Research Article

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Genetic Diversity Assessment in Iraqi Local Goat Breeds by Using Molecular Markers

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Abstract

Goats play a significant role in the economy of Iraq through livestock production, income generation, employment opportunities, and the conservation of valuable genetic resources. Genetic diversity of three Iraqi local goat breeds were studied by using two molecular markers, fifteen microsatellite (SSR) and fifteen RAP DNA markers. Individual blood samples were collected and individual genomic DNA were extracted from 30 Black, 10 Hybrid, and 20 Meriz goat breeds. PCR amplification was conducted. The results revealed that out of 15 SSR primers, 11 were amplified and showed 847 total bands, 53 were polymorphic with 6.58 percentage of polymorphic bands. All the fifteen RAPD primers amplified 6085 total bands, in which 273 were polymorphic bands with 4.33 percentage of polymorphic bands. Different unique bands were detected for each breed. Both SSR and RAPD gave moderate polymorphism 66.67% and 61.52%, respectively. Besides, this value was consistent with the moderate value of the mean of polymorphism information content 0.19 and 0.28, respectively. Meriz and Hybrid breeds revealed the longest genetic distance (0.114 and 0.316). While, Black and Meriz breeds revealed the highest closeness (0.956 and 0.831) for SSR and RAPD markers, respectively. Furthermore, the UPGMA dendrogram for both of SSR and RAPD markers classified the three goat breeds into two main clusters. The first one contained Black and Hybrid breeds. While, the second one contained only Meriz breed. The results of the current study will be helpful for future researchers as a key guide to better understanding the genetic relationships and breed differences in Iraqi goat breeds for planning strategies for the future genetic improvement program.

Keywords: Genetic diversity, Iraqi goat, polymorphism, RAPD, SSR.

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Introduction

Goat (*Capra hircus*) was domesticated from wild goat, belongs to the animal family Bovidae and the tribe Caprini. There are over 300 goats breeds all over the world (1). According to the historical evidence Meriz (Kurdi) goat is one of the oldest domesticated animal species raised at high altitudes in Zagros mountains of Iraqi Kurdistan at 10,000 years ago (2). The goat's population in Iraq is about 1.5 million heads (3), distributed all over the country, of which l2.5% in the southern, 44.2% central and 43.3% northern parts of the country (4). Goats wherever in Iraq usually forage along together with sheep, graze on natural pasture for more than six months annually. This important genetic resource is raised primarily for meat and milk. While, hair is of secondary importance, they are well adapted environmentally, low price, and high production animal (5) It is known as "Poor man's cow" contribute up to 19.9% of the national income.

The two Iraqi indigenous goat breeds are Black (Native or Rashoky) and Meriz (Kurdi) goats, Black or Native goat is a medium size, hair is coarse and medium length (10-15 cm) (4). Males usually have long horns. While, most females are polled. The ears are long and pendulous. The head is narrow and facial profile is slightly concave. Males usually have medium to short beards. The average body weight of an adult male is 49.7 kg and adult female is 39 kg (6). Meriz goat is raised mostly because of its fine fur (8-12 cm). It is smaller than black goat in size. It's either white, red, or brown. The horn of males reach 50 cm in length and female are polled. The ears are Long and directed laterally. The head is straight or slightly concave. The average body weight of an adult male is 40 kg and of an adult female is 35 kg (5). Hybrid goat: is the cross breed between Black (Native) and Meriz goats that have moderate characterization between both parents (4). There are lots of types of hybrid goats in Kurdistan, but this one is a result of combining of black and Meriz goats. It is known as Dura goats. There are two types of Dura. First: if a male black goat combined with a female Meriz goat, the breed features would be more similar to black goats. Also, its color mostly would be between ivory and white, which known as Kazh goat. Second type: if a male Meriz goat combined with a female black goat, their breed's features would be more similar to Meriz, such as its size, head, ears and horn shape. But its hair is coarser than Meriz goats. However, Hybrid goats have a medium size between Black and Meriz goats, they are more active and more ability to walk farther and climbing mountains more than other goat breeds. Although genetic research recently focuses on the conservation of biodiversity in domestic animals, these great efforts still cannot solve the problem of loss in local animal breeds (7). Goat population in Iraq had diminished (8) because of the successive droughts and their impact on feed wheat and barley production. Therefore, a good and speed conservation program for development of these breeds would be very significant. The plan should include a genetic analysis within and between population levels in these local breeds. Molecular markers play a crucial role in

assessing genetic diversity in goat populations and have several important implications for goat breeding, conservation, and research. The objective of the current study was to evaluate the genetic diversity in Iraqi local goat breeds (Black, Hybrid, and Meriz breeds) using two most powerful molecular markers such as microsatellite (SSR) and RAP DNA markers, which haven't been used together by any researcher in Iraqi goat breeds to assess genetic diversity.

Materials and Methods

Animal samples and locations:

Three Iraqi local goat breeds, 30 Black (native or rashoky), 10 Hybrid, and 20 Meriz (Kurdi) were used in the current study. The Native Black goat breed that symbolled (B1-B30) in this study was found at several various locations in Sulaimani province. Hybrid goat breed, in which symbolled (H1-H10), found in the most locations that samples were collected. Also, Meriz goat breed that symbolled (M1-M20), found only at Raparin administration.

