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MOLECULAR EVALUATION OF THREE POPULATIONS OF FARAFRA SHEEP IN COMPARISON TO OSSIMI AND RAHMANI SHEEP BREEDS

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Abstract

Molecular markers are the most ideal approach to study genetic diversity. Consequently, we utilized both ISSR and RAPD markers to assess genetic diversity and relationships among three different populations of Farafra, Ossimi and Rahmani Egyptian sheep breeds. Both ISSR and RAPD gave moderate polymorphism 41.3% and 48.51%, respectively. Besides, this value was consistent with the moderate value of the mean of polymorphism information content (0.16 and 0.20, respectively). Farafra-F and Farafra-D populations had the highest similarity which was 0.92 for ISSR and 0.90 for the RAPD marker. Furthermore, ISSR and RAPD constructed dendrogram separated all the studied sheep into two main clusters. All the three populations of Farafra breed combined into one main cluster, while the second cluster contained both Rahmani and Ossimi breeds. The used molecular markers were able to discriminate among evaluated sheep and displayed that Farafra breed more closely related to Ossimi than Rahmani breed.

Keywords: dendrogram, Farafra sheep, genetic diversity, ISSR, molecular markers, RAPD

INTRODUCTION

Sheep are the most prevalent small ruminant in the world. It is one of the most important suppliers of animal protein, milk and wool. In Egypt, the total number of sheep is about 5,488,000. They are distributing almost all over the country regions (Elshazly and Youngs, 2019). They were classified into three major breeds, Rahmani, Ossimi and Barki. Additionally, eight minor breeds: Farafra, Saidi, Abidi, Abudeleik, Maenit, Kanzi, Sanabawi and Sohagi, which are located mainly in the southern part of the country and the oases (Elbeltagy, 2012). Ossimi sheep were found in Ossim village, Giza governorate. They having a white colour fleece, brown or dark brown head, semi pendulous ears and fat tail (weighing 2.5–4.0% of the animal's body weight) and males have horns, while females do not have (Dehoux and Verhulst, 1994; Elshazly and Youngs, 2019). Concerning Rahmani sheep, resides in northern Syria and southern turkey. It was first introduced into Egypt in the 19th century in Rahmania in Beheira governorate, so the breed was named Rahmani. Rahmani sheep have reddish brown colour which fades with advancing age, small ears that are often vestigial, large tail weighing 4% of live weight and large horns in

male but females are polled or have small horns (Dehoux and Verhulst, 1994; Elshazly and Youngs, 2019). On the other hand, among the minor breeds, Farafra is a native breed that dominates in Oases of El-Farafra, El-Dakhla and El-Kharga of the Egyptian Western Desert. It has a white fleece with a brown face, along cylindrical tail, males and females are typically polled and sometimes rams carry small size scurs on her body and carry small size ear (Elshazly and Youngs, 2019).

To assess a genetic variation within and between different sheep populations, numerous markers have been utilized. However, DNA molecular markers are the best tools, because they are not influenced by environmental alterations. Also, they are providing accurate information covering all genomic regions (Prasad *et al*., 2009). Among various PCR based molecular markers, Inter Simple Sequence Repeat (ISSR), and Randomly Amplified Polymorphic DNA (RAPD) have commonly been used in sheep (Elmaci *et al.*, 2007; Mahfouz *et al.*, 2008; Zamani *et al.*, 2015; Mohammadabadi *et al.*, 2017). ISSR is a dominant marker that can amplify DNA fragments between two simple sequence repeat regions without any prior sequence information (Zietkiewicz *et al.*, 1994). Otherwise, RAPD is a simple and easy technique that uses single short primers usually 10 bases long to amplify random DNA fragments. Also, it does not need any information about the target sequence (Williams *et al.*, 1990; Bardakci, 2001).

Indeed, Farafra sheep is adapted very well for the difficult desert environmental conditions, especially heat stress. Therefore, it is a good candidate could use in sheep genetic improvement programs. According to a literature survey, several papers concerning its morphological characterization (Mousa *et al.*, 2006; Galal, 2007; Elbeltagy, 2012; Elshazly and Youngs, 2019). Moreover, only one available molecular study was performed by El-Hamamsy *et al.* (2018). They characterized three Egyptian sheep populations, collected from Siwa, El-Dakhla and El-Farafra Oases of the Egyptian Western Desert, using microsatellite marker and electrophoretic protein. Their study was performed on general sheep which lies in those regions and did not directed to Farafra sheep. Our study was the first investigation used two different DNA markers for assessing genetic diversity in Farafra breed and the relationship with other breeds.

