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Genetic diversity assessment of some Iraqi Sheep breeds using micro satellite DNA markers

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ABSTRACT

To assess genetic diversity among some Iraqi sheep breeds (Karadi, Jaff, and Awassi) randomly selected from three different locations of Sulaimani governorate (Jeshana, Halabjai Shahid and Kirkuk) respectively. A total of 150 blood samples were collected (50 animals per breed from both sexes) using 10 microsatellite markers, 7 were amplified and showed bands. Four primers were polymorphism and three were monomorphism. The results demonstrated that the total fragment number (TFN) for the 7 primes was 14 fragments. The overall polymorphic fragments number (PFN) was 6, the highest PFN found at locus OarJMP29 which had 3 bands, whereas the lowest PFN found at locus HUJ616, OarFCB20 and OarFCB304 which was 1 band. The overall mean percentage of polymorphic loci was 35.71 %. The OarJMP29 locus showed the highest polymorphism which was 100% and the lowest polymorphism was 0.0% for ILSTS5, MAF214, and OarJMP58 loci. In the current study mean value of number of alleles (Na) was 2 and effective number of alleles (Ne) was 1.5214. Nei's gene diversity and Shannon index are respectively averaged of 0.3296, and 0.5063. The smallest genetic distance recorded between Jaff and Awassi which was 0.0554, while the farthest distance was observed between Karadi and Awassi 0.1168.The results of genetic identity showed that the Jaff and Awassi populations are more genetically alike (0.9461) while the Karadi and the Awassi populations were the least genetically identical (0.8898). The dendrogram tree separated the studied breeds into two clusters, the first one including Jaff and Awassi breeds, and the second cluster includes Karadi breed. Finally, moderate value for both of polymorphism and genetic diversity were observed among the studied sheep breeds, which will help in developing a suitable approach for the genetic improvement, utilization and conservation of Iraqi sheep breeds.

تقيم التنوع الوراثي لعدد من سلالات الأغنام العراقية باستخدام واسمات التتابعات الدقيقة (Microsatellite)

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الخلاصة

لتقيم التنوع الوراثي لعدد من سلالات الأغنام العراقية (الكرادي، الجاف، و العواسي) اختيرت عشوائياً من ثلاثة مناطق مختلفة في محافظة السليمانية (جيشانة، حلبجة الشهيد, وكركوك) على التوالي. تم جمع150 عينة دم (50 حيوان \ سلالة من كلا الجنسين). استخدمت 10 واسمات التتابعات الدقيقة **(Microsatellite Markers)**، 7 منهم اعطت نتيجة، 4 واسمات أعطت حزم مختلفة و 3 واسمات أعطت حزم متشابهة. أضهرت النتائج ان مجموع عدد الحزم (TNF) للواسمات السبعة كانت 14 حزمة. مجموع عدد الحزم المختلفة (PNF) كانت 6، اعلى (PNF) وجدت في مايكروستلايت OarJMP29 والتي كانت 3 حزم، بينما أقل (PNF) كانت لواسمات HUJ616، OarFCB20 و OarFCB304 والتي كانت 1 حزمة. نسبة المتوسط الكلي للواسمات المتعددة الاشكال وصلت الى 35.71% , أعلى تنوع كانت لواسمة OarJMP29 حيث وصلت الى 100% وأقل تنوع كانت 0% للواسمات ILSTS5، MAF214، و OarJMP58 . ان ظاهرة التنوع عادة لها علاقة بوجود تعدد الاليلات في الجين. في الدراسة الحالية متوسط عدد الاليلات الظاهرة (Na) كانت 2 و العدد الفعال للاليل (Ne) كانت 1.5214. متوسط التنوع الوراثي (Nei"s) و الدليل (Shannon) كانت 0.3296 و 0.5063 على التوالي. التباعد او المسافة الوراثية مابين السلالات الثلاثة كانت مابين 0.0554 الى 0.1168. أقل مسافة وراثية سجلت فيمابين الجاف والعواسي التي وصلت 0.0554 بينما أبعد مسافة لوحظت فيمابين الكرادي والعواسي 0.1168.نتائج التشابه الوراثي أضهرت أن سلالتي الجاف والعواسي أكثر تشابهاً (0.9461) بينما السلالتي الكرادي والعواسي أقل تشابهاً من الناحية الوراثية 0.8898. نتائج شجرة النسب (dendogram) أضهرت ان الشجرة قسمت السلالات المدروسة الى قسمين، القسم الأول يشمل سلالتي الاغنام الجاف والعواسي، بينما القسم الثاني يشمل سلالة الكرادي. أخيراً ان لهذه النتائج المتحصل عليها لكل من التنوع و الاختلافات الوراثية فيما بين سلالات الأغنام المدروسة يساعد في تطوير نهج مناسب لتطوير الناحية الوراثية، والاستخدام والحفاظ على سلالات الاغنام المحلية العراقية, إضافةً تعطي الوعي للمزارع في الارياف أهمية السيطرة على نظام التربية المناسبة لحقولهم الحيوانية.

