MOLECULAR GENETIC EVALUATION OF SIX RABBIT BREEDS BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)-PCR

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ABSTRACT

The present study was conducted to assess the genetic variability among six rabbit breeds in Egypt. Eight primers (OPA-10, OPB-05, OPC-01, OPC-02, OPC-08, OPE-11, OPE-19 and OPX-02) were selected. The similarity coefficients and genetic distances were 0.648, 37.0 between New Zealand White/Black Rex; 0.685, 34.0 between New Zealand White/Hyplus; 0.648, 37.0 between New Zealand White/Line V; 0.721, 31.0 between New Zealand White/Line M; 0.807, 23.0 between New Zealand White/Sinai Gabali; 0.807, 23.0 between Black Rex/Hyplus; 0.855, 18.0 between Black Rex/Line V; 0.873, 16.0 between Black Rex/Line M; 0.855, 18.0 between Black Rex/Sinai Gabali; 0.765, 27.0 between Hyplus/Line V; 0.786, 25.0 between Hyplus/Line M; 0.807, 23.0 between Hyplus/Sinai Gabali; 0.924, 10.0 between Line V/Line M; 0.709, 32.0 between Line V/Sinai Gabali and 0.797, 24.0 between Line M/Sinai Gabali. Dendrogram revealed similar results

INTRODUCTION

Rabbits play an important role in meat and fur production. Rabbit also is an ideal laboratory animal in biomedical, genetic researches and production of recombinant proteins. Domestic rabbits have been classified into many breeds based on coat coloration, body size, external body measurements and origin ***(Sandford, 1996)***. It is important to differentiate between these rabbit breeds using different genetic tools. Molecular markers allow the assessment of genetic variability among genotypes at the DNA level. Molecular markers includes random amplified polymorphic DNA (RAPD), restriction fragment length pol-ymorphism (RFLP), amplified fragment length polymorphism (AFLP), microsatellites, single nucleotide polymorphisms (SNPs), allozyme markers, mitochondrial DNA (mtDNA) markers

RAPD-PCR has been used as a tool to assess the genetic variability in hare populations (*Lepus europaeus*) in Greece and European countries (***Mamuris et al., 2002***), in captive

populations of Volcano rabbit (*Romerolagus diazi*) in Mexico (***Soto et al., 2005***) and between domestic rabbit bre-eds (*Oryctolagus cuniculus*) in India (***Rangoju et al., 2007***). So this study aims at assessing the genetic variability and phylogenetic relationships among six rabbit breeds (New Zealand White, Black Rex, Hyplus, Spanish Line V, Sinai Gabali and Moshtohor or Line M) using RAPD-PCR.

MATERIALS AND METHODS:

1. RABBIT BREEDS:

Thirty-six individuals (3 males and 3 females) from each of six pure rabbit breeds (New Zealand White, Black Rex, Hyplus, Spanish line V, Sinai Gabali and Moshtohor or line M) were used in this study.

2. DNA EXTRACTION:

DNA was extracted from 36 blood samples (collected from central ear artery) using salting out protocol (***Miller et al., 1988***) with some modifications. DNA concentr-ation and purity were meas-ured using NanoDrop ® ND-1000 Spectrophotometer. Pooled DNA was prepared for both males and females of each breed (***Iman, 2006***) and then checked for integrity on 1% agarose gel.

3. RAPD-PCR:

Twenty random 10mer primers were used as shown in Table 1. The PCR reaction was carried out in 25 µl volume and was composed of the following: 0.3 µl Taq DNA polymerase containing 1.5 unit (Alliance Bio, USA), 2.5 µl 10X buffer (provided with Taq DNA polymerase), 1 µl MgCl2 (25 mM), 1.3 µl primer (100 µM), 5 µl DNA template and completed with sterile distilled water (nuclease-free) to 25 µl.

