

## **STUDY OF SOME MOLECULAR MARKERS ASSOCIATED WITH REPRODUCTIVE PERFORMANCE OF APRI LINE, BALADI BLACK AND NEW-ZELAND WHITE RABBITS**

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### **SUMMARY**

*Random amplified polymorphic DNA (RAPD), using 15 arbitrary primers, was used to investigate three rabbit genotypes namely Animal Production Research Institute (APRI) line, Baladi Black breed (BB) and New-Zealand White breed (NZW) to detect molecular genetic markers which could be used to distinguish among the studied genotypes. Moreover associations between these markers and the studied genotypes reproductive performance traits (Litter size, living litter size, birth weight and birth litter size) were performed. DNA banding pattern indicated that APRI line did not give any band reaction with primers OPA2 and OPA7, while the other two genotypes gave two distinct bands at least. So, these primers could be used as associated markers for litter size, living litter size, and birth litter size traits. The result of RAPD patterns generated, in total, 104 bands, out of them 62 were polymorphic. APRI line and (BB) genotypes shared most of the bands. This support their similar origin, since they have the same common ancestors. However, the other genotype (NZW) has different pattern than other genotypes. The phylogenetic tree indicated that both genotypes APRI line and (BB) were highly similar than (NZW) which was highly diverged than other genotypes. This investigation indicates that RAPD markers could be used to characterize genetically different rabbits and indicate association with some reproductive performance.*

**Keyword:** *rabbit, molecular genetics marker, RAPD, reproductive traits, phylogenetic tree.*

### **INTRODUCTION**

Rabbits are considered as a source of meat production in Egypt through developing animal production using semi-ruminant species. FAO (1981) stated that rabbits, which fall under the previous group, as short-cycle animals, have the ability to be a source of meat, by increasing their production.

Reproduction is essential for all species as it is the key for survival and evolution. Overall processes of sexual reproduction are relatively conserved and well defined, but the underlying molecular and genetic basis of each of the numerous steps involving these general processes is much less known. The complexity and transient nature of specific reproductive processes are the cause of reduced knowledge concerning their molecular basis.

Recently, a synthetic paternal rabbit line, named Alexandria, was originated by crossing a V-line with a Baladi Black rabbits. Individual selection for daily gain from weaning (28 days) to slaughter age (63 days) was used as a selection criterion for the genetic improvement for this line (El-Raffa, 2005 and 2007).

From a productive point of view, reproductive efficiency is one of the most important factors affecting productivity in livestock industries. Reproductive traits, especially those related with fertility, litter size and pre-weaning viability, are important

components for reducing the costs of producing animal meat (De Vries, 1989; Rothschild and Bidanel, 1998). Therefore, much effort is made to identify the ways for improving these traits. This improvement of reproductive traits is related to different fields, with management, nutrition and genetics having a great impact.

(RAPD) analysis has become common technique for identifying the markers linked to traits of interest without the necessity for mapping the entire genome (Bardakci, 2001). These markers have been used for genotype identification (Tinker *et al.*, 1993), construction of genetic maps (Maddox and Cockett, 2007), analysis of the genetic within and among populations (Tsuda *et al.*, 2001; Hardy *et al.*, 2006), and in detecting polymorphism in different poultry species (Salem *et al.*, 2005).

In this concept, Williams *et al.* (1990) introduced the DNA polymorphisms amplified by arbitrary primers as genetic markers to be used in genetic maps and the use of this type of markers increased rapidly. Simplicity, applicability and low cost of the RAPD technique gave this technique wide range of applications in many areas of genetics and molecular biology. Also, RAPD technique provides a useful approach for evaluating genetic differences particularly in species that are poorly known genetically (Sale *et al.*, 1996 and Wen and Hsiao, 1999), in genetic identification

(Kresovich *et al.*, 1992), in introgression studies (McCoy and Echt, 1993), in parental test (Welsh *et al.*, 1991).

The aim of this study was to investigate the association between markers and reproductive performance in three rabbit breed APRI line, (BB) and (NZW) breed using RAPD technique.

## MATERIALS AND METHOD

The present study was conducted at the rabbit of the Animal Production Research Station Sakha, Animal Production Research Institute, Agriculture Research Center, Egypt. This study aimed to evaluate the reproductive and molecular traits in the APRI line, (BB) and NZW rabbit breed.

