Molecular Characterization for Karadi Sheep Breed Using Random Amplified Polymorphic DNA Markers

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Abstract:

RAPD-DNA markers were used to study genetic characterization among four locations for Karadi sheep breed in Sulamania governorate. A total of 40 samples were typed using twenty RAPD primers. Nine out of the twenty primers had clear bands, which used to investigate the genetic variations among four locations(Halabjai Taza, Penjwen, Halabja and Sharbazer) of same breed. Out of the nine primers 8 of them are polymorphisms. A total of 119 bands were scored, of which 27 bands (25.05%) were polymorphic and seven of polymorphic band were unique bands. For all location, Nei's gene diversity, Shannon index and percentage of polymorphic loci are respectively averaged of 0.2361, 0.3415 and 34.60. Using unweighted pair-group method with arithmetic average (UPGMA) dendrogram, the three clusters, the 1st cluster branch consisted of the Karadi sheep in location 1 and 3, the 2nd cluster was including Karadi sheep from location 2 and the 3rd one including sheep from location 4. These results indicated that the Karadi sheep in location 1 and 3 is most genetically distant from the Karadi sheep in location 2 .The dendrograms show that there are moderately genetic diversity among Karadi sheep breed, were ranged from 0.251to 0.541. Based on the high degree of genetic distance among the four locations it is concluded that there are widely area for selection in this breed of sheep.

Keyword: Karadi sheep breed, Genetic distance, RAPD-PCR**.**

I. Introduction:

There are nearly 863 sheep breeds in the world [1]. The sheep population in Iraq was about 7 million heads [2]. Most of this population (99.8%) is owned by the private sector and is distributed all over the country. The native breeds include the Karadi (20%), Awassi (58.2%) and Arabi sheep (21.8%). They are all fat-tailed, carpet-wool with some potential to produce milk. Native sheep of Iraq are adapted to the harsh environmental of the region and have developed into well-differentiated breeds [3]. Identification and characterization of breeds is necessary to identify the genetic resources and also to prioritize breeds for conservation and development. Assessing genetic variability within and among populations, allelic variation, gene diversity, relationship and genetic distance are also essential for analyzing complete population structure. The complete population structure helps to plan strategies for conservation and

development of a breed [4]. Genetic characterization is the raw material for the animal breeders, which is used to mold domestic animal species to people's needs. The increasing data on genetics of sheep breeds using different genetic markers will help to understand the evolutionary history of sheep better. In addition, it will help to refine the definition of breed [5-6]. Recently, the use of molecular markers, revealing polymorphism at the DNA level, has been playing an increasing role in animal genetics studies. Amongst others, the Random Amplification Polymorphism DNA (RAPD) marker has been widely used, due to its easy use by simple PCR, followed by a denaturing gel electrophoresis for number of fragments and fragments size determination [7]. Therefore, the objective of this study is to determine the genetic characterizations among Karadi sheep breed from four locations in Sulaimani governorate using RAPD-PCR technique.

II. Materials and Methods:

A. Experimental animals and locations:

This study was undertaken on four locations for Karadi sheep breed. The blood samples were collected from regions where breed is predominantly found in Sulamania governorate to ensure that each population was a fair representative of the breed. Before sampling, each breeder in all locations was asked about the nature of the breeding system in order to determine the purity of the animals. A total of forty of the selected indigenous sheep of both sex were sampled. A total of ten samples of Karadi sheep were collect from Halabjai Taza (location 1: 46 km of Sulaimani), Penjwen (Location 2: 99 km of Sulaimani), Halabja (Location 3: 84 km of Sulaimani) and finally ten samples of Karadi sheep were collected from Sharbazer (Location 4: 50 km of Sulaimani).