The current study aims to evaluate genetic diversity and relationships within three different populations of Farafra breed and among all the studied sheep breeds using both the ISSR and RAPD molecular markers.

MATERIALS AND METHODS

Three populations of Farafra (from different locations of New Valley governorate, Egypt), Rahmani and Ossimi sheep breed were utilized in the current study (Tab. I).

Blood Collection

Two ml of whole blood were collected from the jugular vein of each animal, (50 individuals for each population of Farafra breed, 10 for each both Rahmani and Ossimi pure breeds) into 5 ml vacutainer tubes containing EDTA (ethylenediaminetetraacetic acid) as ananticoagulant. The blood samples were stored at -20 °C in the Genetics Department, Faculty of Agriculture, New Valley University. The experiment was carried out during November and December 2019.

DNA Extraction

The collected samples for each group were mixed to be one pooled sample per group. Genomic DNA was extracted from each pooled sample using animal DNA blood isolation kit (Wizard® Genomic DNA Purification) according to the manufacturer's instructions.

PCR Amplification and Electrophoresis

Both ISSR and RAPD PCR amplifications were performed in total reaction mixture volume of 25 µl containing: 12.5 µl of green PCR Master Mix, 2X (50 units/ml Taq DNA polymerase, 400 µM of each dATP, dGTP, dCTP and dTTP and 3 mM $MgCl_2$), 2 µl of 10 µM primer, 2 µl of DNA (50 ng) and 8.5 µl of ddH_2O. Amplifications were performed in a thermal

I: *The name, original place and morphological characteristics of the studied sheep*

No.	Name	Original place of collection	Morphological characteristics
1	Rahmani	Rahmania, Beheira governorate, Egypt	Dehoux and Verhulst, 1994; Elshazly and Youngs, 2019.
2	Ossimi	Ossim, Giza governorate, Egypt	Dehoux and Verhulst, 1994; Elshazly and Youngs, 2019.
3	Farafra-Kh	El-Kharga, New Valley, Egypt	Creamy white, creamy white with brown, dark brown or black spots body colour, brown or white head, long and thin tail and medium or small ears.
4	Farafra-D	El-Dakhla, New Valley, Egypt	Creamy white, creamy white with brown spots body colour, brown or white head, long and thin tail and medium or small ears.
5	Farafra-F	El-Farafra, New Valley, Egypt	Creamy white, creamy white with brown spots body colour, brown or white head, long and thin tail and medium or small ears.

II: *Primer ID and sequences of both ISSR and RAPD*

ISSR Primers	Sequence (5´ to 3´)	RAPD Primers	Sequence (5´ to 3´)
UBC 807	(AG)8T	OPB12	CCTTGACGCA
UBC 808	(AG)8C	OPF04	GGTGATCAGG
UBC 810	(GA)8T	OPC11	AAAGCTGCGG
UBC 811	(GA)8C	OPC14	TGCGTGCTTG
UBC 812	(GA)8A	OPD08	GTGTGCCCCA
UBC 814	(CT)8A	OPD18	GAGAGCCAAC
UBC 815	(CT)8G	OPL05	ACGCAGGCAC
UBC 818	(CA)8G	OPW06	AGGCCCGATG
UBC 823	(TC)8C	S11	GTAGACCCGT
UBC 826	(AC)8C	UBC751	CCCACCACAC
UBC 834	(AG)8TT		
UBC 840	(GA)8TT		

cycler (Labocon, U.K.). Twelve ISSR primers and ten RAPD primers were used (by metabion; Tab. II). The PCR amplifications program starting with initial denaturation at 94 °C for 5 min then, followed by 38 cycles: denaturation at 94 °C for 1 min, annealing at 48 °C for ISSR or at 35 °C for RAPD primers 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 1.5% agarose gels using 1×TBE (Tris-Borate-EDTA) running buffer at 5 V/cm. Then, they were visualized by staining with ethidium bromide.

