

Full Length Research Paper

## Analysis of genetic diversity within and among four rabbit genotypes using biochemical and molecular genetic markers

Ola A. Galal<sup>1\*</sup>, Medhat Rehan<sup>1</sup> and Ragaa E. Abd El-Karim<sup>2</sup>

<sup>1</sup>Genetics Department, Faculty of Agriculture, 33516, Kafrelsheikh University, Kafr El-Sheikh, Egypt.

<sup>2</sup>Animal Production Research Institute, Agricultural Research Center (ARC), Dokki, Giza, Egypt.

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Genetic variations were detected in four different rabbit genotypes; Animal Production Research Institute (APRI) line, New-Zealand White (NZW), Baladi Black (BB) and Gabali (GAB) breeds. Ten individuals from the first three genotypes and seven individuals from GAB, were blood sampled. Isozymes (esterase and peroxidase) and sodium dodecyl sulphate (SDS)-protein markers were used to detect the genetic variations within these genotypes, whereas the random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) analysis using six random arbitrary primers were employed to assess the genetic variations and phylogenetic relationships among the four genotypes. The results show a variation in biochemical activity levels since, esterase profiles showed higher percentage of polymorphism (67.21%) than peroxidase (34.78%) and SDS-protein profiles (39.11 %). Moreover, the mean of the genetic similarity within the genotypes based on overall biochemical markers were 0.81 (APRI), 0.91 (NZW), 0.89 (BB) and 0.86 (GAB), which indicates high homogeneity within each of the four genotypes. The polymorphism percentage based on overall RAPD primers was 35.44% among these genotypes, which presents a kind of genetic diversity. From the dendrogram tree, the genotypes BB and GAB appeared to be closely related, while the APRI genotype was the most different.

**Key words:** Rabbits, genetic diversity, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), isozymes, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR).

### INTRODUCTION

Rabbit as a livestock animal, has its importance as supplier of meat, and it is widely accepted thought out the world for human consumption (Colin and Lebas, 1996). Assessment of genetic diversity forms an integral part of any successful breeding program. The genetic diversity

found in domestic breeds allows breeders to develop new characteristics in response to changes in environment, diseases or market conditions. A considerable number of genetic diversity studies for several livestock species have been carried out during recent years by research

\*Corresponding author. E-mail: [ola.galal@agr.kfs.edu.eg](mailto:ola.galal@agr.kfs.edu.eg) or [olagalal2002@yahoo.com](mailto:olagalal2002@yahoo.com), Tel/Fax: +2-0479102930.

**Abbreviations:** APRI, Animal Production Research Institute; NZW, New-Zealand White; BB, Baladi Black; GAB, Gabali; SDS, sodium dodecyl sulphate; RAPD-PCR, random amplified polymorphic DNA-polymerase chain reaction; FR, fertility rate.

teams from all over the world (Rendo et al., 2004; Zho et al., 2005; Cervini et al., 2006; Teneva et al., 2007; Cinkulov et al., 2008; Loukou et al., 2009). The genetic variability can be assessed by genetic markers. Genetic markers are distinguished on three principle classes: based on visually evaluated traits (morphological and productive traits), based on gene product (biochemical markers), and founded on DNA analysis (molecular markers). Morphological features are indications of the genotype but are represented by only a few loci because there are not a large enough number of characters available. Moreover, they can also be affected by environmental factors and growth practices. To overcome the limitations associated with morphological markers, various biochemical and molecular marker techniques have come up in recent years.

Protein electrophoresis has been an effective technique for the detection of genetic polymorphism for over three decades. The polymorphism of blood protein markers gives some useful information in studies of animal breeding such as the relationships among breeds and their evolution. Variation in proteins reflects changes in the genes that code for them. Protein electrophoresis has been widely used in studying genetic diversity (Atta et al., 2009). In this case, many researchers employed classical biochemical polymorphic markers such as polymorphic proteins (Machado et al., 2000; Han and Yuzhu, 2002) and isozymes (Menrad et al., 2002; Nyamsamba et al., 2003) to characterize and estimate the genetic diversity within and among livestock breeds.