B. Blood collection and DNA extraction

Blood samples were collected from the four locations (10 samples / locations). Whole blood (5 ml) was collected from each animal from jugular vein into 10 ml Vacutainer tubes containing the anticoagulant, Ethylene diaminetetra-acetic acid (EDTA) and blood samples stored at –20 °C until DNA extractions. The ten samples for each location were mixed together to made it as one sample/ location (pooled samples). All laboratory work was done in the Duhok research center at the Faculty of Veterinary, Duhok University, Duhok, and at Biotechnology laboratory at the Faculty of Science, Biology Department, Sulaimani University, Sulaimani. DNA was extracted from each of the blood sample using QIAamp® DNA Blood Mini Kit (QIAGEN GmbH Qiagenstr.1 40724 Hilden Germany). The quantity of DNA was checked and the quantification was done by Nanodrop spectrophotometer. The quality of DNA was determined using 1% agarose gel electrophoresis.

C. RAPD primers

In the present study, a total of 20 RAPD primers which were obtained from (CinnaGen Inc.)**,** Iran were used**.** The descriptions of primers regarding their names, primer sequences, GC percentages are given in “Table. I”.

D. PCR amplification of RAPD primers

Amplifications were performed using a thermal cycler with the final reaction volume of 25 µl. A master mix for minimum of 4 samples was prepared and an aliquot of 22 µl filled in each PCR tube.

Three µl sample DNA was added to each tube to make the final volume (25 µl). Each reaction volume contained: 8µl of Green Master Mix(25 Units/ml Taq polymerase, each dNTPs is 200µM and MgCl2 is 1.5 mM), 2.5 µl of RAPD primer (197.13µM– 599.26 µM), 3 µl (30 ng) of DNA Template and 11.5 µl of DNase free water. In this study three different protocols were used. The 1st for Primer (UBC−775): programmed for 46 cycles of denaturation at 95°C for 1 min, annealing at 35°C for 1 min and extension at 72°C for 2 min. An initial denaturation step of 1 min at 95°C and a final extension step of 5 min at 72°C were included in the first and last cycles, respectively. The 2nd protocol for (OPA-10, OPA-17, OPA-18, OPB-1, OPB-3, OPB-8 and OPB-17) used the above program with change only in annealing with 40°C and the 3rd protocol for Primers (Moh-13 and Moh21) annealing with 36°C. The amplification products were size-fractionated in a 1.5% agarose gel containing ethidium bromide in Tris-borate EDTA buffer and visualized under UV transillumination. In order to detect any DNA contamination, control reactions were set up without genomic DNA. *E. Genotypic analysis* The RAPD bands were scored as present (1) or absent (0) in each pattern. All genetic parameters in present study were calculated by using GENEPOP software (version, 3.3) (Raymond and Rousset, 1995).

Table.I: Primers name, sequence and GC % for 20 primers.

No.	Primer name	Primer Sequence 5’ to 3’	GC %
1	UBC−771	CCC TCC TCC C	80
2	UBC−775	GGT TTG GTG G	60
3	UBC−751	CCC ACC ACA C	70
4	Primer 1	CAG CCC AGA G	70
5	Primer 7 M02	ACA ACG CCT C	60
6	Primer 4 N01	CTC ACG TTG G	60
7	MOH4	GCA TGC GAT C	60
8	OPA-7	GAA ACG GGT G	60
9	OPA-10	GTG ATC GCA G	60
10	OPA-17	GAC CGC TTG T	60
11	OPA-18	AGG TGA CCG T	60
12	OPB-1	GTT TCG CTC C	60
13	OPB-3	CAT CCC CCT G	70
14	OPB-5	TGC GCC CTT C	70
15	OPB-8	GTC CAC ACG G	70
16	OPB-17	AGG GAA CGA G	60
17	OPB-19	ACC CCC GAA G	70
18	OPB-20	GGA CCC TTA C	60
19	Moh-13	GCT GCT CGA GT	70
20	Moh-21	AAC CGC GGT CT	70

III. Results and Discussion:

A. Total fragment numbers (TFN)

A total of nine primers out of the twenty random primers amplified showed clear bands and applied to investigate the genetic variations among the four locations of Karadi sheep breed. Out of the nine primers eight of them are polymorphism in the four locations "Fig. 1-3". This results show the high differences among four locations for TFN. The 119 fragments from 9 primers found in this study was higher than the result reported by [9] in five Egyptian sheep breeds (TFN was 57), [10] in Mengali, Balochi, Beverigh and Harnai sheep breeds (TFN was 92) and [11] in Barbarine, the Western thin tail sheep breeds (TFN was 62). On the other hand the present results was lower than reported by [12] where TFN was 121 in Karayaka sheep breed using thirteen RAPD primers.

