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Molecular Characterization of Iraqi Local Goat Breeds Using Random Amplified Polymorphic DNA Markers

Research Article

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ABSTRACT

Cashmere is only produced from Maraz (Kurdi) goat breed in Iraqi Kurdistan Region. The objective of this study was to assess the genetic diversity of different Maraz color types, black goat and Shami breeds using random amplified polymorphic DNA (RAPD) markers. Fourteen primers were used and nine out of them were selected based on their number of bands (NB) and polymorphic characteristics. These primers generated a total of 154 bands ranged from 100 to 2800 bp. Out of the total bands detected only 50 bands were found to be polymorphic. Thirteen unique bands were found in Maraz goat, whereas the highest unique band was obtained in primer 7-MO2 locus. The overall Nei's gene diversity (gene diversity/heterozygosity) averaged 0.40, while the Shannon diversity index value was 0.58 ranged from 0.45 to 0.69. Phylogenetic dendrograms showed that three clusters, the 1st cluster branch consisted of the black goat breed, the 2nd cluster includes Shami goat with both black and light brown Maraz goat. The 3rd cluster includes both white and dark brown Maraz goats. Maraz color types grouped in one cluster that contains white and dark brown types and the black with light brown types were included in another cluster. It was concluded that the Maraz goat breed was closer to Shami goat than to the black goat breed.

KEY WORDS black and Shami goats, Cashmere, genetic diversity, Maraz.

INTRODUCTION

More than 1153 of domestic goat breeds are listed in the Domestic Animal Diversity Network (DAD-IS) of the Food and Agriculture Organization (FAO, 2009). Moreover, more than 850 million goats exist in the world with more than 95% found in the developing countries (FAO, 2007). From this population, Asia has the highest share at 65.3%, followed by Africa with 29.2% and Central America with 1.3% (Oliver *et al.* 2005). Nevertheless, only 60% of the breeds are found in the developing countries (Scherf, 2000). Europe is the broadest in goat genetic resources (33%) with only of 4% of the world's goat population (Galal, 2005).

Approximately 31% of the goats are dairy goats in the developed countries compared with only 19% for the developing world (Oliver *et al.* 2005). Therefore, worldwide, most goats are valued primarily for their meat. Some goats are raised not only for their meat or milk, but also for their hair. There are two types of goats raised for their hair, the Angora goat, and Cashmere goat. Maraz (Kurdi) goat is raised at high altitudes in the mountains of Iraqi Kurdistan and belong to the Cashmere bearing goat breeds. Undercoat fibers of this breed are ignored due to the unfamiliarity of the breeders with the importance of Cashmere and the possibility of its processing (Mason, 1981). Identification and characterization of breeds are a must to identify the genetic

resources and also to prioritize breeds for conservation and development. Assessing genetic variability within or among breeds is essential for genetic diversity among the animal populations. The complete population structure helps to plan strategies for conservation and development of a breed (Steele, 1996). Genetic variation is the raw material for the animal breeders, who utilize domestic animal species to people's needs. Furthermore, the increasing genetic data on goat breeds using different genetic markers will help to understand the evolutionary history of goat. In addition, it will help to refine the definition of breed (Henderson, 1984; Bearden and Fuquay, 2000). Recently, molecular markers, revealing polymorphism at the DNA level, has been playing an essential role in animal genetics studies. The random amplification polymorphism DNA (RAPD) marker has been widely used, due to its easy utilization by simple PCR, followed by a denaturing gel electrophoresis for number of fragments and fragments size determination (Marle-Koster and Nel, 2003).

RAPD markers are adopted as a powerful molecular fingerprinting technique which allows distinction even between closely related genotypes. Therefore, the objective of this study was to determine the genetic relatedness and characterization among Black, Shami (both of them reared for meat and milk) and Maraz (reared for cashmere, meat and milk production) goat populations in Duhok governorate / Kurdistan Region of Iraq using Random amplified polymorphic DNA markers technique.