On the other hand, random amplified polymorphic DNA (RAPD) is a technology of molecular genetics marker. As the technology is simple, quick, sensitive, little DNA consumption and low cost, it has been widely utilized in genetics analysis in animal, plant and microorganism and shown good results in population genetics diversity, genetic map construction, gene mapping and forecast of genetic distance and heterosis (Huber et al., 2002; Tarik et al., 2010). RAPD technology is principally used to study blood relationship between varieties/species in the rabbit. For instance, Liping et al. (2000) analyzed three domestic rabbit varieties/species; Rongqing et al. (2000) analyzed far or close evolutionary blood relationship among five rabbit populations; Liping et al. (2005) analyzed genetic relationship among three varieties/species and Rangoju et al. (2007) assessed the genetic variability and phylogenetic relationship among rabbit breeds.

Therefore, the present investigation was aimed to estimate the genetic diversity within (using isozymes and sodium dodecyl sulphate (SDS)-protein markers) and among (using RAPD markers) four rabbit genotypes received from Animal Production Research Institute in Sakha Station, Kafr El-Sheikh, Egypt.

## MATERIALS AND METHODS

### Animal material

The experimental materials involved in this study were four different genetic groups of rabbit: one line (APRI) and three breeds; New-Zealand White (NZW), Baladi Black (BB) and Gabali (GAB).

### *Animal Production Research Institute (APRI) line*

This line was developed in the Animal Production Research Institute in Egypt. The first step of development was to get F<sub>1</sub> coming from crossing Red Baladi bucks with dose of V line. The procedure continued getting F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub> and so on. After F<sub>3</sub>, the animals were named APRI line. In this line, the proportion of genes is 50% from V line and 50% from Red Baladi. The V line is housed at the stations of Sakha and Gimmiza and selected for litter weight at weaning. The Institute also keeps the APRI line at Sakha Station and was selected with the same criterion that was in V line.

### *New Zealand White (NZW)*

A foreign breed actually originated in the United States and wide spread overall the world.

### *Baladi Black (BB)*

These are Egyptian traditional breed that after their formation have not been followed by a program of genetic improvement (Khalil, 2002).

### *Gabali (GAB)*

Egyptian breed, recently studied by Afifi (2002) and Iraqi et al. (2007) showed very promising results.

A total of thirty seven mating rabbits were randomly selected for the trial; ten individuals from each of APRI line, NZW and BB breeds, in addition, seven individuals from GAB breed. All these rabbit genotypes were received from Animal Production Research Institute in Sakha Station, Kafr El-Sheikh, Egypt.

### Recorded traits

Four reproductive performance traits were measured on the selected individuals in each rabbit genotype. These traits are: litter size at birth and weaning, litter weight at weaning and fertility rate.

### Biochemical analyses

#### *Blood sample collection*

Approximately 1.5 ml blood sample was collected from each individual (from the central artery vein of the ear) in centrifuge tubes containing heparin sodium as anticoagulant reagent and then preserved in a -20°C freezer. Plasma serum has been obtained by centrifugation at 5000 rpm for 15 min at 4°C and the plasma protein (supernatant) has been transferred to clean tubes and stored at –

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

Primer code	Sequence (5'→3')
OP-A9	GGGTAACGCC
OP-B5	TGCGCCCTTC
OP-B7	GGTGACGCAG
OP-B8	GTCCACACGG
OP-B10	CTGCTGGGAC
OP-B14	TCCGCTCTGG

20°C until the time of electrophoretic analysis.

### Isozymes

The collected blood serum samples were used for detecting isozyme variation among the selected individuals within each rabbit genotype. Two isozyme systems (that is, esterase and peroxidase) were applied in non-denaturing polyacrylamide gel electrophoresis. Esterase isozymes were localized on the gel using  $\alpha$  and  $\beta$ -naphthyl acetate (40 mg of each) and 250 mg Fast blue RR dissolved in 98 ml of 0.1 M phosphate buffer (pH 6.5). However, peroxidase isozyme patterns were determined using the staining solution composed of 250 mg of benzidine dihydro chloride (moistened with 4 drops of glacial acetic acid) in 100 ml H<sub>2</sub>O and 10 drops of 1% H<sub>2</sub>O<sub>2</sub> was added immediately before being used according to Scandalios (1964).

### Sodium dodecyl sulphate (SDS)-protein

The same collected samples were used to detect protein polymorphism within each genotype. Samples were applied to 12% SDS-polyacrylamid gel electrophoresis. Gel preparation, electrophoresis conditions, staining and destaining gels were done according to Laemmli (1970). Protein fractionations were performed exclusively on vertical slab gel using pre-stained high molecular weight standard marker with molecular weight ranged from 175 to 15 KDa. The stained gels were photographed and examined for the presence and absence of visualized bands.

