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Genetic Relationship and Similarity of Some Chicken Strains

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A B S T R A C T

Blood samples were collected from the four chicken strain (twenty males and twenty females)**.** (Ross, Indian River as meat type and, ISA Brown, Hy Line Brown as egg type chicken) . Blood samples were collected in 3 ml tubes containing EDTA. Genomic DNA was extracted from 300 µl of blood. DNA purity and concentrations have been measured by Nano Drop® spectrophotometer. Random amplification of polymorphic DNA (RAPD-PCR) was done by using 35 primers from GenScript USA company. A total of 21 Primers gave results to find a complementary DNA Genomic sites. The PCR program included an initial denaturation step at 94 °C for 5 minutes followed by 40 cycles with 94 °C for 1 minutes for DNA denaturation, annealing as mentioned with each primer, extension at 72 °C for 1 minutes and final extension at 72 °C for 5 minute were carried out. The PCR products were tested with electrophoresis on 2 % agarose gels in 1x TBE buffer stained by Ethidium bromide. The amplified pattern was visualized on a UV trans and photographed .Statistical Analysis of RAPD bands were scored for their presence (1) or absence (0). The index of similarity between each two population's genetic distances was calculated. Polymorphism of each primer was calculated. The highest number of bands was 134 bands among all groups used and which was created by the OPA-13 Primer, and the lowest number of bands was 5 bands, which was created by OPA-03 Primer. The total number of bands created by all the Primers was 1724 and the total number of polymorphic band created by all the Primers was 216. The Primer OPQ-O4 had the highest number of polymorphic bands being 18 bands. While the Primer OPA-15 possessed the lowest number of polymorphic band being 2 bands. The average number was 12.77 of polymorphic bands per primer. The highest percentage of the Polymorphisms observed in the primer OPA-19, was 29.09% when compared with other primers in this study, where the lowest percentage of Polymorphisms was of primer of OPA-15 being 4.55%. A high level of genetic similarity was also observed between the meat type chickens and it was 0.741 between Ross ♀ and Indian ♀. A low level of genetic similarity was also observed between the meat and eggs chickens being 0.587 between Ross ♀ and ISA ♀.

1. INTRODUCTION

Meanwhile, in the category of commercial chickens, the commercial layer is a type of chicken that is bred for egg production under strong artificial selection . Both groups of broilers and layers are maintained under strong artificial selection [13]. Currently, the breeding schemes for commercial chickens are focused on specialized production lines, derived by an intense selection from a few breeds and very large populations, with a great genetic uniformity of traits under selection [12].

Effectiveness of RAPD in detecting polymorphism between chicken populations and their applicability in population studies and

establishing genetic relationships among chicken populations has been reported by[16]. [2] and [4] have also presented some preliminary data showing molecular differences between Egyptian chicken strains, and indicating the potential use of RAPD markers for a wide range of applications in poultry breeding. Characterization at the molecular level is undertaken mainly to explore genetic diversity within and between animal populations, and to determine genetic relationships among such populations. The estimation of genetic variability of a species is an important criterion for its conservation and further genetic improvement [14].

Molecular markers derived from polymerase chain reaction (PCR) amplification of genomic DNA are an important part of the tool kit of evolutionary geneticists [10]. By detecting genetic variation, genetic markers may provide useful information at different levels; population structure, levels of gene flow, phylogenetic relationships, patterns of historical biogeography and the analysis of parentage and relatedness [9]. PCR-based multi-locus DNA fingerprints represent one of the most informative and cost-effective measures of genetic diversity [5]. Randomly Amplified Polymorphic DNA (RAPD) technique, described firstly by [20], is a simple, fast and comparatively low cost assay that uses short oligonucleotide primers of arbitrary sequences to amplify anonymous fragments of genomic DNA [19], and no prior knowledge of the genome under investigation is necessary to perform the assay [8]. The objective of this study was to assess the genetic diversity and phylogenetic relationship among fore chicken breeds (Ross ,Indian River , ISA Brown and Hy Line Brown) using Random Amplified Polymorphic DNA (RAPD) Markers.

2. MATERIALS AND METHODS

2.1 DNA isolation

The experimental materials consisted of twenty males and twenty females. The four chicken strain (Ross, Indian River, ISA Brown and Hy Line Brown). Blood samples from chicken were collected in 3 ml tubes containing EDTA, and stored at –20 °C until DNA extraction. Genomic DNA was extracted from 300 µl of blood following the instruction of the [6]. Wizard Genomic DNA Purification Kit Promega USA . All laboratory work was carried out in the medical research center in Erbil / Hawler Medical University.