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

This line was developed at the Animal Production Research Institute in Egypt. The first step of development was to get an F1 from crossing Red Baladi bucks with does of V line. The procedure continued getting F1, F2, F3 and so on. After F3, 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 maintained at the stations Sakha and Gimmiza and selected for litter weight at weaning. The Institute also keeps the APRI line at Sakha Station and was selected for the high litter weight at weaning.

### *Baladi Black (BB):*

It is a local Egyptian breed; it was founded at the Poultry Breeding Section stations, Ministry of Agriculture (Egypt) through crossbreeding for several generations between native rabbits and Flemish Giant (Badawy, 1975 and Galal & Khalil, 1994).

### *New Zealand White (NZW):*

A foreign breed actually originated in the United States and it is wide spread all over the world.

### *Reproductive data recording:*

Doe reproductive performance and the data of 256 litter (69 litters from APRI strains, 87 litters from New Zealand white (NZW) and 100 litters from Baldi Black (BB) were recorded throughout the breeding seasons. Day of delivery, gestation period, number of total born and born-alive per delivery, litter weight of born alive, litter size and weight at 21 d of age, pre-weaning mortality, and both litter size, and weight at weaning were also recorded.

The following data were then calculated: conception rate (number of service pregnancy), fertility rate (percentage of deliveries/mating), viability rate at birth (number of live born/total number of kids X 100), individual weight of born alive, at 21 d of age, and at weaning.

### *Blood sample collection and DNA extraction:*

Individual blood samples were collected from the vein near the ear edge in 3ml vacuotainer tube included EDTA K3. DNA of collected blood samples were extracted from the whole blood using the E.Z.N.A. Blood DNA Midiprep Kits following the manufacture instructions (Omega Bio-tek 400 Pinnacle Way, Suite 450 Norcross, GA 30071 USA).

### *PCR reaction and separation of RAPD product:*

The PCR reactions using 15 arbitrary oligonucleotide primers (10-mer) that were used in this study are shown in Table (1). The reactions were optimized and mixtures were prepared according to (Keliang *et al.*, 2008) The PCR products were separated by electrophoresis using 1.2% agarose gel in 0.5 x TBE buffer against 100 bp DNA Ladder as a size marker. Bands were detected with ethidium bromide staining and documented on Gel Documentation.

**Table 1. List of Primers used for RAPD analysis and their nucleotide sequences**

S. No.	Primer code	Sequence
1	OPA1	5'-ACA GGT GCT G-3'
2	OPA2	5'-ACG CCA GAG G-3'
3	OPA3	5'-ACG GCG TAT G-3'
4	OPA4	5'-CAG ACA AGC C-3'
5	OPA5	5'-CAG CTC ACG A-3'
6	OPA6	5'-CCC AGC TAG A-3'
7	OPA7	5'-TGG CGC AGT G-3'
8	OPA8	5'-CTG GGC ACG A-3'
9	OPA9	5'-GAC TAG GTG G-3'
10	OPA10	5'-GGT CTA CAC C-3'
11	OPA11	5'-GTC GCC GTC A-3'
12	OPA12	5'-TGC GCC CTT C-3'
13	OPA13	5'-CTT CCC CAA G-3'
14	OPA14	5'-TTC CGC CAC C-3'
15	OPA15	5'-TTC GAG CCA G-3'

**Data Analysis:**

Amplification profiles for the tested genotypes as a result of RAPD application were compared with each other and DNA fragments were scored as a binary data. The electrophoretic patterns of the reproducible banding patterns of each primer which were produced by RAPD were chosen for analysis. Each band was scored as present (1) or absent (0), and pairwise comparisons between individuals were made to calculate the Jukes-Cantor coefficient using PAST program (Paleontological Statistics Version 1.94b) adapted by Hammer *et al.* (2001). Cluster analysis was performed to produce a dendrogram using unweighted pair-group method with arithmetical average (UPGMA) algorithm (Santi, 2002).

**RESULTS AND DISSECTION****Reproductive performance of does:**

Data presented in Table (2) show the reproductive performance of the three rabbit breeds (APRI Line, Baldi Black (BB) and New Zealand White (NZW)). Total litter size, born alive, total weight at birth, litter size at 21d and total weight at 21d were significantly ( $P>0.001$ ) higher in APRI line than in other two breed, however the same traits were significantly ( $P>0.001$ ) higher in (NZW) than in Baldi Black rabbit. While, weight at birth was significantly ( $P>0.042$ ) higher in (NZW) (54.09 g) than in APRI line (51.94 g) and Baldi Black (52.01 g) Table (2).

