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Finger Printing of Rahmani, Chios Sheep Breeds and Their Crosses Using Random Amplified Polymorphic Dna (Rapd)

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ABSTRACT

RAPD-PCR was performed using ten random primers to identify the genetic diversity among Rahmani, Chios and their crosses. The appearance of bands on gels would reflect the differences between genotypes of the examined animals. The differences would be detected by number and size of present or absent bands with each primer which could be used as positive or negative genetic markers to distinguish between breeds and their crosses. Primers A7, C7 and C1 were used for fingerprinting to identify the Rahmani breed, where they produced bands of 454 and 724 bp, respectively only with Rahmani breed. Presence of band at 410 bp with primer A9 would be used as a genetic marker for Chios breed. The presence of bands 1634, 1105, 223 and 187 bp with primer B17 and also bands 735, 683, 425 and 395 bp with primer A9 could be used as a fingerprinting for cross (½ C ½ R). Presence of bands 173, 132 and 122 bp with primer A5 would be used as a genetic marker for the reciprocal cross (½ R ½ C). The results of molecular DNA of the experimental sheep breeds and their crosses, showed that polymorphism within crosses is higher than polymorphism within the pure breeds of Rahmani and Chios. Fragments generated by primers showed a polymorphism ratio of 10.8 % between Rahmani and Chios and 25 % between crosses (½ C ½ R and ½ R ½ C). Also, the similarity between Rahmani and Chios breeds was 89.1 %, while it was 75 % between crosses. The results asserted that fingerprinting (RAPD-PCR genetic marker) technique would be a useful tool to differentiate sheep breeds and their crosses.

Keywords: Fingerprinting, RAPD-PCR, Genetic marker, Rahmani and Chios.

INTRODUCTION

Based on the phenotypic character, Egyptian sheep were classified into five breeds (Salah, *et al.,* 2010) some of them are Rahmani and others are Chios. Genetic improvement of farm animal is of economic importance, especially in reproductive performance and quantity of meat and wool (Ali, 2003). Information of genetic relationships in sheep within and between species has several important applications for genetic improvement and in breeding programs (Rao, *et al.,* 1996). Application of the random amplified polymorphic DNA (RAPD) technique has greatly increased the ability to understand the genetic relationships within species at the molecular level. Polymorphism at the DNA level is frequently used as genetic markers for herd studies in domestic animals. Genetic marker and variation detected at the loci reflect the level of variation in the entire genome (Okumus and Mercan, 2007). The DNA-RAPD assay which is short oligonucleotide primers of arbitrary sequence to amplify genomic DNA by PCR enables the approach for identifying genetic markers (Eman, *et al.,* 2008). RAPD was utilized in breeds genetic studies in most species, including farm animals like cattle (Sharma, et al., 2004), buffalo (Saifi, *et al.,* 2004), goat (Chen, *et al.,* 2001), chicken (Okumus and Kaya, 2005) and sheep (Okumus and Mercan, 2007). The investigation of genetic diversity and similarity among and within breeds is necessary be providing useful genetic information essential for developing affective management plans for the conservation and improvement of these genetic resources. Furthermore, there is a worldwide recognition of the need for the conservation of livestock diversity (FAO, 1995) and for the genetic characterization of breeds and populations including their genetic differentiation and relationships. Genetic analysis of livestock species could be performed by the use of polymorphic markers such as restriction fragment length polymorphisms (RFLPs) and microsatellites (Rincon, *et al.*, 2000 and Dalvit, *et al.,* 2008). However, their use is limited since designation of these genetic markers is expensive technically demanding (Beuzen, *et al.,* 2000). Markers have been used for genotype identification (Tinker, et al., 1993), construction of genetic maps (Maddox and Cockelt, 2007), analysis of the genetic relationships within and among species (Salhi-Hanachi, *et al.,* 2006) and measurement of genetic diversity (Hussein, *et al.,* 2005). The RAPD technique has also been used in analysis of genetic variations between different breeds of fish (Ambak, *et al.,* 2006), chicken (Okumus and Kaya, 2005), ducks (Gholizadeh, *et al.,* 2007), cattle (Devrin and Kaya, 2006 and Hassen, *et al.,* 2007), buffalo and goat (Abdel-Rahmen and Elsayed, 2007 and Yadav and Yadav, 2007) and sheep (Kumar, *et al.,* 2008 and Kunene, *et al.,* 2009). Genetic diversity of indigenous sheep breeds in Egypt has not been sufficiently studied. The objective of this study was to provide information on the molecular level about genetic structure and diversity of some Egyptian breeds employing RAPD-PCR molecular assay.