B. The size range of fragments (bp)

The size range of fragments for 9 primers over all the sheep breed, ranged from 200 to 1400 bp, while the size range of fragments ranged from 200 to 1400, 200 to 1400, 200 to 1400 and 280 to 1300 bp in location1, 2, 3 and 4 respectively "Table. II". The smallest size fragments were recorded at OPB-1 (200 bp in location 1,2 and 4) and UPA-18 loci (200 bp in location 3). These results were in agreement with that reported by [13-16] where size of fragments ranged between 228 to 1871, 339 to 2536, 200 to 2000 and 250 to 2000 bp, respectively.

C. Polymorphic fragment numbers (PFN)

A total of 27 polymorphic fragments were obtained out of 119 TFN from 9 primers "Table. III". The highest PFN found at locus OPB-18 (5 bands), whereas the lowest PFN found at locuse OPB-8 (0 band), apart all other loci have more than 2 PFN "Table. III". These results indicate that it is possible to depend upon these loci for genetic characterization among present sheep breed. The PFN in this study was lower than that reported by by [9, 10,11, 17 and 18] where their PFN values were 56, 56, 44, 133, and, 104 respectively.

D. The percentage of polymorphic loci

The overall mean percentage of polymorphic loci for ten primers in the present study was 25.05 % "Table. III". The Moh-21 was the highest polymorphic locus arrived 50% and lowest polymorphism arrived 0.0% for OPB-8 locus "Table. III". The mean percentage of polymorphic loci in present study was lower than that reported by [19] in Coopworth, Merino, Perendale, Romney and Texel sheep breeds which ranged between 65 to 96%. [20] found polymorphic loci were 79.66% in Tunisia sheep breed. [18] reported that a percentage of polymorphism was 97.20% in Baladi, Sagri, and black Najdi.

E. Unique of band

"Table. IV" revealed that out of 119 bands overall sheep breeds 7 were unique bands. The highest unique bands were obtained from OPA-18 locus, which have 5 TFN, two of them (200 and 1000 bp) in location 3. Therefore, OPA-10, Moh-21, and OPB-3 shows one unique band in location 2, on the other hands OPB-1 and OPA-17 also show one unique band in location 1 and location 3, respectively. These results indicate that these loci can be used to analyze the genetic diversity between or among breeds. Moreover, it shows that there are genetic distances among Karadi sheep breed. The number of unique band in the present study was lower

Table.II: Band numbers and fragments size range (bp) in four locations for Karadi sheep breed in Sulamania governorate.

Primer name	Locations								Overall	
	1		2		3		4			
	No. of amplified band	Size range bp	No. of amplified band	Size range bp	No. of amplified band	Size range bp	No. of amplified band	Size range bp	Total No. of amplified band	Size range bp
OPA-17	2	700-1250	2	700-1250	1	650	2	700-1250	7	650-1250
Moh-21	2	550-750	3	350-750	0	-	1	750	6	350-750
OPA-10	4	300-700	5	250-700	5	300-750	5	300-750	19	300-750
OPA-18	6	250-1100	6	250-1100	6	200-1100	4	400-1100	22	200-1100
OPB-1	5	200-800	4	200-800	1	800	3	200-800	13	200-800
UBC-775	4	250-1300	4	250-1300	5	280-1300	5	280-1300	18	250-1300
OPB-3	3	600-1400	4	600-1400	2	600-850	2	600-850	11	600-1400
OPB-8	2	500-600	2	500-600	2	500-600	2	500-600	8	500-600
OPB-17	5	400-1400	5	400-1400	4	400-1400	1	400	15	400-1400
Total	33	200-1400	35	200-1400	26	200-1400	25	280-1300	119	200-1400

than that reported by [9] in Egyptian sheep breeds.

F. Nei's gene diversity

The Nei's gene diversity (gene diversity/ heterozygosity) overall sheep breeds averaged 0.2361 "Table. V". This result indicates the genetic diversity between sheep breeds is moderately medium. The gene diversity value in this study was higher than (0.0962 and 0.050) was reported by [21] in Dorper and Pedi sheep breeds respectively. On the other hand the present result was lower than reported by [9] in Ossimi sheep breed (0.2529).

