

Genetic Diversity and Population Structure of Bread Wheat (*Triticum aestivum* L.) Genotypes Using SSR Markers

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ABSTRACT

Bread wheat (*Triticum aestivum* L.) is one of the most important staple crops worldwide, yet its genetic diversity remains under continuous evaluation to support breeding and conservation efforts particularly in semi-arid regions. In this study, twenty-six bread wheat genotypes were analyzed using twenty-one simple sequence repeat (SSR) markers to assess genetic diversity, population structure, and clustering patterns. The UPGMA dendrogram based on Nei's genetic distance revealed six distinct genetic clusters, with one genotype forming a unique lineage. SSR marker analysis amplified between two and seven alleles per locus, with a mean of 3.52 alleles per marker. Diversity parameters, including effective allele number (N_e), expected heterozygosity (H_e), polymorphism information content (PIC), and Shannon's index (I), confirmed substantial allelic richness and high marker informativeness, with an average Polymorphism information content (PIC) value of 0.63. Population structure analysis partitioned the genotypes into two major populations ($K=2$), with three varieties exhibiting admixed ancestry, indicating gene flow and potential historical hybridization events.

The results showed clear variation among the studied genotypes. The SSR markers revealed a relatively high level of polymorphism, such as Xgwm33-1A, and the presence of admixed genotypes provide valuable insights for germplasm management and breeding strategies.

Overall, molecular data helped to provide a clearer picture of genetic relationships among the studied bread wheat genotypes. These results suggest that the local germplasm contains useful diversity that could be exploited in breeding programs aimed and enhance the efficiency of wheat improvement programs.

Keywords: bread wheat, genetic diversity, population structure, dendrogram, SSR markers.

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is a cornerstone of global food security, contributing nearly 20% of the world's caloric intake and serving as a primary protein source for billions of people (FAO, 2022). Its hexaploid genome harbors extensive genetic diversity that underpins breeding programs aimed at enhancing yield, resilience to climatic stresses, and nutritional quality. Conservation and characterization of wheat genetic resources are therefore critical for sustaining productivity under increasing biotic and abiotic pressures (Shewry and Hey, 2015; Rouzi, 2024). Molecular marker systems, particularly simple sequence repeat (SSR) markers, have been widely used to assess genetic diversity and varietal purity in

wheat. Some studies (Ciucă et al., 2010; Vasile et al., 2023) demonstrated that SSR markers are effective tools for assessing varietal purity and genetic diversity in wheat, confirming their high informativeness and usefulness for distinguishing wheat cultivars and maintaining genetic integrity in breeding programs.

The Fertile Crescent, including Iraq, represents one of the primary centers of wheat domestication and genetic diversity. Local landraces and traditional varieties, shaped by centuries of farmer selection under diverse agro-ecological conditions, represent valuable resources for resilience to stresses such as drought and temperature fluctuations (Al-Mamari, 2013). However, most studies conducted in Iraq have focused on agronomic performance or limited trait evaluations

(Nwry et al., 2023), while molecular characterizations remain scarce. Early efforts using RAPD markers (Al-Kaab et al., 2016) provided preliminary insights but lacked resolution and geographic coverage.

A clear gap persists: no study has systematically applied robust molecular markers such as SSRs to quantify genetic diversity and population structure in Iraqi wheat germplasm. This gap limits the identification of marker–trait associations relevant to local adaptation and end-use quality, and constrains the development of targeted conservation and breeding strategies. Addressing this limitation is essential for improving breeding efficiency and developing climate-resilient wheat cultivar suited to semi-arid environments.

The present study therefore employs SSR markers to provide a comprehensive assessment of genetic diversity and population structure in bread wheat genotypes. The findings are expected to

inform breeding programs and contribute to the conservation of valuable genetic resources.