### Molecular analysis

#### DNA extraction

Genomic DNA was isolated from approximately 200  $\mu$ l of blood samples using Biospin Whole Blood Genomic DNA Extraction Kit (Bioflux Corporation, Arrow Plaza, Tokyo, Japan). The genomic DNA of all collected samples of each genotype was mixed in a bulked sample according to Lukyanov et al. (1996).

#### Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR)

Six decamer RAPD primers (OP-A9, B5, B7, B8, B10 and B14) were screened on pooled rabbit DNA. All primers were purchased from Bio Basic Inc, Canada. The list of primers and their sequences are presented in Table 1.

The optimization of PCR conditions for each primer was performed in a 25  $\mu$ l reaction volume including 0.5  $\mu$ l of isolated DNA template. Final concentrations were 1 x buffer (Mg<sub>2</sub><sup>+</sup> free), 1.5 mM MgCl<sub>2</sub>, 200  $\mu$ M dNTPs mix, 0.8  $\mu$ M primer, 1 U Taq DNA Polymerase (ROVALAB, Germany). Amplifications were carried out in a thermal cycler according to manufacture instructions as follow: the initial amplification program started with denaturation at 94°C for 5 min, followed by 35 cycles consisting of 30 s of denaturation at 94°C, 45 s of annealing at 30°C and 1.5 min of elongation at 72°C. The program ended with a final elongation step at 72°C for 2 min. Amplification products were separated on 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light. A known DNA Ladder (50 bp DNA Ladder ready-to-use, Cat-no: 300003, GeneON) was run against the PCR products.

### Data analyses

#### Quantitative traits

The recorded data for reproductive traits were subjected to a one-way ANOVA using Statistical Package for the Social Sciences (SPSS) program and the significance level adopted was 95%. Results were expressed as mean  $\pm$  standard errors (SE).

#### Genetic relationships

The data generated from isozymes (native gel) and protein banding patterns, in addition; the banding patterns of the six RAPD primers were introduced to SPSS package program according to binary values of (1) and (0) for the presence and absence of bands, respectively. The genetic similarity within each genotype was assessed based on biochemical analyses on the basis of the Dice similarity coefficient. The phylogenetic relationship among rabbit genotypes was conducted based on the similarity coefficients of RAPD primers data using UPGMA analysis.

