# Assessment of genetic variability among some rabbit breeds using RAPD-DNA technique

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**Abstract**

Random Amplified Polymorphic DNA (RAPD) markers were employed to assess the genetic diversity and phylogenetic relationships among three Egyptian rabbit breeds (Gabali, Baladi Red and Baladi Black) along with New Zealand White breed as a reference population. A total of 120 animals were used in this study, 30 animals from each breed. Genomic DNA was extracted and PCR amplification was performed and the amplification products were resolved by electrophoresis. A total of 14 RAPD primers of arbitrary sequence were used to amplify pooled genomic DNA from these breeds, and only 10 primers generated reproducible, score able and polymorphic bands. Out of 131 bands scored, 74 bands (56.48%) were recognized as polymorphic and 57 bands (43.52%) as monomorphic. The highest percentage of polymorphic bands was recognized by primers OPB02 (94%) and OPB07 (92%), and the lowest percentage of polymorphic bands was recognized by primers OPA02(16%) and OPF12 (28%). One marker of OPA19 was identified as specific for New Zealand White, three markers detected by OPB02, OPA19 and OPA02 were specific for Baladi Black, four markers detected by OPA19, OPA20, OPB05 and OPB07 in Gabali, and five markers of OPB05, OPB02, OPA19, OPF12 and OPB14 were specific for Baladi Red. The band sharing frequencies (BSF) were found to be the highest between Baladi Red and Baladi Black (0.80± 0.038), followed by Gabali and Baladi Black (0.71±0.079 ), New Zealand White and Baladi Black (0.70±0.096), New Zealand White and Baladi Red (0.69±0.088) and the least BSF was found between New Zealand White and Gabali (0.64±0.081). Overall, there was no significant difference (P>0.05) in BSF values between breeds. The closest genetic distance was found between Baladi Red and Baladi Black (0.87), while the lowest genetic distance was found between New Zealand White and Baladi Black (0.73).

**Key words:** Rabbits breeds, genetic diversity, RAPD markers, band sharing frequency, phylogenetic relationships, genetic distance.

## Introduction

Recent reports of FAO and CIHEAM have indicated the lack of information and rapid loss in animal genetic resources, including rabbits. Most of the local breeds of rabbits as well as lines synthesized recently in Egypt appear to be in a real danger of extinction due to the low number of animals, while some breeds are currently being disappeared **(Khalil and Baselga, 2002)**. Characterization of native rabbit breeds at the molecular level is currently considered an important criterion for breed conservation and for further genetic improvement (**Al-Saef *et al*, 2012**).The FAO and CIHEAM working groups proposed a global programme for characterization of *Animal Genetic Resources* (AnGR), including the molecular genetic characterization, with the recommendations of applying molecular analysis of domestic animal diversity on a global scale via a research programme (**FAO, 2011**). Therefore, assessment of genetic variability in rabbits will be an important issue to preserve the genetic resources and propose future breeding plans in order to satisfy the demands of changeable markets (**Groeneveld *et al.,* 2010**). However, genetic diversity among breeds provides us with valuable information, to understand domestication and evolution history, and this will lead us to suggest the conservation and breeding plans.

Molecular analyses performed for economic traits in rabbits are very limited to be used in further genetic improvement programs. RAPD technology has been principally used to study the genetic relationships between varieties/species in the rabbit. For instance, **Liping *et al.* ( 2000 and 2005 )** analyzed the genetic relationships among three domestic rabbit breeds; **Rongqing *et al.* (2000)** analyzed evolutionary relationships among five rabbit populations; **Rangoju *et al* (2007)** assessed the genetic variability and phylogenetic relationships among three rabbit breeds.RAPD technique is one of the most widely techniques used in the applications of molecular biology to identify the markers linked to traits of interest without the necessity of mapping the entire genome **(Bardakci, 2001; Khalil *et al.,* 2008).**

The objective of the present study was to assess the genetic diversity and phylogenetic relationships within and among four rabbit breeds (New Zealand White, Gabali, Baladi Red and Baladi Black) using random amplified polymorphic DNA markers (RAPD).