MATERIAL AND METHODS

Plant Materials and Experimental Site

A collection of twenty-six bread wheat (*Triticum aestivum* L.) genotype, including landraces, improved varieties, and those preferred by local farmers, was assembled in collaboration with the Kurdistan Agricultural Research Institutes. The names of twenty-six bread wheat genotypes and their codes are shown in (Table 1) with their pedigree, the study was conducted during the growing winter season 2024-2025 at the Sulaimani Agriculture Research Center, Sulaymaniyah-Iraq (latitude 35.5570° N, longitude 45.4350° E; elevation ~850 m), the representative site for the region's temperate, rainfed cereal production systems.

Table 1. Bread wheat genotypes name along with their codes, pedigree, and origin used in the present study

Genotypes code	Genotypes name	Pedigree	Year of release	Origin
V1	Gihan 99	Certifies varieties / Import / Entry approved by the Ministry of Agriculture	2016	Turkey
V2	Monaliza	Certifies varieties / import	2021	Turkey
V3	Sulaimani 2	KAUZ*2/YACO//KAUZ/3/OASIS*2/BORL95 CMSS97M05852S-020Y-030M-202Y-040M-14Y-2M-0Y	2016	CIMMYT
V4	Hawler 2	Florkwa2/6/Sakers/5//YMH/TOB/4/BOWS-LC96-0180-030APS-2AP-DAPS-OPA	2018	CIMMYT
V5	Bakrajo 2	Sup152/BAJ#1/4BAJ#1/3KIRITATI//ATTILA*2/PASTOR/5/ CMSS12Y00919T-099TOPM-099Y-099M-OSY-13M-OWGY	2020	CIMMYT
V6	Hawler 4	Baj#1/3/KirjAI/A*2/Pastor CMSSOYOO288S-OS8-099Y-99M-099Y3M-OWGY	2018	CIMMYT
V7	Arass	Sentaelena*(Sonara64* Lerma Rojo64)	1982	Mexico
V8	Denk	Certified varieties / Al-Rif Gazraa company/ Kauz*Pastor	2018	Not available
V9	Koya 18	BHRIKUTI NL623/KAUZ//ALTRA84AOS Selection history: KARS09-05-0KO-0KO-0KO-136KO-28KO	2021	CIMMYT
V10	Hawler 8	FRET2*2/4/SN1/TRAP#1/3KAUZ*2/TRAP//KAUZ/5/2*FRNCLN CMSSO8Y00665T- 099TOPM-099Y-099M-099NJ-19WGY-0B	2021	CIMMYT
V11	Arabia	Certified varieties / Import / Dabana company	---	Not available
V12	Bizanzo	Certified varieties / Erbil - Agriculture research center	---	Not available
V13	Azmar	CMH 83/ ELVIRA// CMH 79/ AGA/ INIA (Rad.)	2020	Iraq
V14	Hsad	SNB//CMH79A955/3*CNO79/3/ATTILA	2014	Iraq
V15	Alaa	ESDA/VEE*10	2014	Iraq
V16	Babil	AIVATAB3/1.08C/3/KUKUNA/WHEAR//2*WBLI*2/4CMS08Y00803T- 099Y099MX112-13/M47IBWSN/306	2019	Iraq
V17	Rzgary	Certified varieties /Erbil - Agriculture research center	2010	ICARDA
V18	Qalla	Certified varieties Erbil - Agriculture research center	2023	Not available
V19	Adana	PFAU/SERI-M-82/BOBWHITE	2012	Turkey
V20	Russian	Certified varieties / import	---	Russia
V21	Kalar 2	CH75479/SARDARI-HD74-	2018	Iraq
V22	Kalar 1	ID800994.W/VEE/5/CA8055/4/ROMTAST/BON/3DIBO//SU-	2018	Iraq
V23	Koya 4	PASTOR/3/KAUZ*2/KAUZ//ALTRA84AOS Selection history: KARS09-02-0KO-0KO-0KO-29KO-36KO	2021	CIMMYT
V24	Wafia	Certified varieties / Import - ICARDA	2015	France
V25	Bora	H31 / Trapf21 / Enesco Certified varieties / Import / Dabana company	2014	Not available
V26	Panda	Certified varieties / Import / Dabana company	2014	Not available

Genomic DNA isolation

After harvesting, twenty-six bread wheat (*Triticum aestivum* L.) genotypes were cultivated in plastic pots (20 cm height × 12 cm diameter) filled with peat moss under controlled conditions at the laboratory of the College of Agricultural Engineering Sciences. Genomic DNA was isolated from young leaves of two-week-old seedlings using the cetyltrimethylammonium bromide

(CTAB) protocol as described by (AACC International, 2000; Majeed et al., 2024).