MATERIALS AND METHODS

Experimental animals and locations

This study was conducted on three goat breeds Maraz (four coat colors: White, Black, Dark brown and Light brown), Black and Shami. Blood samples were collected from commercial flocks at different regions in Duhok governorate. A total of 40 indigenous goats (unrelated females) were sampled, ten samples of each of Black and Shami goats as well as 20 samples of Maraz goat (five samples of each color) were sampled (Figure 1). Five mL of whole blood was collected from each animal from jugular vein into 10 mL vacutainer tubes containing the anticoagulant, ethylenediaminetetra-acetic acid (EDTA) and blood samples were stored at -20 ˚C until DNA extractions. The DNA samples for each breed mixed together to make one sample (pooled sample) except the four color of Maraz goat each of them mixed together to made four separated pooled samples. All laboratory work was done in the biotechnology laboratory at the Department of Animal Resources, College of Agriculture, University of Salahaddin. DNA was extracted from each of the blood sample using QIAamp® DNA Blood Mini Kit (QIAGEN GmbH Qiagenstr.1 40724 Hilden Germany). The quantity and quality of DNA were determined by Nanodrop spectrophotometer and 1% agarose gel electrophoresis, respectively.

RAPD primers

In the present study, a total of 14 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 1.

PCR amplification of RAPD primers

Amplifications were performed using a thermal Cycler (Applied Biosystems® Veriti® 96-Well) with the final reaction volume of 25 µL. A master mix for minimum of 6 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 contained: 8 µL of Green Master Mix (ADM7122 00000311719, Promega-USA), (25 Units/mL Taq polymerase, each dNTPs is 200 µ*M* and $MgCl_2$ was 1.5 m*M*), 3 µL of RAPD primer (197.13 µ*M*–599.26 µ*M*), 3 µL (30 ng) of DNA template and 11 µL of DNase free water. In this study many protocols were used but four of them give clear bands. The 1st for primers (OPA-18, OPB-01, OPB-03, OPB-08, OPB-17 and OPB-20): programmed for 40 cycles of denaturation at 95 ˚C for 1 min, annealing at 40 ˚C for 1 min and extension at 72 ˚C for 2 min. An initial denaturation step of 2 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 (UBC-775) used the above program with change only in annealing with 35 ˚C and the 3rd protocol for primers (Moh-13 and Moh-21) were annealing with 36 ˚C and 4th protocol for primers (UBC-751, primer 7 MO2, OPB-19 and OPB-20) annealing with 38 ˚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.

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).

RESULTS AND DISCUSSION

Number of bands (NB)

A total of nine primers out of the fourteen random primers amplified showed clear bands and were to investigate the genetic variations among the three goat breeds (Marza, Black and Shami Goats).

Figure 1 Black, Shami and Maraz (with different coat colors) goats image

Table 1 Name, sequence and percentage content of GC for all used primers

No.	Primer name	Primer sequence 5′ to 3′	GC %
1	UBC-751	CCC ACC ACA C	70
2	UBC-775	GGT TTG GTG G	60
3	Primer 7 M02	ACA ACG CCT C	60
4	OPA-10	GTG ATC GCA G	60
5	OPA-17	GAC CGC TTG T	60
6	OPA-18	AGG TGA CCG T	60
7	OPB-1	GTT TCG CTC C	60
8	OPB-3	CAT CCC CCT G	70
9	OPB-8	GTC CAC ACG G	70
10	OPB-17	AGG GAA CGA G	60
11	OPB-19	ACC CCC GAA G	70
12	OPB-20	GGA CCC TTA C	60
13	Moh-13	GCT GCT CGA GT	70
14	Moh-21	AAC CGC GGT CT	70

All of the nine primers were polymorphic in the three breeds (Figures 2 and 3). The NB for the 9 primes over all the goat breeds was 154 bands, ranged from 5 bands in primer 7 MO2 to 36 bands in OPB-08, but among the breeds the highest NB found in white Maraz (34 fragments) and the lowest was detected in dark brown Maraz (17 fragments), (Table 2). The NB bands (154) that found in the 9 primers used in our study was higher than that reported by Oliveira *et al.* (2005) in seven populations of the Moxotó goat breed, Anous *et al.* (2009) in Zaraibi goat in Egypt, Sulaiman, (2012) in Iraqi local goat breed, Kumari *et al.* (2013) in Black Bengal goat and Jharkhand Black goat in India, Adam *et al.* (2015) in Ardi, Jabali and Shami goat breeds in Saudi and El-Tarras *et al.* (2015) in Najadi, Harri and Aradi goat breeds.