## Materials and Methods

### Experimental animals:

Three Egyptian rabbit breeds namely Baladi Red (BR), Baladi Black (BB) and Gabali (G) along with

New Zealand White breed as a reference breed were used in the present study. A total of 120 rabbits viz. 30 rabbits from each breed were selected from four accredited farms viz. the rabbitry of the Department of Animal Production, Faculty of Agriculture, Benha University, Egypt; Inshas, Gimmeza and Sakha experimental rabbitries belong to Animal Production Research Institute (APRI), Ministry of Agriculture, Egypt. Baladi Red and Baladi Black rabbits are Egyptian traditional breeds that have not been subjected to followed by a program of genetic improvement after their formation **(Khalil and Baselga, 2002).** These breeds were developed by crossing local Baladi rabbits with Flemish Giant (G) for several generations in rabbitries of Animal Production Research Institute (APRI), Ministry of Agriculture (**Galal and Khalil, 1994; Khalil, 1999; Khalil and Baselga, 2002**). While the Gabali rabbits are raised by the Egyptian Bedouins in Sinai and the north coast of western desert **(Khalil, 1999; Afifi, 2002)**. This breed was extensively studied in the last two decades by **Afifi (2002)** and **Iraqi et al. (2007)**. On the other hand, New Zealand White (NZW) is a foreign breed actually originated in the United States and wide spread globally in the world and has been raised in Egypt in alarge scale of commercial production for several decades. New Zealand White exhibits outstanding maternal abilities as related to maternal behavior, fecundity, lactation, and pre weaning growth and survival (**Khaliland Baselga, 2002**).

**Blood samples and DNA extraction**:

The blood samples were taken randomly from pedigreed animals with the least relationship to decrease the genetic similarity and to have more chance in showing more polymorphism in marker alleles. The animals were identified depending on their pedigrees and full-sib and half-sibs were avoided. In addition, these relationships were checked by analyzing the polymorphism after genotyping to insure that there is no error in their original farm pedigree.

DNA of each breed were extracted from the whole fresh blood samples, approximately 5 *ml* blood was collected from the central artery vein of the ear of animal into tubes containing ethylene di amine 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 using the Promega Wizard Genomic DNA Purification Kit (Cat No. A 1120) using the manufacture protocol.

**PCR amplification and RAPD analysis:**

A total of fourteen random primers (Operon Technologies Inc, USA) of arbitrary sequence with 60-70% GC content were screened on pooled rabbit DNA Ten of the primers generate reproducible, score able and polymorphic bands and were used alone to amplify random sequences from genomic DNA(Table 1). These primers amplified on average 2 to 14 bands of sizes varying from 200 bp 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***). However, the DNA amplification protocol was performed as described by **Welsh and McClelland (1990)** and **Williams *et al* (1990)**.

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

| Primer code | Nucleotide sequence (5`→3`) | GC (%) |
|---|---|---|
| OPA09 | 5'-GGGTAACGCC-3' | 70% |
| OPB05 | 5'-TGCGCCCTTC-3' | 70% |
| OPB07 | 5'-GGTGACGCAG-3' | 70% |
| OPB14 | 5'-TCCGCTCTGG-3' | 70% |
| OPA19 | 5'-CAAACGTCGG-3' | 60% |
| OPA20 | 5'-GTTGCGATCC-3' | 60% |
| OPF09 | 5'-CCAAGCTTCC-3' | 60% |
| OPF12 | 5'-ACGGTACCAG-3' | 60% |
| OPB02 | 5'-TGATCCCTGG-3' | 60% |
| OPA02 | 5'-TGCCGAGCTG-3' | 70% |

As shown in Table 2, PCR reactions were carried out in a total volume of 25 µl containing 2 *µl* of genomic DNA as a template, 30 pmoles of random primer, 2 mM of dNTP's mix (dATP, dCTP, dTTP and dGTP;ABgene, Surrey, UK), 5X PCR buffer, 25 mM $MgCl_2$, and 1 unitTaq DNA polymerase (MBI Fermentas Inc., Hanover, Madison, Wisconsin, USA). A master mix was prepared in a 1.5 *ml* eppendorf tube according to the number of PCR reactions to be performed, with an extra reaction included to compensate the loss part of the solution due to frequent pipetting. An aliquot of 22 µl master mix solution was dispensed in each PCR tube (0.2 *ml* eppendorf tube); containing 2 µl of the appropriate template DNA PCR reaction components.

