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Assessment of Genetic Variability among Some Rabbit Breeds Using Random Amplified Polymorphic DNA Technique (RAPD)

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Abstract

Random Amplified Polymorphic DNA (RAPD) marker was employed to assess the genetic variation and phylogenetic relationship among four rabbit breeds viz. New Zealand White, Gabali, Baladi Red and Baladi Black reared in Egypt. Initially, a total of 14 random primers of arbitrary sequence were used but 10 of them generated reproducible, scoreable and polymorphic bands. Out of 131 bands scored using these primers, 74(56.48%) were recognized as Polymorphic and 57 (43.52%) as monomorphic bands. The highest percentage of polymorphic bands was recognized for primers OPB-02 (94%) and OPB-07 (92%). While, the lowest percentage of polymorphic bands was recognized for primers OPA-02(16%) and OPF-12 (28%). The band sharing frequencies (BSF) was found higher between Baladi Red -Baladi Black (0.80± 0.038) , followed by Gabali - Baladi Black (0.71±0.079), New Zealand White- Baladi Black (0.70±0.096), New Zealand Whit- Baladi Red (0.69±0.088)and the least BSF was found between New Zealand Whit- Gabali (0.64±0.081) . Overall, there was no significant difference (P>0.05) in BSF values between breeds. The highest genetic distance was found between Baladi Red -Baladi Black (0.87) followed by Gabali, Baladi Red (0.86), Gabali, Baladi Black(0.82), New Zealand White, Gabali (0.80), New Zealand White,Baladi Red (0.75) and the lowest genetic distance was found between New Zealand White,Baladi Black(0.73). One primer (OPA-20) in Gabali , two primers(OPA-02,OPB-14) in Baladi Red, three primers (OPA-02,OPB-14, OPA-20) in New Zealand White and Baladi Black were found to be specific for these breeds. The study suggests that RAPD can be successfully utilized for detecting genetic variation among the studied rabbit breeds.

Key words: Rabbit, breed, RAPD markers, genetic diversity, phylogenetic, BSF (Band sharing frequency)

Introduction

Egypt is a country that produces rabbit meat in family farms; tries to develop industrialized rabbit production and has a very important research structure related to rabbit science and technology (**El-Raffa et al.,2005**). The genetic researches in Egypt concerning rabbits had concentrated mainly in the studies describing the local and exotic breeds, Gabali, Baladi Black and Baladi Red were described as local breeds adapted to hot climate and somewhat resistant to diseases **CIHEAM ,(2002)** In the last decade, popular meat breed (New Zealand White) of rabbit was introduced in Egypt, being used in large scale of commercial production throughout Egypt. New Zealand White exhibits outstanding maternal abilities as related to maternal behavior, fecundity, lactation, and preweaning growth and survival (**Khalil, 1993**). Recently, the rabbit has attracted more attention from the biotechnology community. The rabbit genome is estimated to be 3 billion base pairs long, almost equal to the size of the human genome. Also, rabbits have similar lipid metabolism to humans, making them good models of atherosclerosis (**Dove, 2000**).

Characterization at the molecular level is undertaken mainly to explore genetic diversity within and between animal populations, and to determine genetic relationships among these populations. The estimation of genetic variability of a species is an important criterion for its conservation and for further genetic improvement (**Rahimi et al., 2005**). Molecular markers derived from polymerase chain reaction (PCR) amplification of genomic DNA are an important part of the toolkit of evolutionary geneticists (**Holsinger et al., 2002**). By detecting genetic variation, genetic markers may provide useful information at different levels; population structure, levels of gene flow, phylogenetic relationships, patterns of historical biogeography and the analysis of parentage and relatedness (**Feral, 2002**). PCR based on multi-locus DNA fingerprints represent one of the most informative and cost-effective measures of genetic diversity (**Bagley et al., 2001**). Randomly Amplified Polymorphic DNA (RAPD) technique, described firstly by **Williams et al., (1990)**, is a simple, fast and comparatively low cost assay that uses short oligonucleotide primers of arbitrary sequences to amplify anonymous fragments of genomic DNA (**Stepniak et al., 2002**), and no prior knowledge of the genome under investigation is necessary to perform the assay (**Bowditch et al., 1993**). Due to those features, the RAPD analysis has

found many uses in different fields of study in both plants and animals. Polymorphism of RAPD fragments is detected as a band's presence or absence and may result from deletion, insertion or differences in the nucleotide sequences in or between the priming regions (**Clark and Lanigan, 1993**). RAPD markers are the randomly amplified target regions of less functional part of the genome that do not strongly respond to selection on the phenotype level. Such amplified regions may accumulate more mutations thereby offering a wider potential in assessing the interbreed/population genetic differentiation. The objective of this study was to assess the genetic diversity and phylogenetic relationship among four rabbit breeds viz. New Zealand White, Gabali, Baladi Red and Baladi Black using Random Amplified Polymorphic DNA (RAPD) Markers.