The size range of bands (bp)

The bands size range of the 9 primers over all the goat breeds, ranged from 100 to 2800 bp (Table 2). The smallest size of bands was recorded for OPA-18 (100 bp in white Maraz breed), while the highest size bands range was recorded for primer 7 MO2 locus (2800 bp in white Maraz breed). These results were in agreement with that reported by Anous *et al.* (2009) in Zaraibi goat in Egypt, Vahidi *et al.* (2014) in five Iranian goat breeds and Moradi *et al.* (2014) in Iranian mohair goat.

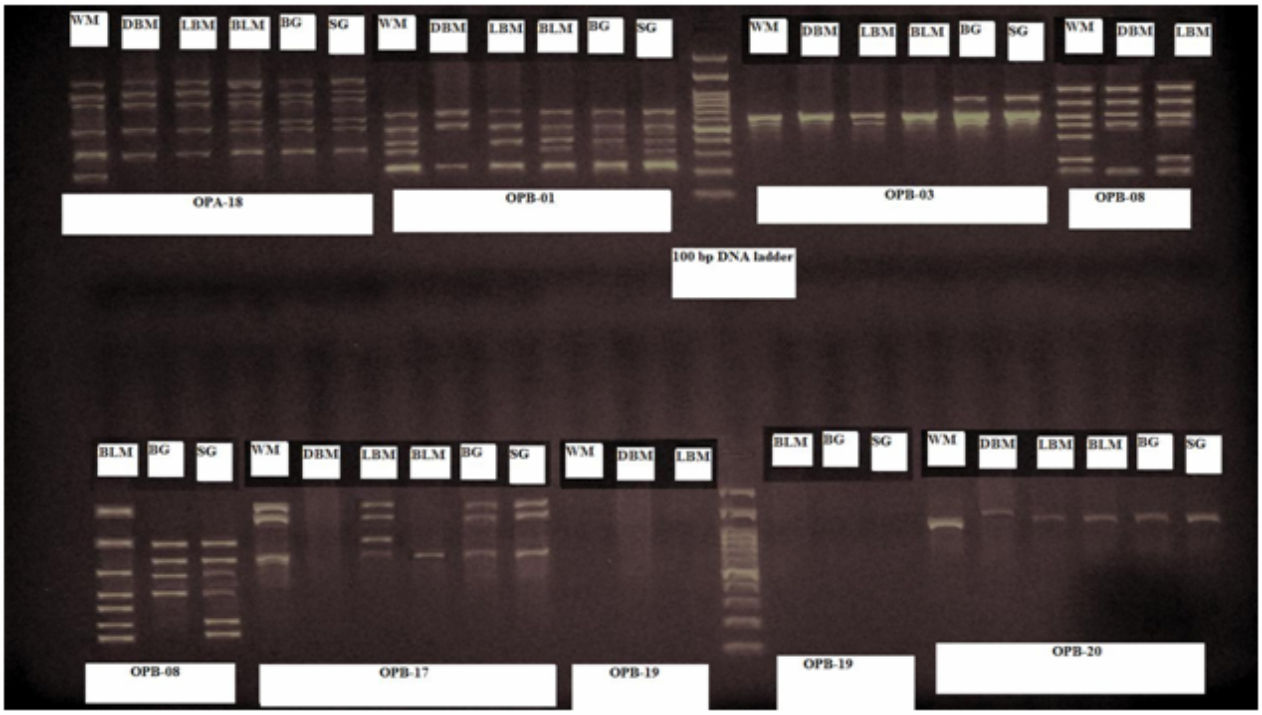

Figure 2 Gel electrophoresis for seven RAPD primers for six pooled goat samples

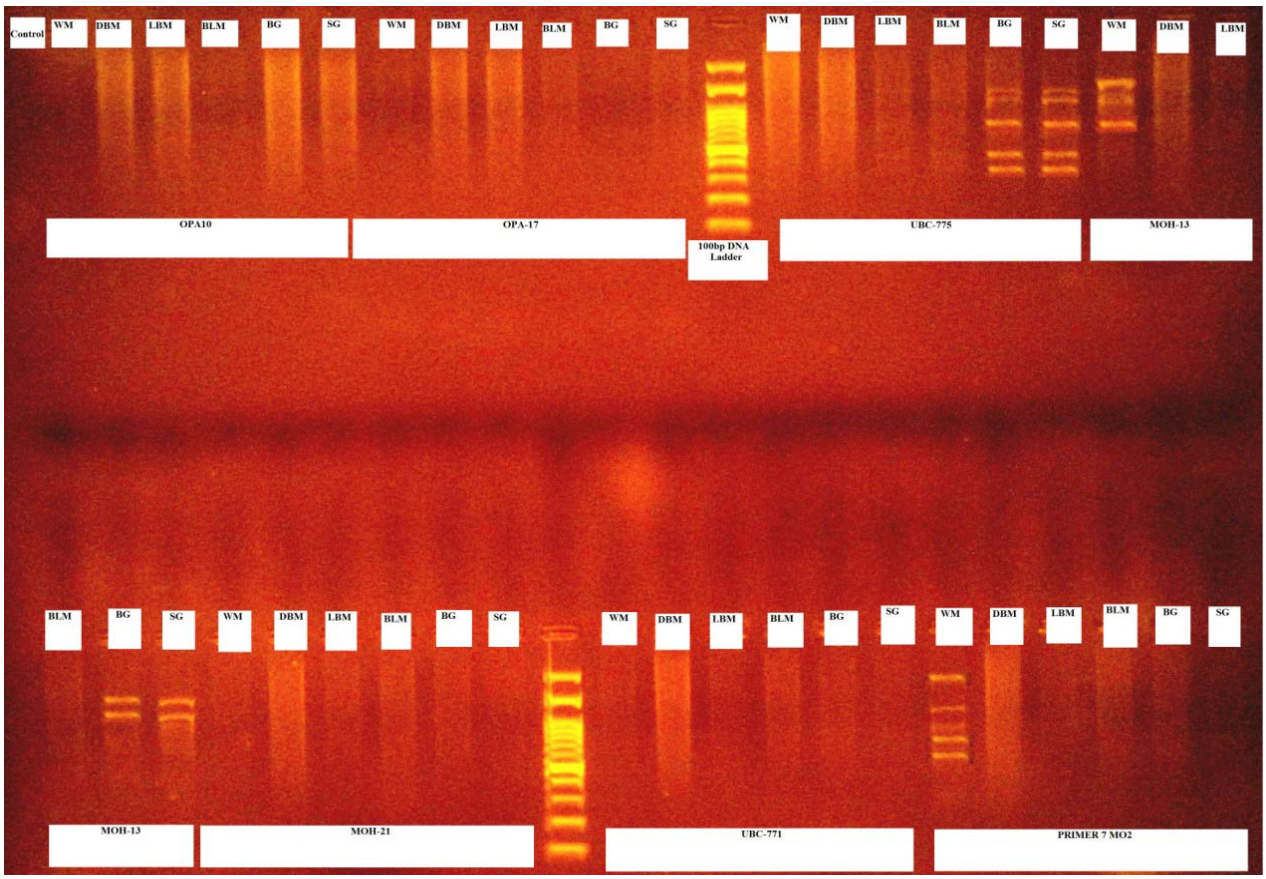

Figure 3 Gel electrophoresis for seven RAPD primers for six pooled goat samples.
Where:
BG: Black goat; SG: Shami goats; WM: White Maraz; BLM: Black Maraz; DBM: Dark brown Maraz and LBM: Light brown Maraz goat and control