**Table 2.** Components of RAPD-PCR reaction**.**

| PCR Component | Amount of one PCR reaction (1X) |
|---|---|
| 5X PCR buffer | 5µl |
| MgCl2 (25mM) | 2µl |
| dNTP's mix (2mM) | 2.5 µl |
| Primer (10 pmoles/µl) | 3 µl |
| Taq (5 U/µl) | 0.2µl |
| DNA (10ng/µl) | 2µl |
| d.dH2O | 10.3µl |
| Total volume | 25µl |

PCR amplification was performed in a Gene Amp® PCR System 9700 (Applied Bio systems, Foster City, California, USA), programmed to fulfill 35 cycles after an initial denaturation cycle for 4 minutes at 94ºC, in this cycle dsDNA converted to ssDNA and Taq DNA polymerase was activated.

Each PCR amplification cycle consisted of three steps, a denaturation step at 94ºC for 40 seconds, an annealing step at 36ºC for one minute, and an elongation or extension step at 72ºC for one minute. After the last cycle, the primer extension segment was extended to 10 minutes at 72ºC in the final extending cycle and was followed by soaking at 4ºC until reaction removed from PCR machine.

**Electrophoresis and visualization of RAPD- PCR products**

The amplification products were separated by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 µg/*ml*) in 1X TBE buffer.A 10µl of each PCR product was mixed with 2 µl of loading buffer (tracking dye), and loaded into the wells of the gel. The gel was run at 95 volts until tracking dye reached to the end of the gel. After electrophoresis, the amplified RAPD-PCR products (amplicons) were visualized with 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.

RAPD banding patterns on gels scrutinized for variation in presence/absence variation of aband at a specific position on the gel, presume to reflect priming sequence variation or large insertion/deletion variants that preclude successful amplification.

**Statistical analysis:**

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. BSF was calculated as an expression of animals from either the same or different breeds using the following formula:

$$BSF=2N_{ab}/(N_a + N_b)$$

Whereas, $N_{ab}$ is the number of bands common to a and b individuals, $N_a$ is the number of bands present in the animal a, while $N_b$ is the number of bands present in the animal b. The BSF values were statistically analyzed by the ANOVA procedure using SAS program **(SAS, 2004)**.Significant differences between means were detected using Duncan's multiple range test (**Duncan, 1955).**

The genetic distances (D) were 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 populations have no allele in common at any locus the distance may be set to its maximum value. The genetic distances were calculated by POPGENE software (***Yeh et al.,1999***) using standard genetic distance equation ***(Nei, 1972)***.The phylogenetic relationships among rabbit breeds were analyzed by generating the phylogenetic tree for different genetic distances using POPGENE software ***(Nei 1972;Yeh et al.,1999)***.

**Results and Discussion**

**Number of bands detected in the breeds:**

The number of detectable bands per primer (TDB), number of polymorphic bands (NPB) and percentages of polymorphic bands (PB) are presented in Table 3. A total of 131 bands were scored in the RAPD profiles and were amplified by 10 primers. Among these 131scoreable bands, 74 bands (56.5%) were recognized as polymorphic and 57 bands (43.5%) as monomorphic. ***El-Bayomi et al. (2013)*** detected a total of 120 bands within RAPD profiles amplified by 14 primers, and 39 bands (33%) were recognized as polymorphic and 81 bands (67%) as monomorphic bands.

The average number of polymorphic bands per primer varied from 2 to 17 (Table 3). The highest percentage of polymorphic bands was recognized for primers OPB02 (94%) and OPB07 (92%),and the lowest percentage of polymorphic bands was recognized for primers OPA02(16%) and OPF12 (28%). ***Khalil et al. (2008)*** detected five polymorphic RAPD markers of OPA12, OPA19, OPA20, OPF09, and OPF12 at molecular weights of 1500, 1100, 1200, 700 and 900 bp, respectively. ***Mamuris et al. (2002)*** used five RAPD primers (OPA02, OPA09, OPA10, OPA20 and OPF01) to assess the genetic variability