G. Shannon's information index (I)

The Shannon diversity index value in present study averaged 0.3415 "Table. V". This value was computed to provide relative estimation of variability. Such value show the diversity among sheep breed studied. The Shannon index value in present study was higher than those reported by others. [18] reported a Shannon index for Jordanian local Awassi, Baladi, Sagri, Blackface and black Najdi sheep breeds values ranged 0.19 to 0.22. Similarly, [10] found Shannon index values ranged from 0.1449 to 0.2217 for four Balochistan sheep breeds.

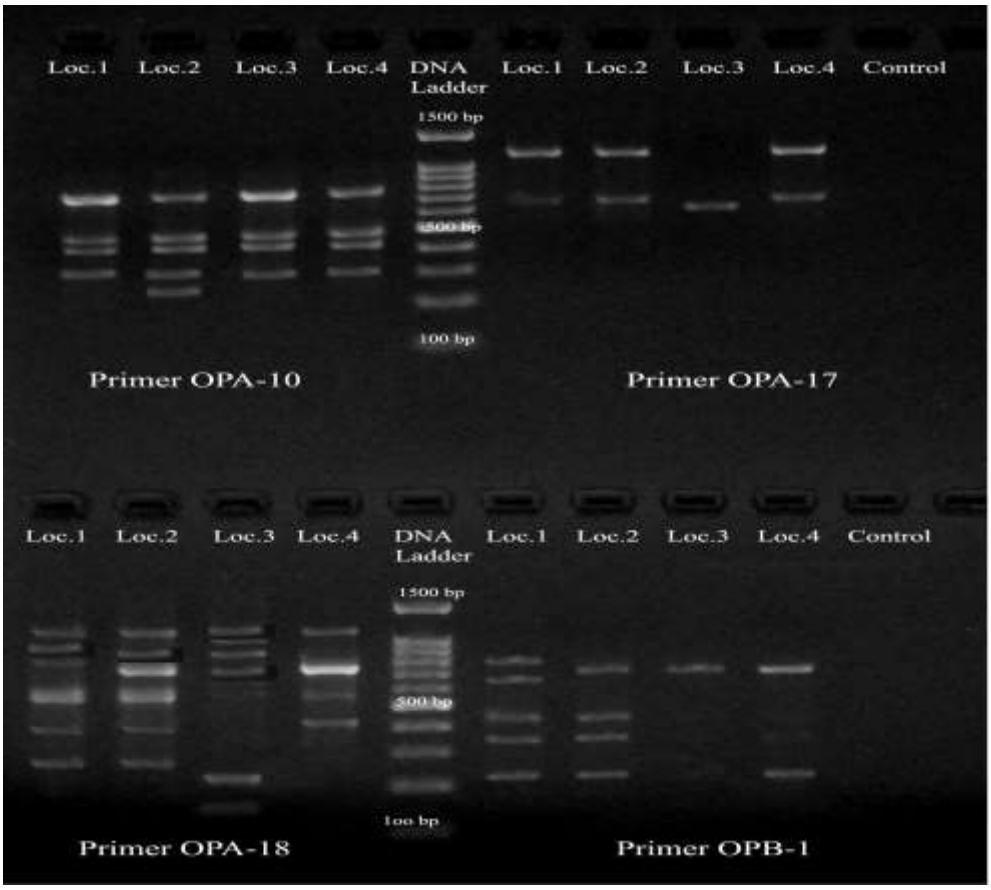

Fig. 1 Gel electrophoresis for four RAPD primers for four pooled sheep locations.