## RESULTS AND DISCUSSION

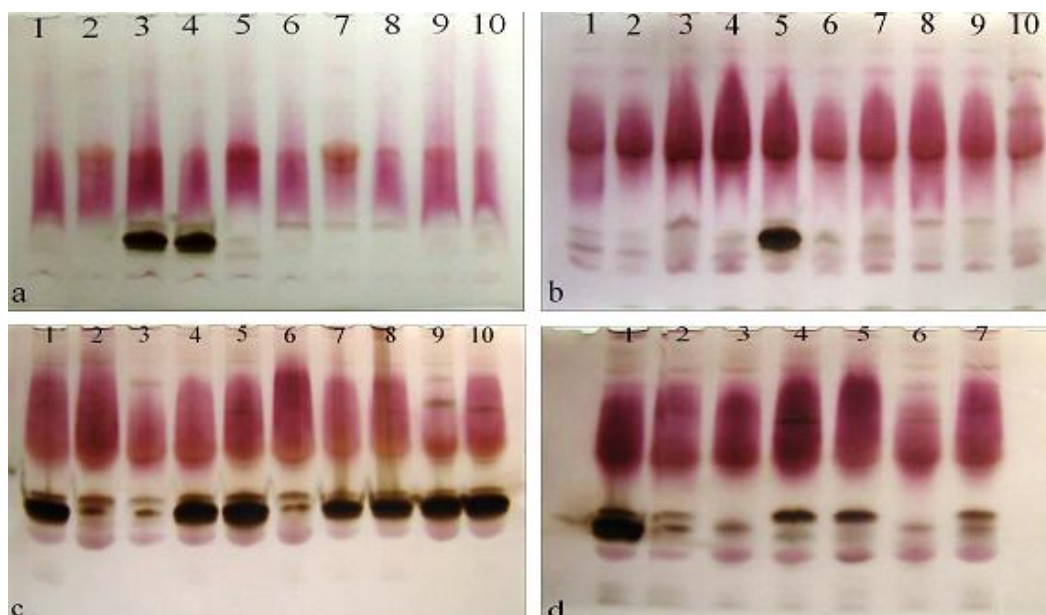
### Reproductive performance analyses

The indicative data of reproductive performance in the four rabbit genotypes were taken not to compare performances between them but to know the level and range of variation of the traits among the four genotypes. Table 2 shows the reproductive performance of the four rabbit genotypes used. Data showed that there were no significant differences for all studied traits among the four genotypes, except the litter size at weaning. Analysis of variance showed significant differences for this trait between the two genotypes (APRI and NZW) and the other genotypes (BB and GAB). Similarly, Argente et al. (2010) did not find relevant differences in fertility rate (FR) between two lines of rabbits divergently selected for uterine capacity. A similar FR was also found in previous results in the same lines (Moce et al., 2004; Peiro et al., 2007) and by other authors using other lines with different embryo survival (Bolet and Theau-Clement, 1994).

**Table 2.** Means of the four reproductive performances traits of different rabbit genotypes.

Genotype	Litter size (No)		Litter weight at weaning (g)	Fertility rate (%)
	At birth	At weaning		
APRI (10)	7.20 ± 0.99	5.60 ± 1.06 a	360.00 ± 49.47	57.50 ± 10.57
NZW (10)	5.50 ± 0.92	4.55 ± 0.78 a	227.50 ± 38.81	67.50 ± 9.89
BB (10)	5.20 ± 1.56	1.95 ± 0.97 b	260.00 ± 78.19	42.50 ± 10.57
GAB (7)	4.75 ± 2.49	0.25 ± 0.25 b	237.50 ± 124.37	62.50 ± 16.14

These traits are produced from ten individuals from APRI, NZW, BB and seven individuals from GAB. Different letters indicate significant differences ( $p < 0.05$ ) between mean values.



**Figure 1.** Esterase electrophoretic patterns of the four rabbit genotypes in non-denaturing gel electrophoresis. a, b and c, Ten individuals from APRI, NZW and BB genotypes, respectively; d, seven individuals from GAB genotype.

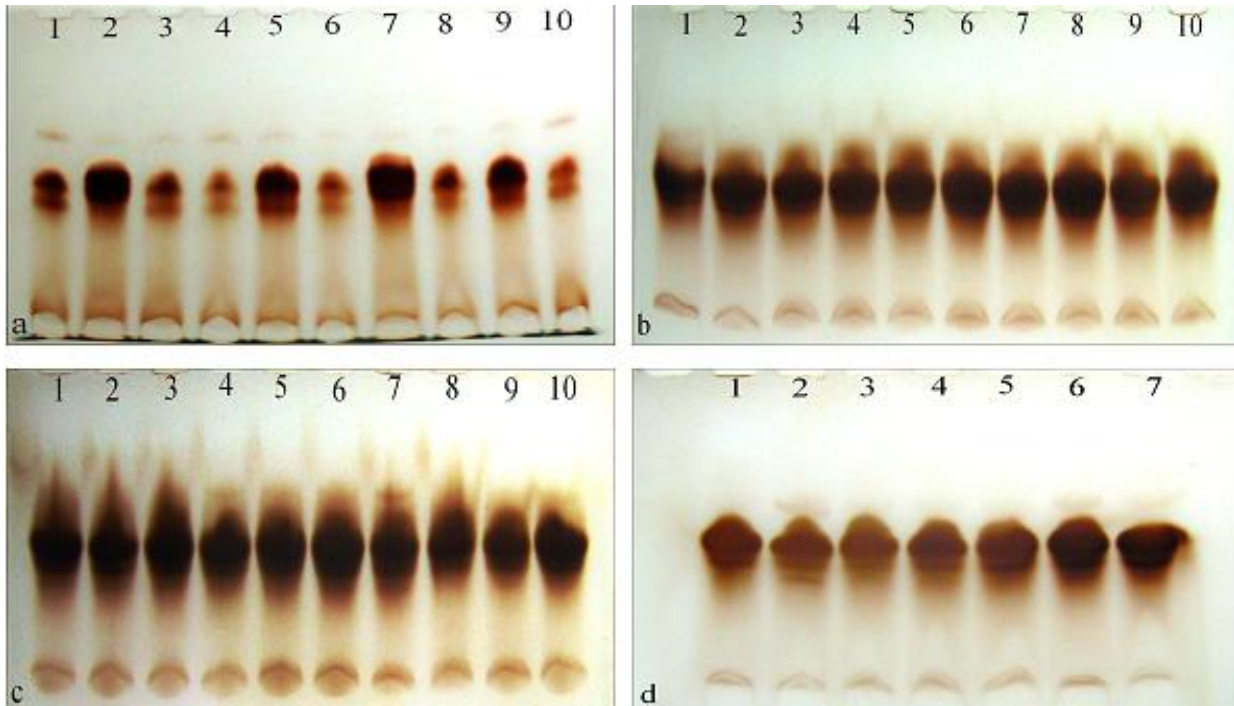
### Genetic variations within rabbit genotypes based on biochemical markers