Materials and Methods

This research was performed in the Department of Genetics and Genetic Engineering (Biotechnology Services Unit), Faculty of Agriculture, Benha University.

1. Experimental Animal Used:

A total of sixty rabbits viz. 15 rabbits each in New Zealand White (NEW), Gabali (G), Baladi Red (BR) and Baladi Black (BB) were taken for this study. The animals selected from four accredited farms viz. The rabbitry of the Department of Animal Production, Faculty of Agriculture, Benha University, Egypt; Inshas, Gimmeza and Shakha Experimental Rabbitries, which belong to Animal Production Research Institute (APRI) Agricultural Research Center (ARC), Ministry of Agriculture, Egypt. The animals were taken randomly from pedigreed animals with the least relationship to decrease the genetic similarity and to have more chance to have more polymorphism in markers alleles. We identified the animals depending on their pedigrees to be sure that these animals not full-sib or half-sibs. In addition, we check these relationships by analyzing the polymorphism after genotyping to insure that there is no error in their original farm pedigree.

2. Blood samples and DNA extraction:

DNA of each breed were extracted from whole fresh blood samples, approximately 5 ml blood was collected from the central artery vein of the ear of animal into tubes containing Ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The blood samples were kept in Ice tank till reaching the laboratory and were then preserved in a freezer at -20°C. Until extraction of DNA. Genomic DNA was extracted from leukocytes using the Promega Wizard Genomic DNA Purification Kit (Cat No. A 1120) using the manufacture protocol.

3. RAPD-PCR conditions and Electrophoresis:

A total of fourteen random primers (Operon Technologies Inc, USA) of arbitrary sequence with 60-70% GC content were screened on pooled rabbit DNA but ten of them generated reproducible, scoreable and polymorphic bands Table (1) . The amplification reaction was carried out in 25 µl final volume containing 2 µl template DNA ,3µl primer (10 pmol/µl), 5µl (5X PCR buffer),0.2 µl Taq polymerase(5U/µl), 2.5 µl dNTP's mix(2mM) ;(dATP, dCTP, dTTP and dGTP), 2 µl MgCl2 (25mM) and 10.3 µl dd-H2O. PCR amplification was performed in a GeneAmp® PCR System 9700 (Applied Biosystems, Foster City, California, USA), with the following amplification conditions: initial denaturation at 94°C for 4 min, followed by denaturation at 94°C for 40 sec, annealing at 36°C for 1 min, extension at 72°C for 1 min for 35 cycles and final extension at 72°C for 10min. The PCR products were run on 1.5% agarose gel. Gel Documentation System (Gel-Doc 2000 with Diversity Database software Ver. 2.1, Bio-Rad Laboratories, Hercules, California, USA) was used for gel documentation and gel analysis.

Table 1. List of random amplified polymorphic DNA primers (RAPD) and their nucleotide sequence.

Primer code	Nucleotide Sequence (5`→3`)	G+C (%)	Primer code	Nucleotide Sequence (5`→3`)	G+C (%)
OPA-09	5'-GGGTAACGCC -3'	70%	OPA-20	5'-GTTGCGATCC-3'	60%
OPB-05	5'-TGCGCCCTTC -3'	70%	OPF-09	5'-CCAAGCTTCC-3'	60%
OPB-07	5'-GGTGACGCAG -3'	70%	OPF-12	5'-ACGGTACCAG-3'	60%
OPB-14	5'-TCCGCTCTGG -3'	70%	OP-B2	5'-TGATCCCTGG-3'	60%
OPA-19	5'-CAAACGTCGG-3'	60%	OP-A2	5'-TGCCGAGCTG-3'	70%

4. Analysis of RAPD data:

Only distinct and clear bands of RAPD products on agarose gel were scored. The presence and absence of band was recorded as "1" and "0", respectively. The binary coded characters (1,0) were used for the genetic analysis. Band Sharing Frequency (BSF) was used to estimate the genetic similarity for each primer (***Lynch, 1990***) and a simple expression of similarity measured in terms of sharing bands between breeds.