MATERIALS AND METHODS

Animals: A total of 32 ewes and one ram of Rahmani (R) and 30 ewes and one ram of Chios (C) sheep were used as parent of animals. Crosses and reciprocal crosses were made between the two breeds of sheep. Ewes of each breeds were divided at random into two main groups, ewes of the first group of each breed were housed and mated to rams of Rahmani breed, while the second group of each breed were housed and mated to rams of Chios breed. According to the system of mating applied, four mating groups were obtained. Two of them produced purebred lambs of Rahmani (RR) and Chios (CC), while the other two produced crossbred lambs of Rahmani rams X Chios ewes (½R ½C) and Chios rams

X Rahmani ewes (½C ½R). All animals were apparently healthy and free from any clinical disorders or diseases.

DNA Extraction: Total genomic DNA was extracted from whole sheep blood samples of the four groups of mating: 4 blood samples were collected from each Rahmani (RR), Chios (CC), and their progenies (½R ½C) and (½C ½R) with total 16 samples. Total genomic DNA was extracted using a modification of Helms method (Helms, 1990) as follows: 5-10 ml of venous blood was obtained from every selected animal. Blood samples were taken in standard heparinized vacutainer tubes. The tubes were vertically stored in the refrigerator to separate the plasma as a yellow layer on the top of the tubes. About 400-500 µl of buffy coat was taken in 2.0 ml Eppendorf tube, then 1.5 ml of cold buffer-A (0.32 M sucrose, 10 mM Tris-HCl, pH 7.6, 5 mM $MgCl_2$, 1% Triton-x-100) was added and mixed well. The tubes were spined at 1500 rpm for 15 minutes at 4 ^oC, then keep the pellet. One ml of cold buffer-B (25 mM EDTA, pH 8.0, 75 mM/1L NaCl), 1/10 volume of 10% SDS and 1/100 volume of proteinase-K were added and gently mixed and incubated at 37 ^oC overnight. One third volume of NaCl was added and centrifuged at 6000 rpm for 15 minutes. The supernatant was taken in new tube and an equal volume of buffer-equilibrated phenol was added and centrifuged at 7000 rpm for 10 minutes. The upper aqueous phase was transferred into a new tube, and an equal volume of chloroform/isoamylalcohol was added and centrifuged at 7000 rpm for 15 minutes. The upper aqueous phase was transferred into a new 1.5 ml Eppendorf tube and 1/10 volume of 3M Sodium acetate and 1.5 volumes of cold Isopropanol were added and incubated at -20 ^oC for 2 hours, then centrifuged at 7000 rpm for 20 minutes and the pellet was washed twice by 500 µl cold 70% Ethanol then dried and dissolved in 30-100 µl TE-buffer. Samples concentration was adjusted at 25 ng/µl using TE buffer pH 8.0.

PCR conditions for RAPD analysis (Finger printing): PCR for RAPD analysis was performed using 25 ng of pooled genomic DNA of each four mating group samples. The PCR mixture and amplification conditions were prepared according to Williams *et al.,* (1990) with minor modifications. The contents of PCR mixture are shown in Table (1).

Table 1: contents of PCR mixture for RAPD analysis

2.5 mM dNTPs	2.5 µl
10X reaction buffer + $MgCl_2$	2.5 µl
15 ng primer	3.0 µl
25 ng template DNA	1.0 µl
5 units Taq (super thermal)	1.0 µl
H_2O	15.0 µl
Total volume	25.0 µl

Ten decamer random primers, obtained from Operon Technologies Inc. USA, were used for DNA amplification. Primer codes and sequences are listed below:

RAPD analysis Primer AO6: (5'-GGTCCCTGAC-3')
RAPD analysis Primer AO7: (5'-GAAACGGGTG-3')
RAPD analysis Primer AO9: (5'-GGGTAACGCC-3')
RAPD analysis Primer BO3: (5'-CATCCCCCTG-3')
RAPD analysis Primer B17: (5'-AGGGAACGAG-3')
RAPD analysis Primer CO1: (5'-TTCGAGCCAG-3')
RAPD analysis Primer CO7: (5'-GTCCCGACGA-3')
RAPD analysis Primer C12: (5'-TGTCATCCCC-3')
RAPD analysis Primer EO5: (5'-TCAGGGAGGT-3')
RAPD analysis Primer GO3: (5'-GAGCCCTCCA-3')