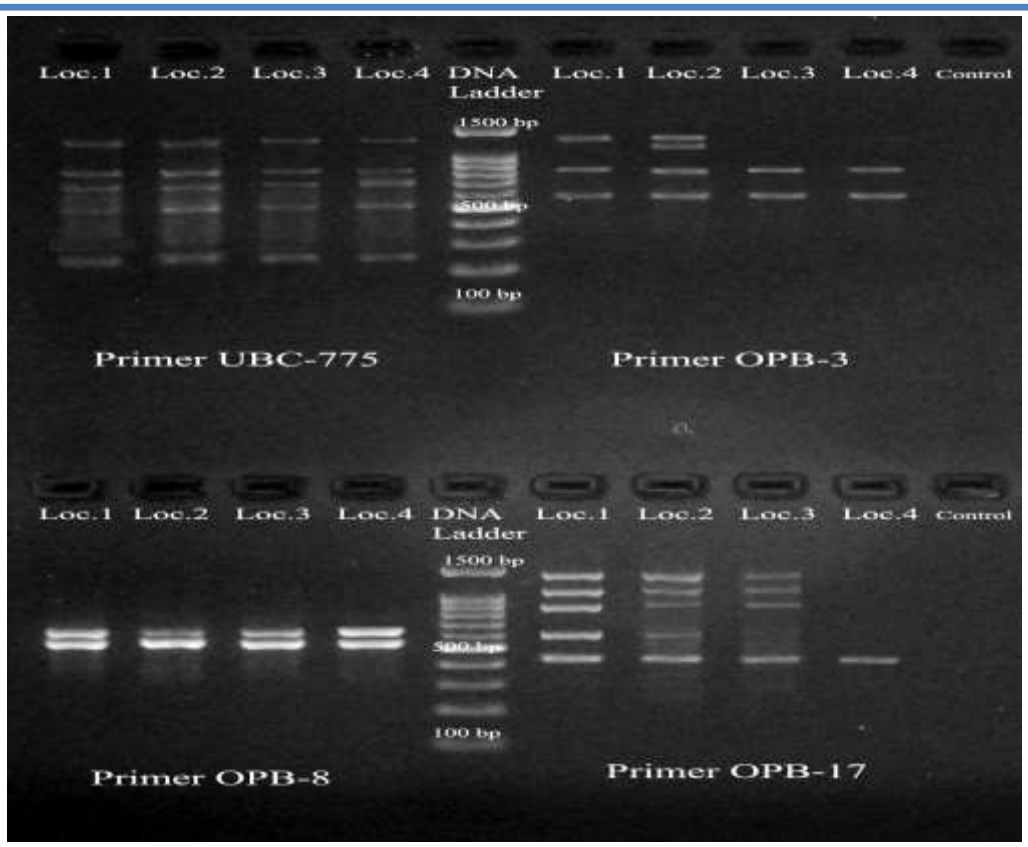

Fig. 2 Gel electrophoresis for four RAPD primers for four pooled sheep locations.