Table 1. List of primer code, sequence, G+C content (%) and melting temperature (Tm):

Primer Code	Sequence (5'to3')	G+C Content (%)	T_m	Primer Code	Sequence (5' to3')	G+C content (%)	T_m
OPA-01	CAGGCCCTTC	70%	34°C	OPC-08	TGGACCGGTG	70%	34°C
OPA-08	GTGACGTAGG	60%	32°C	OPE-01	CCCAAGGTCC	70%	34°C
OPA-09	GGGTAACGCC	70%	34°C	OPE-06	AAGACCCCTC	60%	32°C
OPA-10	GTGATCGCAG	60%	32°C	OPE-07	AGATGCAGCC	60%	32°C
OPA-18	AGGTGACCGT	60%	32°C	OPE-11	GAGTCTCAGG	60%	32°C
OPB-03	CATCCCCCTG	70%	34°C	OPE-16	GGTGACTGTG	60%	32°C
OPB-05	TGCGCCCTTC	70%	34°C	OPE-19	ACGGCGTATG	60%	32°C
OPC-01	TTCGAGCCAG	60%	32°C	OPE-20	AACGGTGACC	60%	32°C
OPC-02	GTGAGGCGTC	70%	34°C	OPX-02	TTCCGCCACC	70%	34°C
OPC-06	GAACGGACTC	60%	32°C	OPX-03	TGGCGCAGTG	70%	34°C

The reaction was carried out in the thermal cycler (Little genius TC-25/H, Bioer Techn-ology Co., Ltd., Japan) which uses a heated lid in the following conditions: initial denaturation at 94°C for 2 min, denatura-

tion at 94°C for 1 min, annealing at 32-34°C for 1 min, extension at 72°C for 2 min for 40 cycles and final extension at 72°C for 5 min. The PCR products were separated on 2% agarose gel and were visualized on UV transilluminator and bands were scored "1" and "0" for their presence and abse-nce respectively.

4. ANALYSIS OF RAPD DATA:

DNA-fingerprint similarity (S) was calculated using the following formula according to

Lynch (1990). $S_{xy} = 2n_{xy} / (n_x + n_y)$

Whereas: n_{xy} is the number of common bands shared by individuals x and y, n_x and ny are the number of bands present in the individuals x and y.

Genetic distances were calculated as the total number of RAPD band differences after using different primers by ***Statistica 5.0 (1995)***. The matrix of similarity was analyzed by the unweighted pair group methods using arithmetic average (UPGMA) as suggested by ***Sneath and Sokal (1973)***. Dendrogram was constructed using ***Statistica 5.0 (1995)*** based on squared Euclidean distance.

RESULTS:

Eight (OPA-10, OPB-05, OPC-01, OPC-02, OPC-08, OPE-11, OPE-19 and OPX-02) of twenty primers (Table 1) were selected according to their positive results. Eight primers produced a total of 71 bands with a molecular size ranging from 150 to 2000 bp. The banding patterns of RAPD bands produced with primers OPA-10, OPB-05, OPC-01, OPC-02, OPC-08, OPE-11, OPE-19 and OPX-02 in six rabbit breeds; New Zealand White (**NZW**), Black Rex (**R**), Hyplus strain (**H**), Spanish line V (**V**), Line M (**M**), and Sinai Gabali (**SG**) are illustrated in Plates 1, 2, 3 and 4. 100 bp DNA ladder was loaded in the 1st lane (MAR lane).

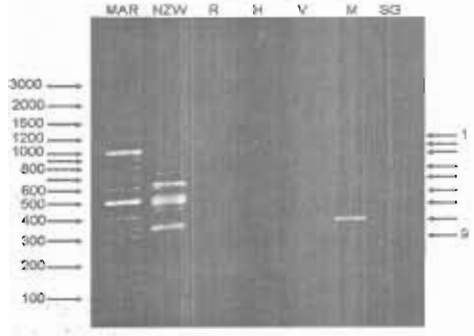


Plate 1, A: RAPD pattern produced by primer OPA–10 on genomic DNA of six breeds.