The Genomic DNA was isolated from 26 bread wheat genotypes by using Bioscience plant DNA preparation Kit Beta Bayern DNA preparation Kit (Beta Bayern GmbH .90453 Bayern, Germany). The isolated DNA was electrophorized in 1.5% Agarose gel. As shown in (Figure 1).

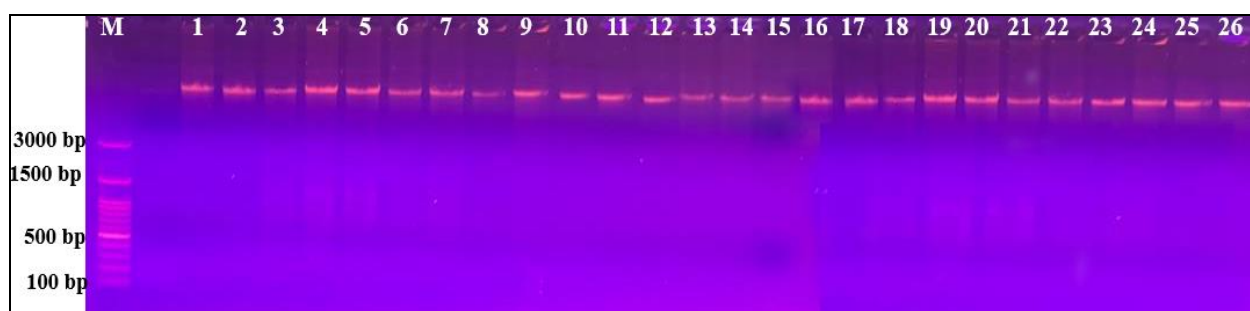


Figure 1. Genomic DNA isolated from young leaves of twenty-six bread wheat genotypes

PCR amplification and Electrophoresis

Twenty-one primers were tested in the present study as shown in (Table 2), and the primer sequences were obtained from the study of (Röder et al., 1998). The primer genes were used for 26 bread wheat genotypes, which were Synthesized by

Micro-gene Company (South Korea). Each primer could yield a band of the expected size bp. The PCR product was electrophoresed and visualized on a 2% agarose gel. The PCR produces as shown in the (Figure 1).

Table 2. Details of 21 primers along with number of amplicons produced and annealing temperatures