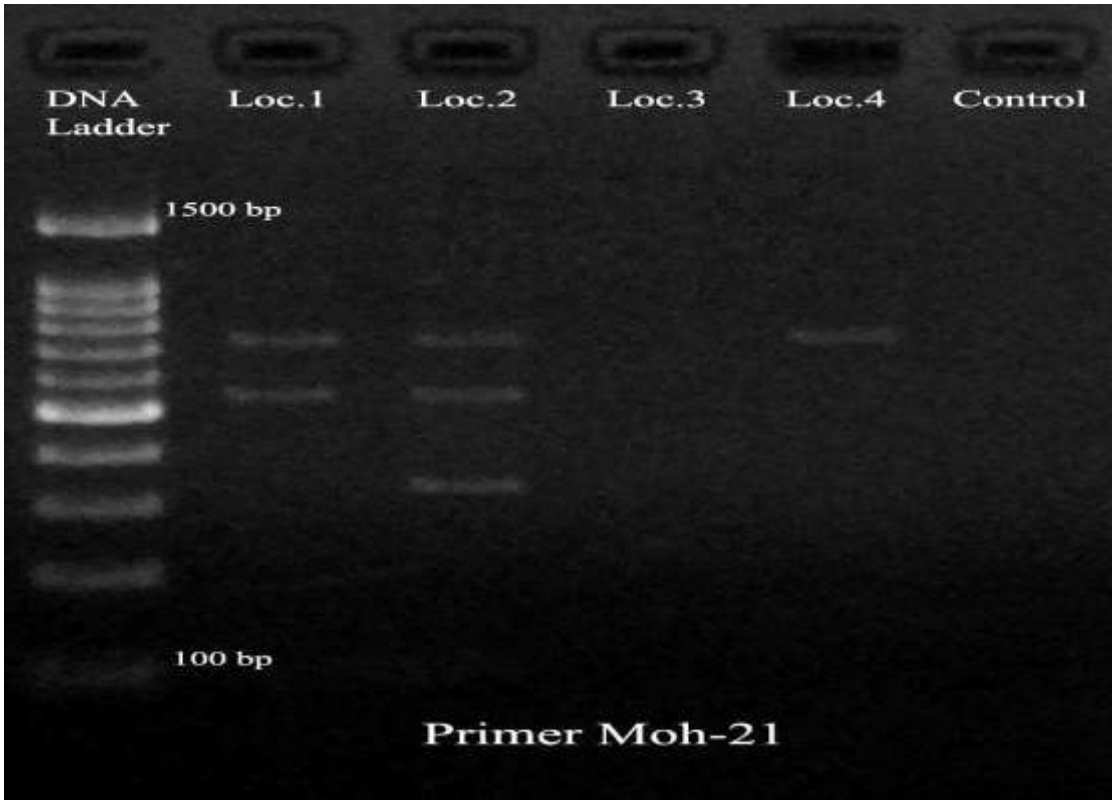

Fig. 3 Gel electrophoresis for one RAPD primer for four pooled sheep locations.

Table.III: Polymorphic fragment numbers and percentage polymorphic fragments for nine primers.

Primer name	No. of polymorphic fragments	Polymorphic fragments (%)
OPA-17	3	42.85
Moh-21	3	50.0
OPA-10	3	17.64
OPA-18	5	22.72
OPB-1	4	30.76
UBC-775	3	16.66
OPB-3	2	18.18
OPB-8	0	0.0
OPB-17	4	26.66
Mean	3.0	25.05

Table. IV: Unique band numbers and fragments size in four locations of Karadi sheep breed in Sulamania governorate.

Primers name	Locations								Over all	
	1		2		3		4			
	No. of unique band	Fragments size (bp)	No. of unique band	Fragments size (bp)	No. of unique band	Fragments size (bp)	No. of unique band	Fragments size (bp)	Total of unique band	Fragments size (bp) range
OPA-17	-	-	-	-	1	650	-	-	1	650
Moh-21	-	-	1	350	-	-	-	-	1	350
OPA-10	-	-	1	250	-	-	-	-	1	250
OPA-18	-	-	-	-	2	200 1000	-	-	2	200-1000
OPB-1	1	650	-	-	-	-	-	-	1	650
UBC-775	-	-	-	-	-	-	-	-	-	-
OPB-3	-	-	1	1300	-	-	-	-	1	1300
OPB-8	-	-	-	-	-	-	-	-	-	-
OPB-17	-	-	-	-	-	-	-	-	-	-

H. Genetic distance

The Nei's genetic distances among four locations sheep breed in this study are present in "Table. VI". The genetic distance among four locations ranged from 0.251 to 0.541. The lowest genetic distance recorded between location 1 and 3 was 0.251, and highest genetic distance recorded among location 1 , 3 and 2 "Table. VI". These results show the effect of location and breeding system on genetic diversity among same breed of sheep. The genetic distances among sheep breed in present study were higher than that reported by [9] in Rahmani, Ossimi, Barki, Saidi and Sohagi sheep breeds. Whereas, genetic distance ranged from 0.0253 (between Ossimi and Rahmani) to 0.1825 (between Sohagi and Rahmani). [10] in Balochistan sheep breeds, found the genetic distance ranged from 0.003 (between Beverigh and Harnai) to 0.085 (between Mengali and Balochi).

I. Nei's Genetic Identity

"Table. VI" show Nei's genetic identity among Karadi sheep breed from four locations. The genetic identity among four locations ranged from 0.459 to 0.749, the lowest genetic identity recorded between location 1, 3 and location 4 arrived 0.459, and highest genetic identity recorded between location 1 and location 3 arrived 0.749, where the genetic identity between the location 4 with both location 1 and 3 arrived 0.595 "Table. VI". The results of the present study were lower than that reported by [22] for Barki, Rahmani, Baladi and Suffolk sheep breeds (0.819 to 0.957). [23] reported that genetic similarity arrived 0.9631 among Lesvos, Chios and Karagouniko sheep breeds, reported that. [10] found that similarity ranged from 0.91 between Balochi and Mengali breeds to 0.996 between Harnai and Beverigh breeds.