Blood collection and DNA extraction:

Goats individually were sampled 5 ml of whole blood from jugular vein into vacutainer tubes (10 ml) containing the (EDTA) as anticoagulant, from regions where each breed is predominantly found in Sulaimani province to ensure that each population was a fair representative of the breed. Before sampling, each breeder was asked about the nature of the breeding system in order to determine the purity of the animals, at the same time, we collected blood from various goats color, age and gender within each breed, the blood samples were immediately transferred in a cooler box to the Biotechnology Laboratory at the College of Agricultural Engineering Sciences -Animal Science department, Sulaimani University, during the period from January to May of 2022. Blood samples were stored at –20 °C until DNA extractions. DNA was extracted from each of the blood sample using SinaPureTM DNA Blood Mini Kit (SINACLON EX6001) (SinaClonBioScience, Tehran, Iran), according to the manufacturer`s instructions. The quantity and quality of DNA was calculated by nanodrop spectrophotometer (Thermo Fisher Scientific, 2000, USA) later the concentration of DNA was written on each tube individually (26).

Microsatellite (SSR) and RAPD primers:

In the present study, a total of 15 of SSR and 15 of RAPD primers which were obtained from (Macrogen-LIGO) in Korea were used**.** The descriptions of primers regarding their names, primer sequences, annealing temp. (°C) are given in Table (1).

Table 1: List of SSR and RAPD markers used in this study with their sequences and annealing temperatures.

No.	SSR primers	Primer sequence (5' -- 3')	Annealing temp.	References
1	**OarFCB128**	ATTAAAGCATCTTCTCTTTATTTCCTCGC CAGCTGAGCAACTAAGACATACATGCG	55	(9)
2	**SRCRSP9**	AGAGGATCTGGAAATGGAATC GCACTCTTTTCAGCCCTAATG	55	(10)
3	**ILSTS5**	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	55	(10)
4	**ILSTS11**	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	55	(11)
5	**SRCRSP5**	GGACTCTACCAACTGAGCTACAAG GTTTCTTTGAAATGAAGCTAAAGCAATGC	56	(12)
6	**OarFCB20**	AAATGTGTTTAAGATTCCATACAGTG GGAAAACCCCCATATATACCTATAC	56	(13)
7	**OarFCB304**	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	56	(14)
8	**OarJMP29**	GTATACACGTGGACACCGCTTTGTAC GAAGTGGCAAGATTCAGAGGGGAAG	56	(15)
9	**OarHH47**	TTTATTGACAAACTCTCTTCCTAACTCCACC GTAGTTATTTAAAAAAATATCATACCTCTTAAGG	58	(9)
10	**MAF214**	GGGTGATCTTAGGGAGGTTTTGGAGG AATGCAGGAGATCTGAGGCAGGGACG	58	(9)
11	**OarJMP58**	GAAGTCATTGAGGGGTCGCTAACC CTTCATGTTCACAGGACTTTCTCTG	58	(16)
12	**BM1824**	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	58	(17)
13	**INRA063**	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	58	(18)

14	**MAF65**	AAAGGCCAGAGTATGCAATTAGGAG CCACTCCTCCTGAGAATATAACATG	60	(10)
15	**MAF70**	CACGGAGTCACAAAGAGTCAGACC GCAGGACTCTACGGGGCCTTTGC	60	(19)
	RAPD			
1	**RAP1**	CAGCCCCTTTC	42	
2	**RAP2**	AATCGGGCTG	42	
3	**RAP3**	GGGAGGGTGTT	42	(20)
4	**RAP4**	AGCGTGTCTG	42	
5	**RAP5**	TCACGTCCAC	42	
6	**RAP6**	GAAACACCCC	42	
7	**RAP7**	TCTCCGCCCT	42	
8	**RAP8**	CCCACACGCA	42	
9	**OPP03**	CTGATACGCC	36	
10	**OPP08**	ACATCGCCCA	36	(21)
11	**OPP14**	CCAGCCGAAC	36	
12	**OPP15**	GGAAGCCAAC	36	
13	**OPQ04**	AGTGCGCTGA	36	
14	**OPQ06**	GAGCGCCTTG	36	
15	**OPQ12**	AGTAGGGCAC	36	

PCR Amplification and Electrophoresis:

Both SSR and RAPD PCR amplifications were carried out in 25 µl as total reaction mixture volume containing: 10 µl of master mix 2X, 2 µl of primers (20 Pmol), 2 µl of each individual DNA sample (50 ng) continued the volume by 11 µl of deionized distilled water. The PCR amplifications program were performed starting with initial denaturation at 94 °C for 2 min. Then, followed by 35 cycles: denaturation at 94 °C for 30 sec, annealing at (55-60°C) for SSR or at (36 and 42°C) for RAPD primers for 30 sec., extension at 72 °C for 30 sec. with a final extension at 72 °C for 5 min. Products were then separated on 1.5% agarose gels using 1 × TBE (Tris-Borate-EDTA) running

buffer at 5 V/cm. and visualized by staining with ethidium bromide.

Data Analysis:

An overall of 60 goat individuals were DNA sampled and the data was used to calculate: a total number of fragments, monomorphic, polymorphic fragments, a percentage of polymorphic band (PPB) and unique bands for both SSR and RAPD markers.

The genetic analysis of molecular variance (AMOVA) and pairwise estimates across goat populations (Black, Hybrid, and Meriz) were performed using the GenALEx ver. 6.51 software (22). POPGENE 32 (23) software package was used for calculating: number of alleles (Na), number of effective alleles (Ne), observed (Ho) and unbiased expected heterozygosity (uHe) values, Nei's gene diversity value (h), Shannon's Information index (I), genetic distance values and Polymorphic Information content (PIC) among three goat populations. (ANOVA) and comparison test among goat`s genotypes were calculated by XLSTAT software. Based on microsatellite and RAPD genotypes at multiple loci, the program STRUCTURE uses a model-based Bayesian clustering method to infer the number of distinct populations (*K*) from which samples have been drawn and to infer the genetic ancestry of the individuals sampled (24).