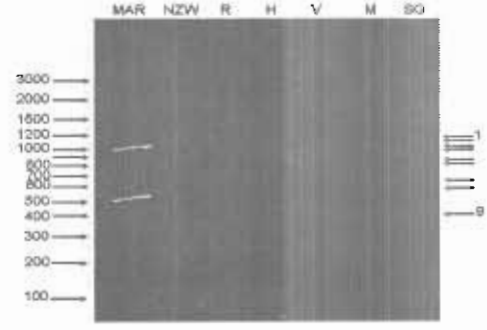


Plate 1, B: RAPD pattern produced by primer OPB-05 on genomic DNA of six breeds.

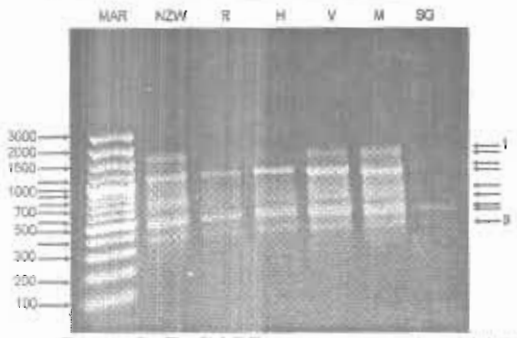


Plate 2, B: RAPD pattern produced by primer OPC-02 on genomic DNA of six rabbit breeds.

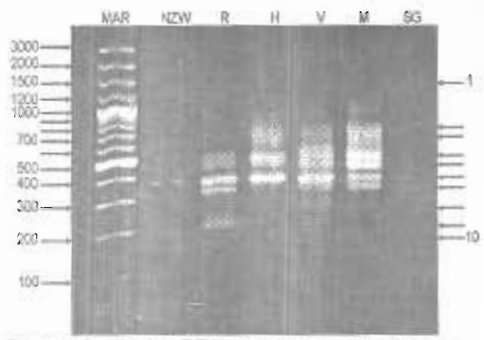


Plate 2, A: RAPD pattern produced by primer OPC-01 on genomic DNA of six breeds.

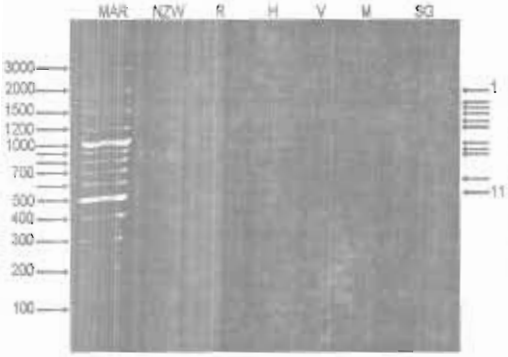
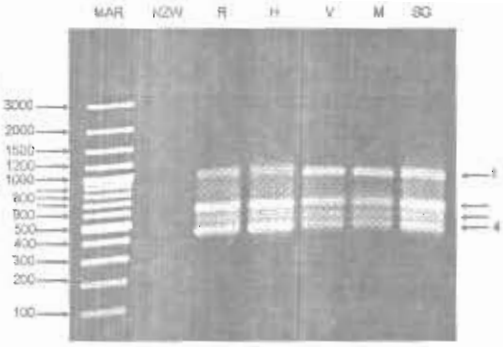


Table 2 shows DNA-fingerprint similarity among the six studied rabbit breeds based on band polymorphism generated by RAPD-PCR after using eight primers.



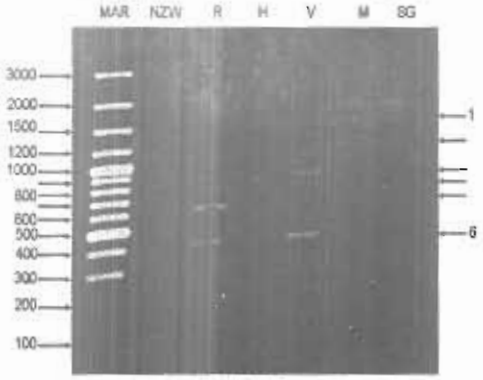


Plate 4, A: RAPD pattern produced by primer OPE-19 on genomic DNA of six breeds.