among brown hare populations (L.europaeus) from different geographical regions. It was found that all the primers produced polymorphic bands in the range of 5 to 11. ***Rangojuet al. (2007)*** used six random primers (OPA01, OPA08, OPA10, OPA18, OPB03 and OPB05) to screen the genomes of three rabbit breeds. All bands were polymorphic and the maximum number of bands was 13.2 ± 0.4, while the minimum number of bands were 6.4 ± 0.2. Also, ***El-Bayomi et al. (2013)*** used 13 RAPD primers for three rabbit breeds (OPA01, OPA06, OPA10, OPB05, OPB13,OPB14,OPC01,OPC02,OPC08,OPE19,OPE 11,OPF9,OPF12 and OPX02) and found that, the highest percentage of polymorphic bands was recognized for primers OPA10 and OPA06 (56%),and the lowest percentage of polymorphic bands was recognized for primers OPE19 (7%) and OPF12 (14%).

**Table 3.**The total number of detectable bands (TDB), number of polymorphic bands (NPB) and percentages of polymorphic bands (PB %) for the ten random primers used (across the breeds).

| Primer | TDB | NPB | PB% |
|---|---|---|---|
| OPA09 | 13 | 9 | 69% |
| OPB05 | 9 | 5 | 55% |
| OPB07 | 13 | 12 | 92% |
| OPB14 | 14 | 9 | 64% |
| OPA19 | 15 | 6 | 40% |
| OPA20 | 8 | 4 | 50% |
| OPF09 | 15 | 6 | 40% |
| OPF12 | 14 | 4 | 28% |
| OPB02 | 18 | 17 | 94% |
| OPA02 | 12 | 2 | 16% |
| | Total= 131 band | Total= 74 band | Average = 56.5 % |

**Number of bands amplified within each breed**

Among the four rabbit breeds, the number of bands amplified per primer was variable (Table 4). Using the primer OPF09, the maximum numbers of bands detected in New Zealand White rabbits were 14 bands, followed by Gabali, Baladi Black and Baladi Red (13 bands each). The maximum number of bands given by primer OPF09 was 53 band, while the minimum number of bands was 18 bands and were obtained using primer OPB05.Using eight RAPD primers (OPA10, OPB05, OPC01, OPC02, OPC08, OPE11, OPE19 and OPX02),***El Sayed (2010)***found that, all the primers yielded informative and identifiable bands revealing genetic differences between New Zealand White, Black Rex, Hyplus, Spanish line V, Moshtohor line and Sinai rabbits.

**Table 4.** Number of bands amplified per primer in each rabbit breed.

| RAPD primer | New Zealand White | Gabali | Baladi Black | Baladi Red | Total |
|---|---|---|---|---|---|
| OPA09 | $6{\pm}0.57^{b}$ | $12{\pm}0.57^{a}$ | $11{\pm}0.57^{a}$ | $7{\pm}0.57^{b}$ | 36 |
| OPB05 | $2{\pm}0.57^{b}$ | $3{\pm}0.57^{b}$ | $6{\pm}0.57^{a}$ | $7{\pm}0.57^{a}$ | 18 |
| OPB07 | $2{\pm}0.57^{c}$ | $9{\pm}0.57^{a}$ | $9{\pm}0.57^{a}$ | $7{\pm}0.57^{b}$ | 27 |
| OPB14 | $8{\pm}0.57^{b}$ | $9{\pm}0.57^{b}$ | $9{\pm}0.57^{b}$ | $14{\pm}0.57^{a}$ | 40 |
| OPA19 | $12{\pm}0.57^{a}$ | $11{\pm}0.57^{a}$ | $12{\pm}0.57^{a}$ | $10{\pm}0.57^{a}$ | 45 |
| OPA20 | $6{\pm}0.57^{a}$ | $7{\pm}0.57^{a}$ | $7{\pm}0.57^{a}$ | $4{\pm}0.57^{b}$ | 24 |
| OPF09 | $14{\pm}0.57^{a}$ | $13{\pm}0.57^{a}$ | $13{\pm}0.57^{a}$ | $13{\pm}0.57^{a}$ | 53 |
| OPF12 | $11{\pm}0.57^{a}$ | $11{\pm}0.57^{a}$ | $12{\pm}0.57^{a}$ | $12{\pm}0.57^{a}$ | 46 |
| OPB02 | $3{\pm}0.57^{d}$ | $6{\pm}0.57^{c}$ | $12{\pm}0.57^{b}$ | $14{\pm}0.57^{a}$ | 35 |
| OPA02 | $10{\pm}0.57^{a}$ | $9{\pm}0.57^{a}$ | $11{\pm}0.57^{a}$ | $10{\pm}0.57^{a}$ | 40 |
| Total | 74 | 90 | 102 | 98 | 364 |

