeding programs.

In hexaploid wheat, just a small portion of the *T. monococcum* genome is present. Therefore, in breeding tetraploid and hexaploid cultivars, exploiting genetic variation in the *T. monococcum* genome can be a valuable source for discovering new and extra characteristics (Wang et al. 2014). In this context, knowing the range of variation present in a germplasm collection will majorly affect wheat improvement.

It is critical that the techniques of crop improvement and development make advantage of the native varieties that are available (Pachauri et al. 2017). According to a study by Demirel and Eren (2020), diploid and tetraploid types of wheat are still grown in Türkiye. Few farmers still grow these species in some limited areas, as they adapt better to the local environment than commercial hybrids (Gurcan et al. 2017). The fact that these species are still being cultivated is hopeful for the sustainability of genetic resources.

Conclusion

The wheat genotypes used in this study showed a high level of polymorphism. This is promising in terms of sustaining diversity. These results may indicate the potential of the SSR marker technique to discriminate among the wheat genotypes. This is very useful in choosing the parental genotypes used in hybridization and selection breeding programs. The use of these markers in association mapping studies may yield findings related to some characters to be examined in wheat. This investigation was an important study that revealed relationships between ploidy groups of *Triticum* spp. genotypes using SSR markers. It was observed that the genotypes were grouped clearly according to their ploidy levels. This study can contribute to wheat breeding programs and other breeders worldwide interested in wheat genetic resources.

Author contributions SD and FD conceptualized and established the methodology, while SD performed molecular characterization. FD analyzed the statistical data. The manuscript was written by SD and FD.

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Data availability All data needed to conduct this study is provided within the manuscript. Sample spike pictures for einkorn wheat and emmer wheat are included in the supplement.

Declarations

Conflict of interest The author's confirm that this article content has no conflict of interest.

Consent to participate and Publish The authors reviewed the manuscript and expressed a desire to publish this study.

Ethics approval This article does not contain any animal or human participant investigations conducted by the authors.

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