DNA amplification cycles: The Thermocycler (MWG-BIOTECH, Germany) was programmed as follows: Pre-Denaturation (one cycle) at 94 ^oC for 5 min, followed by 40 cycles of: Denaturation at 94 ^oC for 1 min, Annealing at 40 ^oC for 50 seconds, Extension at 72 ^oC for 1 min and Final Extension at 72 ^oC for 10 min. then the samples were held at 4°C. The amplified DNA fragments were separated on 1.5% agarose gel, stained with Ethidium bromide, visualized on a UV Transilluminator and photographed.

Molecular analyzes: RAPD-PCR profiles were analyzed using Gene profiler 3.1 software. The banding profiles were scored (using 1kb DNA Ladder RTU, promega) in binary manner (1,0) where (1) indicates band presence, while (0) indicates band absence. The scored binary profiles were analyzed by unweighted pair-group method based on arithmetic mean (UPGMA) to estimate similarity, genetic distances and reconstruct the dendogram.

RESULTS

Genetic diversity of Rahmani, Chios and their crosses was studied using Random Amplified Polymorphic DNA (RAPD) technique. Ten oligodecamers arbitrarily primers A6, A7, A9, B3, B17, C1, C7, C12, E5 and G3 were used in the present investigation to generate RAPD profiles from four samples [Rahmani RR (1), Chios CC (3), cross CR (4) and cross RC (6)]. All primers were amplified successfully on the genomic DNA from taken pooled samples yielding distinct RAPD patterns (Figures 1, 2 and Table 2). The number of the amplified fragments per primer varied between 8 (C7) and 24 (B17), with an average 15.3 bands per primer. Each of these fragments has a size ranged from 1634 to 122 base pairs (bp). A total number of 129 bands were amplified, with 66 bands being monomorphic (51.2%) and the other 63 bands were polymorphic with a polymorphism ratio of 48.8%. For Rahmani and Chios a total number of 110 bands were amplified, with 99 bands being monomorphic (90.0%) and the other 11 bands were polymorphic (10.0%). While, the crosses between Rahmani and Chios have a total number of 124 bands with 93 bands being monomorphic (75%) and the other 31 bands were polymorphic (25%). Primer B17 generated the largest number of fragments (24), while primer C7 generated the lowest number of fragments (8). The ten primers produced a total number of 346 bands in the four samples. Primer G3 produced 34 fragments in all four samples. It reacted with samples generating 13 bands ranged in size from 862 to 223 bp. The number of bands generated by this primer varied among samples, where the highest number was ten observed in sample 6 (RC), while the lowest number was seven in sample 1 (RR). Primer A7 produced 41 fragments in the four samples. It generated 15 bands ranged in size from 562 to 151 bp. The lowest number of fragments generated by this primer was eight observed in samples 1 and 3, while the highest number was 13 in sample 6. It generated fragment with size 454 bp present only in sample number 1.