Results and Discussion

The development and finding a reliable molecular marker for genetically distinguishing among and within breeds (populations) has become increasingly important due to the increasing demand for understanding the genetic background of our local animals especially goat, because of its economic importance. For the purpose of the identification of animals and breeds, microsatellites (SSR) and Random Amplified Polymorphic DNA (RAPD) are widely used as molecular markers. For the first time two molecular markers have been used to study the genetic distance within and among three populations of Black, Hybrid and Meriz Iraqi local goat breeds, using fifteen SSR and fifteen RAPD molecular markers (Table 1). The parameters measured in the current study including individual identification and genetic population diversity. Previous studies have shown that all 15 SSR and RAPD markers were polymorphic in goat (Table 1). About the SSR markers, out of fifteen markers, eleven were amplified and showed bands in the current study, a total of 847 bands were scored with size varied from 125 bp to 400 bp. 53 bands were polymorphic, with 6.58% Percentage of Polymorphic Bands (PPB). On the other hand, RAPD markers showed 6085 bands with size ranged from 150 bp to 1900 bp, 273 bands among them were polymorphic, with 4.33% Percentage of Polymorphic Bands (PPB). These results were higher than those recorded by (25, 26, 27, 28) in Iraqi goat breeds. These differences between our study and others because they used pooled sample to represent the population. While the current study used individual samples. The SSR marker detected high Percentage of Polymorphism Bands (6.58%) more than which detected by RAPD molecular marker (4.33%). Reflecting, that the SSR markers

are highly specific due to their design, which involves amplifying specific repeat motifs. This specificity allowed for better discrimination between individuals and detection of genetic variations. RAPD markers, being random, may amplify non-targeted regions that are less variable, resulting in fewer bands.

Both SSR and RAPD markers detected 7 and 50 unique bands in the three studied goat breeds, respectively (Table 2). The unique bands detected by RAPD in the current study were higher than those previous studies. It has been found that the unique bands detected by RAPD was 13 and 20 unique bands in Iraqi goat breeds (25, 27). The SSR marker (SRCRSP9) detected 1 unique band in Hybrid, and ILSTS5 showed 2 unique bands in Black and Meriz goats. Each of OarJMP29, MAF65, and MAF70 markers showed (1, 2, and 1) unique bands, respectively in Black goat. On the other hand, the highest unique band for RAPD markers was recorded by 2 markers. First, RAP1 (6 bands) which 3 of them for Black and 3 for Meriz. The second marker was OPQ12 (5 unique bands), which 3 of them for Black and 2 for Meriz breed, the rest showed different unique bands from different breeds for instant RAP2 detected 4 unique bands (2 Black and 2 Meriz). Each of RPA3 and RAP4 detected 4 bands (3 Black and 1 Meriz). RAP5 detected 2 unique bands in Black goat. RAP6 detected 4 unique bands (1 Black and 3 Meriz). RAP8 detected 2 unique bands in Meriz. OPP03 detected 4 unique bands (1 Black and 3 Meriz). OPP08 detected 4 bands (3 Black and 1 Meriz). OPP14 detected 4 bands (2 Black, 1 Meriz and 1 Hybrid). OPP15 detected 1 unique band in Black goat. OPQ04 recorded 3 unique bands (1 Black, 1 Hybrid and 1 Meriz). OPQ06 detected 3 bands (2 Black and 1 Hybrid). These results were higher than those recorded by Al-Barzinj *et al.,* (25) in Iraqi goat breeds because of the differences in the type of primers and individual sample analysis in the current study in comparison to pooled samples in their study. These results indicate that these loci can be used to analyze the genetic diversity between breeds. Moreover, SSR markers are designed to amplify specific repetitive DNA sequences, which are known to be highly variable. However, due to their targeted nature, SSR markers may detect variations only within specific regions of the genome. If the genetic diversity in the goat population is concentrated in regions that are not targeted by the SSR markers used in the study, it may lead to the detection of fewer unique bands.

The polymorphic information content (PIC) value can be used as one tool among many to assess the genetic diversity within goat populations, contributing to breeding programs, conservation efforts, and understanding the genetic structure of goat populations. The set of SSR loci analyzed in this study had moderate to relatively high PIC values, ranging from 0.09 (OarFCB304) to 0.35 (MAF70), with a mean value of 0.19 (Table 2), indicating the availability of high allelic variation in the marker loci and their distribution within the populations under study. While, PIC in RAPD marker ranged from 0.13 (RAP2) to 0.42 (OPP03) with an average of 0.28, which can be considered relatively high. RAPD markers tend to have lower PIC values compared to SSR markers

and typical PIC values for RAPD markers range from 0 to 0.3. Therefore, a PIC value of 0.28 suggests a relatively good level of polymorphism and genetic diversity at the RAPD marker locus. This study recorded lower PIC value as compared to reports of 0.78 and 0.756 by (29) and (30), respectively based on SSR loci. The observed number of alleles (Na) is a measure of genetic diversity within a population or a sample of individuals. Specifically, in the context of goat genetic diversity, Na represents the number of different alleles observed at a particular genetic locus or marker. A higher value of Na indicates a greater number of alleles and, consequently, a higher level of genetic diversity at that locus within the goat population. In the current study, the mean observed number of alleles (Na) was 1.365 and 1.336 for SSR and RAPD markers, respectively (Table 3). Lower than which recorded by (31) in Iranian mohair goat (1.82). While, higher than recorded by (30) (0.98) in Markhoz goat.