Table 2 Band numbers and bands size range (bp) in goat breeds

No.	Primers name	Goat breeds												Overall	
		White Maraz		Dark brown Maraz		Light brown Maraz		Black Maraz		Black goat		Shami goat			
		No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	No. of band	Size range bp	Total No. of band	Size range bp
1	OPA-18	6	100-1500	5	300-1500	5	300-1500	6	350-1500	6	350-1500	6	350-1500	34	100-1500
2	OPB-01	5	230-700	3	230-700	4	230-750	5	230-700	4	230-700	6	230-700	27	230-750
3	OPB-03	2	550-600	2	550-600	2	550-600	2	550-600	3	500-900	3	500-900	14	500-900
4	OPB-08	8	150-1250	6	150-1250	7	150-1250	7	150-1250	3	400-750	5	150-750	36	150-1250
5	OPB-17	4	550-2250	0	-	4	700-2250	1	600	3	600-2250	3	600-2250	15	550-2250
6	OPB-20	1	1100	1	1500	1	1250	1	1250	1	1250	1	1250	6	1100-1500
7	UBC-775	0	-	0	-	0	-	0	-	5	350-1500	5	350-1500	10	350-1500
8	MOH-13	3	800-2000	0	-	0	-	0	-	2	1250-1500	2	1250-1500	7	800-2000
9	PRIMER 7 MO2	5	600-2800	0	-	0	-	0	-	0	-	0	-	5	600-2800
Total		34	100-2800	17	150-1500	23	150-2250	22	150-1500	27	230-2250	31	150-2250	154	100-2800

Number of polymorphic bands (NPB)

By the screening of the 9 primers, 154 bands were found, only 50 out of them were polymorphic (Table 3).

Table 3 Overall NPB and PPB for nine primers used

No.	Primer name	No. of polymorphic bands	Polymorphic bands (%)
1	OPA-18	9	90.0
2	OPB-01	7	87.5
3	OPB-03	3	75.0
4	OPB-08	8	88.89
5	OPB-17	6	100
6	OPB-20	3	100
7	UBC-775	5	100
8	MOH-13	4	100
9	PRIMER 7 MO2	5	100
Mean		5.56	93.49

Overall mean of NPB was 5.56 with the highest value found at locus OPA-18 (9 bands), whereas the lowest NPB found at locus OPB-3 and OPB-20 (3 bands). Apart from OPB-03 and OPB-20 loci all other loci have more than 3 NPB (Table 3).

These results indicated the possibility of depending on these loci for genetic characterization or genetic distances between/among the present goat breeds.

The NPB in present study was higher than that reported by Anous *et al.* (2009) in Zaraibi goat and Adam *et al.* (2015) in three Saudi goat breeds (Ardi, Jabali and Shami). On the other hands, the NPB in present study was lower than those reported by Vahidi *et al.* (2014) in five Iranian goat breeds, Moradi *et al.* (2014) in Iranian mohair goat, Kanaan *et al.* (2014) in Shami goats in Syria and El-Tarras *et al.* (2015) in Najadi, Harri and Aradi goat breeds.

Percentage of polymorphic bands (PPB)

The overall mean PPB for nine primers in the present study was 93.49% (Table 3).

As in the results four primers have 100% PPB, the lowest polymorphism (75%) was detected in OPB-03 locus (Table 3).

The mean of PPB in present study were higher than that reported by Moradi *et al.* (2014) in Iranian mohair goat. On the other hands, the PPB in present study was lower than that reported by Kanaan *et al.* (2014) in Shami goats and Adam *et al.* (2015) in three Saudi goat breeds.

Gene frequency

The mean of gene frequency for all loci for allele 0 was 0.35 ranged from 0.17 for (OPB-03 and OPB-17) loci to 0.67 for (OPB-20) locus and allele 1 reached 0.65, ranged from 0.33 for (OPB-20) locus to 0.83 for (OPB-03 and OPB-17) loci (Table 4).