#### *Isozymes and SDS-PAGE polymorphism within rabbit genotypes*

In the present study, two isozyme systems of esterase and peroxidase were used to detect the genetic variability within the four studied rabbit genotypes (Figures 1 and 2). One band has assumed to be corresponded to one locus (allele). The electrophoretic patterns of esterase enzyme revealed marked polymorphism within the studied rabbit genotypes as shown in Table 3. The four genotypes contained 61 alleles, APRI line contained 14 alleles, and the two breeds NZW and BB had 15 alleles, while GAB breed contained the highest number of

esterase alleles (17 alleles). Percentage of polymorphic loci within the four genotypes ranged from 53.33 to 85.71%. APRI line showed the highest polymorphic bands (85.71%); 12 out of the 14 loci scored were polymorphic, while NZW breed revealed the lowest polymorphic bands (53.33%); 8 out of the 15 loci scored were polymorphic. Deza et al. (2000) studied esterase variations among some native goats from different locations and found individual differences among goat samples using this isozyme marker. Moreover, esterase had a value to discriminate among (El-Seoudy et al., 2005) and detect individual variations within (Anous et al., 2008) goat populations.

Concerning peroxidase marker, levels of polymorphism differed a little within genotypes. A total of six loci ranged from 4-6 were detected for APRI line as well as NZW and



**Figure 2.** Peroxidase electrophoretic patterns of the four rabbit genotypes in non-denaturing gel electrophoresis. a, b and c, Ten individuals from APRI, NZW and BB genotypes, respectively; d, seven individuals from GAB genotype.

GAB breeds. However, out of these loci, four loci were monomorphic and presented in all individuals whereas only two loci were polymorphic (33.33% polymorphism). On the other hand, the profile of BB breed blood serum exhibited only five loci ranged from 3-5 with 40.00% polymorphism. These results indicated that this enzymatic system could not be used alone to differentiate among the tested individuals. The same trend was observed by Nyamsamba et al. (2003) since they could not obtain any clear genetic differences among eight native goat populations by studying their isozyme variations.

The protein banding patterns of the four rabbit genotypes revealed wide variations of different bands as shown in Table 3 and Figure 3. The maximum number of bands (55 bands) was found in GAB breed, while the minimum numbers of bands (38 bands) were obtained in BB breed which showed the lowest polymorphic ratio (21.05%). The total number of bands of BB breed were only 38 bands, out of them 30 bands were monomorphic. These bands which were observed in all samples are common bands in this breed. On the other hand, the highest polymorphic ratio was found to be 75.61% in APRI line, while NZW and GAB breeds revealed moderate polymorphic bands (33.33 and 29.09%, respectively). Recently many authors have reflected on this point such as Anous et al. (2008) who assessed the

genetic structure within each of three Egyptian goat populations using serum protein marker. They concluded that protein analysis is a sensitive method for studying the genetic structure of goat populations.

Concerning all biochemical markers, most of polymorphisms were due to esterase alleles. Esterase had 20 monomorphic loci and 41 polymorphic loci (67.21% polymorphism) indicating high esterase variation. However, peroxidase exhibited the lowest level of polymorphism among the studied genotypes, only 8 out of the 23 loci scored were polymorphic (34.78%) and 15 loci were monomorphic indicating low peroxidase variation. A total of 179 bands were generated using protein analysis, 70 bands were polymorphic (39.11%), however, 109 bands were monomorphic (common) for all genotypes indicating moderate protein variation. The results suggested that esterase marker was a powerful method to evaluate genetic variability within rabbit genotypes. Similar result was observed by El-Seoudy et al. (2008) who detected lower genetic differences using native protein marker than six isozyme systems within the examined animals of three Egyptian camel breeds.

#### **Genetic similarity within rabbit genotypes**

The ranges and averages of similarity values within each

**Table 3.** Polymorphism percentages generated by esterase and peroxidase isozymes as well as protein marker in the four rabbit genotypes.