No.	Primers	Forward 5' 3' Reverse 5' 3'	Repeat	AT c°	Opata (bp)	Synth. (bp)
1.	Xgwm30-2D F	ATC TTA GCA TAG AAG GGA GTG GG	(AT)19(GT)15	60°	—	156
	Xgwm30-2D R	TTC TGC ACC CTG GGT GAT				
2.	Xgwm33-1A F	GGA GTC ACA CTT GTT TGT GCA	(GA)19	60°	116	—
	Xgwm33-1A R	CAC TGC ACA CCT AAC TAC CTG C				
3.	Xgwm55-6D F	GCA TCT GGT ACA CTA GCT GCC	(TC)3(T)3(CT)17	60°	128	132
	Xgwm55-6D R	TCA TGG ATG CAT CAC ATC CT 3				
4.	Xgwm66-4B F	CCA AAG ACT GCC ATC TTT CA	(CA)30(TA)21	60°	—	218
	Xgwm66-4B R	CAT GAC TAG CTA GGG TGT GAC A				
5.	Xgwm71.1-2A F	GGC AGA GCA GCG AGA CTC	(GT)20	60°	126	124
	Xgwm71.1-2A R	CAA GTG GAG CAT TAG GTA CAC G				
6.	Xgwm77-3B F	ACA AAG GTA AGC AGC ACC TG	(CA)10(GA)40imp	60°	—	135
	Xgwm77-3B R	ACC CTC TTG CCC GTG TTG				
7.	Xgwm165-4A F	TGC AGT GGT CAG ATG TTT CC	(GA)20	60°	188	193
	Xgwm165-4A R	CTT TTC TTT CAG ATT GCG CC				
8.	Xgwm194-4D F	GAT CTG CTC TAC TCT CCT CC	(CT)32imp	50°	136	131
	Xgwm194-4D R	CGA CGC AGA ACT TAA ACA AG				
9.	Xgwm213-5B F	TGC CTG GCT CGT TCT ATC TC	(GA)35	60°	162	198
	Xgwm213-5B R	CTA GCT TAG CAC TGT CGC CC				
10.	Xgwm273-1B F	ATT GGA CGG ACA GAT GCT TT	(GA)18	55°	171	165
	Xgwm273-1B R	AGC AGT GAG GAA GGG GAT C				
11.	Xgwm292-5D F	TCA CCG TGG TCA CCG AC	(CT)38	60°	214	188
	Xgwm292-5D R	CCA CCG AGC CGA TAA TGT AC				
12.	Xgwm304-5A F	AGG AAA CAG AAA TAT CGC GG	(CT)22	55°	202	208
	Xgwm304-5A R	AGG ACT GTG GGG AAT GAA TG				

No.	Primers	Forward 5' 3' Reverse 5' 3'	Repeat	AT c°	Opata (bp)	Synth. (bp)
13.	Xgwm333-7B F	GCC CGG TCA TGT AAA ACG	(GA)19	55°	154	166
	Xgwm333-7B R	TTT CAG TTT GCG TTA AGC TTT G				
14.	Xgwm369-3A F	CTG CAG GCC ATG ATG ATG	(CT)11(T)2(CT)21	60°	184	—
	Xgwm369-3A R	ACC GTG GGT GTT GTG AGC				
15.	Xgwm374-2B F	ATA GTG TGT TGC ATG CTG TGT G	(GT)17	60°	210	192
	Xgwm374-2B R	TCT AAT TAG CGT TGG CTG CC				
16.	Xgwm383-3D F	ACG CCA GTT GAT CCG TAA AC	(GT)27	60°	188	199
	Xgwm383-3D R	GAC ATC AAT AAC CGT GGA TGG				
17.	Xgwm437-7D F	GAT CAA GAC TTT TGT ATC TCT C	(CT)24	50°	109	111
	Xgwm437-7D R	GAT GTC CAA CAG TTA GCT TA				
18.	Xgwm494-6A F	ATT GAA CAG GAA GAC ATC AGG G	(CA)13	60°	194	196
	Xgwm494-6A R	TTC CTG GAG CTG TCT GGC				
19.	Xgwm642-1D F	ACG GCG AGA AGG TGC TC	(GT)14	60°	187	179
	Xgwm642-1D R	CAT GAA AGG CAA GTT CGT CA				
20.	Xgwm644-6B F	GTG GGT CAA GGC CAA GG	(GA)20	60°	152	—
	Xgwm644-6B R	AGG AGT AGC GTG AGG GGC				
21.	Xgwm666-7A F	GCA CCC ACA TCT TCG ACC	(CA)13	60°	87	—
	Xgwm666-7A R	TGC TGC TGG TCT CTG TGC				

Data Analysis

Genetic Diversity Statistics

Following fragment amplification, banding patterns were manually scored as present (1) or absent (0) to generate a binary data matrix. Pairwise genetic similarities were calculated using the Jaccard coefficient and subsequently converted into a dissimilarity matrix. Cluster analysis of the genotypes was performed using the unweighted pair group method with arithmetic mean (UPGMA). Polymorphism information content (PIC), allele frequencies, and other genetic diversity parameters were estimated based on SSR allele data scored according to fragment size. These parameters were calculated using GenAlEx, treating SSR markers as co-dominant loci. Gene diversity indices were further computed PowerMarker version 3.25 was used (Laidò et al., 2013).

