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ASSESSMENT OF GENETIC VARIABILITY AMONG FAYOUMI, RHODE ISLAND RED AND THEIR CROSSES USING RAPD-PCR

Samar, H. Baloza[1], S. A. Hemeda[2], G. A. Sosa[3] and Asmaa, Abo Shady[4]

[1] Department of Animal Wealth Development, Faculty of Vet. Medicine, Benha University. [2]Department of Animal Wealth Development, Faculty of Vet. Medicine, Alex. University.[3] Department of Theriogenology, Faculty of Vet. Medicine, Benha University.[4] Department of Genetics, Faculty of Agriculture, Ain Shams University.

ABSTRACT

This study was carried out to evaluate the genetic diversity and phylogenetic relationships using randomly amplified polymorphic DNA (RAPD) in four chicken populations; Fayoumi × Fayoumi (FF), Fayoumi × Rhode Island Red (FR), Rhode Island Red× Fayoumi (RF) and Rhode Island Red× Rhode Island Red (RR). Fifteen decamer (RAPD) primers were screened on pooled genomic DNA of four chicken populations produced a total of 154 bands. The number of polymorphic bands was 55.0 with an average of 3.66 per primer. The level of polymorphism among the four chicken genotypes was 36.34%. Genetic similarity between crossbred populations (FR and RF) was (80.28%) while, the similarity between the purebred populations (FF and RR) was (76%). RAPD data showed a clear distinction of Fayoumi from RIR.

KEY WORDS: Genetic diversity, Phylogenetic, Fayoumi, RAPD

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1.INTRODUCTION

Amongst the poultry species, chicken is the most extensively exploited species to meet the demands of egg and meat production. However, in order to meet the present diverse demand for poultry products, there is a need to explore the other poultry germplasms, which in fact, has received rather less attention such as indigenous chicken in Egypt [1]. Fayoumi is the most important Egyptian native chicken breed and spread recently to many countries worldwide. It was found mainly in Fayoum city known as the first man-made agricultural oasis in ancient Egypt, Fayoumi chickens might share basically in the original germplasm of chickens in Egypt. Moreover, it is assumed that the natively established Fayoumi chickens showed ancient historic genetic relationships to some European chickens may be as early as 600 B.C [2]. Rhode Island Red chicken breed (RIR) is an exotic American dual-purpose breed which has been adapted to the environmental circumstances in Egypt for more than 20 years [3]. Crossbreeding carried out between the improved exotic and the local breed aiming mainly to combine the better production capacity of the former with the later adaptability to harsh environment. This system also maximizes the expression of heterosis in the cross, normally reflected in improved the fitness characteristics [4].

Molecular markers provide useful information on genetic variability, either within or between populations. Random amplified polymorphic DNA (RAPD)

markers used PCR to amplify the segments of nuclear DNA and do not require prior molecular information. RAPD had quickly become the method of choice for genotype identification, population and pedigree analysis, phylogenic studies and genetic mapping because it is simple, fast and comparatively low-cost. In poultry, RAPD has been used to estimate genetic relatedness among various poultry species [5]. As well as for showing molecular differences between Egyptian chicken strains [6] and [7]. The use of RAPD marker technology was recently reviewed in poultry research, especially in some genetic resources of economically important species such as chickens, quails, ducks, goose, turkey and other birds [8].
The aim of this study was to investigate the genetic variation and phylogenetic relationships among four chicken breeds (Fayoumi, Fayoumi × Rhod Island Red, Rhod Island Red× Fayoumi and Rhod Island Red) using Random Amplified polymorphic DNA (RAPD)-PCR.

2. MATERIALS AND METHODS

1. Chicken breeds

Four chicken populations: FF (Fayoumi × Fayoumi), FR (Fayoumi × Rhode Island Red), RF (Rhode Island Red × Fayoumi), and RR (Rhode Island Red × Rhode Island Red) were used in this study. 20 samples per population (about 2-5 ml venous blood sample per bird from 80 birds belonging to 4 populations) were taken from the jugular vein under aseptic conditions using tube containing EDTA as anticoagulant then frozen at -20°C till DNA extraction.