On the other hand, litter size at weaned, weaning weight and total weaning weight were significantly ( $P>0.001$ ) higher in APRI line than in other two breed.

**Table 2. Main effect of breed on rabbit reproductive performance**

Parameters	New Zealand White (NZW)	APRI Line	Baldi Black (BB)	Level of significant
Total litter size at birth (n)	6.82±0.13 <sup>b</sup>	7.65±0.22 <sup>a</sup>	6.25±0.18 <sup>c</sup>	0.001
Born alive (n)	6.56±0.12 <sup>b</sup>	7.43±0.21 <sup>a</sup>	5.99±0.16 <sup>c</sup>	0.001
Weight at birth (g)	54.09±0.79 <sup>a</sup>	51.94±0.80 <sup>b</sup>	52.01±0.48 <sup>b</sup>	0.042
Total weight at birth (g)	356.24±9.14 <sup>b</sup>	383.69±9.93 <sup>a</sup>	310.32±8.52 <sup>c</sup>	0.001
Litter size at 21d (n)	5.24±0.13 <sup>b</sup>	6.23±0.17 <sup>a</sup>	4.61±0.17 <sup>c</sup>	0.001
Weight at 21d (g)	235.9±4.27 <sup>b</sup>	240.22±6.55 <sup>ab</sup>	253.89±4.61 <sup>a</sup>	0.024
Total weight at 21d (g)	1228.85±34.13 <sup>b</sup>	1482.85±51.78 <sup>a</sup>	1118.55±32.99 <sup>c</sup>	0.001
Litter size at weaning (n)	4.44±0.15 <sup>b</sup>	5.36±0.17 <sup>a</sup>	4.07±0.16 <sup>b</sup>	0.001
Weaning weight (g)	544.0±10.19 <sup>a</sup>	479.8±11.18 <sup>b</sup>	565.01±10.72 <sup>a</sup>	0.001
Total weaning weight (g)	2415.36±65.63 <sup>b</sup>	2571.73±96.97 <sup>a</sup>	2299.59±78.07 <sup>b</sup>	0.012

**Detection of genetic variation among rabbit genotypes using RAPD's markers:**

On the basis of these characteristics, two different approaches were used to extract statistical information from the RAPD data. The first one corresponds to the direct study of RAPD bands presence or absence. We will refer to it as the 'band-based' approach. The second one, that we call the 'allele frequency-based' approach, consists of estimating allelic frequencies, at each locus, which appear from polymorphic percentage. This will be used to survey genetic diversity or differentiation with classical population genetics methods.

Arbitrary primers complement different parts of the genomic DNA. PCR products differ in band number and size. In this study, 15 primers were used to assess the genetic variability among the three rabbit genotypes (Figure 1) it generated a total of 104 bands. Sixty two bands (59.61 %) were polymorphic; however, forty two bands were monomorphic (common) for all genotypes. The highest level of polymorphism (100%) was observed in primer OPA2 and OPA7. The APRI genotype did not give any band reaction with this primer. However, the other two genomes gave at least two distinct bands. Thus, we could use these primers for litter size traits and associated marker for this character. Moreover, the lowest

level of polymorphism was 40%, associated with primer OPA14 as shown in Table (3). 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).

So, we could consider the RAPD as primary molecular marker to study the association between rabbit populations and some reproductive traits like litter size, living litter size, and birth litter size. This is indicated by the absence and presence of some bands.