Means with the same letters in the row are not significantly different ($p \leq 0.05$).

**The RAPD profiles and breed specific markers:**

The RAPD profiles of New Zealand White, Gabali, Baladi Black and Baladi Red breeds generated by 10 random primers identified breed- specific markers (Table 5), i.e. the marker unique to a particular breed only. As presented in Table 5, one band of OPA19 at molecular weight of 360 bp was identified as specific for New Zealand White. Three bands of OPB02, OPA19and OPA02 at molecular weights of1500, 1500 and 2050 bp were identified as specific for Baladi Black. Four bands of OPA19, OPA20, OPB05 and OPB07 were identified as specific for Gabali, and five markers of OP05, OPB02, OPA19, OPF12 and OPB14were identified as specific for Baladi Red. Therefore, these breed specific markers can be used in identification of the breeds. ***El-Bayomi et al. (2013)*** used 14 RAPD primers and reported that one marker of OPE-11 was identified to be specific for *New Zealand White*, Three bands of OPA10, OPC02 and OPF09 were identified to be specific for *Californian*, five bands of OPA01, OPA06, OPA10, OPB14 and OPF09 were identified as specific for Flander rabbits.

**Table 5.** Specific RAPD markers identified in the four rabbit breed studied.

| Breed | Molecular marker | Number of bands | Molecular weight (bp) |
|---|---|---|---|
| New Zealand White | OPA19 | 14 | 360 |
| Baladi Black | OPB02 | 16 | 400 |
| | OPA19 | 3 | 1500 |
| | OPB02 | 3 | 1500 |
| | OPA02 | 1 | 2050 |
| Gabali | OPA19 | 9 | 720 |
| | OPA20 | 7 | 550 |
| | OPB07 | 8 | 620 |
| | OPB07 | 4 | 970 |
| | OPB07 | 2 | 1250 |
| | OPB05 | 7 | 790 |
| | OPB05 | 6 | 860 |
| Baladi Red | OPB14 | 12 | 440 |
| | OPB14 | 1 | 1880 |
| | OPA19 | 6 | 920 |
| | OPB02 | 7 | 1000 |
| | OPB02 | 4 | 1400 |
| | OPB02 | 2 | 1750 |
| | OPB02 | 1 | 2000 |
| | OPF12 | 2 | 1600 |
| | OPB05 | 1 | 1880 |

**Band sharing frequency (BSF)**

BSF is an indicator of the relationships between breeds (***Nei and Li, 1979)***. The sharing frequencies given in Table 6 showed that there were no significant differences (P>0.05) in BSF values among the breeds. Among the primers used, the highest overallBSF was between Baladi Black and Baladi Red rabbits (0.807±0.038), followed by Gabali and Baladi Black rabbits (0.711±0.079) and the lowest BSF wasbetween New Zealand White and Gabali (0.649±0.081). ***Rangoju et al. (2007)*** used 40 RAPD primers to study the genetic variation among three rabbit breeds viz. White Giant (WG), Soviet Chinchilla (SC) and Grey Giant (GG).The BSF was the highest in SC-GG rabbits, followed by WG-SC and WG-GGrabbits. ***El-Bayomi et al. (2013)*** found that the highest sharing frequency was between *New Zealand White* and *Californian* rabbits (0.88±0.029), followed by that between Californian-*Flander* rabbits (0.87±0.024) and between New *Zealand White-Flander* rabbits (0.84±0.034).