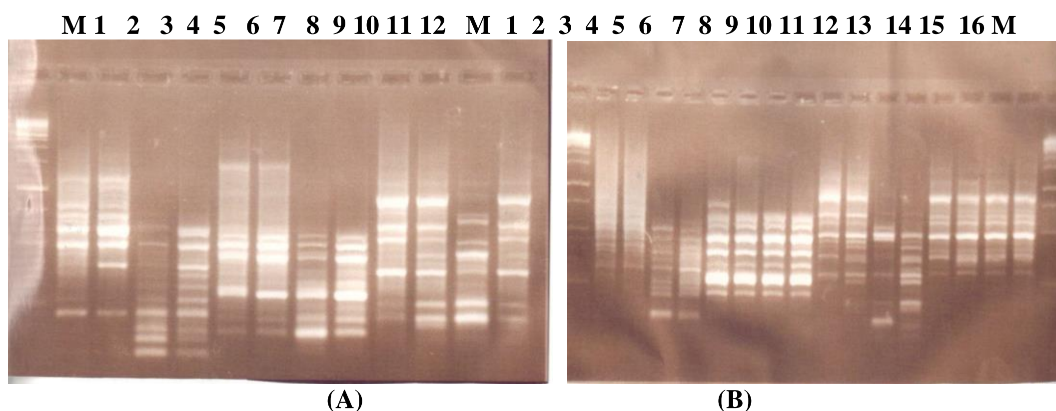

Figure 1: Agarose-gel electrophoresis of RAPD products generated in the examined samples: M = DNA marker. (A) Primers B3 (lanes 1, 2, 3 and 4), A6 (lanes 5, 6, 7 and 8) and A9 (lanes 9, 10, 11 and 12). (B) Primers C12 (lanes 1, 2, 3 and 4), C7 (lanes 5, 6, 7 and 8), E5 (lanes 9, 10, 11 and 12) and C1 (lanes 13, 14, 15 and 16)

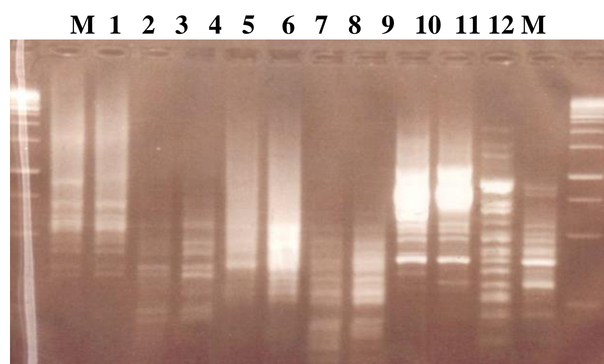

Figure 2: Agarose-gel electrophoresis of RAPD products generated by primers G3 (lanes 1, 2, 3 and 4), A7 (lanes 5, 6, 7 and 8) and B17 (lanes 9, 10, 11 and 12) in the examined samples. M = DNA marker.

Primer B17 produced 59 fragments in the four samples. It reacted with samples generating 24 bands ranged in size from 1634 to 187 bp. The lowest number of fragments generated by this primer was 12 observed in sample 6, while the highest number was 18 in sample 4. Fragments of size 1634, 1105, 223 and 187 bp were generated only with sample 4 and absent in all other three samples. Also, bands with size 672 and 318 bp were only present in samples 1 and 6, respectively. Meanwhile, fragments with size 1418, 1274, 747 and 721 bp were only absent in sample 6. Also, band with size 862 bp was only absent in sample number 4.

The results of RAPD analysis obtained by primer E5 are illustrated in Figure (1-B) and Table 2. Primer E5 produced 44 fragments in the four samples. It generating 20 bands ranged in size from 1067 to 122 bp. The lowest number of fragments generated by this primer was nine observed in sample 1, while the highest number was 14 in sample 6. This primer was only generated fragments in size of 334, 297, 218, 173, 132 and 122 bp with sample number 6. The results obtained by primer B3 are illustrated in Figure (1-A) and Table 2. Primer B3 produced 48 bands in the four examined samples. It generating 18 fragments ranged in size from 1098 to 137 bp. The number of fragments generated by this primer varied among samples where the lowest number was ten observed in sample 6 (cross RC♀), while the highest number was 14 in sample 1(Rahmani RR♀). Primer C12 produced 45 bands in the four samples (Figure 1-B and Table 2). It generating 16 bands ranged in size from 783 to 148 bp.

The lowest number of fragments generated by this primer was ten observed in samples 1 and 3, while the highest number was 13 in sample 6. The fragment with size 574 bp was absent only in sample number 4 and present in all other three samples. The results of RAPD analysis of four examined samples, obtained by primer A6, are illustrated in Figure 1-A and Table 2. Primer A6 produced 49 bands in the four samples. It generating 15 fragments ranged in size from 1270 to 177 bp. The number of fragments generated by this primer varied among samples from ten in samples 4 (cross CR♀) and 6 (cross RC♀) to 15 in sample 1 (Rahmani RR♀). Meanwhile, only one fragment with molecular weight 295 bp generated only with sample number (1) and no fragment in the same size was detected with the other three samples. Primer A9 produced 43 bands in the four samples. It reacted with samples generating 15 fragments ranged in size from 1058 to 212 bp. The lowest number of fragments generated by this primer was ten observed in samples 1 (Rahmani RR♀), while the highest number was 12 in sample 4 (cross CR♀). Meanwhile, this primer produced fragments with size 735, 683, 425 and 410 bp only with sample number 3. Moreover, fragments with size 762 bp and 635 bp were absent only in sample number 4. Primer C1 produced 35 bands (Figure 1-B and Table 2). It generated nine fragments ranged in size from 846 to 297 bp. The number of bands generated was eight in sample 1 and nine in samples 3, 4 and 6. Fragment with size 334 bp was absent only with sample number 1. Primer C7 produced 29 bands (Figure 1-B and Table 2). It generated eight fragments ranged in size from 724 to 210 bp. The number of fragments generated by this primer was eight in sample 1 and seven in samples 3, 4 and 6. Fragment with size 724 bp was detected only with sample number 1. The summary of bands, produced by different primers, could be used as genetic markers (fingerprinting) to distinguish Rahmani, Chios and their reciprocal crosses are illustrated in table (3).