Data Analysis

We recorded 1 for the present bands and 0 for absent band. Genetic similarity was estimated using Jaccard's similarity coefficient (Jaccard, 1908). 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) were calculated using the software computational package MVSP 3.1. program. Different indices namely, polymorphic information contents (PIC), resolving power (Rp) and marker index (MI) were calculated according to Anderson *et al.* (1993); Prevost and Wilkinson (1999); Powell *et al.* (1996), respectively.

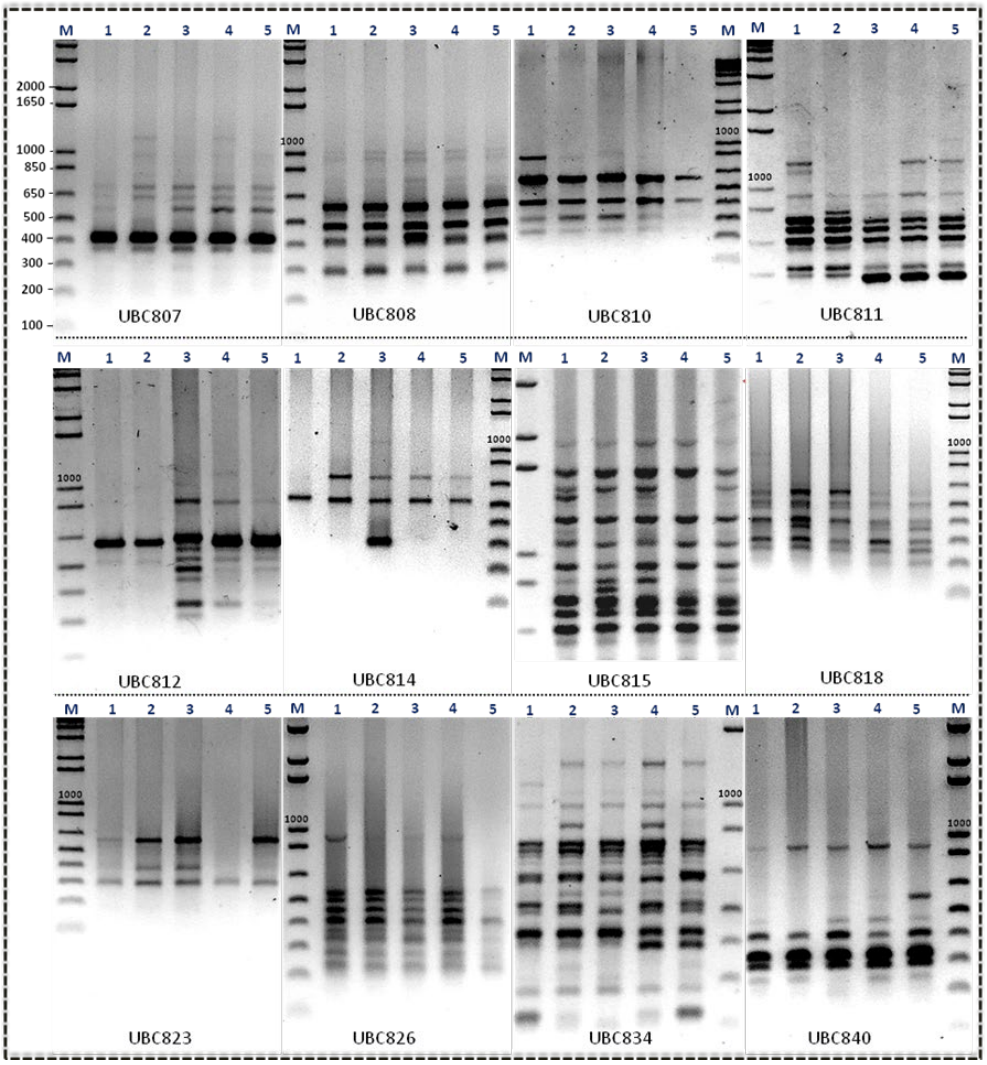
RESULTS AND DISCUSSION

A- Polymorphism Assessment

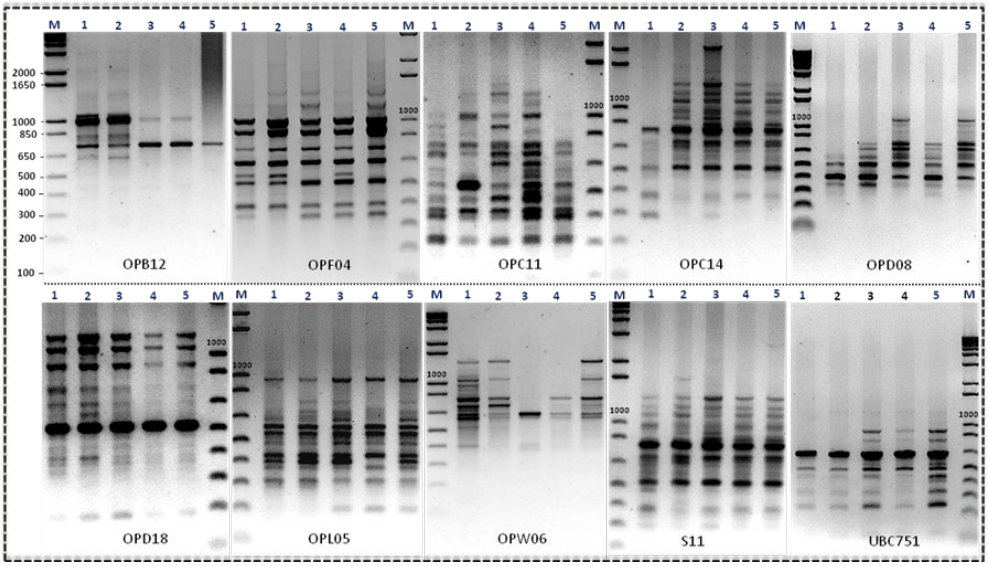
To study the genetic distance among the three populations of Farafra, Rahmani and Ossimi breeds, twelve ISSR and ten RAPD molecular markers were used (Figs. 1 and 2, respectively). About the ISSR marker, a total of 92 bands were scored with size varied from 200 bp to 1344 bp. Only 38 bands were polymorphic, with 41.3% polymorphism (Tab. III). On the other hand, RAPD markers detected 101 bands having size ranged from 178 bp to 1910 bp, 49 bands among them were polymorphic. However, the estimated polymorphism was 48.51% (Tab. III). ISSR and RAPD molecular markers have been widely used in the detection of genetic diversity in sheep (Elmaci *et al.*, 2007; Mahfouz *et al.*, 2008; Zamani *et al.*, 2015; Mohammadabadi *et al.*, 2017). The ISSR marker detected moderate polymorphism percentage (41.3%) close to but less than which detected by RAPD molecular marker (48.51%). Reflecting, the RAPD marker is more effective in the detection of molecular polymorphism. This may be due to the RAPD is less specific than ISSR and screens all most the genome. This result is similar to the findings of Guasmi *et al.* (2012), Haider *et al.* (2012), Elmeer *et al.