2. DNA extraction

100µl of blood was added in 1.5 ml appendorf tube to 30 µl SDS 20%, 10 µl proteinase k and 100µl lysis buffer, then the genomic DNA was isolated through phenol-chloroform extraction using the protocol adopted by [9] with some modifications. After extraction, DNA pellet was resuspended in 50-100 µl 1x T E buffer. DNA was stored at 4 °C till further step or at –20°C for long time storage.

3. DNA quantification and preparation of pooled DNA

Using the spectrophotometer, the concentration of DNA and its relative purity was determined on the basis of the absorbance at 260 and 280 nm respectively. The purity of DNA samples was ranged from 1.2 up to 1.9. Pooling of DNA samples was made by mixing equal amount of genomic DNA of the twenty samples of each population to obtain four pooled DNA samples for the four populations.

4. Random Amplified Polymorphic DNA-Polymerase Chain reaction (RAPD-PCR)

Amplifications were performed in a DNA thermal cycler using a set of fifteen arbitrary 10-mer primers having GC content of 60–70% designed by Operon Technologies, The primer sequences are listed in (Table1). The amplification protocol adopted by [9], was carried out in a total volume of 25 µl in 0.2 ml thin walled PCR tube containing 10 ng of genomic DNA as a template, 10 pmoles of random primer, 2mM of dNTP's mix (dATP, dCTP, dTTP and dGTP), 10 X PCR buffer, 25 mM MgCl2, and 2 units Taq DNA polymerase (Fermentas). PCR reaction was carried out under the following conditions: an initial denaturation step for 5 minute at 94°C, followed by 40 cycles of denaturation for 1 minute at 94°C; annealing for 1 minute at 36ºC and an extention for 2 min at 72ºC. The PCR products were visualized on 2% agarose gel after staining with ethidium bromide on a UV transilluminator. A 1-kb DNA ladder marker was used as a molecular size standard.

5. Scoring and Data analysis

The data were transformed into a similarity matrix based on the Jaccard's

coefficient (Sj). Phylogenic dendrogram was constructed as the unweighted pair-group mean arithmetic method analysis (UPGMA). All these analyses were also made with the software program Community Analysis Package."CAP 4.0", developed and produced by [10].

3. RESULTS

From analysis of the banding patterns of RAPD bands (Figure 1 and Table 2), total of 154 bands were produced with an average of 10.26 per primer with molecular weights ranging from 190 to 1500 bp while the total number of bands in each population was 130, 124, 132 and 134 in FF, FR, RF and RR respectively(Table 3). The highest numbers of band were 134 this observed in RR population. Primer OPC-11 produced the highest number of bands (50) while the lowest number of bands was produced by the primer OPB-19(15). Level of polymorphism (36.34 %), total number of polymorphic bands was 55.0 with an average of 3.66 per primer. These observations revealed a comparatively low level of genetic diversity between the chicken breeds.

The results showed that the 14 primers were developed unique molecular markers for the four chicken populations (Table 4): For Fayoumi population: 12 unique markers were developed by 9 primers; OPA-02 (1050 bp), OPA-07 (1100 bp), OPA-10 (320 and 850 bp), OPC-11 (320 bp), OPG-04 (350 bp), OPE-05 (750 bp), OPG-08 (1000 and 1200 bp), OPA-06 (450 and 800 bp) and OPZ-17 (220 bp). For FR population: 9 unique markers were developed by 6 primers; OPA-16 (440 bp), OPA-07 (700 and 1000 bp), OPB-19 (900 bp), OPE-05 (220 and 230 bp), OPA-06 (240 bp), OPZ-13 (250 and 290 bp). For RF population: 5 unique markers were developed by 2 primers; OPG-08 (320, 870 and1100 bp) and OPZ-13 (500 and 600 bp). For Rhode Island Red population: 9 unique markers were developed by 5 primers; OPA-02 (220 bp), OPA-13 (200 bp), OPA-10 (400 bp), OPC-11 (240, 700,770, 850 and 950 bp), OPG-06 (750 bp). No characteristic molecular genetic markers were developed by primer OPA-14 for any population. However, primer OPC-11 gave the highest number of molecular markers (6 markers) either positive or negative ones.