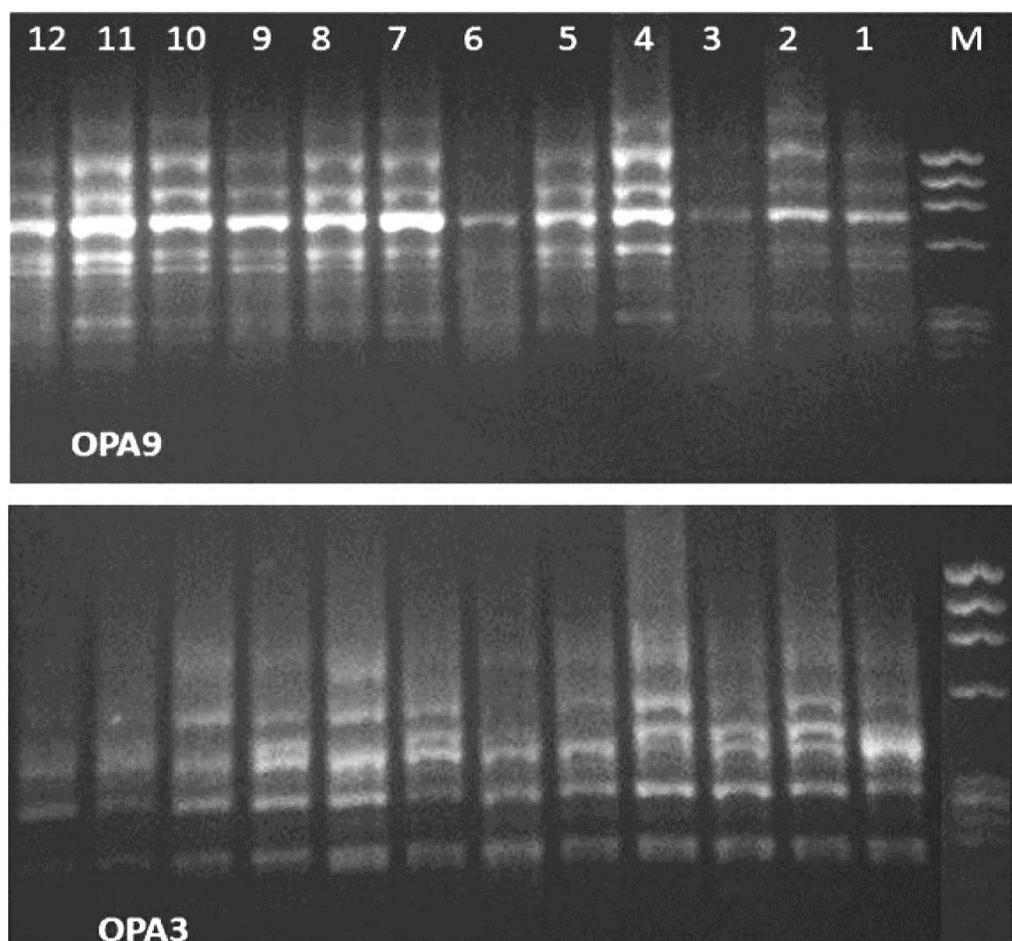
Primers OPA2 and OPA7 showed the highest polymorphism while primer OPA14 showed the lowest polymorphism. TNB, total number bands; PB, polymorphic bands; MP, monomorphic bands; P%, polymorphism

**Genetic relationships among the studied rabbit genotypes:**

The obtained variations from RAPD analysis was used to construct phylogenetics tree (Table 4 and Figure 2) based on the amount of shared alleles between all pairs of individuals. The results indicated that the similarity index was so high within each group for example it was 97% within BB group followed by 94% within NZW group and 93% within APRI group. Furthermore,

**Table 3. Numbers and types of the amplified DNA bands as well as the total polymorphism percentage obtained by using the 15 selected RAPD primers**

Primer code	TNB	PB	MB	% P
OPA1	6	5	1	83.30
OPA2	6	6	0	100.0
OPA3	6	3	3	50.00
OPA4	4	2	2	50.00
OPA5	12	5	7	41.00
OPA6	7	4	3	57.00
OPA7	4	4	0	100.0
OPA8	9	4	5	44.4.0
OPA9	9	4	5	44.4.0
OPA10	9	6	3	66.66
OPA11	7	5	2	71.42
OPA12	6	4	2	66.66
OPA13	7	4	3	57.14
OPA14	5	2	3	40.00
OPA15	7	4	3	57.00
Total	104	62	42	59.61