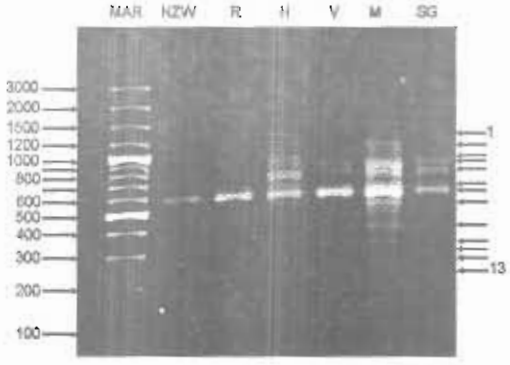


Plate 4, B: RAPD pattern produced by of primer OPX-02 on genomic DNA of six breeds.

Table 2. Estimated DNA-fingerprint similarity coefficients between the six rabbit breeds under the study using data from all primers.

Breeds	NZW	R	H	V	M	SG
New Zealand White (NZW)	1.000	0.648	0.685	0.648	0.721	0.807
Black Rex (R)		1.000	0.807	0.855	0.873	0.855
Hyplus (H)			1.000	0.765	0.786	0.807
Line V (V)				1.000	0.924	0.709
Line M (M)					1.000	0.797
Sinai Gabali (SG)						1.000

Genetic distances calcu-lated as the total number of RAPD band differences after using eight primers among the six rabbit breeds under the study are shown in Table 3.

Table 3. Genetic distances, calculated as the total number of RAPD band differences after using eight primers among the six rabbit breeds:

Breed	NZW	R	H	V	M	SG
New Zealand White (NZW)	00.0	37.0	34.0	37.0	31.0	23.0
Black Rex (R)		00.0	23.0	18.0	16.0	18.0
Hyplus (H)			00.0	27.0	25.0	23.0
Line V (V)				00.0	10.0	32.0
Line M (M)					00.0	24.0
Sinai Gabali (SG)						00.0

Dendrogram in Plate 5 based on the data obtained from 8 primers showed similar results to that in Table 3. Line V and M were close to each other while New Zealand White and Hyplus were more distant breed

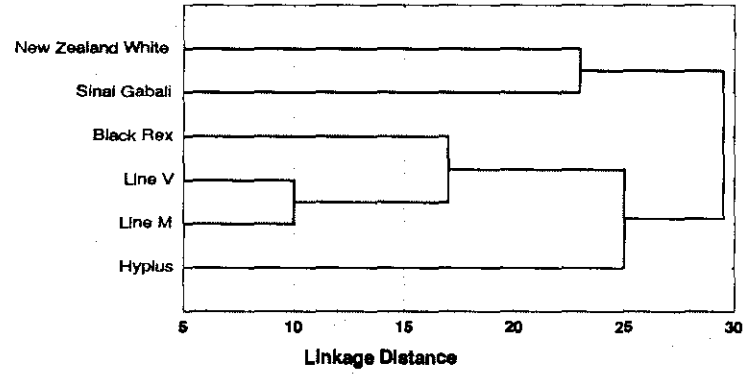


Plate 5. Dendrogram using average linkage based on RAPD data obtained by eight primers between six rabbit breeds under the study.

DISCUSSION

Eight random 10-mer primers produced a total of 71 bands with a molecular size ranging from 150 to 2000 bp. These results are similar to those obtained by ***Fatma (2004), Soto et al., (2005) and Rangoju et al., (2007)***. The primer OPA-09 didn't produce any bands in any breed and this agrees with the results of ***Rangoju et al., (2007)***. The genetic distance was higher as compared to the results shown by ***Rangoju et al. (2007)***, which indicated less genetic distances in three rabbit breeds in India. These variations might be due to the different breeds and different geographical and climatic conditions, which cause variability in the gene pool.