* (2017). Moreover, to study further the capacity of the markers for detecting polymorphism, so the PIC value was estimated. In ISSR this value ranged from 0.06 to 0.32 with an average of 0.16, while in RAPD marker varied from 0.06 to 0.35 with an average of 0.20. Actually, the RAPD marker exhibited a higher level of PIC average, as it also reported by Guasmi *et al.* (2012), Elmeer *et al.* (2017). The PIC average values of both ISSR and RAPD were moderate, thus showed moderate detectable polymorphism. Additionally, the primer resolving power (Rp) was calculated to determine the preferable primers in each marker. The Rp for ISSR primers fluctuated from 0.4 for both UBC 810 and UBC 823 primer to 5.6 for UBC 834 primer, while this value varied from 0.8 to 5.2 for OPD18 and OPC11, respectively. Moreover, to evaluate the efficiency of the two markers for the detection of variations, the marker index (MI) was calculated. The results showed that the MI value was higher in RAPD (1.12) than ISSR (0.67). Thus, the RAPD had more effectiveness in comparison to the ISSR marker. Furthermore, Shannon's information index was used for further detection of genetic diversity among all the breeds. Among the used ISSR primers, UBC 834 displayed the highest value (2.51), but UBC 823 had the least value (0.18) with an average of 0.89. On the other hand, in RAPD marker ranged from 0.37 to 2.53 for OPD18 and OPC11, respectively, with an average of 1.38. Shannon index value for both ISSR and RAPD markers revealed low genetic diversity among all studied sheep this may be due to high inbreeding mating in those studied populations. Almost all the previous indices were higher in RAPD than in the ISSR marker this finding is in agreement with several studies (Guasmi *et al.*, 2012; Tonk *et al.*, 2014; Elmeer *et al.*, 2017).