**Figure 1. RAPD banding patterns of the three rabbit genotypes for the tow primers OPA9 and OPA3 Lanes from: 1 to 4 represent APRI line (lane 1 Sir, 2,3,4 daughters) , 5 to 8 NZW breed (lane 5 Sir, 6,7,8 daughters) and from 9 to 12 BB breed (lane 9 Sir10,11,12 daughters) and (M) stander marker 100 bp ladder.**

the lowest similarity was between APRI and NZW with similarity 68%. APRI genotypes (male and its related females) were closely related to each other and core close to the BB breed, while the NZW genotypes were the most

different and highly diverged from the other two breeds. This may be due to the fact that the BB and APRI are Egyptian genotypes. This result is constant out with the performance results for litter size at weaning. This is 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 the genetic improvement (Ceron and Angel, 2001). The similarity index was 68% as the lowest values between APRI and NZW genotypes indicating

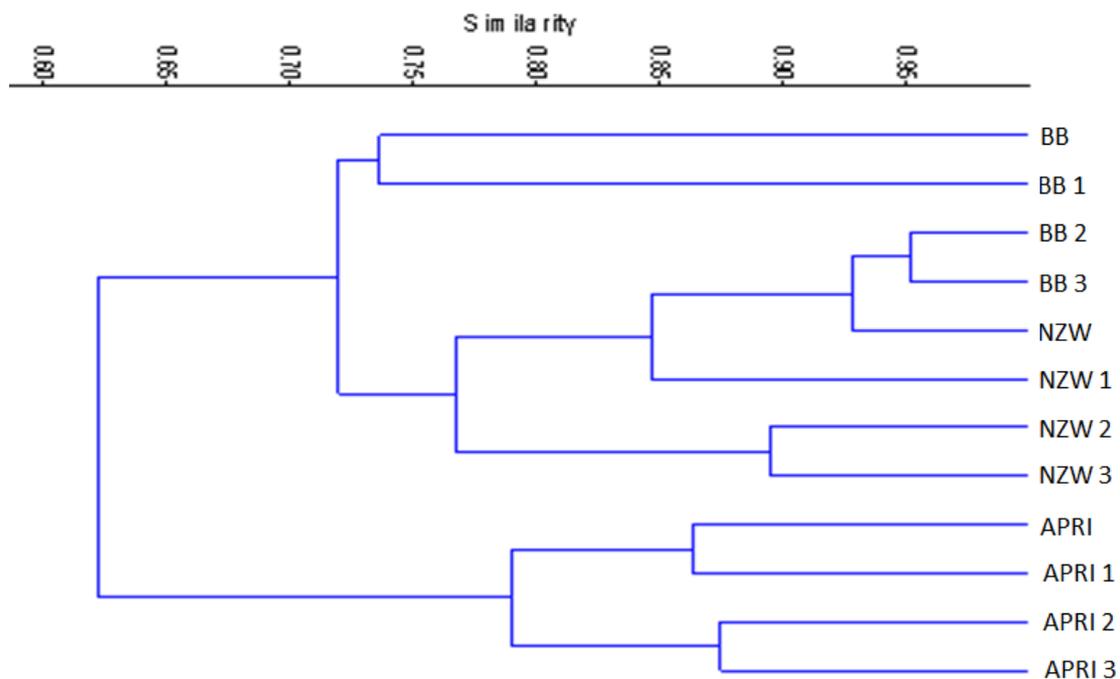
that they are highly diverged. However the highest value was 97% between BB and NZW as showed in Table (4) which they are mixed clustering in the dendrogram Figure (2).

Similarity among the three rabbit genotypes represent: APRI (Sir), (APRI 1, APRI 2 and APRI 3 daughters), BB (Sir), (BB 1, BB 2 and BB 3 daughters) and NZW (Sir), (NZW 1, NZW 2 and NZW 3 daughters).

RAPD based dendrogram of the three rabbit genotypes constructed using unweight pair-group arithmetic average (UPGMA) and similarity matrices.

**Table 4. Similarity matrix among the rabbit genotypes based on RAPD analysis**

0	APRI	APRI 1	APRI 2	APRI 3	BB	BB 1	BB 2	BB 3	NZW	NZW 1	NZW 2	NZW 3
APRI 1	0.88372	1										
APRI 2	0.92683	0.85	1									
APRI 3	0.91304	0.93333	0.88372	1								
BB	0.85	0.76923	0.86486	0.80952	1							
BB 1	0.7027	0.69667	0.76471	0.69667	0.84848	1						
BB 2	0.71429	0.78049	0.71795	0.81818	0.84211	0.85714	1					
BB 3	0.74419	0.7619	0.75	0.84444	0.87179	0.83333	0.97561	1				
NZW	0.71429	0.78049	0.71795	0.81818	0.84211	0.8	0.95	0.97561	1			
NZW 1	0.82609	0.84444	0.7907	0.91667	0.85714	0.76923	0.91909	0.93333	0.90909	1		
NZW 2	0.73171	0.75	0.68421	0.74419	0.81081	0.82353	0.92308	0.9	0.92308	0.83721	1	
NZW 3	0.76923	0.73684	0.72222	0.68293	0.85714	0.875	0.86486	0.84211	0.86486	0.78049	0.94444	1