الكلمات المفتاحية: اغنام عراقية، تنوع وراثي ، سلالات.

INTRODUCTION

Sheep *(Ovis aries)* are the most important and earliest domesticated species of livestock, currently the global sheep population stands at more than 1 billion head with 19 per cent found in Asia and Africa. There are more than 850 breeds of sheep in the world (Rege and Gibson, 2003), in Iraq there are three native sheep breeds and some sub breeds, because of random crossing with other breeds they do not consider as pure breeds. However they differ among themselves in the phenotypic and productive characteristics, these breeds are Awassi (Naami and Shefali) (58.2%), Karadi (Kurdi, Hamdani, Jaff, Harki and Dzaie) (20%) and Arabi sheep (21.8%) (AL-Kudsi, *et al*., 2012, Oramari, *et al*., 2014). Awassi is a dominant type of sheep in Iraq and the most famous species, it is mainly raised for the production of meat, milk and wool, which are sheep wool carpet (AL-Dabbagh, 2009). Naimi which is similar to Awassi produce fine wool, white color and light fleeces (Al-Sabea, *et al*., 2020). Karadi like the other Iraqi breeds of sheep which is fatty tail and

gives carpet fleece (Zin-ALabidin and Ayhan, 2017). The selections which made by farmers were toward meat production, as they are considered low in milk and wool production compared to international breeds (Al-Sabea, *et al*., 2020). As genetic diversity, it is wide due to the large number of breeders in different regions. This diversity led to the emergence of multiple breeds and genetic lines, and one of the most famous breeds in the Sulaimani Governorate is Karadi sheep (Juma & Alkass, 2000), from which the Jaff sheep descended (relative to the Jaff trips that raised these sheep).

To assess genetic variation within and among different sheep populations, numerous markers have been used. However, DNA molecular markers are the best tools, because they are not influenced by environmental alterations. In addition, they are providing accurate information covering all genomic regions (Prasad *et al*., 2009). Among various PCR based molecular markers, Microsatellites, or Simple Sequence Repeat (SSR) has commonly been used in sheep, which is a dominant marker that can amplify DNA fragments between two simple sequence repeat regions without any prior sequence information (Alnajm, 2020).

Over the past decades, Iraqi sheep have been widely classified based on morphological (Al-Sabea, *et al*., 2020), productive characteristic (Alkass, *et al*., 2021), but in the area of genetic markers using PCR technology very little information is available (Al-Barzinji and Ali, 2014) and (Hadi, *et al*., 2020), therefore the present study was designed to assess genetic diversity of three sheep breeds (Karadi, Jaff, and Awassi) in Sulaimani governorate, using microsatellite DNA markers as recommended by FAO (FAO, 2011), with the aim whether these markers can be useful for evaluation of genetic diversity of Iraqi local sheep breeds and to generate the useful data for further analysis and comparison as well as designing strategies for breed conservation.