2.2 DNA Quantification

DNA purity and concentrations have been measured by Nano Drop® spectrophotometer. The purity of DNA samples ranged from 1.5 up to 1.8 . Samples were then diluted to 30 ng/µl for using RAPD PCR. In research center in Erbil /University of Salahadden Erbil.

2.3 RAPD PCR

Random amplification of polymorphic DNA (RAPD-PCR) was done by using 35 primers are Series (Table 1) from GenScript USA company. A total of 21 Primers out of the 35 Primers gave results to find a complementary DNA Genomic sites. The mixture of the PCR reaction had a final volume of 25 µl and contained 30 ng of genomic DNA, 10 µM of each primer . The annealing temperatures of the cycling parameter were calculated Tm based on the GC sequence composition. The PCR program included an initial denaturation step at 94°C for 5 minutes followed by 40 cycles with 94°C for 1 minute for DNA denaturation, annealing as mentioned with each primer, extension at 72 °C for 1 minutes and final extension at 72 °C for 5 minutes were carried out. The PCR products were tested with electrophoresis on 2 % agarose gels in 1x TBE buffer (Promega, USA) stained by ethidium bromide. The amplified pattern was visualized by a UV trans and photographed.

2.4 Statistical Analysis

The RAPD bands were scored for their presence (1) or absence (0). The index of similarity between each two populations was calculated using the formula: Similarity = 2nxy / nx + ny. and using for, genetic distance = 1- (2nxy / nx + ny). Polymorphism of each primer was calculated based on the following formula:- Polymorphism % = (Np/Nt) x 100 , Np = the # of polymorphic bands of random primer Nt = the total number of bands of the sample primer [7].

3. RESULTS AND DISCUSSION

In this study a total of (35) RAPD Primers were used from GenScript USA company. A total 21 Primers of them gave results to find a complementary DNA Genomic sites. The highest number of bands was 134 bands among all groups used and which was created by the OPA-13 Primer, and the lowest number of bands was 5 bands, which was created by OPA-03 Primer. The results of the OPA-13 suggests that this primer should be used in the future in other birds because it gave the highest number of bands. The total number of bands created by all the Primers was 1724 and the total number of polymorphic bands created by all the Primers was 216. The Primer OPQ-O4 had the highest number of polymorphic band being 18 bands. While the Primer OPA-15 possessed the lowest number of polymorphic band being 2 bands. The average number was 12.77 of polymorphic bands per primer. The highest percentage of the polymorphisms observed in the primer OPA-19, a 29.09 when compared with other primers in this study, where the lowest percentage of Polymorphisms was for the primer of OPA-15 being 4.55 . The highest range of the molecular weight was (150 - 1900 bp) for the primer OPQ-11, and was over in less primer OPQ-15 which is (120 - 900 bp) all this are Series in (Table 2). The analysis of genetic diversity and relatedness between or within species, populations and individuals is a prerequisite towards effective utilization and protection of animal genetic resources. With DNA being the only basis of genetic differences between distinct organisms, DNA fingerprinting presently is the ultimate method of biological individualization. [15] observed an average number of 9.2 polymorphic bands per primer using RAPD-DNA fingerprinting between meat and layer pure line of chickens. In this study it was found an average number of 12.77 polymorphic bands per primer. In a similar study involving native egg and meat type strains [1], the genetic similarity within egg and meat type chickens were 0.79 and 0.89, receptively. [18] for the White Leghorne population (21.9%). Based on the results obtained, the existence of high levels of polymorphism may indicate the accuracy of the used selection program and also the large enough effective population size in this breeding flock. Therefore, there is enough genetic variation left to generate further progress in the years ahead.

Additionally, the use of RAPD markers represents a useful and efficient method and thus provides a potential tool for detection of genetic variability among individuals in poultry breeder flocks. In addition,[3] also reported that a high level of genetic similarity was observed among the commercial chickens from different localities. One of the reasons that could have led to the high level of genetic variation among the chickens was they breeds from meat and eggs chickens. Similarly in this study, a high level of genetic Similarity was also observed between the meat chickens and its 0.741 between Ross ♀ and Indian ♀. a low level of genetic Similarity was also observed between the meat and eggs chickens and its 0.587 between Ross ♀ and ISA ♀ giving in (Fig. 1). [21] reported that a great difference of genetic variation was observed between the broiler and layer chicken breeds. Hence, the finding of this study is compatible with the study by [21]. Nevertheless, this study was found to be inconsistent with the study conducted by [3] who observed a high level of genetic similarity between the commercial broiler and layer chickens from different localities. The reasons that could have led to the high level of genetic variation among the commercial broiler and layer chicken were the different breeds of chicken, whereby the broilers were bred for meat production and the

commercial layers were bred for egg production [16] ; [17]. The genetic similarity between the two egg-producing strains (White Leghorn and White Rock) and **(**Rhode Island Red and Barred Plymouth Rock) were between 81.3 to 89.3 %.