J. Phylogenetic tree construction

As in the dendrograms below "Fig. 4" there are three clusters, the 1st cluster branch consisted of the location 1 and 3, the 2nd cluster was including location 4 and the 3rd one including only location 2. These results indicated that the location 2 is most genetically distant from the both locations 1 and 3 (0.541). The location 1 and 3 in the 1st cluster indicating a close relationship between them and the results indicate that the sheep in location 1 and 3 were closer to location 4 than to the sheep in location 2. The dendrograms show that there are genetic diversity among sheep breed, were ranged from 0.251 to 0.541.

IV. Conclusion:

The high genetic diversity among the four location of Karadi sheep breed in this study, explain that there is widely area for selection among this sheep breed depended on molecular marker to speed up selection process per generations and this results show the effect of locations on genetic diversity in this breed of sheep.

Acknowledgment

This work was supported by MSc project at the Department of Animal Production, Faculty of Agriculture, University of Sulaimani, Kurdistan Region of Iraq. The authors thank for Dr. Jasim from the University of Duhok, Dr. Dlnea and Dr. Paywast from Sulaimani University, Kurdistan for all kindly help for the experiment.

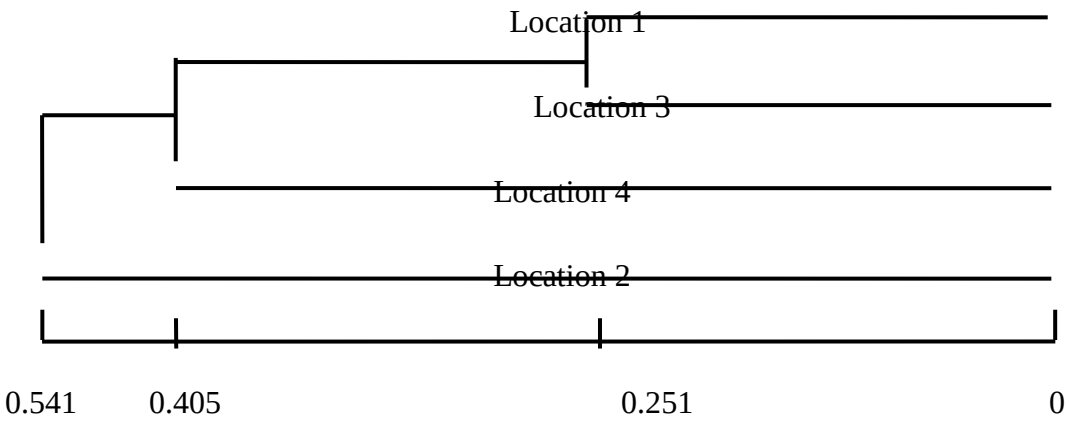

Fig. 4 UPGMA dendogram showing differentiation between the four sheep breeds based on [24] genetic distance.

Table.V: Estimation of heterozygosity and Shannon's information index for nine primers used.

Locus	h*	I≠
OPA-17	0.5000	0.6931
Moh-21	0.0000	0.0000
OPA-10	0.3750	0.5623
OPA-18	0.3750	0.5323
OPB-1	0.3750	0.5323
UBC-775	0. 0000	0.0000
OPB-3	0. 0000	0.0000
OPB-8	0.0000	0.0000
OPB-17	0.5000	0.6931
Mean	0.2361	0.3415

* h = Nei's gene diversity [25], *≠I = Shannon's Information index* [26]

Table.VI: Genetic identity (above diagonal) and genetic distance (below diagonal) among four locations.

Sheep breeds	Location 1	Location 2	Location 3	Location 4
Location 1	***	0.459	0.749	0.595
Location 2	0.541	***	0.459	0.669
Location 3	0.251	0.541	***	0.595
Location 4	0.405	0.331	0.405	***

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