Band Sharing Frequency (BSF)

Band Sharing Frequency (BSF) was calculated as an expression of animals from either the same or different breeds using the following formula (***Jeffery and Morton, 1987***).

BSF=2Nab/ (Na + Nab)

Whereas, Nab is the number of bands common to a and b individual, Na is the number of bands present in the animal a, while Nb is the number of bands present in the animal b. The BSF values between breeds will be statistically analyzed by analysis of variance procedure using **SAS 2004** program. Significant differences between means will be detected using Duncan's multiple range test (***Kramer, 1957***).

Genetic Distance (D)

Genetic distances are designed to express the genetic differences between two populations as a single number. If there are no differences, the distances could be set to zero, whereas, if the population have no allele in common at any locus the distance may be set equal to its maximum value. The genetic distances (D) were calculated by POPGENE software (***Yeh et al.,1999***) using ***Nei (1978***) standard genetic distance equation.

Phylogenetic relationship

The phylogenetic relationship among rabbit breeds was analyzed by generating the phylogenetic tree by ***Nei (1972)*** genetic distances using UPGMA analysis through POPGENE software (***Yeh et al.,1999***).

Results and Discussion

In the present study, RAPD technique was used to assess the genetic variability and phylogenetic relationship among four rabbit breeds. Fourteen random primers were tested to amplify pooled genomic DNA from these breeds. Ten of them were chosen for further analysis, on the basis of the presence of reproducible and distinct RAPD profiles in one or more rabbit breeds (Figure 1). These primers amplified on average 2 to 14 bands of sizes varying from 200bp to 2000 bp. This observed range of products presumably due to limitations in the resolving power of the agarose gels at lower molecular weights as well as inefficiency of the extension reaction under the described PCR conditions at higher molecular weights (***Bowditch et al., 1993***). A total of 131 diagnostic bands were scored within RAPD profiles amplified by these 10 primers. The number of detected bands (TDB) per primers, number of polymorphic bands (NPB) and percent of polymorphic bands (PB) are presented in Table (2). Among 131 scorable bands 74(56.48%)were recognized as polymorphic and 57 (43.52%) as monomorphic bands Table (2). The average number of polymorphic bands per primer varied from 2 to 17. The highest percentage of polymorphic bands was recognized for primers OPB-02 (94%)and OPB-07(92%) while the lowest percentage of polymorphic bands was recognized for primers OPA-02(16%) and OPF-12 (28%), Table (2).

Table 2. Summary of the results of RAPD analysis with 10 random primers: total number of detected bands (TDB), number of polymorphic bands (NPB) and percentage of polymorphic bands (PB %).

Primers	TDB	NPB	PB	Primers	TDB	NPB	PB
OPA-09	13	9	69%	OPA-20	8	4	50%
OPB-05	9	5	55%	OPF-09	15	6	40%
OPB-07	13	12	92%	OPF-12	14	4	28%
OPB-14	14	9	64%	OPB-02	18	17	94%
OPA-19	15	6	40%	OPA-02	12	2	16%
		TOTAL			131	74	56.48%

The number of bands amplified per primer was variable among the four rabbit breeds Table (3). The maximum numbers of bands were found in New Zealand White (14) followed by Gabali, Baladi Black and Baladi Red (13) using primer OPF-9. Primer OPF-9 gave the maximum numbers of bands (53) while the minimum numbers of bands were obtained using primer OPB-5 (18). ***Khalil, et al., (2008)*** studied association between RAPD markers and some reproductive traits in rabbits. They found five polymorphic fragments at molecular weight of 1500, 1100, 1200, 700 and 900 bp, respectively for five RAPD markers (OPA-12, OPA-19, OPA-20, OPF-09, and OPF-12) linked to these reproductive traits. In a similar study conducted by ***El Sayed (2010),*** he used eight RAPD primers (OPA-10, OPB-05, OPC-01, OPC-02, OPC-08, OPE-11, OPE-19 and OPX-02) to assess the genetic diversity among six rabbit breeds viz; New Zealand White, Black Rex, Hyplus strain, Spanish line V, Moshtohor or Line M, and Sinai. He found that, all primers yielded informative and identifiable bands revealing genetic differences between breeds.