Genotype	Total band	Range of band product	Monomorphic band	Polymorphic band	Polymorphism (%)
Esterase marker					
APRI (10)	14	5 – 10	2	12	85.71
NZW (10)	15	9 – 12	7	8	53.33
BB (10)	15	8 – 14	6	9	60.00
GAB (7)	17	8 – 14	5	12	70.59
Total	61	5 – 14	20	41	67.21
Peroxidase marker					
APRI (10)	6	4 – 6	4	2	33.33
NZW (10)	6	4 – 6	4	2	33.33
BB (10)	5	3 – 5	3	2	40.00
GAB (7)	6	4 – 6	4	2	33.33
Total	23	3 – 6	15	8	34.78
Protein marker					
APRI (10)	41	23 – 33	10	31	75.61
NZW (10)	45	37 – 42	30	15	33.33
BB (10)	38	32 – 38	30	8	21.05
GAB (7)	55	46 – 50	39	16	29.09
Total	179	23 – 50	109	70	39.11

Esterase enzymes showed the highest ratio of polymorphism followed by protein marker and peroxidase enzyme.

**Table 4.** Similarity ranges and averages within each of the four genotypes based on biochemical markers.

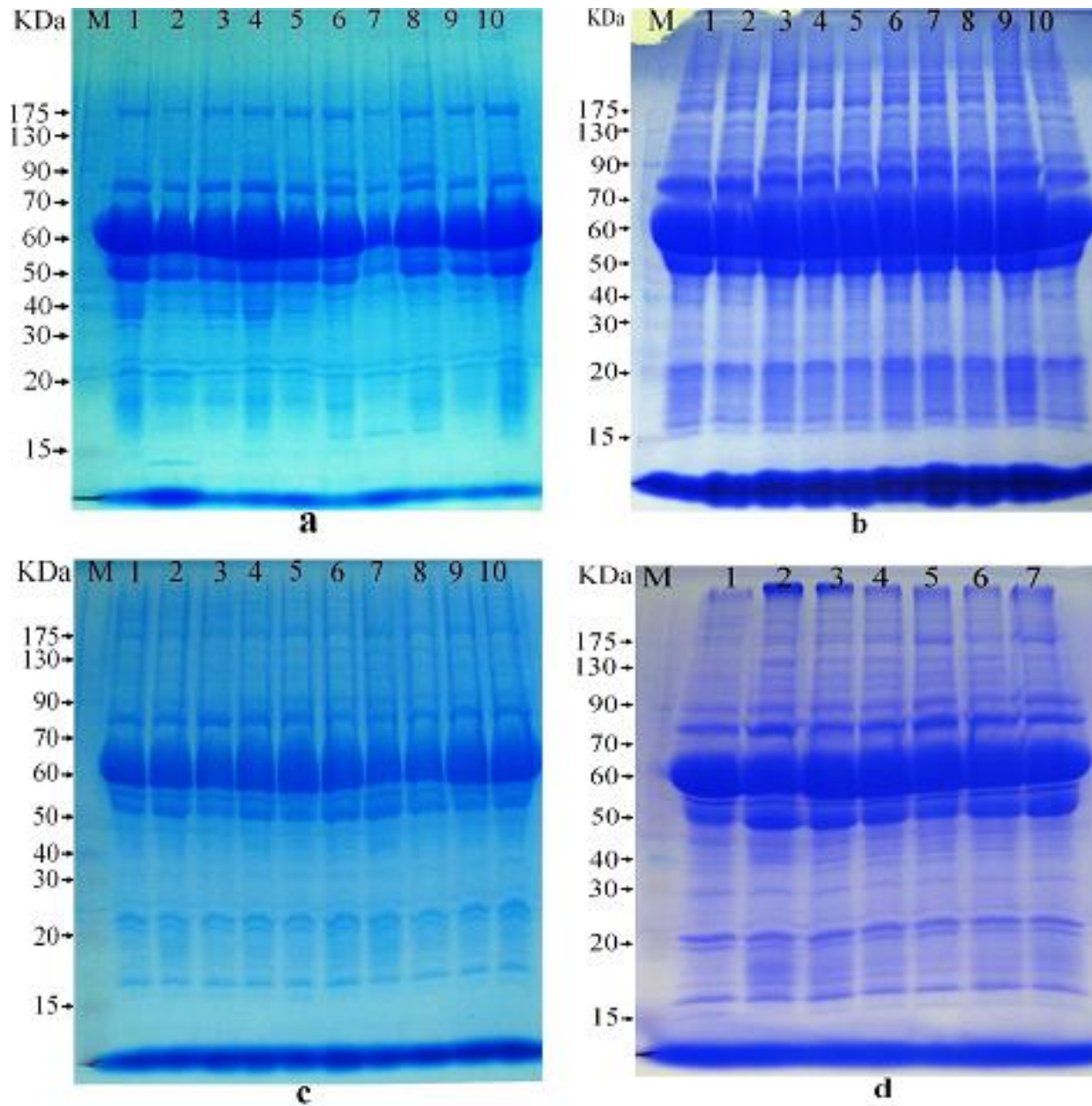
Genotype	Esterase marker		Peroxidase marker		Protein marker		All markers average
	Range	Average	Range	Average	Range	Average	
APRI	0.43 – 0.90	0.73	0.80 – 1.00	0.90	0.64 – 0.96	0.80	0.81
NZW	0.70 – 1.00	0.86	0.80 – 1.00	0.93	0.87 – 1.00	0.93	0.91
BB	0.67 – 0.95	0.80	0.75 – 1.00	0.91	0.91 – 1.00	0.95	0.89
GAB	0.61 – 0.88	0.77	0.80 – 1.00	0.88	0.90 – 0.99	0.94	0.86