The effective number of alleles (Ne) provides a more accurate estimation of genetic diversity because it accounts for the unequal distribution of alleles within a population. When a population has a few dominant alleles with high frequencies, Ne tends to be lower than Na. Conversely, when a population has a more even distribution of allele frequencies, Ne can be closer to Na. Ne values in the present study were 1.283 and 1.341 for SSR and RAPD markers, respectively. This finding weas lower than those previous studies (31 and 29). in Iranian mohair goat was 1.62, and Markhoz goat was 4.12.

Furthermore, Shannon's information index (I) was used for further detection of genetic diversity among all the breeds. Black goats showed higher I value for both SSR and RAPD markers were 0.343 and 0.415, respectively. While, Hybrid showed the least value for both markers (0.223 and 0.188) with an average of 0.276 and 0.306 for SSR and RAPD markers all over the breeds, respectively (Table 3). Shannon index value (I) for both SSR and RAPD markers recorded moderate genetic diversity among all studied goat breeds this may be due to high inbreeding mating in those studied populations. These results were higher than those reported by (25) in Iraqi goat I=0.10. However, lower than reported by each of (30, 31) 1.5 and 0.5, respectively.

Both expected heterozygosity and unbiased heterozygosity provide valuable insights into the genetic diversity and variability of a population. They are commonly used in population genetics studies to compare genetic diversity among populations, assess the impact of genetic drift, evaluate breeding programs, and monitor changes in genetic diversity over time. In the current study, the overall expected heterozygosity (He) and unbiased heterozygosity (uHe) were (0.176 and 0.181) and (0.202 and 0.208) for SSR and RAPD marker respectively (Table 3). RAPD markers showed higher values indicate greater genetic diversity within the population. Black goat showed the highest observed heterozygosity (uHe). While, Hybrid showed the lowest value for both of SSR and RAPD. (He) in both methods (SSR and RAPD) is equal or very close to (uHe), it suggests that the observed heterozygosity in the population matches the expected

heterozygosity based on the allele frequencies and follows the expectations of Hardy-Weinberg equilibrium. In Hardy-Weinberg equilibrium, the frequencies of alleles and genotypes in a population remain constant over generations in the absence of factors such as mutation, migration, selection, and genetic drift. The average unbiased heterozygosity (uHe) in our study was lower than that reported by (11) (0.385) in Nubian goats in Brazil. On the other hand (9) obtained mean heterozygosity higher than 0.91, in the local Saudi Arabia goat breed

A higher Polymorphism percentage for SSR and RAPD markers indicates a greater proportion of loci with different banding patterns, suggesting higher genetic diversity within the population. The highest percentage was recorded by Black breed (88.68%) and (85.29%) for both SSR and RAPD marker, respectively. While, the lowest percentage was recorded by Hybrid (45.28% and 33.46%). In addition, the Meriz breed showed medium percentage value (66.04% and 65.81%) with an average percentage value (66.67% and 61.52%) for both SSR and RAPD markers, respectively. This study recorded higher polymorphic percentage compared with previous studies (46% and 55.29%), respectively (28 and 26). However, lower percentage (93.4%) in comparison to previous study (25). The differences in the percentage of polymorphism between the present study and the other studies could be attributed to the nature of the samples used in this study which used individual sample for the analysis not pooled sample like other studies did in Iraqi goat breeds. Analysis of molecular variance (AMOVA) of the 3 Iraqi local goat breeds (Black, Hybrid and Meriz) genotypes analysis by using 11 SSR and 15 RAPD markers demonstrated 76% and 62% of the total variation within the populations. While, 24 and 38% was recorded to differences between populations, respectively (Table 4). When genetic diversity within a population is greater than among populations, it suggests that there is a higher level of genetic variation and differentiation within individuals of the same population compared to the differences observed between different populations.

Genetic distance and similarity among goat breeds refer to the degree of genetic divergence or similarity between different breeds of goats. These measures help to assess the genetic relationships and population structure among goat breeds. In the current study, the level of genetic distance (dissimilarity) was estimated by using Jaccard's similarity coefficient depending on SSR and RAPD data (Table 5). Both of SSR and RAPD markers showed the longest genetic distance 0.114 and 0.316 between Hybrid and Meriz breed, respectively. The most closeness (similarity) recorded between Black and Meriz breed (0.956 and 0.831) for both SSR and RAPD, respectively. The same result was recorded by Mohammed (32) which observed a close genetic distance between Black (Native) and Meriz goats (D=0.1667) in Iraqi local goats. Moreover, the UPGMA dendrogram based on Nei`s genetic distance values for both of SSR and RAPD markers classified the three studied goat breeds into two main clusters (Figure 1 & 2).

Table 2: Total number of fragments, monomorphic, polymorphic fragments, Percentage of Polymorphic Bands, and unique band obtained by SSR and RAPD markers in Iraqi local goat breeds (Black, Hybrid, and Meriz).