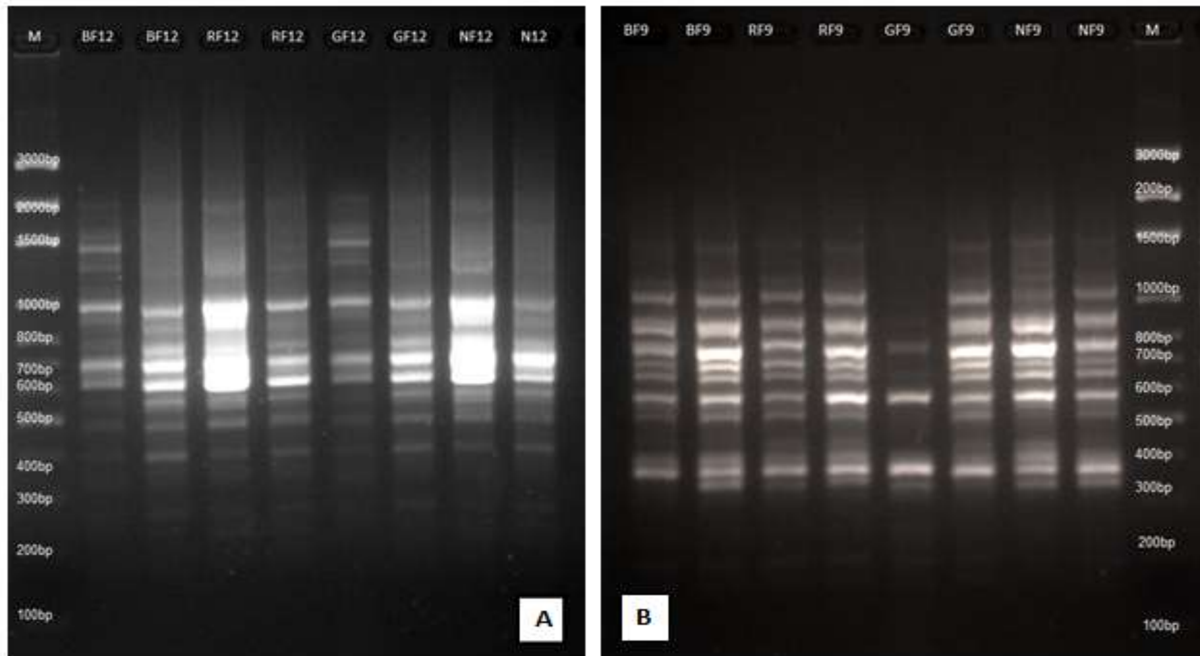
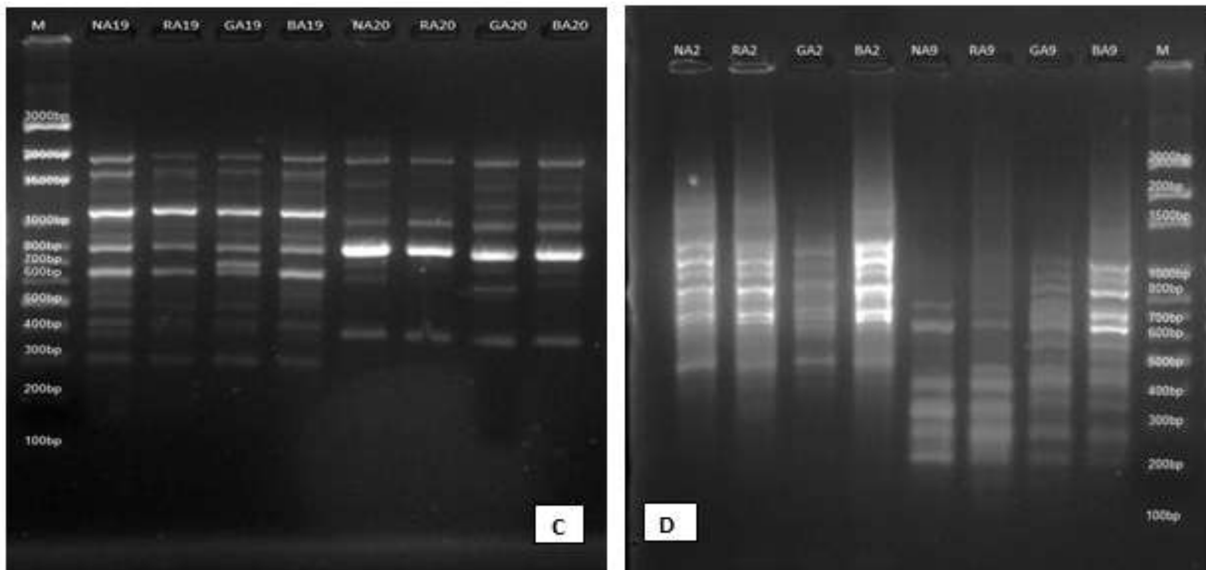