**Genetic distances between the breeds**

The genetic distances (D) between the four rabbit breeds are shown in Table 7. The genetic distances ranged from 0.87 between Baladi Black and Baladi Red (more related) to 0.73 between New Zealand White and Baladi Black (distantly related).High value of genetic distance was found between New Zealand White and Gabali rabbits (0.80). ***Rangoju et al. (2007)*** found that the highest genetic distance was between *White Giant and Grey Giant* rabbits (D=0.1605), followed by between *White Giant and Soviet Chinchilla rabbits* (D=0.1403) and between *Soviet Chinchilla and Grey Giant* rabbits (D=0.1295), explaining the difference between the breeds to reflect the survival under different geographical and climatic conditions. ***El Sayed (2010)*** reported that the highest genetic distance was 0.37 between New Zealand White and Black Rex and between New Zealand White and Spanish V line rabbits, while the lowest value was 0.10 between Spanish V line and Moshtohor line rabbits.

**Phylogenetic relationships**

The phylogenetic relationships among the studied four rabbit breeds based on genetic distance were given in Figure 1. As shown in this dendro gram, two separate clusters were formed in the four rabbit breed used. The local breeds are clustered together in one cluster, while New Zealand White breed clustered alone. In agreement with these findings, **Grimal*et al.* (2012)** reported that the four Egyptian breeds (Baladi Black, Baladi Red, Giza White and Gabali) were structurally separated from the Spanish New Zealand White line. These variations might be due to that these breeds are raised in different geographical regions and/or rasied from different races, which cause variability in the gene pool. In India ***Rangoju et al. (2007)*** illustrated the phylogenetic relationships among many rabbit breeds and revealed that Soviet Chinchilla and Grey Giant are close to each other , while White Giant and Grey Giant are distant from each other. In Egypt, ***El Sayed (2010)*** showed that Spanish V line and Moshtohor line were close to each other, while New Zealand White and Hyplus were more distant breeds. ***El-Bayomi et al.(2013)*** showed also that New Zealand White and Californian breeds were close to each other, while Flander breed was distant to them.

**Table 6.** Estimates of band sharing frequency (BSF) among the rabbit breed studied [+].

| RAPD primer | NZW-G | NZW-BB | NZW-BR | G-BB | G-BR | BR-BB |
|---|---|---|---|---|---|---|
| | BSF±SE | BSF±SE | BSF±SE | BSF±SE | BSF±SE | BSF±SE |
| OPA09 | 0.55± 0.005[d] | 0.47± 0.005[e] | 0.92± 0.005[b] | 0.95± 0.005[a] | 0.63± 0.005[c] | 0.55± 0.005[d] |
| OPB05 | 0.40± 0.041[b] | 0.50± 0.041[b] | 0.44± 0.041[b] | 0.22± 0.041[c] | 0.20± 0.041[c] | 0.92± 0.041[a] |
| OPB07 | 0.18± 0.005[e] | 0.36± 0.005[d] | 0.44± 0.005[c] | 0.55± 0.005[b] | 0.37± 0.005[d] | 0.75± 0.005[a] |
| OPB14 | 0.58± 0.005[d] | 0.94± 0.005[a] | 0.72± 0.005[c] | 0.55± 0.005[e] | 0.78± 0.005[b] | 0.78± 0.005[b] |
| OPA19 | 0.86± 0.005[b] | 0.91± 0.005[a] | 0.81± 0.005[c] | 0.86± 0.005[b] | 0.85± 0.005[b] | 0.81± 0.005[c] |
| OPA20 | 0.76± 0.005[d] | 0.92± 0.005[a] | 0.8± 0.005[c] | 0.85± 0.005[b] | 0.72± 0.005[e] | 0.72± 0.005[e] |
| OPF09 | 0.88± 0.005[b] | 0.88± 0.005[b] | 0.88± 0.005[b] | 0.84± 0.005[c] | 0.84± 0.005[c] | 0.92± 0.005[a] |
| OPF12 | 0.90± 0.005[b] | 0.95± 0.005[a] | 0.86± 0.005[c] | 0.95± 0.005[a] | 0.86± 0.005[c] | 0.91± 0.005[b] |
| OPB02 | 0.44± 0.024[b] | 0.13± 0.024[c] | 0.11± 0.024[c] | 0.44± 0.024[b] | 0.40± 0.024[b] | 0.76± 0.024[a] |
| OPA02 | 0.94± 0.23[a] | 0.95± 0.23[a] | 1.00± 0.23[a] | 0.90± 0.23[a] | 0.94± 0.23[a] | 0.95± 0.23[a] |
| Overall± S.E | 0.649± 0.081 | 0.701± 0.096 | 0.698± 0.088 | 0.711± 0.079 | 0.659± 0.075 | 0.807± 0.038 |

[+]NZW= New Zealand White, G= Gabali, BB= Baladi Black and Baladi Red (BR).
Means with the same letters in the row are not significantly different ($p \leq 0.05$).

