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Assessment of Genetic Polymorphisms in Commercial Broiler Breeds using RAPD-PCR as Molecular Markers

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ABSTRACT

RAPD-PCR was applied to evaluate the phylogenetic relationship and genetic variation among three chicken breeds; Cobb, Avian, and Ross. A total of seven arbitrary short primers were used and they generated polymorphic, reproducible and score able bands. 42 bands were produced from these primers. Out of these bands, 3 (7.14 %) were recognized as monomorphic and 18 (42.85 %) as polymorphic bands. The highest percentage (67%) of polymorphic bands was generated by OPC-1 primer, while the lowest percentage (20%) of polymorphic bands was generated by OPA-20 primer. Interbreed of band sharing frequencies were 0.82±0.13 between Avian-Cobb**,** 0.76±0.19 between Avian-Ross and 0.85±0.09 between Cobb-Ross. Three primers (OPA-8, OPA-10, and OPA-20) were specified for Ross**,** Avian and Cobb breeds respectively. The study recommends the use of RAPD- PCR to detect the genetic changes and relations among broiler chicken breeds.

Keywords: Poultry, RAPD- PCR, Genetic Variability, Phylogenetic.

INTRODUCTION

Poultry production is a vital sector all over the world. Nowadays, more care has been given to poultry due to quality of poultry meat and its sustainable production (Kaya and Yıldız, 2008). The consumer preference for poultry products (meat and eggs) has been increasing day by day due to their high-quality and the relatively affordable price (Scanes, 2007).

Currently, in the Arabic Republic of Egypt; Egyptian poultry production has become an industry instead of being an agricultural activity. Broiler production is the main component of the poultry industry. Broiler chicken meat production has increased to meet the consumer's need for affordable animal protein. Technology for broiler production varies among the commercial broiler farms, the poultry industry has a pyramid structure where the meat production or broiler sector at the top of the pyramid, the broiler breeders in the middle and the actual meat production birds (broilers) at the bottom (Shatokhin et al., 2017)**.**

RAPD is an important technique used in the molecular analysis of relatedness between genotypes. It was used to evaluate kinship relationships, create specific probes, determine the taxonomic identity and analyze mixed genome samples. In RAPD technique, small inverted repeats scattered throughout the genome were amplified by PCR. It was an efficient technology which could be able to use limited quantities of DNA and work on anonymous genomes (Hadrys et al., 1992).

The objective of this study is to evaluate the phylogenetic relationship and genetic diversity among three broiler breeds; Avian 48, Cobb 500, Ross 308 using RAPD-PCR technique as a Genetic Marker.

MATERIALS AND METHODS

Experimental birds

A total of fifteen broiler chicks from three different breeds (Cobb, Avian and Ross) five birds each were used to conduct the study. The chicks were purchased from a commercial hatchery.

DNA extraction

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Blood samples were taken from the wing vein of each bird on EDTA containing vacutainer tubes. The genomic DNA was extracted from blood samples using DNeasy Blood & Tissue Kit (QIAGEN, Germany Cat. No. 51104) and kept under -20°C until used for RAPD-PCR.

RAPD-PCR

In this study, seven primers (Operon Technologies Inc, USA) listed in (Table 1) is used for RAPD-PCR.

The reaction was carried in a 20 µl consist of:

1-pooled DNA template	2 µl
2- Primer	1.5 µl
3- 2x Taq PCR master mix	10 µl
4- H2O (d.d water)	6.5 µl
Total	20 µl

Data analysis

Table (1): Name, Sequences and G+C (%) of primers used for amplification of RAPD loci

Primers	Primer sequence5′→ 3′	G+C% content
OPA-8	GTGACGTAGG	60%
OPA-10	GTGATCGCAG	60%
OPA-20	GTTGCGATCC	60%
OPB- 1	GTTTCGCTCC	60%
OPB-11	GTAGACCCGT	60%
OPB- 15	GGAGGGTGTT	60%
OPC-1	TTCGAGCCAG	60%

The gel photos were used to assess the following parameters:

1. The presence (1) or absence (0) of DNA bands in the RAPD profile. Only clear distinct bands were recorded. The binary code (1, 0) was used for the genetic analysis.

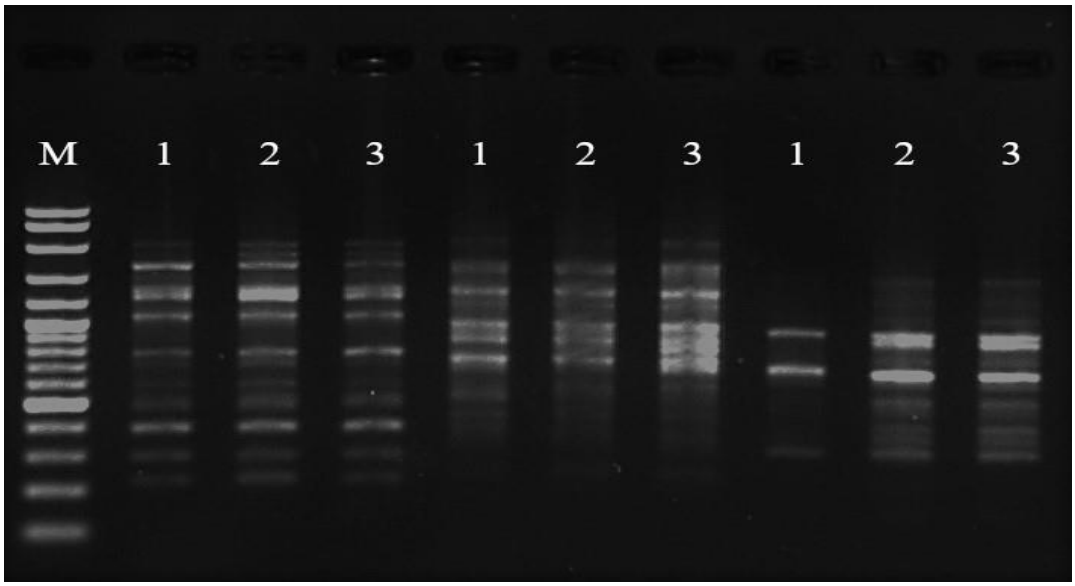

Figure (1): RAPD profile in different three broiler breeds; 1: Avian, 2: Cobb and 3: Ross using primers; OPA-20, OPB-1 and OPA-10. M: Molecular marker (100 bp ladder).