Figure 1. RAPD profile in different rabbit breeds; NZW: New Zealand White, G: Gabali, B: Baladi Black and R: Baladi Red using primers; A: OPF-12 and B: OPF-09 Molecular marker (100 bp plus ladder).



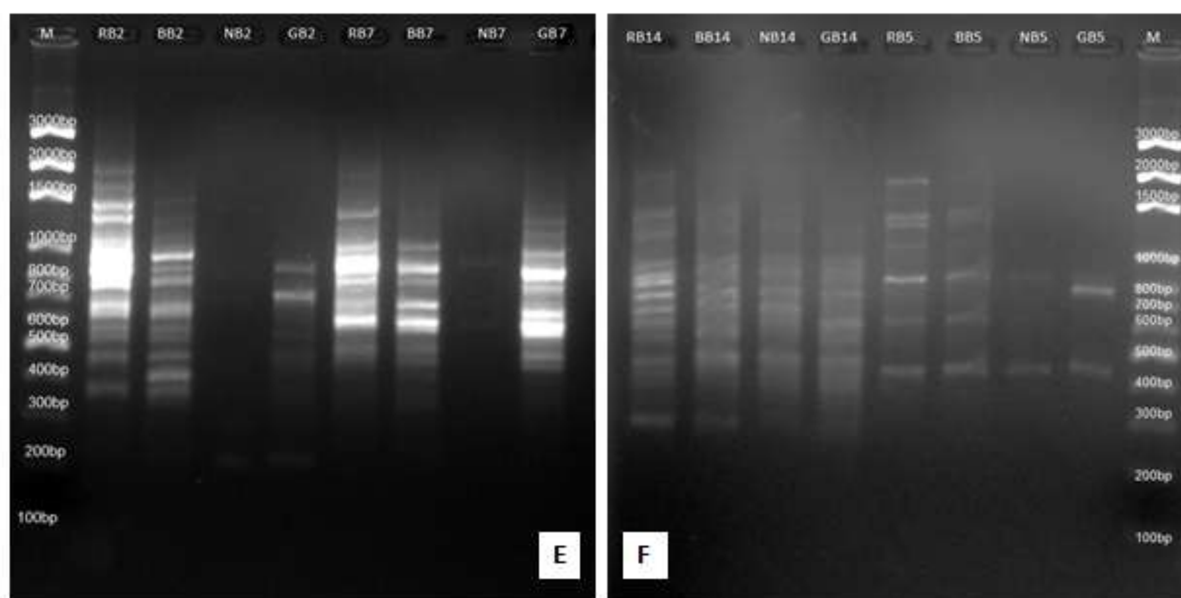


Figure 2. RAPD profile in different rabbit breeds; NZW: New Zealand White, G: Gabali, B: Baladi Black and R: Baladi Red using primers; C: OPA-19 and OPA-20; D: OPA-02 and OPA-09; E: OPB-02 and OPB-07; F: OPB-14 and OPB-5. Molecular marker (100 bp plus ladder).

Table (3): Number of bands per primer in different rabbit breeds.

RAPD Primer	NEW	G	BB	BR	TOTAL
OPA-09	6	12	11	7	36
OPB-05	2	3	6	7	18
OPB-07	2	9	9	7	27
OPB-14	8	9	9	14	40
OPA-19	12	11	12	10	45
OPA-20	6	7	7	4	24
OPF-09	14	13	13	13	53
OPF-12	11	11	12	12	46
OPB-02	3	6	12	14	35
OPA-02	10	9	11	10	36
TOTAL	74	90	102	98	324