Genetic similarity analysis: The highest similarity level (82.19%) was detected between (RF &RR) populations which revealed that they are closely related genotypes followed by 80.28% which was observed between FR and RF populations. While, the genetic similarity was lower (76%) between the purebred populations (FF and RR). Phylogenetic relationships between the four chicken populations: The phylogenetic tree was carried out through analysis of UPGMA cluster analysis of RAPD data (Figure 2), which showed that, Fayoumi (FF) was clearly distinct and separated in one main cluster from other populations. The second cluster was divided into 2 sub-clusters: one represented FR population while the other sub-cluster contained RF and RR.

4. DISCUSSION

Fayoumi has low similarities with Rhode Island Red. This observation is in accordance with [2] who said that Fayoumi and Rohde Island Red breeds are inconsistent strains.

Fayoumi (FF) was clearly distinct and separated in one main cluster from other populations. This may be due to its purity and genetic uniqueness compared with other populations, this agrees with [3] who said that Fayoumi is a unique native breed which has good adaptability to local conditions. The second cluster was divided into 2 sub-clusters: one represents FR population while the other sub-cluster contained RF and RR. RF was very close to RR than FF as they clustered together. This may be attributed to sire effect as RF

population was a result of crossing Rhode Island Red male with Fayoumi female.

RAPD was proved to be a very effective technique in detecting polymorphism at the breed level as seen in this study which agrees with [11] and [12]. They also demonstrated the effective use of RAPD for detecting the polymorphism between breeds in chicken and turkey. RAPD-PCR was used to discriminate animal species other than chicken such as geese [13], quail [9], duck breeds [14] and [15] and turkey breeds [16].

The present results demonstrate the effectiveness of RAPD for the study of genetic diversity and phylogenetic relationships among the four chicken populations under study.

Table1: Sequence of the fifteen decamer arbitrary primers assayed in RAPD-PCR:

No.	Primer	Sequence (5´-3´)
1	OPA-02	TGCCGAGCTG
2	OPA-13	CAGCACCCAC
3	OPA-16	AGCCAGCGAA
4	OPA-07	GAAACGGGTG
5	OPA-10	GTGATCGCAG
6	OPB-19	ACCCCCGAAG
7	OPC-11	AAAGCTGCGG
8	OPG-04	AGCGTGTCTG
9	OPE-05	TCAGGGAGGT
10	OPA-14	TCTGTGCTGG
11	OPG-06	GTGCCTAACC
12	OPG-08	TCACGTCCAC
13	OPA-06	GGTCCCTGAC
14	OPZ-13	GACTAAGCCC
15	OPZ-17	CCTTCCCACT

Table 2: Levels of polymorphism, total number of bands, monomorphic bands, polymorphic bands and percentage of polymorphism as revealed by fifteen RAPD primers for the four chicken genotypes.

No.	Primers	Total number of bands	Monomorphic bands	Polymorphic bands	% of Polymorphism
1	OPA-02	13	10	3	23.07
2	OPA-13	12	11	1	8.33
3	OPA-16	11	8	3	27.27
4	OPA-07	6	2	4	66.66
5	OPA-10	6	3	3	50
6	OPB-19	4	3	1	25
7	OPC-11	17	7	10	58.82
8	OPG-04	12	10	2	16.66
9	OPE-05	11	6	5	45.45
10	OPA-14	9	9	0	0
11	OPG-06	14	9	5	35.71
12	OPG-08	10	4	6	60
13	OPA-06	8	4	4	50
14	OPZ-13	10	4	6	60
15	OPZ-17	11	9	2	18.18
Total		154	99	55	-
Average		10.26	6.6	3.66	36.34%

Table 3: Total number of bands amplified by 15 primers in four chicken populations, where: FF (Fayoumi), FR (Fayoumi× Rhode Island Red), RF (Rhode Island Red× Fayoumi) and RR (Rhode Island Red).