**Table 7.**Genetic distances among the rabbit breeds investigated using the RAPD data

| Breed[+] | **NZW** | **G** | **BR** | **BB** |
|---|---|---|---|---|
| **NZW** | 1 | | | |
| **G** | 0.81 | 1 | | |
| **BR** | 0.75 | 0.86 | 1 | |
| **BB** | 0.74 | 0.82 | 0.87 | 1 |

[+]NZW= New Zealand White, G= Gabali, Baladi Red =BR and BB= Baladi Black.

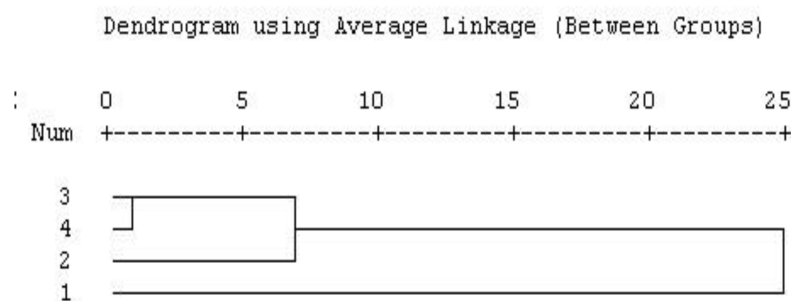


**Figure 1.**Dendrogram of the studied rabbit breeds based on the genetic distances: (1) NEW Zealand White. (2) Gabali. (3) Baladi Black. (4) Baladi Red.

**Conclusions**

1) RAPD can be successfully utilized as a tool for detecting the genetic variation and phylogenetic relationships among the Egyptian rabbit breed studied (Gabali, Baladi Black and Baladi Red).
2) The wide genetic diversity between New Zealand White and local breeds allows the genetists to carry out further research in rabbit breeding programs to obtain unique molecular genetic markers which may be useful in differentiating between these breeds at molecular level.

**References**

**Afifi, E.A. (2002).** The Gabali rabbits. In Khalil MH, Baselga M (Eds). Rabbit Genetic Resources in Mediterranean Countries. Options Méditerranénnes, Series B, No. 38:51-64.

**Al-Saef, A.M., Khalil M.H. and El-Zarie M.F. (2012)**. Identifying the molecular markers of RAPD type linked to resistance to heat stress and daily body gains in rabbits. Proceeding of 1stInternational Conference on Biotechnology Applications In Agriculture, Session of Molecular Genetics and Tissue Culture, Moshtohor and Hurghada 18-22, February 2012, Egypt, pp 7-12

**Bardakci, F. (2001**).Random Amplified Polymorphic DNA (RAPD) Markers. Turkish Journal of Biology, 25, 185-196.

**Bowditch B.M., Albright D.G., Williams J.G. and Braun M.J. (1993):** Use of randomly amplified polymorphic DNA markers in comparative genome studies. Methos Enzymol. 224: 294-309.

**Duncan, D. B. (1955).** Multiple range and multiple *F* tests, *Biometrics*, **11**, 1-42.

**El-Bayomi, K. M, Awad A and Saleh A. A.(2013):**Genetic diversity and phylogenetic relationship among some rabbit breeds using Random Amplified Polymorphic DNA markers.Life Science Journal, 2013; 10 (1)

**El Sayed, A.H. (2010):** Assessment of genetic variability among some rabbit breeds by Random Amplified Polymorphic DNA (RAPD)-PCR. Master thesis, Faculty of Veterinary Medicine, Suez Canal University, Egypt.

**FAO (2011).**Molecular genetic characterization of animal genetic resources. FAO Animal Production and Health Guidelines. No. 9. Rome.