Similar type of the study was conducted by ***Mamuris et al., (2002)*** they used RAPD primers (OPA-02, OPA-09, OPA-10, OPA-20 and OPF-01) for assessment of genetic variability among brown hare (L.europaeus) population from different geographical regions. In their study, all primers produced polymorphic bands in the range of 5 to 11. ***Rangoju et al., (2007)*** assessed genetic variability and phylogenetic relationship among three rabbit breeds using six random primers (OPA-01, OPA-08, OPA-10 ,OPA-18 , OPB-03 and OPB-05). They found that, the maximum number of bands was (13.2 ± 0.4), while minimum number of bands were (6.4 ± 0.2) in all the breeds. In a similar study conducted by ***El-Bayomi Kh. M et al.,(2013)*** they used RAPD primers (OPA-01, OPA-06, OPA-10, OPB-05, OPB-13, OPB-14,OPC-01,OPC-02,OPC-08,OPE-19,OPE-11,OPF-9,OPF-12and OPX-02) for assessment of genetic variability among three rabbit breeds. They found that, The highest percentage of polymorphic bands was recognized for primers OPA-10 and OPA-06 (56%) while the lowest percentage of polymorphic bands was recognized for primers OPE-19 (7%) and OPF-12 (14%).

RAPD markers for breed differentiation:

The RAPD profiles of New Zealand White, Gabali, Baladi Black and Baladi Red breeds generated by 10 random primers were studied for identifying breed specific markers i.e. the marker unique to a particular breed only Table (4). One primer (OPA-19) was identified in New Zealand White. Three primers (OPB-02, OPA-02 and OPA-19) were identified in Baladi Black .Four primers (OPA-19, OPA-20, OPB-05 and OPB-07) were identified in Gabali. Five primers (OPB-05, OPB-02, OPA-19, OPF-12 and OPB-14) were identified in Baladi Red, Table (4). These breed specific primers can be used in identification of the breeds. However, these results need to be validated by using large sample size.

Table 4. Specific RAPD markers in the four rabbit breeds.

No.	Molecular marker	Band number	Molecular weight	Breed
1	OPF-12	2	1600 bp	RB
2	OPA-02	1	2050 bp	BB
3	OPA-19	3	1500 bp	BB
		6	920 bp	RB
		9	720 bp	G
		14	360 bp	NEW
4	OPA-20	7	550 bp	G
5	OPB-14	1	1880 bp	RB
		12	440 bp	RB
6	OPB-05	1	1880 bp	RB
		6	860 bp	G
		7	790 bp	G
7	OPB-07	2	1250 bp	G
		4	970 bp	G
		8	620 bp	G
8	OPB-02	1	2000 bp	RB
		2	1750 bp	RB
		3	1500 bp	BB
		4	1400 bp	RB
		7	1000 bp	RB
		16	400 bp	BB

Band Sharing Frequency (BSF) is an indicator of relatedness between breeds (***Nei & Li, 1979***). Interbreed BSF being the highest between Baladi Black - Baladi Red (0.807±0.038), followed by Gabali- Baladi Black (0.711±0.079) and the lowest between New Zealand White- Gabali (0.649±0.081) Table (5). Similarity between fingerprint patterns expressed by band sharing values provides a reliable method for evaluating genetic distance among population (***Kuhnlein et al., 1989*** and ***Dunnington et al., 1991***).

Table 5. Band sharing frequency between rabbit breeds.

RAPD Primer	NEW-G	NEW-BB	NEW-BR	G-BB	G-BR	BR-BB
OPA-09	0.55	0.47	0.92	0.95	0.63	0.55
OPB-05	0.4	0.5	0.44	0.22	0.2	0.92
OPB-07	0.18	0.36	0.44	0.55	0.37	0.75
OPB-14	0.58	0.94	0.72	0.55	0.78	0.78
OPA-19	0.86	0.91	0.81	0.86	0.85	0.81
OPA-20	0.76	0.92	0.8	0.85	0.72	0.72
OPF-09	0.88	0.88	0.88	0.84	0.84	0.92
OPF-12	0.9	0.95	0.86	0.95	0.86	0.91
OPB-02	0.44	0.13	0.11	0.44	0.4	0.76
OPA-02	0.94	0.95	1	0.9	0.94	0.95
Overall± S.E	0.649±0.0815	0.701±0.096	0.698±0.088	0.711±0.079	0.659±0.075	0.807±0.038

Rangoju et al., (2007) studied genetic variation among three rabbit breeds viz. White Giant (WG), Soviet Chinchilla (SC) and Grey Giant (GG) using 40 RAPD primers. Six primers were found polymorphic and the overall BSF value between breeds was found higher in SC-GG followed by WG-SC and WG-GG. The Nei's genetic distance (D) of the present study was found highest between WG-GG followed by WG-SC and SC-GG. Genetic distances among the studied four rabbit breeds were shown in Table (6). The genetic distances ranged from 0.87 between Baladi Black - Baladi Red (more related) to 0.73 between New Zealand White - Baladi Black (distantly related).High value of genetic distance was found between New Zealand White – Gabali (0.80).