MATERIAL AND METHODS

Animal Sampling and DNA Isolation:

A total of 150 blood samples were collected from three populations of Iraqi sheep breeds (Karadi, Jaff, and Awassi) from different locations of Sulaimani governorate (Jeshana, Halabjai Shahid and Kirkuk) respectively.

Five ml of whole blood were collected from the jugular vein of each animal, (50 individuals for each population, 25 male and 25 female) into vacutainer tubes containing EDTA (ethylenediaminetetraacetic acid) as an anticoagulant. The samples were placed in a cooler bag and transported immediately to a laboratory, they were stored at -20 ℃ prior to DNA isolation in the Biotechnology Lab. at Animal Science Department, College of Agricultural Engineering Sciences, Sulaimani University. The collected samples from each population were mixed to be one pooled sample per population.

Microsatellite Markers

A set of ten microsatellite markers was chosen, seven were amplified and showed bands, taking into consideration their level of allelic polymorphism and the location on different chromosomes, preferably unlinked, following the recommendations of the Food and Agriculture Organization (FAO, 2011) (Table1).

PCR Amplification and Fragment Analysis

An Animal-type kit (Sinaclon, SinaPure TM DNA) according to the manufacturer's instructions, for multiplex analysis of 10 microsatellite markers was used to perform PCR reactions. All PCR reactions were carried out in a total volume of 25 µl that contained: 12.5 µl PCR Master Mix, 2X (50 units/ml Taq DNA polymerase, 400 µM of each dATP, dGTP, dCTP and dTTP and 3 mM MgCl2), 2 µl of 10 µM primer, 2 µl of DNA (50 ng) and 8.5 µl of ddH2O. Amplifications were performed in a thermal cycler (Labocon, U.K.). Out of ten microsatellite primers (Table 1), seven were amplified and showed bands. The PCR amplifications program starting with initial denaturation at 94 °C for 5 min then, followed by 35 cycles: denaturation at 94 °C for 1 min, annealing at (depend on the primer 54-58) °C for 1 min, extension at 72 °C for 2 min, with a final

extension at 72 °C for 7 min. PCR products were separated on 5% agarose gels using 1×TBE (Tris-Borate-EDTA) running buffer at 5 V/cm. Then, they were visualized by staining with ethidium bromide.

Table 1: Information about ten microsatellite markers that used in this study, including marker name, chromosome number, primer sequences, annealing temperature and gene bank accession number.

No.	**Marker Name**	**Chr. no.**	**Primer sequence (5' → 3')**	**Annealing Temp °C**	**Gene bank accession Number**	**Allele Range (bp)**
1.	HUJ616	OAR 13	TTCAAACTACACATTGACAGGG GGACCTTTGGCAATGGAAGG	54	M88250	114-160
2.	SRCRSP	CHI13	TGCAAGAAGTTTTTCCAGAGC ACCCTGGTTTCACAAAAGG	54	L22192	116-148
3.	SRCRSP9	CHI12	AGAGGATCTGGAAATGGAATC GCACTCTTTTCAGCCCTAATG	55	L22201	99-135
4.	ILSTS5	OAR 7	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	55	L23481	174-218
5.	ILSTS11	OAR 9	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	55	L23485	256-294
6.	OarFCB20	OAR 2	AAATGTGTTTAAGATTCCATACA GTG GGAAAACCCCCATATATACCTA TAC	56	L20004	95-120
7.	OarFCB304	OAR 19	CCCTAGGAGCTTTCAATAAAGA ATCGG CGCTGCTGTCAACTGGGTCAGG G	56	L01535	150-188
8.	OarJMP29	OAR 24	GTATACACGTGGACACCGCTTTG TAC GAAGTGGCAAGATTCAGAGGGG AAG	56	U30893	96-150
9.	MAF214	OAR 16	GGGTGATCTTAGGGAGGTTTTG GAGG AATGCAGGAGATCTGAGGCAGG GACG	58	M88160	174-282
10.	OarJMP58	OAR 26	GAAGTCATTGAGGGGTCGCTAA CC CTTCATGTTCACAGGACTTTCTC TG	58	U35058	145-169

Data Analysis

A total number of fragments, monomorphic, polymorphic fragments, and percentage of polymorphic loci (% P) were measured.