Table 2: Summary of all fragments generated by the assay of the ten primers, and their molecular weight in all four samples where (+) means presence and (-) means absence.

Primer code	M.W. (bp)	Samples 1	3	4	6	Primer code	M.W. (bp)	Samples 1	3	4	6
B17	1634	-	-	+	-	E5	552	-	-	+	-
B17	1418	+	+	+	-	A6, A9	549	+	+	+	+
B17	1274	+	+	+	-	B17	543	+	+	+	+
A6	1270	+	+	-	-	C12	511	+	+	+	+
B17	1105	-	-	+	-	B3	510	+	+	+	+
B3	1098	+	+	-	-	A7	505	+	+	+	+
E5	1067	+	+	-	-	G3	505	-	+	-	+
A9	1058	+	+	+	+	E5, A6, C1	492	+	+	+	+
B17	1029	+	+	-	-	G3	488	-	+	-	+
B3	984	+	+	-	-	B17	471	+	+	+	+
B17	925	-	-	+	+	A9	457	+	+	+	+
A6	915	+	+	-	-	C7	455	+	+	+	+
B3	882	+	+	-	-	E5	455	-	-	+	-
B17	862	+	+	-	+	A7	454	+	-	-	-
G3	862	+	+	-	-	B3	441	+	+	-	-
A9	850	+	+	+	+	C12, E5, B17, G3	438	+	+	+	+
C1	846	+	+	+	+	A6	425	+	+	+	+
B3	820	+	+	-	-	A9	425	-	-	+	-
E5	814	+	+	-	-	A7	423	-	+	+	+
B17	803	+	+	-	-	A9	410	-	+	-	-
B3	790	+	+	-	-	E5, C12, C1	405	+	+	+	+
C12	783	+	+	-	-	B3	395	+	+	+	+
A9	762	+	+	-	+	A9	395	-	-	+	-
B17	747	+	+	+	-	A7, B17	394	+	+	+	+
A9	735	-	-	+	-	G3	380	+	+	+	+
C7	724	+	-	-	-	C7	375	+	+	+	+
B17	721	+	+	+	-	A6, A9	367	+	+	+	+
B3	708	+	+	-	-	E5, C12, C1	360	+	+	+	+
C12	697	+	+	-	-	G3, A6, B3	354	+	+	+	+
C1	697	+	+	+	+	A7, B17	342	+	+	+	+
G3	696	+	+	-	-	C1	334	-	+	+	+
A6	683	+	+	-	-	E5	334	-	-	-	+
A9	683	-	-	+	-	G3	330	-	-	+	+
B17	672	+	-	-	-	A7	330	+	+	-	-
E5	645	+	+	+	+	C12	321	+	+	+	+
C12	645	+	+	-	-	B17	318	-	-	-	+
C1	645	+	+	+	+	A9, A6	317	+	+	+	+
B3	635	+	-	+	-	C7	309	+	+	+	+
A9	635	+	+	-	+	A7	307	+	+	+	+
B17	626	+	+	+	+	G3, B17	307	-	-	+	+
G3	626	+	+	-	-	B3	306	-	-	+	+
C7	620	+	+	+	+	C1, C12	297	+	+	+	+
A6	612	+	+	-	-	E5	297	-	-	-	+
C1	597	+	+	+	+	A6	295	+	-	-	-
B3	590	+	+	+	+	A7	286	+	+	+	+
B17	583	+	+	-	-	E5	275	+	+	+	+
C12	574	+	+	-	+	A6	274	+	+	+	+
E5	574	+	+	-	-	C12	264	-	-	+	+
G3	562	+	+	+	+	B17	257	-	-	+	+
A7	562	+	+	-	+	B3, A9	255	+	+	+	+
B17	562	-	-	+	+	C7	254	+	+	+	+
C7	552	+	+	+	+	E5	254	-	+	-	+

Table 2: (Continuo) Summary of all fragments generated by the assay of the ten primers,and their molecular weight in all four samples where (+) means presence and (-) means absence.