Primers	Range of fragment size bp	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	PPB*	Unique band	PIC*
			SSR				
SRCRSP9	150-225	62	57	5	8.06	1	0.12
ILSTS5	190-250	60	56	4	6.67	2	0.18
ILSTS11	275-350	60	56	4	6.67	0	0.16
SRCRSP5	190-250	60	56	4	6.67	0	0.16
OarFCB304	150-400	79	69	10	12.66	0	0.09
OarJMP29	130-150	60	57	3	5	1	0.26
MAF214	225-350	141	135	6	4.26	0	0.25
OarJMP58	150-200	60	57	3	5	0	0.21
BM1824	175-225	60	56	4	6.67	0	0.18
MAF65	125-200	72	67	5	6.94	2	0.15
MAF70	140-200	133	128	5	3.76	1	0.35
Total	-	847	794	53	-	7	-
Average	-	77	72.18	4.82	6.58	-	0.19
			RAPD				
RAP1	250-1900	609	583	26	4.27	6	0.30
RAP2	225-1200	262	241	21	8.02	4	0.13
RAP3	275-1100	431	414	17	3.94	4	0.33
RAP4	500-1500	362	347	15	4.14	4	0.28
RAP5	225-1500	320	364	16	4.21	2	0.26
RAP6	275-1200	561	538	23	0.04	4	0.34
RAP7	275-1150	445	428	17	3.82	0	0.37
RAP8	225-1000	500	480	20	4	2	0.27
OPP03	275-1550	385	372	13	3.38	4	0.42
OPP08	150-1000	327	310	16	4.91	4	0.25
OPP14	425-1600	377	360	17	4.51	4	0.22
OPP15	300-1200	402	388	14	3.48	1	0.33
OPQ04	250-1200	289	270	19	6.57	3	0.18
OPQ06	325-1200	353	334	19	5.38	3	0.21
OPQ12	275-1050	462	442	20	4.33	5	0.29
Total	-	6085	5871	273	-	50	
Average	-	405.67	391.4	18.2	4.33	-	0.28

*** PPB: Percentage of polymorphic bands; *PIC: polymorphic Information Content.**

Table 3: The number of samples (n), observed number of alleles (Na), effective number of alleles (Ne), Shannon's Information index (I), expected heterozygosity (He), unbiased expected heterozygosity (uHe), and Percentage of Polymorphic Loci (%P) of the Iraqi (Black, Hybrid, and Meriz) goat breeds.

Breeds	n	Na	Ne	I	He	uHe	%P
			SSR				
Black	30	1.774	1.313	0.343	0.211	0.214	88.68%
Hybrid	10	0.962	1.259	0.223	0.149	0.157	45.28%
Meriz	20	1.358	1.276	0.263	0.168	0.172	66.04%
Overall		1.365	1.283	0.276	0.176	0.181	66.67%
			RAPD				
Black	30	1.735	1.451	0.415	0.272	0.277	85.29%
Hybrid	10	0.879	1.227	0.188	0.128	0.135	33.46%
Meriz	20	1.393	1.345	0.317	0.207	0.213	65.81%
Overall		1.336	1.341	0.306	0.202	0.208	61.52%

Table 4: Analysis of molecular variance (AMOVA) of the three Iraqi goat local breeds (Black, Hybrid, and Meriz).

Source	df	SSR SS	MS	Est. Var.	%	P-Value
Among Pops	2	87.550	43.775	2.038	24%	0.001
Within Pops	57	365.500	6.412	6.412	76%	0.001
Total	59	453.050		8.450	100%	
		RAPD				
Among Pops	2	929.850	464.925	23.321	38%	0.001
Within Pops	57	2130.017	37.369	37.369	62%	0.001
Total	59	3059.867		60.690	100%	

Table 5: The similarity index (above diagonal) and genetic distance (below diagonal) among the studied goat breeds based on SSR and RAPD analysis.

Genotypes	Black	Hybrid	Meriz	Marker type
Black	***	0.907	0.956	**SSR**
	***	0.822	0.831	**RAPD**
Hybrid	0.098	***	0.892	**SSR**
	0.196	***	0.729	**RAPD**
Meriz	0.045	0.114	***	**SSR**
	0.185	0.316	***	**RAPD**

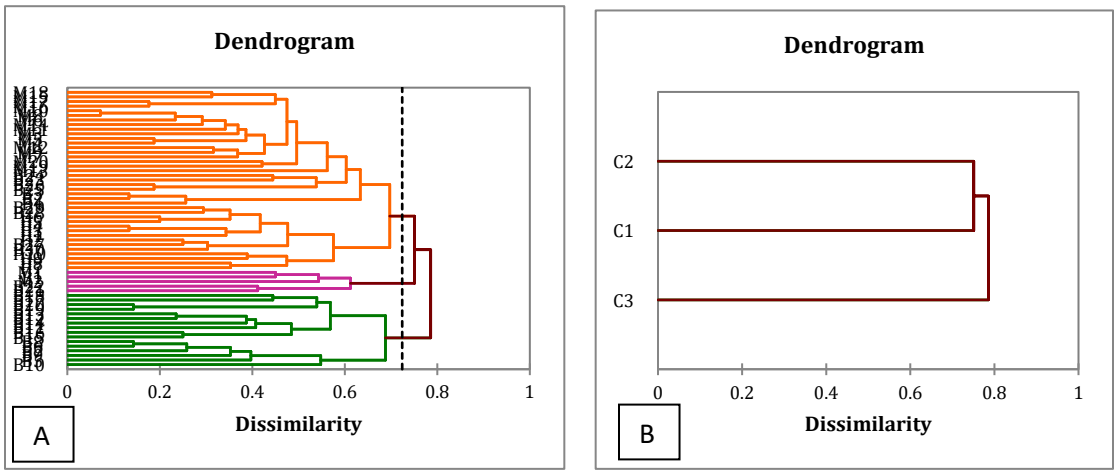


Figure 1: UPGMA dendrogram among individuals (A) of three native goat populations (B) based on SSR marker analysis. C1: Black; C2: Hybrid; C3: Meriz breeds.