**Fig. 2. Dendrogram showing genetic relationships among the three rabbit genotypes based on similarity indices derived from RAPD analysis using UPGMA software**

From the dendrogram APRI was clustered on a separate group, which could indicate that this line has differed out characteristics as showed in Table (4).

RAPD markers have been used for genotype identification (in rabbits: Khalil *et al.*, 2008 and Galal, Ola *et al.*, 2013; in chicken: Ghanem, Hanan *et al.*, 2012).

These results revealed high level of homogeneity among the individuals of these lines on the molecular level since almost all the 15 used primers showed high common bands among the studied individuals. RAPD-PCR provides a highly effective method for identifying the markers linked to traits of interest without the necessity for mapping the entire genome (Bardakci, 2001) and also in the discrimination of genetic resources that are economically important such as poultry and farm animals (Nikkhoo *et al.*, 2011). Similar, results were reported in four different rabbit genotypes (Galal, Ola *et al.*, 2013).

## CONCLUSION

The present study is a preliminary step to generate RAPD markers in a new synthetic paternal rabbit line called APRI and to try to find marker associated with productive performance. These markers represent reliable tools for characterization and genetic improvement of rabbits under Local breeding conditions in Egypt. From DNA banding pattern we can concluded that APRI genotype did not give any band reaction with the OPA2 and OPA7 primers. The other two genomes gave at least two distinct bands. Thus, we could use these primers for litter size, living litter size, and birth litter size traits and associate these markers with these characters. Moreover, RAPD analysis can be successfully applied to study genetic diversity and relationships among the three genotypes. This could have a great impact on rabbit breeding programs.

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## دراسة بعض الواسمات الجزيئية المرتبطة بالصفات التناسلية لخط أرناب الأبرى و سلالتى البلدى الاسود و النيوزيلاندى الابيض

أيمن فؤاد عبدالهادى عاشور و شريف مغاوى شامية

معهد بحوث الإنتاج الحيوانى ، مركز البحوث الزراعية ، وزارة الزراعة ، دقى ، مصر

تم استخدام ١٥ بادىء عشوائى لتكنيك ال RAPD لدراسة ثلاثة تراكيب وراثية بين خط ارانب الابرى و سلالتى البلدى الاسود و النيوزيلندى الابيض لتحديد بعض الادلة الوراثية التى من الممكن ان تستخدم فى التفرقة بين التراكيب الوراثية للسلالات المستخدمة فى الدراسة. علاوة على ذلك محاولة إيجاد ارتباط وراثى بين هذه الواسمات الجزيئية و الصفات التناسلية مثل (عدد الخلفة و عدد الخلفة الاحياء و الوزن عند الميلاد) للتراكيب الوراثية محل الدراسة.

من خلال التفريد الكهربائى للـ DNA باستخدام بوادىء OPA2 و OPA7 لم يعطى الابرى أى حزم مع هذين البادئين و لكنهما مع التراكيب الأخرى (البلدى الاسود و النيوزيلاندى) أعطت على الأقل حزمتين لذلك من الممكن أن تكون هذه البوادىء دليل لبعض الصفات محل الدراسة.

عموماً من خلال عمل RAPD تم الحصول على ١٠٤ حزمة منهم ٦٢ حزمة أظهرت اختلافات واضحة بين التراكيب الوراثية المدروسة. حيث اشترك كل من الأبرى و البلدى الأسود فى معظم الحزم الموجوده و هذا يدعم أن لهما نفس الجدود المشتركة فى تراكيبهما الوراثية و تم تأكيد ذلك من خلال شجرة النسب الوراثية.

هذه الدراسة اظهرت ان الـ RAPD يمكن استخدامه لتوصيف سلالات الارانب و تحديد بعض الادلة المرتبطة بالصفات التناسلية.