Primers	Chicken populations				Total
	FF	FR	RF	RR	
OPA-2	11	11	11	11	44
OPA-13	12	12	12	11	47
OPA-16	10	8	10	11	39
OPA-07	4	3	6	6	19
OPA-10	4	6	6	5	21
OPB-19	4	3	4	4	15
OPC-11	13	13	14	10	50
OPG-4	11	12	11	11	45
OPE-05	8	8	10	9	35
OPA-14	9	9	9	9	36
OPG-06	10	10	12	13	45
OPG-08	8	7	6	8	29
OPA-06	8	4	5	6	23
OPZ-13	8	8	6	9	31
OPZ-17	10	10	10	11	41
Total	130	124	132	134	520

Table 4: unique RAPD molecular markers for the four chicken populations, where: FF (Fayoumi), FR (Fayoumi× Rhode Island Red), RF (Rhode Island Red× Fayoumi) and RR (Rhode Island Red). 1 and 0 mean positive and negative marker respectively.

primer	(bp)	Chicken populations			
		FF	FR	RF	RR
Primer OPA-02	220	0	0	0	1
	1050	1	0	0	0
primer OPA-13	200	1	1	1	0
primer OPA-16	440	1	0	1	1
	700	1	0	1	1
primer OPA-07	1000	1	0	1	1
	1100	0	1	1	1
	320	0	1	1	1
primer OPA-10	400	1	1	1	0
	850	0	1	1	1
primer OPB-19	900	1	0	1	1
primer OPC-11	240	1	1	1	0
	320	1	0	0	0
	700	1	1	1	0
	770	0	0	0	1
	850	1	1	1	0
	950	1	1	1	0
primer OPG-04	350	0	1	1	1
	220	1	0	1	1
primer OPE-05	230	0	1	0	0
	750	0	1	1	1
primer OPG-06	750	0	0	0	1
primer OPG-08	320	1	1	0	1

	870	1	1	0	1
	1000	1	0	0	0
	1100	0	0	1	0
	1200	0	1	1	1
	240	1	0	1	1
primer OPA-06	450	1	0	0	0
	800	1	0	0	0
	240	1	0	1	1
	250	1	0	1	1
primer OPZ-13	290	0	1	0	0
	500	1	1	0	1
	600	1	1	0	1
Primer OPZ-17	220	0	1	1	1

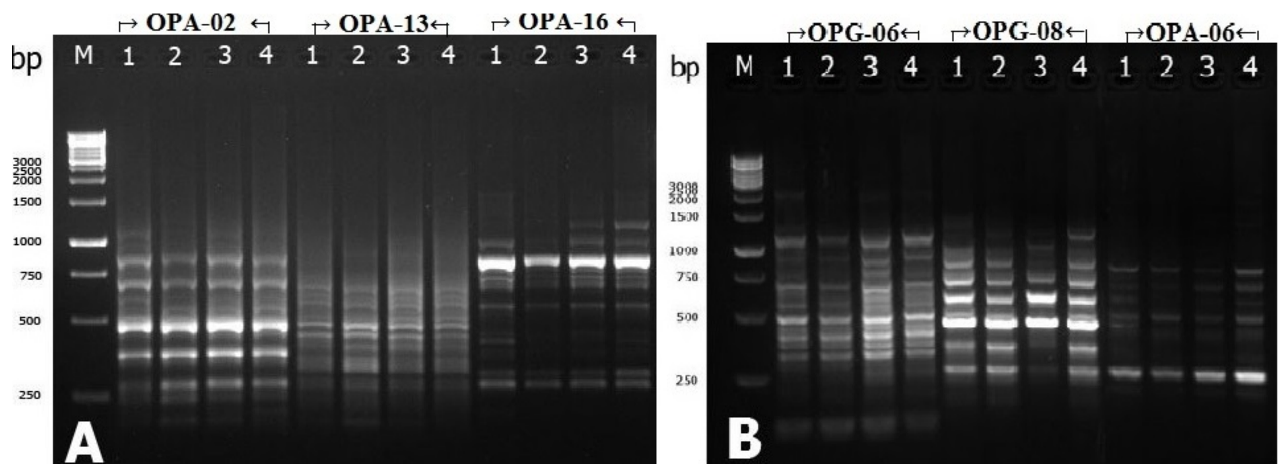

Figure 1. Examples of the banding patterns of RAPD produced with A: primer (OPA-02), (OPA-13) and (OPA-16) and B: primer (OPG-06), (OPG-08), (OPA-06) respectively in: FF (lane1), FR (lane 2), RF (lane 3) and RR (lane 4), M (DNA ladder)