Table 4 Overall gene frequency for all primers used

No.	Locus	Allele 0*	Allele 1**
1	OPA-18	0.33	0.67
2	OPB-01	0.50	0.50
3	OPB-03	0.17	0.83
4	OPB-08	0.50	0.50
5	OPB-17	0.17	0.83
6	OPB-20	0.67	0.33
7	UBC-775	0.33	0.67
8	MOH-13	0.33	0.67
9	PRIMER 7 MO2	0.17	0.83

* Mean the absent of the band.
** Mean the present of the band.

This result was in agreement with that reported by Kumari *et al.* (2013) in Black Bengal goat whose gene frequency ranged from 0.125-0.729 for allele 0 and from 0.271-0.875 for allele 1. Similarly in case of Jharkhand Black, gene frequency ranged from 0.146-0.625 for allele 0 and from 0.375- 0.854 for allele 1.

Unique bands

Table 5 revealed that 13 out of 154 bands were unique, found in Maraz goat only.

Table 5 Unique band numbers and fragments size in goat breeds

No.	Primers name	Goat breeds												Overall	
		White Maraz		Dark brown Maraz		Light brown Maraz		Black Maraz		Black goat		Shami goat			
		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)	No. of unique band	Fragments size (bp)	No. of unique band	Fragments size (bp)	Total No. of unique band	Fragments size range (bp)
1	OPA-18	1	100	0	-	0	-	0	-	0	-	0	-	1	100
2	OPB-01	0	-	0	-	1	750	0	-	0	-	0	-	1	750
3	OPB-17	1	550	0	-	1	900	0	-	0	-	0	-	2	550-900
4	0PB-20	1	1100	1	1500	0	-	0	-	0	-	0	-	2	1100-1500
5	MOH-13	2	800 2000	0	-	0	-	0	-	0	-	0	-	2	800-2000
6	PRIMER 7 MO2	5	2800 1250 900 800 600	0	-	0	-	0	-	0	-	0	-	5	600-2800
Total		10	100-2800	1	1500	2	750-900	0	-	0	-	0	-	13	100-2800

These results can be used in future to make the correlations between these unique band and cashmere production from Maraz goat. Out of nine primers 6 of them gives unique band, the highest unique band was obtained by primer 7-MO2 locus, which had 5 bands, all of them were unique (ranged from 600 to 2800 bp) and found only in white Maraz goat.

These results indicate that these six loci can be used to analyze the genetic diversity between / among breeds. Moreover, it shows that there are genetic distances between goat breeds.

Nei's gene diversity

The Nei's gene diversity (gene diversity/heterozygosity) for overall goat breeds averaged 0.40 (Table 6). This result indicates the genetic diversity among goat breeds is moderately high. The gene diversity value in this study was higher than that reported by Oliveira *et al.* (2005) in Moxotó goat breed, Moradi *et al.* (2014) in Iranian mohair goat. On the other hand this result was lower than that reported by Kumari *et al.* (2013) in Black Bengal and Jharkhand Black goat.

Table 5 reveals of the 9 amplified primers were polymorphic; the OPA-18, OPB-20, UBC-775 and MOH-13 loci give the highest heterozygosity (0.44).

Shannon's information index (I)

The average of the Shannon diversity index values in the present study was 0.58 ranging from 0.45 to 0.69 (Table 6).

These values were computed to provide relative estimation of variability. Such value shows the high diversity among the studied goat breeds. The Shannon index value in present study was higher than that reported by Moradi *et al.* (2014) in Iranian mohair goat. On the other hand this result was lower than reported by Kumari *et al.* (2013) in Black Bengal and Jharkhand Black goat where Shannon's Information Index was 0.6792 and 0.5898, respectively.