Primer code	M.W. (bp)	Samples 1	3	4	6	Primer code	M.W. (bp)	Samples 1	3	4	6
G3	248	-	-	+	+	B17	187	-	-	+	-
C12	245	-	-	+	+	B3	183	-	-	+	+
A6	237	+	+	+	+	C12	179	-	-	+	+
A7	231	-	-	+	+	A6	177	+	+	+	+
B17	223	-	-	+	-	E5	173	-	-	-	+
G3	223	-	-	+	+	A7	168	-	-	+	+
B3	220	+	+	+	+	B3	164	-	-	+	+
E5	218	-	-	-	+	A7	151	-	-	+	+
C12	218	-	-	+	+	C12	148	-	-	+	+
A7	215	-	-	+	+	E5	142	-	-	+	-
A6, A9	212	+	+	+	+	B3	137	-	-	+	+
C7	210	+	+	+	+	E5	132	-	-	-	+
C12	210	-	-	+	+	E5	122	-	-	-	+
A7	208	-	-	+	+	Total = 346	108	85	86	88	87
A7, E5	187	-	-	+	+						

Genetic similarity and cluster analysis based on RAPD results:

Pairwise genetic similarity (GS) was estimated for the ten combined primers as presented in Table 4. The results showed that the highest percentages of similarity were 89.1 % between samples 1 and 3 and 75 % between samples 4 and 6.

A dendogram, indicating genetic relationships between the four sheep samples, was generated by cluster analysis (UPGMA) with the RAPD data (Figure 3). Sheep samples were classified into two groups. The first group containing samples 1 (Rahmani RR♀) and 3 (Chios CC♂) with genetic similarity of 89.1, both are races. The second group containing samples 4 (cross CR♀) and 6 (cross RC♀) with genetic distance of 75, both are heterozygous produced by reciprocal crosses [(½ C ½ R) and (½ R ½ C)].

Table 3: Summary of the molecular marker (Fingerprinting) produced by different primers used with Rahmani, Chios and their reciprocal crosses.

Breed	Primer code	Band size in bp
	A6	295
	C7	724
	A7	454
RR	B17	672
		318
CC	A9	410
		735
		683
	A9	425
		395
		552
	C7	455
		142
CR		1634
		1105
	A7	223
		187
		297
		218
		173
	E5	132
		122
RC	B17	672
		318

Table 4: The similarity matrix in percentage, among the examined sheep samples based on RAPD band pattern analysis and Jaccard index.

Samples	1	3	4	6
1	100			
3	89.1	100		
4	50.4	50.7	100	
6	50.7	56.6	75	100

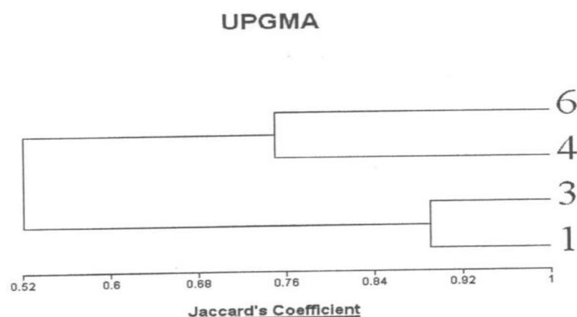

Figure 3: UPGMA dendrogram of the four sheep samples based on values of genetic distances calculated from of all ten primers.