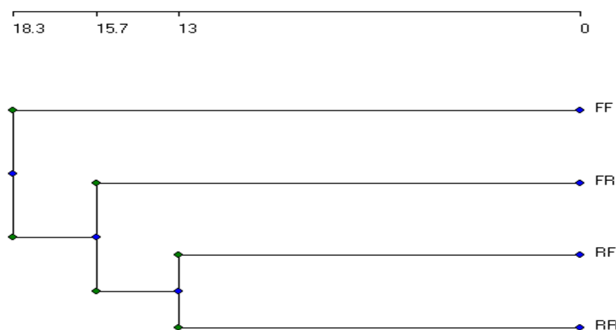

Figure (2): Dendrogram for the four Chicken genotypes constructed from the RAPDs generated data using UPGMA.

5. CONCLUSION

The results obtained from RAPD revealed the effectiveness of RAPD markers in determining the genetic diversity and the phylogenetic relationship among the chicken populations under the study. RF& RR were clustered together which are closely related genotypes. Fayoumi has low similarities with Rhode Island Red.

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تقييم التنوع الوراثي بين الفيومي والرودايلاند الأحمرباستخدام إنزيم البلمرة المتسلسل العشوائي.

سمر حسني بلوزة[1]، شعبان عبد اللطيف حميدة[2] ، جمال عبد الرحيم سوسة[3] أسماء أبوشادي[4]

قسم تنمية الثروة الحيوانية كلية الطب البيطري جامعة بنها[1] ، قسم تنمية الثروة الحيوانية كلية الطب البيطري جامعة الإسكندرية[2]، قسم التوليد والتناسل والتلقيح الاصطناعى كلية الطب البيطري جامعة بنها[3] ،قسم الوراثة كلية الزراعة جامعة عين شمس[4].

الملخص العربى

أجريت هذه الدراسة في مزرعة الدواجن للبحوث التابعة لقسم تنمية الثروة الحيوانية، كلية الطب البيطرى، جامعة بنها، مصر. وذلك لتقييم التنوع الوراثي و درجة القرابة بين أربع سلالات من الدجاج؛ وهي الفيومي، الفيومي ×الرودايلاند الأحمر ، الرود ايلاند الأحمر× الفيومي والرودايلاند الأحمر باستخدام إنزيم البلمرة المتسلسل العشوائي. استخدم 15 بادئ عشوائي في هذه الدراسة علي عينات الحامض النووى المجمعه لكل سلالة من الأربعة؛ وكان الناتج الكلي لعدد الحزم هو 154 حزمة، عدد الحزم المختلفه 55 بمتوسط (3.66%) لكل بادئ. وكانت درجة الاختلاف بين الأربع سلالات هي 36.34%. وكان اعلي مستوي للتشابه الوراثي (82.19%) بين سلالتي الرود أيلاند الأحمر و(الفيومي × الرودايلاند الأحمر) ويليه (80.28%) بين سلالتى الهجين؛ (الفيومي × الرودايلاند الأحمر و الرود ايلاند الأحمر× الفيومي) في حين كان اقل تشابه وراثي بين سلالتى الفيومى والرود أيلاند الأحمر(76%).أوضح التمثيل الشجري لعلاقات القرابة بين السلالات تحت الدراسة أن الرود أيلاند الأحمر، الرود ايلاند الأحمر× الفيومي و الفيومي × الرودايلاند الأحمر كانوا متجمعون معا بينما كان الفيومى منفصلا عنهم.

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