The data obtained from the genetic variation in microsatellite loci in Karadi, Jaff and Awassi sheep populations, were fed into POPGENE software in binary format as ASCII files and analysis were carried out that generated several parameters including observed number of alleles (Na), effective number of alleles (Ne), Shannon's information index (I), homozygosity (h). A dendrogram was constructed using unweighted pair group method with arithmetic average (UPGMA) based on the similarity matrix data, cluster analysis and Shannon's Information index (I).

RESULTS AND DISCUSSION

Genotyping data from 10 microsatellites loci were used to assess the genetic structure and differentiation in Karadi, Jaff, and Awassi sheep breeds (Table 1), out of ten SSR primers seven were amplified and showed bands. Four of the seven primers were polymorphism and three primers were monomorphism in these three local sheep breeds. The total fragment number (TFN) for the 7 primes was 14 fragments, ranged from 1fragments in ILSTS5 and OarJMP58 to 3 fragments in OarJMP29 and MAF214 with fragments size ranged from 95 to 282 bp (Table. 2) These results show the differences among three sheep breeds for TFN. The 14 fragments from 7 primers found in this study was lower than the result reported by (Mahfouz, *et al*., 2008) in five Egyptian sheep breeds (which TFN was 57), (Tariq, *et al*., 2012) in Mengali, Balochi, Beverigh and Harnai sheep breeds (TFN was 92) and (El Hentati, *et al*., 2012) in Barbarine, the Western thin tail sheep breeds (TFN was 62). On the other hand the present results were lower than reported by (Okumus, *et al*., 2007) where TFN was 121 in Karayaka sheep breed using thirteen RAPD primers, these differences may due to the different primer numbers and different markers which used in these different studies.

The overall polymorphic fragments number (PFN) was 6, obtained out of 14 TFN from 7 primers (Table 2). The highest PFN found at locus OarJMP29 which had 3 bands, whereas the lowest PFN found at locus HUJ616, OarFCB20 and OarFCB304 which was 1 band. These results indicate that it is possible to depend upon these loci for genetic characterization among present sheep breeds. The PFN in this study was lower than those reported by (Mahfouz, *et al*., 2008, Tariq, *et al*., 2012, El Hentati, *et al*., 2012, Aytekin and Boztepe, 2010 and Jawasreh, *et al*., 2011) where their PFN values were 56, 56, 44, 133, and, 104 respectively.

The overall mean percentage of polymorphic loci (P %) for seven primers in the present study was 35.71 % (Table 2). The OarJMP29 showed the highest polymorphism which arrived 100% and the lowest polymorphism arrived 0.0% for ILSTS5, MAF214, and OarJMP58 loci. The mean percentage of polymorphic loci in present study was lower than that reported by (Cushwa, *et al*., 1996) in Coopworth, Merino, Perendale, Romney and Texel sheep breeds which ranged between 65 to 96%. It was found that mean percentage polymorphic loci were 79.66% in Tunisia sheep breed (Khaldi, *et al*., 2010). Moreover, it was reported that a percentage of polymorphism was 97.20% in Baladi, Sagri, and black Najdi sheep breeds (Jawasreh, *et al*., 2011).

Table 2. Microsatellite Primer name, range of fragment size (bp), total number of fragments, monomorphic, polymorphic fragments, and percentage of polymorphic loci (% P) in three local Iraqi sheep breeds (Karadi, Jaff, and Awassi).