Table 6. Genetic distances between rabbit breeds using RAPD data

breed	NEW	G	BR	BB
NEW	1	0.808511	0.753927	0.738462
G	0.808511	1	0.860104	0.822335
BR	0.753927	0.860104	1	0.87
BB	0.738462	0.822335	0.87	1

El Sayed, (2010), found that the highest value of the genetic distances (37.0) was between New Zealand White/Black Rex and New Zealand White/ Spanish Line V while the lowest value was 10.0 between Spanish Line V/ Line M (Moshtohor). The phylogenetic relationships among the studied four rabbit breeds based on genetic distance were given in Figure (2). There were two separate clusters formed from the four rabbit breeds. The local breeds clustered together in one cluster however, New Zealand White breed clustered alone. These variations might be due to the different geographical climatic conditions and/or different races , which cause variability in the gene pool.

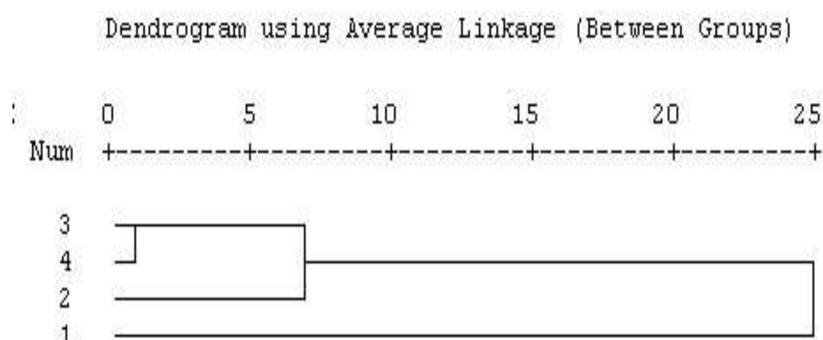


Figure 3. Dendrogram of studied rabbit breeds based on genetic distances: (1) NEW Zealand White. (2) Gabali . (3) Baladi Black. (4) Baladi Red

In this concept, ***Rangoju et al., (2007)*** illustrated the phylogenetic relationship among some rabbit breeds and their dendrogram revealed that Soviet Chinchilla and Grey Giant are closer, while White Giant and Grey Giant are distant to each other. Moreover, ***El Sayed (2010***), showed that Spanish Line V and Line M (Moshtohor) were close to each other while New Zealand White and Hyplus were more distant breeds. and ***El-Bayomi Kh. M et al.,(2013)*** showed that New Zealand White and Californian breeds were close to each other while Flander breed was distant to them.

RAPD analysis has been used to discriminate animal species other than rabbits and to determine the genetic diversity and phylogenetic relationship between animals such as cattle breeds (***Rincón et al.,2000; Ramadan, 2004; Devrim and Kaya, 2006 & Joshi et al., 2007).*** Buffalo ***(Sodhi et al., 2006; & Abdel-Rahman and Hafez, 2007).*** Horse breeds ***(Alves do Egito et al., 2007 & Saleh, 2011).*** Sheep and goat ***(Ali, 2003; Abd Rabou, 2007; Elmaci et al., 2007; Mahfouz, et al., 2008 & Kunene et al.,2009).*** Chicken ***(Zhang et al., 2002; & Ahlawat et al., 2004 & El Araby, 2006)*** .Geese ***(Maciuszonek et al., 2005).*** Quail ***(Sharma et al., 2000***). Duck breeds ***(El-Gendy et al., 2005 & Gholizadeh et al., 2007).*** Turkey breeds ***(Smith et al., 2005)*** and fish species ***(Callejas and Ochando, 2002; Hassanien, 2004 & Abel Wahab, 2009).***

Conclusion

The present study suggests that RAPD can be used as a tool to understand the genetic variability and phylogenetic relationship among rabbit breeds. The wide genetic diversity between New Zealand White and local breeds allows scientists' for further research in rabbit breeding programs to obtain hybrid vigor and improve rabbit production. The study also provided unique molecular genetic markers for the studied rabbit breeds which may be useful in differentiating between these breeds at the molecular level.

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