A total of 105 amplified bands by these seven primers were evaluated using RAPD-PCR profiles. The total number and size of amplified bands (TAB, SAB), number and size of Common bands (NCB, SCB), breed specific band (BSB), and number and percentage of polymorphic bands (NPB, PB %) were summarized in Table (2).

2. Band sharing (BS): comparisons of bands on the same gel were done. The extents of band sharing were calculated using the following formula of Lynch (1990): BS= 2(Bab)/ Ba + Bb.

- **BS** means band sharing.
- **Bab** is the number of the bands shared by a and b samples.
- **Ba and Bb** are the total number of the bands in a and b breeds.

Table (2): RAPD analysis results using 7 arbitrary primers:

Primers	TAB	SAB	NCB	SCB	BSB	NPB	PB %
OPA-8	4	525-1480 bp	3	(1480,910,525 bp)	(1043 bp) (Ross)	1	25%
OPA-10	8	311-2500 bp	3	(841,642,311 bp)	(2500 bp) (Avian)	5	63%
OPA-20	10	234-1600 bp	8	(1330,1080,948,800, 508,406,305,234 bp)	(610 bp) (Cobb)	2	20%
OPB-1	7	580-1650 bp	4	(1300,1103,850,753 bp)	_	3	43%
OPB-11	2	460-550 bp	1	(460 bp)	_	1	50%
OPB-15	5	378-1200 bp	3	(858,506,378 bp)	_	2	40%
OPC-1	6	472-1332 bp	2	(615,472 bp)	_	4	67%

*TAB: total number of amplified bands, SAB: size of amplified bands, NCB: number of common bands, SCB: size of common bands, BSB: breed specific band, NPB: number of polymorphic bands, PB %: percentage of polymorphic bands.

RESULTS AND DISCUSSION

In this current study, phylogenetic relationship and the genetic difference between three broiler chicken breeds (Avian, Cobb, and Ross) were evaluated using RAPD-PCR technique. Seven arbitrary primers were used to amplify pooled DNA sample from these breeds. Amplified fragments produced by these primers ranged from 2 to 10 bands with average sizes from 311 bp to 2500 bp (Figure 1).

From 42 noticed bands, 3 (7.14 %) were identified as monomorphic bands and18 (42.85%) as polymorphic ones. Per primer the number of polymorphic bands varied from 1to 5. The highest polymorphic bands percentage (67%) was detected in OPC-1 primer while the primer as lowest percentage (20%) was detected in OPA-20 primer, (Table 2). Shiau et al., (1996) reported that RAPD-PCR was successfully used to differentiate between chickens, geese and ducks. Tian Fang et al., (2002) and Zhang *et al.*, (2002)also verified polymorphic patterns in amplified DNA bands in various duck and chicken breeds ,respectively. El-Araby and Saleh, (2016) found that RAPD-PCR was a powerful technique to differentiate between four duck breeds.

The number of amplified bands per primer among the three chicken breeds was variable. The highest number of amplified bands was (9) in the three breeds using OPA-20 primer followed by (7) in Avian and Ross breeds using OPB-1 primer and in Cobb breed using OPA-10. Primer OPA-20 had the highest numbers of bands (10) while the lowest numbers of amplified bands were given using primer OPB-11 (2) Table (3).

Breed identification RAPD-PCR markers

The RAPD-PCR profiles of Avian, Cobb and Ross breeds produced by seven

Table (3): Frequency of bands per primer in the three studied breeds

Primers	Breed			
	Avian (A)	Cobb (C)	Ross (R)	Total
OPA-8	3	3	4	10
OPA-20	9	9	9	27
OPB-1	7	4	7	18
OPA-10	5	7	6	18
OPB-11	1	2	2	5
OPB-15	5	5	3	13
OPC-1	4	6	4	14
Total	34	36	35	105

arbitrary primers were used to recognize specific markers (unique to a certain breed only). OPA-8 primer was identified in Ross only, OPA-20 was identified in Cobb only; and OPA-10 was identified in Avian only, Table (2). These remarkable primers can be used to identify the breeds; though, these results need more investigation on large sample to be validated. Hoshi et al., (2002) investigated the genetic similarity and variability within and between populations of various breeds in guinea fowl, chickens and quail depending on the efficiency of RAPD-PCR markers. Singh and Sharma, (2002) verified that the using of RAPD-PCR as genetic markers to reflect the changes in genetic structure and genetic variability of chicken and duck populations.

Band Sharing Frequencies (BSF)

Interbreed BSF was 0.82±0.13 between Avian-Cobb, 0.76±0.19 between Avian-Ross and 0.85±0.09 between Cobb-Ross.

CONCLUTION

We concluded that RAPD- PCR can be successfully used as Molecular Markers to detect the genetic changes and relations among broiler chicken breeds using remarkable primers. More investigations on large number of random primers and sample are needed to validate these results.

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