B- Genetic Similarly and Relationship

To study the level of genetic relationship among all the studied sheep, the level of similarity among them was estimated by using Jaccard's similarity coefficient depending on ISSR and RAPD data (Tab. IV). Both of ISSR and RAPD markers showed the highest similarity (0.92 and 0.90, respectively)



1: *ISSR banding pattern of the studied sheep. 1, Rahmani; 2, Ossimi; 3, Farafra-Kh; 4, Farafra-D; 5, Farafra-F. M, kbp DNA marker*



2: *RAPD banding pattern of the studied sheep. 1, Rahmani; 2, Ossimi; 3, Farafra-Kh; 4, Farafra-D; 5, Farafra-F. M, kbp DNA marker*

III: *Polymorphism, Polymorphism Information Content, Marker index, Resolving power and Shannon's index values obtained by ISSR and RAPD markers in the studied sheep*

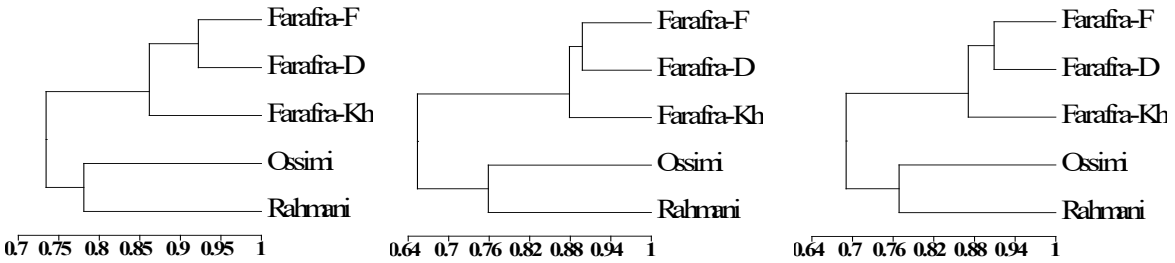
Primer Name	Range of fragment size bp	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	P %	PIC	MI	R p	I
			ISSR						
UBC 807	365–1155	7	4	3	42.86	0.18	0.55	2	0.79
UBC 808	295–1000	7	6	1	14.3	0.07	0.07	0.8	0.37
UBC 810	205–785	5	4	1	20	0.06	0.06	0.4	0.32
UBC 811	490–1344	10	6	4	40	0.16	0.64	2.4	1.26
UBC 812	320–890	8	2	6	75	0.32	1.92	4	1.87
UBC 814	310–710	3	1	2	66.67	0.21	0.42	0.8	0.5
UBC 815	380–987	13	8	5	38.46	0.14	0.70	2.4	1.16
UBC 818	200–575	8	5	3	37.5	0.16	0.48	2.0	0.85
UBC 823	300–575	3	2	1	33.33	0.11	0.01	0.4	0.18
UBC 826	224–950	8	7	1	12.5	0.06	0.6	0.8	0.37
UBC 834	230–1330	14	5	9	64.29	0.26	2.34	5.6	2.51
UBC 840	265–860	6	4	2	33.33	0.13	0.26	1.2	0.49
Total	-	92	54	38	-	-	-	-	9.62
Average	-	7.67	4.5	3.17	40.57	0.16	0.67	-	0.89
			RAPD						
OPB12	640–1080	5	1	4	80	0.35	1.4	2.8	1.28
OPF04	325–1000	8	6	2	25	0.1	0.2	1.2	0.49
OPC11	340–1335	15	6	9	60	0.24	2.16	5.2	2.53
OPC14	290–1910	12	3	9	75	0.27	2.43	4.4	2.21
OPD08	325–1125	10	4	6	60	0.24	1.44	3.6	1.52
OPD18	360–1160	8	7	1	12.5	0.06	0.06	0.8	0.37
OPL05	178–960	13	7	6	46.15	0.19	1.14	3.6	1.52
OPW06	380–1456	11	5	6	54.55	0.25	1.5	4.4	2.03
S11	368–1295	11	8	3	27.27	0.1	0.3	1.6	0.95
UBC751	240–915	8	5	3	37.5	0.18	0.54	2.4	0.92
Total	-	101	52	49	-	-	-	-	13.82
Average	-	10.1	5.2	4.9	47.80	0.20	1.12	-	1.38