No.	Primer Name	Range of fragment size (bp)	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	P %
1	HUJ616	114-160	2	1	1	50
2	ILSTS5	174-218	1	1	0	0
3	OarFCB20	95-120	2	1	1	50
4	OarFCB304	150-188	2	1	1	50
5	OarJMP29	96-150	3	0	3	100
6	MAF214	174-282	3	3	0	0
7	OarJMP58	145-169	1	1	0	0
	Total		14	8	6	-
	Mean		2	1.33	0.86	35.71

The phenomenon of polymorphism is generally related to presence of number of multiples alleles of a gene. In the current study mean value of Na was 2 and Ne was 1.5214 (Table 3), the same result was recorded by (Ahmed *et al*., 2022). and was less than reported values in the previous studies on other sheep breeds in the world (Ali *et al*., 2016 in Harnai breed 2.448±0.869; Ahmad *et*

al., 2014 in Kail sheep was 5.273; Musavi *et al*., 2011 in Hazargi sheep 6.296; Yadav *et al*., 2011 in Indian sheep was 8.64; Kumar *et al*., 2018 was 6.7). The Ne reported by these researchers in respective breeds were also higher than those values found in the present study 1.70 by (Ali *et al*., 2016) 3.94 by (Ahmad *et al*., 2014), 4.394 by (Musavi *et al*., 2011), 4.57 by (Yadave *et al*., 2011), finally, 3.658 by (Kumar *et al*., 2018).

The values of Nei' s gene diversity (gene diversity /heterozygosity) overall sheep breeds averaged 0.3296. This result indicated the genetic diversity among local sheep is moderate. As it is shown in (Table 3) out of 7 amplified primers only three were monomorphism, the rest gives heterozygosity, the OarFCB304 and OarJMP29 loci give the highest heterozygosity which were 0.4717 and 0.4082 respectively. Such results indicate possibility of using these loci more than others one in the future studies. The gene diversity value in this study was higher than (0.0962 and 0.050) which was reported by (Hlophe, 2011) in Dorper and Pedi sheep breeds respectively. On the other hand the present result was also higher than reported by (Mahfouz, *et al*., 2008) in Ossimi sheep breed (0.2529).

The mean value of Shannon diversity index in the current study was 0.5063 (Table. 3). This value was computed to provide relative estimation of variability. Such value show the diversity among local sheep breeds which used in this study. The Shannon index value in the present study was lower in comparison to (Odjakova, *et al*., 2022) in two Bulgarian sheep breeds (I = 1.79), also (Sharma, *et al*., 2020) reported higher index in Indian native sheep breeds (1.56). (Alnajm, 2020) observed higher I value in some Iraqi sheep breeds by ssr marker (I=0.965). While our result was higher than those reported by others. (Jawasreh, *et al*., 2011) reported a Shannon index for Jordanian local Awassi, Baladi, Sagri, Blackface and black Najdi sheep breeds values ranged 0.19 to 0.22. Similarly, Tariq, *et al*., (2012) observed I value ranged from 0.1449 to 0.2217 in four Balochistan sheep breeds.

Table 3. Microsatellite Primer name, number of identified alleles per locus (na*), effective number of alleles (ne*), gene diversity (h*), and Shannon's Information index (I*) of three local Iraqi sheep breeds (Karadi, Jaff, and Awassi).

No.	Primer Name	na*	ne*	h*	I*
1	HUJ616	2.0000	1.4459	0.3084	0.4869
2	ILSTS5	2.0000	1.2082	0.1723	0.3145
3	OarFCB20	2.0000	1.4459	0.3084	0.4869
4	OarFCB304	2.0000	1.8927	0.4717	0.6645
5	OarJMP29	2.0000	1.6897	0.4082	0.5983
6	MAF214	2.0000	1.4459	0.3084	0.4869
7	OarJMP58	2.0000	1.5215	0.3295	0.5060
	Mean	2.0000	1.5214	0.3296	0.5063
	SD	0.0000	0.2372	0.1024	0.1195