Table 1: Nucleotide sequence of selected random primers and % GC content.

NO	Primer name	Sequence	% GC content
1	OPA-01	CAGGCCCTTC	70%
2	OPA-02	TGCCGAGCTG	70%
3	OPA-03	AGTCAGCCAC	60%
4	OPA-04	AATCGGGCTG	60%
5	OPA-05	AGGGGTCTTG	60%
6	OPA-06	GGTCCCTGAC	70%
7	OPA-07	GAAACGGGTG	60%
8	OPA-08	GTGACGTAGG	60%
9	OPA-09	GGGTAACGCC	70%
10	OPA-10	GTGATCGCAG	60%
11	OPA-11	CAATCGCCGT	60%
12	OPA-12	TCGGCGATAG	60%
13	OPA-13	CAGCACCCAC	70%
14	OPA-14	TCTGTGCTGG	60%
15	OPA-15	TTCCGAACCC	60%
16	OPA-16	AGCCAGCGAA	60%
17	OPA-17	GACCGCTTGT	60%
18	OPA-18	AGGTGACCGT	60%
19	OPA-19	CAAACGTCGG	60%
20	OPA-20	GTTGCGATCC	60%
21	OPQ-01	GGGACGATGG	70%
22	OPQ-03	GGTCACCTCA	60%
23	OPQ-04	AGTGCGCTGA	60%
24	OPQ-05	CCGCGTCTTG	70%
25	OPQ-06	GAGCGCCTTG	70%
26	OPQ-08	CCCCGATGGT	70%
27	OPQ-09	CTCCAGCGGA	70%
28	OPQ-10	GGCTAACCGA	60%
29	OPQ-11	TGTGCCCGAA	60%
30	OPQ-12	TCTCCGCAAC	60%
31	OPQ-13	AGTAGGGCAC	60%
32	OPQ-14	GGAGTGGACA	60%
33	OPQ-15	GGACGCTTCA	60%
34	OPQ-16	GGGTAACGTG	60%
35	OPU-01	ACGGACGTCA	60%

Table 2: primers , No. of amplified bands, No. of polymorphic bands , % Polymorphism and Size (bp).

Primer number	No. of amplified bands	No. of polymorphic bands	% Polymorphism	Size (bp)
OPA-01	101	11	10.89	250 – 1600
OPA-02	76	10	13.16	260 – 1700
OPA-03	60	5	8.33	200 – 1000
OPA-04	83	13	15.66	220 – 1300
OPA-10	72	6	8.33	170 -1500
OPA-13	134	16	11.94	120 – 1600
OPA-14	81	9	11.11	200 – 1500
OPA-15	44	2	4.55	470 – 1600
OPA-16	98	10	10.20	230 – 1800
OPA-18	113	12	10.62	130 – 1600
OPA-19	55	16	29.09	200 – 1600
OPQ-01	97	10	10.31	170 – 1600
OPQ-04	120	18	15.00	110 – 1600
OPQ-05	97	12	12.37	200 – 1600
OPQ-06	75	10	13.33	120 – 1800
OPQ-08	60	11	18.33	270 – 1600
OPQ-09	75	12	16.00	280 – 1600
OPQ-11	77	9	11.69	150 – 1900
OPQ-13	87	6	6.90	200 – 1300
OPQ-15	60	7	11.67	120 – 900
OPU-01	59	11	18.64	170 – 1300
Total	1724	216		

Table. 3 : similarity of RAPD profile generated through 21 primers on 40 chicken

Bird No	Ross ♂	Ross ♀	Indian ♂	Indian ♀	ISA ♂	ISA ♀	Hy line ♂	Hy line ♀
Ross ♂	1							
Ross ♀	0.696	1						
Indian ♂	0.64	0.713	1					
Indian ♀	0.663	0.741	0.735	1				
ISA ♂	0.635	0.668	0.665	0.714	1			
ISA ♀	0.597	0.587	0.653	0.631	0.717	1		
Hy line ♂	0.654	0.598	0.622	0.625	0.674	0.722	1	
Hy line ♀	0.598	0.624	0.665	0.709	0.704	0.66	0.655	1

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