P = Polymorphism, MI = Marker index, PR = Resolving power, I = Shannon's Information index

between Farafra-F and Farafra-D populations. Also, they revealed the lowest similarity (0.69 and 0.60, respectively) between Rahmani breed and Farafra-D population. Moreover, the dendrogram of genetic distance in both ISSR and RAPD markers classified all the studied sheep into two main clusters (Fig. 3). the first one contained all the three populations of Farafra breed and divided into two sub-clusters, one of them grouped both Farafra-F and Farafra-D populations, while the second one contained only Farafra-Kh population. On the other hand, the second cluster gathered both Rahmani and Ossimi breeds. Furthermore, the results of combined ISSR and RAPD data also showed that the highest similarity (0.91) was between Farafra and Eldakhla populations. Ossimi breed and Dakhla population had the lowest similarity (0.64). Additionally, the combined data conducted the same dendrogram as in individual ISSR or RAPD marker. The higher similarity between Farafra-F and Farafra-D and grouping them in the same sub-cluster indicated that they have higher homozygosity than Farafra-Kh (Mahfouz *et al.*, 2008). This higher similarity is maybe due to more exchange in animal trade and in all aspects of life between El-Farafra and El-Dakhla region. Furthermore, there are many common and relative human families between them more than El-Kharga. Additionally, they are more isolated geographical regions in comparison to El-Kharga. Moreover, all the three groups of Farafra breed were more closely related to Ossimi than the Rahmani breed, this is fitted with that Farafra

IV: *The similarity index among the studied sheep based on ISSR, RAPD and combined analysis*

Genotypes	Rahmani	Ossimi	Farafra-Kh	Farafra-D	Marker type
Ossimi	0.78				ISSR
	0.76				RAPD
	0.77				Comined
Farafra-Kh	0.73	0.73			ISSR
	0.61	0.68			RAPD
	0.66	0.71			Comined
Farafra-D	0.69	0.76	0.84		ISSR
	0.60	0.69	0.86		RAPD
	0.64	0.72	0.85		Comined
Farafra-F	0.74	0.76	0.89	0.92	ISSR
	0.62	0.72	0.89	0.90	RAPD
	0.67	0.74	0.89	0.91	Comined



A) ISSR B) RAPD C) Combined

3: *The dendrograms of genetic distances among the studied sheep based on ISSR, RAPD marker and combined analysis*

breed may be resulted from crossing between Ossimi and any desert breed (Elshazly and Youngs, 2019). On the other hand, Ossimi and Rahmani breeds were located in one main cluster this result is in agreement with the findings of Mahfouz *et al.* (2008); Othman *et al.* (2015). The detected identity between them in the RAPD marker was less than which was obtained by Mahfouz *et al.* (2008); Abdel-rahman *et al.* (2010) (0.975 and 0.87, respectively). The closeness of Ossimi and Rahmani has been confirmed by using many different types of DNA molecular markers (Abdel-rahman *et al.*, 2010; Ghazy *et al.*, 2013; Othman *et al.*, 2015; Rushdi, 2018). Actually, Rahmani and Ossimi breeds are distributed in close geographical locations this leads to intermixing of genetic material between them. Obviously, the resulted data from both ISSR and RAPD markers are more consistent with their geographic distribution and genetic makeup. The current study could provide further helpful information about genetic diversity within Farafra breed and the level of relatedness with other breeds located in different close geographical regions around.

CONCLUSION

The present study aimed to evaluate genetic diversity and the relationship among three different populations of Farafra breed, in addition to two pure breeds (Rahmani and Ossimi). Moderate value for both of polymorphism and genetic diversity were observed among the studied sheep. The three populations of Farafra have the most closeness. Furthermore, these populations had higher similarity and closer proximity to Ossimi more than to Rahmani breed. The study provided further information regarding Farafra breed.

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