Table 6 Overall Nei's gene diversity and Shannon's information index for nine primers

No.	Locus	h[3]	I[4]
1	OPA-18	0.44	0.63
2	OPB-01	0.50	0.69
3	OPB-03	0.27	0.45
4	OPB-08	0.50	0.69
5	OPB-17	0.27	0.45
6	OPB-20	0.44	0.63
7	UBC-775	0.44	0.63
8	MOH-13	0.44	0.63
9	PRIMER 7 MO2	0.27	0.45
Mean		0.40	0.58
SD		0.09	0.10

h[3]: overall Nei's gene diversity and I[4]: overall Shannon's information index.
SD: standard deviation.

Genetic distance

Table 7 presents the Nei's genetic distances among goat breeds. The genetic distance among the three studied goat breeds ranged from 0.1256 to 0.4917. The lowest genetic distance was recorded between light brown Maraz and black Maraz that was 0.1256 and the highest genetic distance recorded between black goat and all goat breeds were

0.4917. Furthermore, the genetic distance between the Shami goat and each of white, dark brown, light brown and black Maraz goats were 0.3496, 0.3496, 0.2027 and 0.2027, respectively (Table 7).

Table 7 Overall Nei's genetic distance (below diagonal)

Pop ID	WM*	DBM	LBM	BLM	BG	SG
WM	***	-	-	-	-	-
DBM	0.2027	***	-	-	-	-
LBM	0.3496	0.3496	***	-	-	-
BLM	0.3496	0.3496	0.1256	***	-	-
BG	0.4917	0.4917	0.4917	0.4917	***	-
SG	0.3496	0.3496	0.2027	0.2027	0.4917	***

* BG: Black goat; SG: Shami goat; WM: White Maraz; BLM: Black Maraz; DBM: Dark brown and LBM: Light brown Maraz goat.

The genetic distances among goat breeds in the present study were higher than that reported by Kumari *et al.* (2013) in Black Bengal and Jharkhand Black goat, Moradi *et al.* (2014) in Iranian mohair goat. On the other hand these results were lower than that reported by Vahidi *et al.* (2014) in five Iranian goat breeds.

Phylogenetic tree construction

As in the dendrograms below (Figure 4), three clusters were generated, the 1st cluster branch consisted of the black goat breed, the 2nd cluster was including the Shami goat with both black and light brown Maraz goat and the 3rd one includes both white and dark brown Maraz goat breed.

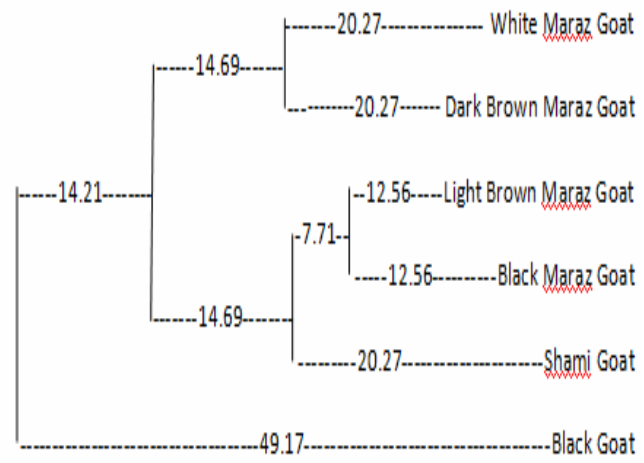

Figure 4 UPGMA dendogram showing differentiation among the goat breeds

These results indicated that the black goat breed is most genetically distant from the Shami and Maraz goat breeds (0.4917). The light brown and black Maraz goat in the 2nd cluster indicate a close relationship between them (0.1256) and the results indicated that the Maraz goat breed was more closer to Shami goat breed than to the black goat breed.

CONCLUSION

The high distance (49.17) and polymorphism (93.49) among three goat breeds found in this study indicates that these goat breeds have the required amount of genetic variation to made genetic improvement in near further. This study helps us to clarify the image of the genetic diversity of the local Iraqi goat breeds in Duhok governorate and the breeders can used it for mating system when need to make the crossing among these goat breeds. Finally, to get the accurate estimation of the genetic distance of these local genetic resources, further studies using different genetic methods and large number of animals from various geographical regions in the KRG-Iraq are needed.

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