**Galal, E.S.E. and Khalil, M.H. (1994):** Development of rabbit industry in Egypt. Options Méditerranéennes, Series Cahiers, 8: 43-56.

**Grimal, A.;Safaa, H.M.; Saenz-de-Juano, M.D.;Viudes-de-Castro, M.P.;Mehaisen, G.M.K.;Elsayed, D.A.A.;Lavara, R.; Marco-Jiménez, F. and Vicente, J.S.(2012):**Phylogenetic relationship among four Egyptian and one Spanish rabbit populations based on microsatellite markers. World Rabbit Congress, 3 – 6September, 2012, Sharm El-Sheikh, Egypt, 177- 181.

**Groeneveld, L.F.;Lenstra, J.A.;Eding, H.; Toro, M.A.;Scherf, B.; Pilling, D.;Negrini, R.;Jianlin, H.; Finlay, E.K.; Groeneveld, E.;Weigend, S. and the GlobalDiv Consortium (2010).**Genetic diversity in livestock breeds. Animal Genetics, 41(suppl. 1): 6–31

**Iraqi, M.M.;Shenana, M.E. and Baselga, M. (2007).**Some factors affecting productive and milk composition characters in a crossbreeding experiment involving Gabali and V- line rabbits in Egypt. World Rabbit Sci. 15(3):151-159.

**Khalil, M. H. (1999).** Rabbit genetic resources of Egypt. *Animal Genetic Resources Information*, *26*, 95-111.

**Khalil, M.H. and Baselga, M. (2002)**.The Baladi rabbits.Rabbit Genetic Resources in Mediterranean Countries. Options Méditerranénnes, Series B, N. 38:37-50.

**Khalil, M.H.; Motawei, M.I.; Al-Saef, A.M.; Al-Sobayil, K.A. and El-Zarei, M.F. (2008):** RAPD markers linked to litter, lactation and growth traits in rabbits. 9th World Rabbit Congress – June 10-13, 2008 – Verona – Italia, 143-147

**Liping, C.; Wei, Z. and HuaChun, Y. (2005).**RAPD analysis of genetic traits in WHBE rabbit. J. Zhejiang University (Edition of Life Science) 31(4):493-498.

**Liping, Y.;Yusheng, Z. and Lianshan, L. (2000).**RAPD genetics analysis on various domestic rabbit varieties/species. Chinese J. Rabbit Farming (3):15-18.

**Lynch, M. (1990):** The similarity index and DNA fingerprinting. MolBiolE. 7: 478-484.

**Mamuris Z.; Sfougaris A.I.; Stamatis C. and Suchentrunk F. (2002**) Assessment of genetic structure of Greek brown hare (Lepuseuropaeus) populations based on variation in random amplified polymorphic DNA (RAPD). Biochem. Genet. 40, 323-338.

**Nei M. (1972):** Genetic distance between populations. Am Nat 106:283-291.

**Nei, M. and Li, W. H. (1979).** Mathematical modeling for studying genetic variations in terms of restriction endonucleases. Proc. NatlAcadSci USA, 76: 5269-5273

**Rangoju, P.K.; Kumar, S.; Kolte, A. P.; Gulyani, R. and Singh, V.K. (2007):** Assessment of genetic variability among rabbit breeds by random amplified polymorphic DNA (RAPD) - PCR. World Rabbit Sci., 15: 3 – 8.

**Rongqing, P.;Zhilong, C.;Chenggong, C. andWanchen, L. (2000).**Analysis of random amplified polymorphic DNA in domestic rabbits. Chinese Journal of Veterinary Science 20(2):195-197.

**SAS (2004).** SAS Procedure Guide "Version 6.12 Ed." SAS Institute Inc., Gary,

**Welsh, J. and McClelland, M. (1990).** Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213-7218.

**Williams J.G.K.; Kubbelik A.R.; Livak K.J.; Rafalski J.A. and Tingey S.V. (1990):** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res., 18, 6531-6535.

**Yeh F.C., Yang R.C., Boyle T. B. J., Ye Z.H., Mao J.X.( 1999):** POPGENE, the user friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre University of Alberta, Canada.