The first one contained the Black and Hybrid breeds. While, the second one contained only Meriz breed. Structure analysis is a statistical method used to infer population structure and individual ancestry based on genetic data, including SSR and RAPD markers. It helps identify genetic clusters within a population and estimate individual admixture proportions. The analysis can reveal the presence of distinct genetic clusters within the studied population. Each cluster represents a group of individuals that share similar genetic characteristics. The population structure and degree of admixture of the three goat breeds were evaluated using Bayesian model-based clustering in the STRUCTURE software. The structure of the studied breeds was determined based on the degree of admixture for each individual using the correlated allele frequencies model implemented in the software STRUCTURE. The obtained DK value was highest at K = 3. On the basis of the 11 SSR and 15 RAPD markers, the results of STRUCTURE revealed the division of the goat breeds into three genetic clusters (Figure 4). This finding suggests significant genetic differentiation within the population. Each cluster represents a group of individuals that share more genetic similarity with each other than with individuals from the other clusters. The three clusters may correspond to subpopulations or genetically distinct groups within the studied population. This substructure could arise due to factors such as geographical isolation, breeding practices, or historical events. On the other hand, the identification of three clusters implies the existence of significant genetic diversity within the population. Each cluster likely represents a unique set of alleles and genetic variants that contribute to the overall genetic variation observed.

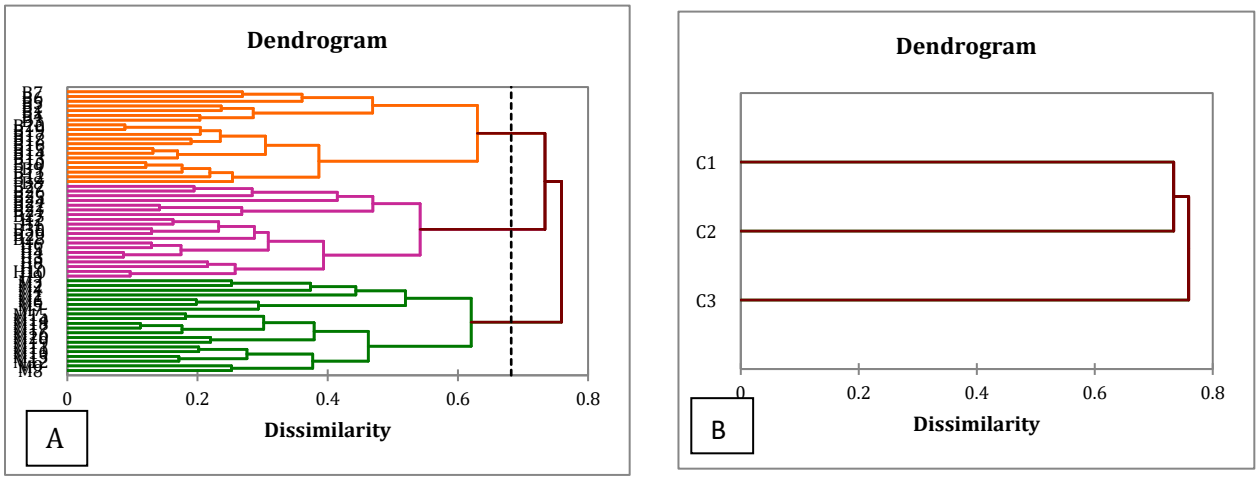
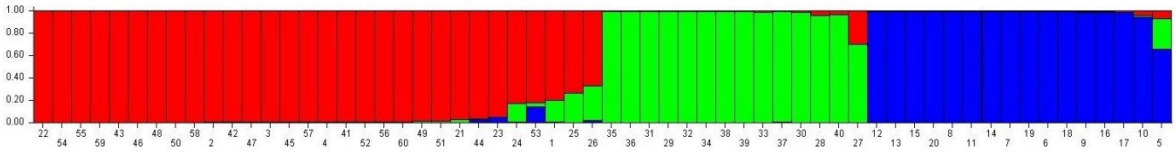
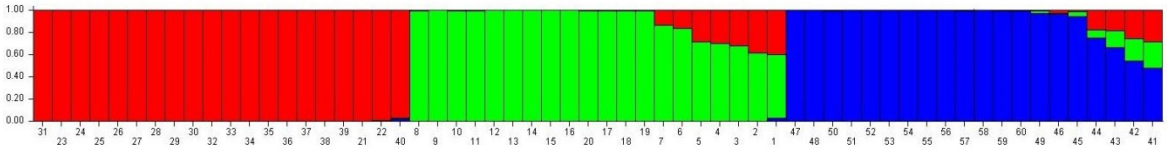


Figure 2: UPGMA dendrogram among individuals (A) of three native goat populations (B) based on RAPD marker analysis. C1: Black; C2: Hybrid; C3: Meriz breeds.

Black **Hybrid** **Meriz**



(A) K=3



(B) K=3

Figure 3: Estimated population structure of 3 Iraqi goat breeds as revealed by (A) 11 SSR and (B) 15 RAPD polymorphic markers for (K = 3), Red indicates Black, green indicates Hybrid, blue indicates Meriz breed.

Conclusions

The aim of the current study was to assess the genetic diversity and the relationship individually and among three different breeds of Iraqi goat by SSR and RAPD markers for the first time. Both markers ensured high polymorphism and moderate genetic diversity among breeds. In addition, the genetic diversity within a population was greater than among populations. Longest genetic distance using both of SSR and RAPD markers was between Hybrid and Meriz breeds. The most closeness (similarity) recorded between Black and Meriz. Furthermore, the UPGMA dendrogram classified the three studied goat breeds into two main clusters. The first one contained the Black and Hybrid breeds. While, the second one contained only Meriz breed. The results of the current study will be helpful for future researchers as a key guide to better understanding the genetic relationships and breed differences in Iraqi goat breeds for planning strategies for the future genetic improvement program.