DISCUSSION

To identify the genetic diversity among Rahmani, Chios and their crosses, RAPD-PCR was performed using ten random primers. After estimation of genetic similarity, cluster analysis and establishment of dendrogram, samples are classified into two distinct groups. The first group containing parent breeds (Rahmani RR and Chios CC) and the second group compromise the two hybrids (CR and RC). These results indicated that polymorphism within genomic of hybrids is higher than polymorphism within genomic of races as indicated by the results of similarity (GS), where similarity between races was 89.1 % and between crosses was 75 %. These results are in agreement with those found by Doloksaribu *et al.,* (2000), Boujenane and Kansari (2002), Hassen *et al.,* (2004) and Abd Allah (2009). Also, fragments generated by primers show a polymorphism ratio of 10.8 % for varieties and 25 % for crosses. Complete analysis of the resulted bands elucidate interesting findings, eight primers out of ten can be used as genetic markers to distinguish between varieties and/or hybrids. Primers A7, C7 and C1 can be used to identify the Rahmani variety, where presence of bands 454 bp and 724 bp used as a positive genetic marker with primers A7 and C7, respectively. While, absence of bands 423 bp and 334 bp can be used as a negative genetic marker with primers A7 and C1, respectively. These results are in harmony with those reported by Rajendra and Ashok (2011) and Sukumar *et al.*, (2012). Presence of band 410 bp with primer A9 can be used as a positive genetic marker to distinguish Chios variety. Also, presence of bands 1634, 1105, 223 and 187 bp with primer B17 and bands 735, 683, 425 and 395 bp with primer A9 can be used as a positive genetic markers to distinguish the hybrid CR. While, presence of bands 173, 132 and 122 bp with primer A5 can be used as a positive genetic markers to distinguish the hybrid RC. So, RAPD-PCR can be used as a tool to distinguish between varieties and / or hybrids in sheep breeds. It is simple, easy, rapid and cheap method to distinguish between individuals in the sheep breeds. Moreover, genetic markers may provide useful information at different levels: population structure, phylogenetic relationships and patterns of historical biogeography.

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البصمة الوراثية لاغنام الرحمانى والكيوس وهجنهما عن طريق دراسه تعدد اشكال الاكثار العشوائي للـ DNA (RAPD)

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لتحديد الاختلافات الوراثية بين اغنام الرحمانى والكيوس والهجن بينهما وكذلك الهجن العكسية لهما تم تحليل الـ DNA المستخلص من دماء كل حيوان على حده وتم استخدام جهاز الـ PCR لعمل PAPD-PCR وذلك باستخدام عدد 10 باديئات. حيث ان ظهور الحزم الوراثية على الجل يعكس التركيب الوراثى للحيوانات المختبره. والاختلافات بين التراكيب الوراثية يمكن تحديدها بواسطة حجم وعدد الحزم الوراثية التى تظهر مع كل باديئ ويمكن استخدامها كبصمة وراثية للتفرقه بين السلالات والهجن بينهما. اظهرت النتائج ان البصمة الوراثية لسلالة الرحمانى أمكن تحديدها بواسطة استخدام الباديئ (A7) وكذلك الباديئ (C7) حيث تظهر الحزم الوراثية بحجم 454 bp و 724 bp (زوج من القواعد) بالتتابع. بينما ظهور الحزم الوراثية 410 bp باستخدام الباديئ (A9) يمكن استخدمها كبصمة وراثية لسلالة الكيوس. كما ان ظهور الحزم الوراثية 1634 bp و 1105 bp و 395 bp و 223 bp باستخدام الباديئ (B17) وايضا الحزم الوراثية 735 bp و 683 bp و 425 bp و 187 bp باستخدام الباديئ (A9) يمكن استخدمها كبصمة وراثية لتحديد الخليط (1/2 كيوس 1/2 رحمانى). كما ان ظهور الحزم الوراثية 173 bp و 132 bp و 122 bp باستخدام الباديئ (A5) يمكن استخدمها كبصمة وراثية للخليط العكسى (1/2 رحمانى 1/2 كيوس). وعموما نتائج الدراسة الجزيئيه للـ DNA لاغنام الرحمانى والكيوس وهجنهما العكسيه اظهرات ان الاختلافات بين الهجن اكبر من الاختلافات داخل السلالات النقيه (الكيوس والرحمانى). كما وجد ان درجة التماثل الوراثي بين سلالات الرحمانى والكيوس كانت 89.1% وبين الهجينين (1/2 كيوس 1/2 رحمانى) و (1/2 رحمانى 1/2 كيوس) كانت 75% وايضا وجد ان درجه اختلاف الاشكال للشظايا الناتجة كانت بنسبة 10.8% بين سلالتى الرحمانى والكيوس وبين الهجينين (1/2 كيوس 1/2 رحمانى) و (1/2 رحمانى 1/2 كيوس) كانت بنسبه 25%.بذلك نستخلص ان البصمة الوراثية بواسطة تقنية تعدد اشكال الاكثار العشوائي للـ DNA (RAPD) ذات فائدة لتمييز السلالات وهجنهما العكسية