Population structure analysis

Population structure analysis was conducted using the software STRUCTURE, version 2.3.4, with the number of assumed populations (K) ranging from 1 to 10. Each run was replicated three times to ensure consistency of the results. In addition, genetic distances and accession groupings were

determined using UPGMA implemented in XLSTAT.

RESULTS AND DISCUSSION

Genetic Diversity based on Microsatellite Markers

The UPGMA dendrogram based on Nei's genetic distance revealed six distinct genetic clusters (Figure 2). Cluster 1 contained a single genotype V12, which appeared as distinct and representing a unique lineage. Cluster 2 comprised two closely related genotypes (V13 and V14). Cluster 3 was the largest, grouping contains nine genotypes (V3-V11). Cluster 4 also contained nine genotypes (V18-V26). Cluster 5 comprised of three genotypes (V15-V17), while Cluster 6 included two genotypes (V1, and V2).

The genetic distance scale ranged from 0 to 6, where 0 indicates identity and 6 represents maximum divergence. The greatest genetic distances were observed between Cluster 1, V12, and the other clusters (~4.5-5.2), highlighting their unique genetic backgrounds. Within-cluster distances were notably smaller (0.8-1.5), indicating a higher genetic similarity among members of the same cluster.

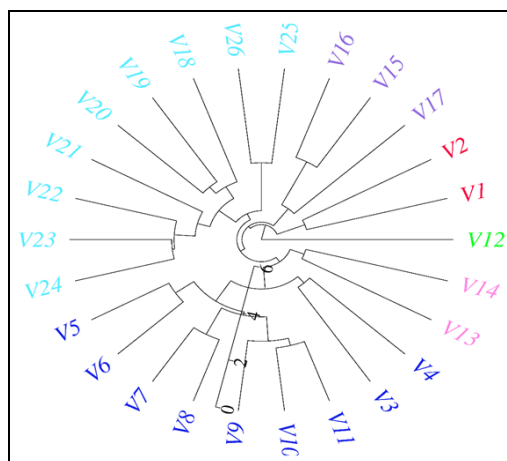


Figure 2. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of twenty-six bread wheat varieties based on simple sequence repeat (SSR) markers

SSR Marker Diversity Analysis

A total of twenty-one SSR marker were used to assess the genetic diversity among twenty-six bread wheat genotypes. The markers amplified between two and seven alleles per locus, with a total mean of 3.52 alleles per marker (Table 3). The highest number of alleles ($N_a=7$) was observed for marker Xgwm33-1A, whereas the lowest ($N_a=2$) was detected for Xgwm494-6A and Xgwm666-7A. The effective number of alleles (N_e) ranged from 1.98 (Xgwm494-6A) to 3.84 (Xgwm33-1A), with an average of 2.73, indicating substantial allelic diversity across loci. Expected heterozygosity (H_e) varied from 0.49 to 0.74, with a mean of

0.63. Similarly, polymorphism information content (PIC) values ranged from 0.49 to 0.74, averaging 0.63, reflecting a high level of marker informativeness.

The Shannon information index (I) ranged from 0.69 to 1.70, with a mean value of 1.20, further supporting the presence of considerable genetic variation among the studied varieties. Among all markers, Xgwm33-1A exhibited the highest diversity parameters (N_a , N_e , H_e , and PIC), whereas Xgwm494-6A showed the lowest values, indicating limited polymorphism. Overall, the SSR markers demonstrated a high capacity to detect genetic variability in the wheat germplasm.