Nei's genetic distance change between 0 and 1. "0" stands for identical populations and "1" is for populations that share no alleles. The genetic distance among Karadi, Jaff, and Awassi sheep breeds in this study is presented in (Table. 4). The genetic distance among three breeds ranged from 0.0554 to 0.1168. The lowest genetic distance recorded between Jaff and Awassi which was 0.0554, and the highest genetic distance recorded between Karadi and Awassi 0.1168. These results show the effect of different breeds on genetic diversity among the studied sheep breeds. The genetic distances among sheep breeds in the current study were in agreement with results published previously between Sipsu and Tsirang (0.041) and lower than between Tsirang and Karakul (0.705) (Dorji, *et al*., 2010) The genetic relationships between the sheep breeds in Iran using five microsatellite markers ranged from 0.04 for Kermani and Kermani-Pakistani to 0.25 for Kermani and Lori-Bakhtiari-Pakistani (Ebrahimi, *et al*., 2017) The studied genetic distance in four native

Turkish sheep breeds using 21 microsatellites that lowest genetic distance was found between Norduz and Guney Karaman (0.088) and the highest between Kangal and Karakas (0.135) (Karsli, *et al*., 2020).

The results of genetic identity (Table 4) indicates that the Jaff and Awassi populations are more genetically alike (0.9461) while the Karadi and the Awassi populations were the least genetically identical (0.8898). The close similarity of Jaff and Awassi can be the result of gene flow from a common source but the distance between Karadi and Awassi indicates that these two breeds are relatively distant from each other. This estimate indicates that these breeds are genetically different. These results are higher than in Turkish sheep breeds using 15 microsatellites (Oner, *et al*., 2014), but lower than in local Chile sheep using 17 microsatellite markers (Bravo, *et al*., 2015).

As it is shown in the dendrogram (Fig. 1). The tree separated the surveyed breeds into two clusters, the first one including Jaff and Awassi sheep breeds, and the second cluster includes Karadi breed. These results indicated that Karadi breed is most genetically distant from the both breeds Jaff and Awassi (0.1168). The two breeds Jaff and Awassi in the 1st cluster indicating a close relationship between them. Migration has a large effect on the decrease of genetic differentiation between populations. The dendrogram shows that there are genetic diversity among sheep breeds, which were ranged from 0.0554 to 0.1168 (Table 4).

Table 4: Nies genetic identity (above diagonal) and genetic distance (below diagonal) among sheep breeds.

Population	Karadi	Jaff	Awassi
Karadi	***	0.9430	0.8898
Jaff	0.0587	***	0.9461
Awassi	0.1168	0.0554	***

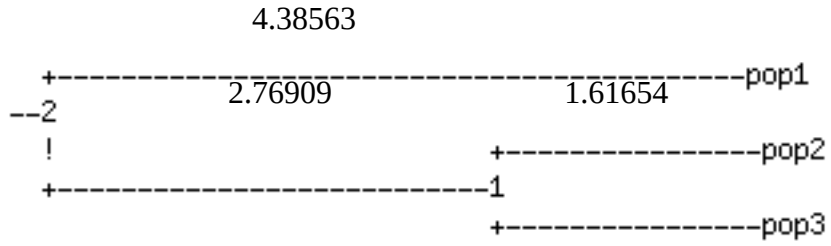

Figure 1: UPGMA dendogram showing differentiation between three local Iraqi sheep breeds: population 1 (Karadi breed), population 2 (Jaff breed), and population 3 (Awassi breed) based on Nei's (1972) genetic distance

CONCLUSION

The present study aimed to evaluate genetic diversity and the relationship among some Iraqi sheep breeds (Karadi, Jaff, and Awassi), Moderate value for both of polymorphism and genetic diversity were observed among the studied sheep breeds. The two breeds Jaff and Awassi have the most closeness. Furthermore, Karadi breed had higher genetic distance to Awassi breed. This study demonstrated the usefulness of the microsatellite markers assay in detecting genetic diversity and similarity within and among selected sheep breeds, also provided further information regarding these breeds, which will help in developing a suitable approach for the genetic improvement, utilization and conservation of local Iraqi sheep breeds. In addition, it will give awareness to the rural-based farmers of the importance of controlling breeding on their farms.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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