The highest genetic distance (37.0) between New Zealand White/Black Rex and New Zealand White/Line V may be due to the geographical distance between the countries of origin of these breeds where New Zealand White, Black Rex and Line V originated in America, France and Spain respecttively. These results are consistent with that observed by ***Mamuris et al. (2002)*** who found that reared hare population was similar to the individuals from the European countries as compared to the six wild populations of Greece. The lowest genetic distance (10.0) between Line V and M may be because Line M is a synthetic line between the Egyptian Sinai Gabali bucks (50%) and the Spanish V-Line does (50%) according to (***Iraqi et al., 2008***). Dendrogram in Plate 5 showed that the similarity between line M and V was the highest while the lowest simi-larity was between NZW and Black Rex, and NZW and Line V

Seven primers produced unique molecular markers for 5 breeds:

For New Zealand White: 13 unique markers were devel-oped by 6 primers; OPA-10 (275 and 1162 bp), OPB-05 (470, 616 and 900 bp), OPC-01 (1500 bp), OPC-02 (307 and 1400 bp), OPC-08 (700 bp) and OPE-11 (449, 524, 656, 1100 bp), For Black Rex: one unique marker was developed by OPX-02 (1200 bp), For Hyplus: 8 unique markers were prod-uced by 3 primers; OPB-05 (820, 1010 and 1050 bp), OPC-08 (750, 1364 and 1737 bp) and OPX-02 (920 and 1303 bp), For Line V: 4 unique markers were gener-ated by 2 primers; OPA-10 (457, 530 and 1017 bp) and OPC-08 (1382 bp), For Line M: one marker was produced by one primer; OPX-02 (200bp) but no characteristic markers were developed in the 6th rabbit breed; Sinai Gabali.

CONCLUSION:

The results of this study can be helpful in designing rabbit breeding programs. The wide genetic diversity among New Zealand White/Black Rex and New Zealand

White/Line V allows scientists' further research in rabbit breeding programs to obtain hybrid vigor and improve rabbit pro-duction in Egypt. The less divergent breeds were Line V and M which increases the probability of inbreeding depression if they are crossed. The study also provided unique molecular markers for five of the six studied breeds which may be useful in discriminating these breeds at the molecular level.

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الملخص العربي

التقييم الوراثي ألجزيئي لست سلالات من الأرانب باستخدام تفاعل إنزيم البلمرة العشوائي

أجريت هذه الدراسة لتقييم التباين الوراثي ودرجات القرابة بين ستة أنواع من الأرانب باستخدام تفاعل إنزيم البلمرة العشوائي (RAPD-PCR) باستخدام 20 بادىء عشوائي يحتوى على 60-70% جوانين + سيتوزين تم اختيار 8 منهم (OPA-10, OPB-05, OPC-01, OPC-02, OPC-08, OPE-11, OPE-19 OPX-02) لإجراء باقي الدراسة، وكان متوسط معامل التشابه والمسافة الوراثية بين سلالتى النيوزيلاندى الأبيض والركس الأسود هو 0.648 و 37.0، وبين سلالتي النيوزيلاندي الأبيض وهاى بلس هو 0.685 و 34.0 بينما بين سلالتى النيوزيلاندي الأبيض و لاين فـي هو 0.648 و 37.0 وبين سلالتى النيوزيلاندي الأبيض ومشتهر هو 0.721 و 31.0 وبين سلالتى النيوزيلاندى الأبيض وجبلى سيناء 0.807 و 23.0و بين سلالتى الركس الأسود وهاى بلس 0.807 و 23.0 بينما كان بين سلالتى الركس الأسود و لاين فـي 0.855 و 18.0وبين سلالتى الركس الأسود ومشتهر 0.873 و 16.0 وبين سلالتى الركس الأسود وجبلى سيناء 0.855 و 18.0 ، وكان متوسط معامل التشابه والمسافة الوراثية بين سلالتى هاى بلاس و لاين فـي 0.765 و 27.0 وبين سلالتى هاى بلاس ومشتهر 0.786 و 25.0 وبين سلالتى هاى بلاس وجبلى سيناء 0.807 و 23.0 وبين سلالتى لاين فـي ومشتهر 0.924 و 10.0 بين سلالتى لاين فـي وجبلى سيناء هو 0.709 و 32.0 بينما كان متوسط معامل التشابه والمسافة الوراثية بين سلالتى مشتهر وجبلى سيناء هو 0.797 و 24.0، وكذلك بين التمثيل الشجري لعلاقات القرابة بين السلالات المدروسة نتائج مماثلة.