Table 3. Genetic diversity parameters of 21 SSR markers in 26 bread wheat genotypes, including number of alleles (N_a), effective number of alleles (N_e), expected heterozygosity (H_e), Shannon's information index (I), and polymorphism information content (PIC)

Marker	N_a	N_e	H_e	I	PIC
Xgwm30-2D	4	2.91	0.66	1.32	0.66
Xgwm33-1A	7	3.84	0.74	1.79	0.74
Xgwm55-6D	3	2.45	0.59	1.05	0.59
Xgwm66-4B	4	2.76	0.64	1.28	0.64
Xgwm71.1-2A	4	2.68	0.63	1.24	0.63
Xgwm77-3B	4	2.89	0.65	1.30	0.65
Xgwm156-4A	3	2.52	0.60	1.08	0.60
Xgwm194-4D	3	2.63	0.62	1.12	0.62
Xgwm213-5B	3	2.41	0.58	1.03	0.58
Xgwm273-1B	4	2.70	0.63	1.25	0.63
Xgwm292-5D	4	2.55	0.61	1.19	0.61
Xgwm304-5A	3	2.83	0.65	1.21	0.65
Xgwm333-7B	3	2.74	0.64	1.18	0.64
Xgwm369-3A	5	3.21	0.69	1.49	0.69
Xgwm374-2B	3	2.87	0.65	1.22	0.65
Xgwm383-3D	4	2.69	0.63	1.26	0.63
Xgwm437-7D	3	2.71	0.64	1.17	0.64
Xgwm494-6A	2	1.98	0.49	0.69	0.49
Xgwm642-1D	3	2.30	0.56	0.98	0.56
Xgwm644-6B	3	2.85	0.65	1.20	0.65
Xgwm666-7A	2	2.00	0.50	0.69	0.50

Analysis of population structure

Population structure was conducted to assess the genetic stratification among bread wheat genotypes. The results revealed that the twenty-six genotypes were partitioned into two main genetic populations (Figure 3). The first population (red) consisted of eleven varieties, whereas the second population (green) comprised twelve varieties. The remaining three genotypes exhibited admixed ancestry, indicating genetic contributions from both populations. Based on simple sequence repeat (SSR) marker data, the number of genetic clusters was determined using the K-value approach.

The optimal number of populations (K) was identified by plotting the likelihood values against different K levels, where the highest peak was observed at K=2 (Figure 3A). This result suggests the presence of two distinct genetic populations, designated as Population 1 (red) and Population 2 (green), as illustrated in (Figure 3B). Similar approaches for determining population structure and optimal K values have been widely applied in genetic diversity studies using Bayesian clustering methods (Safner et al., 2011).

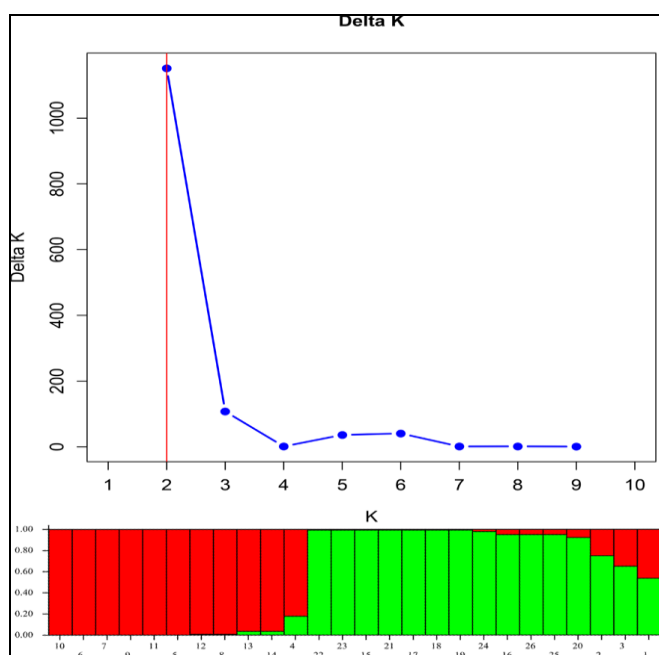


Figure 3. Determination of the optimal number of populations (K) based on the ΔK method, showing a clear peak at K = 2 resulting from the SSR dataset