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References:

1. Hirst, K. (2023, April 5). The Domestication of Goats. ThoughtCo, thoughtco.com/the domestication-history-of-goats-170661.
2. Zeder, M.A., Hesse, B. (2000). The initial domestication of goats (Capra hircus) in the Zagros mountains 10,000 years ago. *Science 287,* 2254–2257. https://doi.org/10.1126/science.287.5461.2254.
3. FAO (Food and Agriculture Organization). (2014). Global information and early warning system on food and agriculture (GIEWS) Special Alert. http://www.fao.org.. *332*(3):1-45.
4. Alkass, J. E., Juma, K.H. (2005). Small Ruminant Breeds of Iraq. In: Characterization of Small Ruminant Breeds in West Asia and North Africa (ed. L. Iniguez). ICARDA (International Center of Agriculture Research in the Dry Areas), Aleppo, Syria
5. Alkass, J. E. (2012). Performance of black and Meriz goats raised on pasture conditions in Kurdistan region of Iraq. 2nd OHAC, Hamburg/Trenthorst, Germany, Sep., 12-14.
6. Taha, S. A. (1990). *The Effect of Castration and Fattening Period on Quantitative and Qualitative Meat Production in Local Goats*. (Doctoral dissertation, College of Agriculture, University of Baghdad). (in Arabic).
7. Bélanger, J., & Pilling, D. (2019). The State of the World's Biodiversity for Food and Agriculture; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy.
8. Juma K.H., Alkass J.E. (2005). Native goats of Iraq: A Review. *Dirasat, Agricultural Sciences*, *32*, No. 2.
9. Mahmoud, A. H., Farah, M. A., Rady, A., Alanazi, K. M., Mohammed, O., Amor, N., Alarjani, K. M. (2020). Molecular characterization of goats from Saudi Arabia using microsatellite markers. *Journal of King Saud Univ. Sci*. *32*:1681-1686. https://doi.org/10.1016/j.jksus.2020.01.001.
10. Visser, C. E., Van, M. K., Friedrich, H. (2011). Parentage verification of South African Angora goats, using microsatellite markers. *South African Journal of Animal Science*, *41* (3).
11. Silvestre, E. A., Costa, M. S., Silva, P. O., Bajay, M. M., Pinheiro, J. B., Zucchi, M. I., Campelo, J. E. G., Britto, F. B. (2015). A note on the distribution of genetic diversity of Anglo-Nubian goats in central-northern farms of Piauí, Brazil.

Revista Brasileira De Zootecnia, *44*(4), 155–160. https://doi.org/10.1590/S1806-92902015000400004.

12. Raghavendra, C., Saireddy, S., Amareswari, P., Raghunandan, T. (2017). Molecular characterization of Mahabubnagar goats based on microsatellite markers. *The Pharma Innovation*, *6*, 130.

13. Yang, S.L., Meerow, A.W. (1999). Genetic variation in Chinese cycad population. In: Chen CJ. ed, Biology and conservation of cycads. Proceedings of the Fourth International Conference on Cycad Biology. Beijing: International Academic Publishers, 175–186.

14. Verma, N., Mishra, P., Aggarwal, R., Dixit, S., Dangi, P. Dash, S. (2015). Characterization, performance and genetic diversity among goats of Odisha. *Indian Journal of Animal Sciences*, *85*, 165-171. https://epubs.icar.org.in/index.php/IJAnS/article/view/46609.

15. Mishra, P., Ali, A. S., Kuralkar, S., Dixit, S., Aggarwal, R., DangI, P., Verma, N. (2013). Analysis of genetic diversity in berari goat population of Maharashtra State. *Iranian Journal of Applied Animal Science*, *3*(3), 553-559.

16. Bhat, R. A., Uddin R. M., Beigh, S. A., Ahad, W. A., Padder, T. A., Ganai, N. A., Andrabi, M., Shah, R. A. (2017). Microsatellite marker-based DNA fingerprinting for parentage verification in goat breeds of Kashmir. *International Journal of Current Microbiology App Sci*, *6*, 1131-1134. https://doi.org/10.20546/ijcmas.2017.611.134.

17. Tulafu, G., Qiong, W., Tuersun, N., Wu-Jun, L. (2013). The Study on Polymorphism of Microsatellites and Its Association with Economic Traits in Qinggeli Cashmere Goats. *Xinjiang Agricultural Sciences*, *50*, 726.

18. Bindu, K.A., Raghavan, K.C., Antony, S. (2011). Genetic diversity in Malabari goats. In: Pérez-Cabal, M.Á., Gutiérrez, J.P., Cervantes, I., Alcalde, M.J. (eds) Fibre production in South American camelids and other fibre animals. Wageningen Academic Publishers, Wageningen. https://doi.org/10.3920/978-90-8686-727-1_12.

19. Shu, C., Han, X., Li, L. (2016). Genetic Diversity Analysis of Guizhou Black Goat Using Microsatellite DNA Markers. *Agricultural Biotechnology, 5*, 30.

20. Esmaeel khanian, S., Aliabad A.J., Seyedabadi, H. (2007). Genetic relationships among six Iranian goat populations based on random amplified polymorphic DNA markers. *Pakistan Journal of Biological Sciences*, *10*(17):2955-2959. DOI: 10.3923/pjbs.2007.2955.2959.

21. Li, B., Du, M., Guo, X., Zhou, Z. (2002). Genetic analysis of Shanxi native goats using RAPD markers. 7th World Congress on Genetics Applied to Livestock Production, Montpellier, France:26-50.

22. Smouse, P.E., Banks, S.C., Peakall, R., 2017. Converting Quadratic Entropy to

Diversity: Both Animals and Alleles Are Diverse, but Some Are More Diverse than Others. *PLoS ONE,* 12, e0185499. [CrossRef] [PubMed].