These values reflect high ratio of homogeneity and inbreeding in all studied genotypes.

of the four genotypes based on isozymes and protein markers are listed in Table 4. The average of similarity indices reflects the range of homogeneity and inbreeding within each tested genotypes. Esterase and protein markers revealed that APRI line had the lowest similarity average among the ten selected individuals within the line. These results are constant with the results of polymorphism which showed that APRI line had the highest polymorphism based on esterase and protein markers. Based on esterase marker, the highest genetic similarity average was found within NZW breed (0.86), while the lowest one was observed within APRI line (0.73). How-

ever, BB and GAB breeds showed genetic similarity averages with 0.80 and 0.77, respectively. Concerning peroxidase marker, all genotypes revealed high similarity averages ranging from 0.88 (GAB) to 0.93 (NZW), which indicated high homogeneity within each genotype. Using protein marker also gave high similarity averages within the tested genotypes. However, the lowest genetic similarity averages were found within APRI line (0.80).

The general mean of the genetic similarity within the studied genotypes based on biochemical markers overall was 0.91 (NZW), 0.89 (BB), 0.86 (GAB) and 0.81 (APRI), which indicated the high degree of homogeneity and in-



**Figure 3.** The protein banding patterns of the four rabbit genotypes in SDS-PAGE. a, b and c, Ten individuals from APRI, NZW and BB genotypes, respectively; d, seven individuals from GAB genotype. M, Protein molecular marker ranged from 15 to 175 KDa.

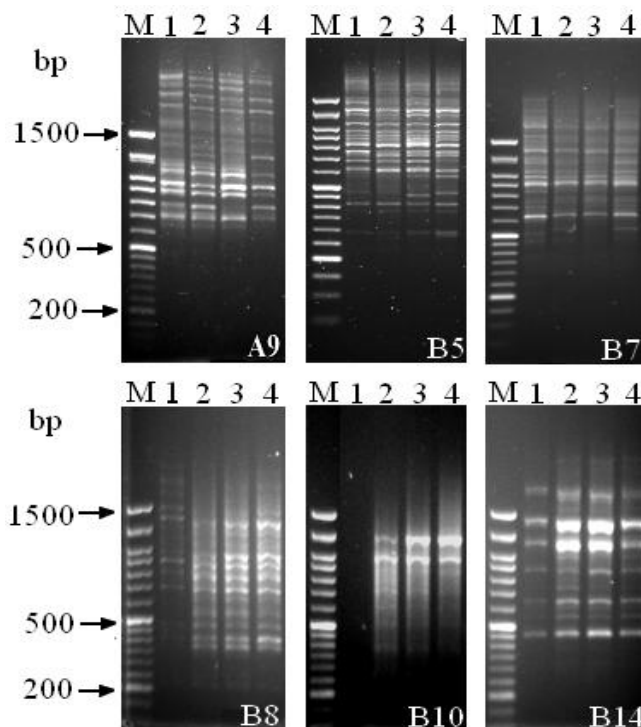
inbreeding within each one of the four studied genotypes.