Genetic Diversity and population Structure

The SSR analysis revealed a clear population structure along with a moderate to high level of genetic diversity among the studied bread wheat genotypes. The presence of two main genetic clusters (K=2), together with a small number of admixed genotypes, suggests a combination of genetic differentiation and some level of gene flow among the materials. This pattern likely reflects differences in breeding history and selection practices (Evanno et al., 2005; Jaradat, 2014). In general, modern improved genotypes tend to be more genetically similar due to the use of common parental lines, whereas landraces

usually show greater diversity as a result of long-term adaptation to local environmental conditions. Similar observations have been reported in previous studies, where intensive breeding has contributed to genetic narrowing, while traditional germplasm has helped preserve wider variability (Kara et al., 2017).

Although modern genomic tools such as SNP markers are increasingly used in diversity studies, SSR markers still provide a reliable and cost-effective alternative, especially in regions where resources are limited. Their high level of polymorphism, reproducibility, and co-dominant nature make them particularly useful for assessing genetic diversity and

population structure in crop germplasm (Röder et al., 2002; Strugala et al., 2003).

The genetic differentiation observed among clusters indicates the presence of distinct gene pools that could be effectively exploited in breeding programs. Genotypes that are genetically more distant are especially valuable for hybridization, as they may increase heterosis and help broaden the genetic base of future cultivars. In this context, using diverse parental lines is important for improving adaptability, yield stability, and resilience under semi-arid conditions (Laidò et al., 2013).

SSR Marker-Based Diversity Analysis

The SSR marker analysis indicated a moderate to high level of genetic diversity among the evaluated bread wheat genotypes from the Kurdistan Region of Iraq. The average number of alleles per locus ($N_a=3.52$) falls within the range commonly reported in wheat studies, suggesting that the selected markers captured a reasonable level of variability within the germplasm (Laidò et al., 2013).

The mean polymorphism information content ($PIC = 0.63$) further confirms that the SSR markers used were highly informative. Values above 0.50 are generally considered good indicators of marker efficiency, which in this case supports their suitability for varieties discrimination, diversity assessment, and potential use in breeding applications. Similarly, the relatively high expected heterozygosity ($H_e=0.63$) points to substantial genetic variation within the studied materials, which is important for selection and long-term genetic improvement (Chao et al., 2007; Song et al., 2023).

Differences in diversity observed across individual loci are likely related to variation in mutation rates, genomic location, and historical selection pressure. For instance, Xgwm33-1A showed the highest level of polymorphism, making it particularly useful for genetic diversity studies and breeding purposes. In contrast, Xgwm494-6A displayed lower variability, which may be due to allele fixation or reduced genetic

diversity in that specific genomic region (Röder et al., 2002).

The SSR results were in good agreement with the population structure analysis, where the Varieties were grouped into two main clusters. Markers with higher informativeness contributed more strongly to group separation, while the presence of admixed individuals suggests shared ancestry and gene flow among some of the genotypes. Such patterns are commonly reported in wheat breeding materials, where germplasm exchange and selection practices shape the genetic structure of populations (Kara et al., 2017).

CONCLUSIONS

The evaluation of twenty-six bread wheat (*Triticum aestivum* L.) genotypes revealed clear variability at the molecular level, confirming the presence of significant genetic diversity within the studied germplasm.

SSR marker analysis demonstrated a well-structured population with moderate to high genetic diversity among the genotypes. The presence of distinct genetic clusters, along with some admixed individuals, likely reflects differences in breeding history, selection practices, and local adaptation. Overall, these results point to a genetically diverse but structured germplasm pool that can be effectively used in future breeding efforts.

This study highlights the dual importance of conserving unique genetic lineages and leveraging admixed genotypes to enhance breeding efficiency and provides the first comprehensive multi-level assessment of bread wheat diversity in the Kurdistan Region of Iraq. By integrating molecular marker data with population structure analysis, breeders can design more effective selection programs, ultimately contributing to the development of resilient wheat varieties capable of meeting future agricultural and food security challenges.

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