23. Yeh, F.C., Yang, R., & Boyle, T. (1999). POPGENE. Version 1.31. Microsoft Window-based Freeware for Population Genetic Analysis. University of Alberta, Edmonton, AB, Canada.

24. Kim, K.S., Sappington, T.W. (2013). Microsatellite data analysis for population genetics. *Methods Mol Biol.*, *1006*:271-95. doi: 10.1007/978-1-62703-389-3_19. PMID: 23546798.

25. Al-Barzinj, Y.M.S., Oramari, R.A., Al-Sanjury, R.A. (2016). Molecular characterization of Iraqi Local goat breeds using random amplified polymorphic DNA markers. *Iranian Journal of Applied Animal Science*, 6(3), 671-678.

26. Yousif, A.N., Abdalla, M.R. (2022). Genetic diversity of Iraqi local goat breeds by RAPD DNA markers. *Jurnal Kedokteran Hewan*. *16*(4), 127-131. DOI: https://doi.org/10.21157/j.ked.hewan.v16i4.28579.

27. Al-Barzinj, Y.M.S., Hamad, A.O. (2017). Characterization of local goat breeds using RAP DNA markers. *AIP Conference Proceedings* 1888:020010. Doi:10.1063/1.5004287.

28. Sulaiman, B.K. (2012). Evaluation of the Genetic Diversity in Iraqi Local Breeds of Goat Using Random Amplified Polymorphic DNA Markers. *Al-Anbar J. Vet. Sci*., : *5(*1)

29. Karsli, T., Eymen, D., Huseyin, G. F., Mehmet, A., Bahar, A. K., Ibrahim, Z. A., Emine, S. S., Kemal, K., Murat, S. B. (2020). Determination of genetic variability, population structure and genetic differentiation of indigenous Turkish goat breeds based on SSR loci. *Small Ruminant Research*,*190*, 106147. https://doi.org/10.1016/j.smallrumres.2020.106147.

30. Asroush, F., Mirhoseini, S.Z., Badbarin, N., Seidavi, A., Tufarelli, V., Laudadio, V., Dario, C., Selvaggi, M. (2018). Genetic characterization of Markhoz goat breed using microsatellite markers. *Arch Anim Breed*, 6, *61*(4):469-473. doi: 10.5194/aab-61-469-2018. PMID: 32175455; PMCID: PMC7065388.

31. Moradi, M.H., Rostamzadeh, J., Rashidi, A., Vahabi, K., Farahmand, H. (2014). Analysis of genetic diversity in Iranian Mohair goat and its color types using Inter Simple Sequence Repeat (ISSR) markers. *Journal of Agricultural Communication*, *2*(1), 55-62.

32. Mohammed, A. B. (2013). Characterization of two domestic goat breeds in Duhok province / Iraq using microsatellites. *Journal of University of Zakho*, *1*, (A)1:. 101-107.

تقييم التنوع الوراثي لسلالات من الماعز المحلى العراقي باستخدام المؤشرات الجزيئية

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الخلاصة

يلعب الماعز دورا مهما فى الاقتصاد الوطنى للعراق من خلال إنتاجيته العالية، وزيادة توليد الدخل، وتوفير فرص العمل، و الحفاظ على الموارد الوراثية القيمة. تمت دراسة التنوع الوراثى لثلاث سلالات من المعز المحلى العراقي باستخدام اثنين من المؤشرات الجزيئية، حيث تم استخدام ١٥ SSR و ١٥ RAPD موءشر، جمعت عينات من الدم بشكل فردى من كل حيوان على حده، واستخلص الحامض النووى الرايبوزى (DNA) من كل عينة من ال ٣٠ ماعز الاسود، ١٠ هجين، و ٢٠ المرعز. تم أجراء عملية تضخيم الدنا بتقنية ال PCR. أظهرت النتائج انه من مجموع ١٥ بادئات ال SSR و ١١ بادئة أعطت النتائج موجبة حيث اظهرت ٨٤٧ حزمة، ٥٣ منها كانت حزماً متعددة الأشكال بنسبة ٦,٥٨%. مجموع ال ١٥ البادئات RAPD أظهرت ٦٠٨٥ حزمة، ٢٧٣ كانت حزماً متعددة الأشكال بنسبة ٤,٣٣%. كل سلالة على حده اظهرت عدد مختلف من الحزم خاصة بالسلالة، كل من SSR و RAPD أظهرت تعدد الاشكال المعتدل ٦٦,٦٧% و ٦١,٥٢% على التوالى. هده النتائج كانت متزامنة مع معدل قيمة PIC ٠,١٩ و ٠,٢٨ على التوالى. أعلى المسافة الوراثية سجلت مابين ماعز المرعز والهجين (٠,١١٤ و ٠,٣١٦) و أعلى قيمة التشابه الوراثى سجلت بين سلالة الماعز الاسود والمرعز حيث كانت (٠,٩٥٦ و ٠,٨٣١) لل SSR و RAPD على التوالى. بالأضافة الى ان نتائج المستحصلة عليه من قبل الشجرة الوراثية (dendrogram) صنفت السلالات الثلاثة الى مجموعتين, المجموعة الاولى احتوت الماعز الاسود والهجين, بينما المجموعة الثانية احتوت على سلالة المرعز فقط. نتائج الدراسة الحالية قد يساعد الباحثين فى المستقبل كدليل مفتاح لفهم افضل للعلاقات الوراثية والاختلافات الموجودة بين سلالات المعز المحلى العراقى لكى نستفاد منها لوضع خطط ستراتيجية مهمة لتطويرها وراثيا.

الكلمات المفتاحية: تنوع الوراثى, SSR, RAPD, تعدد الأشكال, الماعز العراقي.