#### **Genetic variations among rabbit genotypes based on RAPD markers**

##### ***RAPD polymorphism among rabbit genotypes***

RAPD amplifies the target genomic DNA, with a short

arbitrary primer (commonly 10 bp) in a PCR reaction, can be used to produce relatively complicated DNA profiles for detecting amplified fragment length polymorphisms between organisms. Since the arbitrary primers complement different parts of the genomic DNA, PCR products will differ in number and size (polymorphism). In this study, six primers were used to assess the genetic variability among the four rabbit genotypes (Figure 4)



**Figure 4.** DNA-RAPD patterns using six arbitrary primers with the four rabbit genotypes; APRI (lane 1), NZW (lane 2), BB (lane 3) and GAB (lane 4). M refers to the DNA ladder.

**Table 5.** Level of polymorphism among the four rabbit genotypes based on RAPD analysis.

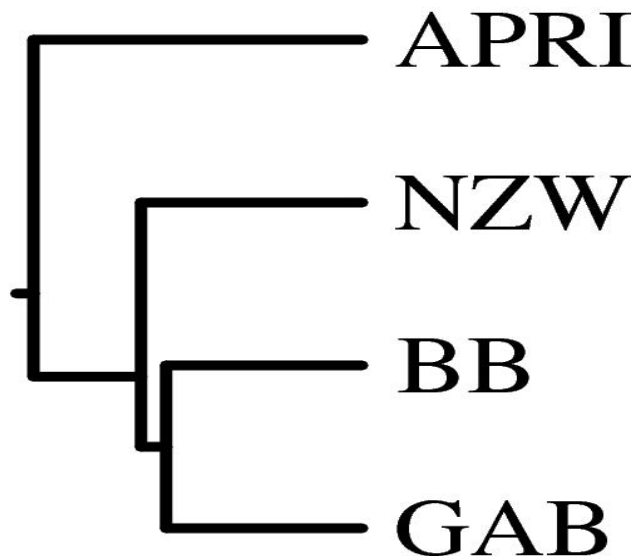
Primer	TAF	PB	MP	P %	AF			
					APRI	NZW	BB	GAB
OP-A9	17	7	10	41.18	16	17	15	12
OP-B5	19	6	13	31.58	16	17	16	14
OP-B7	16	4	12	25.00	16	13	13	14
OP-B8	14	6	8	42.86	11	10	11	12
OP-B10	3	3	0	100.0	0	3	3	3
OP-B14	10	2	8	20.00	8	10	9	10
Total	79	28	51	35.44	67	70	67	65
					269			

Primer B10 showed the highest value of polymorphism while primer B14 showed the lowest value of polymorphism. TAF, Total amplified fragment; PB, polymorphic bands; MP, monomorphic bands; P%, polymorphism %; AF, amplified fragment.

which generated a total of 269 DNA fragments. Twenty eight bands (35.44 %) were polymorphic, however, fifty one bands were monomorphic (common) for all genotypes. The highest level of polymorphism (100%) was observed in primer OP-B10; since the APRI genotype did not give any band reaction with this primer while the other

three genomes gave two distinct bands at least. Moreover, the lowest level of polymorphism was 20% in primer OP-B14 as shown in Table 5. RAPD-PCR fingerprints have been successfully used in defining genetic diversity among different species of horse, buffalo, beef, venison, rabbit, and kangaroo (Yang et al., 2013).





**Figure 5.** Dendrogram showing genetic relationships among the four rabbit genotypes based on similarity indices derived from RAPD analysis using UPGMA software.

### Genetic relationships among rabbit genotypes

The obtained variations from RAPD analysis was used to construct a genetic distance tree (Figure 5) based on the amount of shared alleles between all pairs of individuals. The results indicated that BB genotype was closely related with GAB breed, while the APRI genotype was the most different. This may be due to the fact that BB and GAB are Egyptian genotypes. This result is in constant with performance results for litter size at weaning. This was in agreement with Keliang et al. (2008) who found that RAPD marker correlated with reproductive performances in Rex rabbit. These results indicated that RAPD primers revealed a kind of genetic diversity among these genotypes, which suggested that RAPD markers can be used as a tool to understand the genetic variability and phylogenetic relationship among rabbit genotypes. Knowledge of the genetic distances among different genotypes is very useful for genetic improvement (Ceron and Angel, 2001).

In conclusion, the results of this study indicated that there is a low genetic variation within each of the four rabbit genotypes based on biochemical markers. Esterase enzymes showed the highest ratio of polymorphism followed by protein marker and peroxidase enzyme. Moreover, RAPD analysis can be successfully applied to study genetic diversity and relationships among the four genotypes. This could have a great impact in rabbit breeding programs.

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