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RESEARCH PAPER

Genetic Similarity of two different color Local Guinea Fowl (*Numida Meleagris*) By Using RAPD-PCR Technique

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A B S T R A C T:
The aim of present study to determine DNA polymorphism, between and within two different color (white) and (black) guinea fowl lines. Blood samples were collected about 3 ml of 30 white guinea fowl and 30 black guinea fowl, in six different local farms. DNA was isolated, the purity of the DNA samples ranged from 1.8 to 1.9. RAPD – PCR was used to evaluate genetic similarity between the lines of guinea fowl. Using eighteen primers for amplification of RAPD, from Gen Script USA. A total of (14) primers out of (18) primers gave results to find Genomic DNA complementary sites. After electrophoresis the largest number of bands were appeared with OPA-03 primers, but the smallest was obtained when OPQ-15 primer used. Determined Genetic similarity as the mean for the primers used, between the black and white guinea fowl, was 0.76. The coefficient of genetic similarity within white guinea fowl line, was 0.78 and 0.73 within black guinea fowl line.

KEY WORDS: Genetic similarity, Guinea fowl, RAPD – PCR, Polymorphisms.
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INTRODUCTION

The most important type of poultry in Iraq is domestic fowl. The domestication of poultry was originally started from the wild types that collect the eggs from it for hatching and rearing young birds, but keeping the birds permanently in captivity later. The term poultry is used collectively for those species of birds that have been domesticated to reproduce and grow in captivity and render product of economic value such as meat, eggs manure. The term poultry is applied to birds of several species like fowls or chicken, guinea, duck, turkeys etc.

The second most exceedingly eaten globally from of meat is the birds and along with eggs, because it has high-quality protein with low proportion of fat which supply nutritionally helpful food (Fadhil et al., 2016). Around the world in different avian spices the genetic variation has been investigated, locate the degree of relatedness or to identify genes responsible for interesting traits. The molecular nature of the precise differences in the nucleotide sequences within gene is. The genetic identity is related to check relationships between species and breeds. The genetic improvement of poultry has created new possibilities because of the molecular development. Build on DNA markers (RAPD, RFLP, VNTR, CRI, SSR – PCR), they enable to be studied whole genomes or the polymorphic of DNA fragments. The rapid and simple technique is RAPD (Randomly Amplified Polymorphic DNA) to determining relatedness and to

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identifying responsible of genes for avian advantage trait. In the study of similarity or variation the RAPD – PCR has been used repeatedly in chickens populations (Abdulrazaq and Suliaman.,2016, Dehghanzadeh, et, al., 2009, Smith, et, al., 1996, Sharma, et, al.,2001, Ali, et, al., 2003, Sharma and Singh, 2002, Semenova, et al., 2002), ducks (Dolmatova, et al., 2000a, b), turkey (Smith, et, al., 1996), animal (Abdulrazaq, et, al., 2019).guinea birds (Sharma, et, al., 1998, Nahashon, et, al., 2010, Daham and Sharma, 2007). In many countries the basis of genetic improvement in guinea birds species to improve meat production efficiency. Due to the nutritional value and the gorgeous taste of their meat, guinea birds are a valuable though undervalued species of gallinaceous poultry. Guinea birds farming was popular in the 1980s and 1990s, in Iraq, when guinea birds multiplication farms and breeding were instituted. Today, yet, there are no reproduction programmers and organized breeding for guinea bird, which are mainly kept under backyard systems in Iraq. Understanding better of guinea birds lines may help to increase their popularity. Determine the genetic similarity within and between two different color local guinea fowl are the aim of this study.

1. MATERIALS AND METHODS

1.1 SAMPLES COLLECTION

The blood samples were collected from the wing near the elbow joint into tubes contain a EDTA, and DNA analysis was performed directly in the laboratory. Blood samples were collected about 3 ml of 30 black guinea birds and 30 white guinea birds, in 6 different local farms.

1.2 GENOMIC DNA EXTRACTION

The DNA Extracted by Using Kit Promega USA (Beutler et., 1990). The laboratory analyses of DNA were performed in Erbil Medical Research Center / Hawler Medical University. The DNA quality isolated by the Nano Drop® spectrometere, purity of DNA samples ranged from 1.8 to 1.9. Were diluted the samples to 30 ng /µl for use of RAPD – PCR in the Research Center of Salahaddin University - Erbil.

1.3 RAPD- PCR ANALYSIS

Using 18 primers for amplification of RAPD, from Gen Script USA. A total of (14) Primers out of (18) Primers gave results to find Genomic DNA complementary sites, OPA-07, OPA-10, OPA-03, OPA-06, OPA-12, OPA-14, OPA-15, OPA-19, OPA-20, OPQ-10, OPA-04, OPQ-01, OPQ-12, OPQ-15, (Table 1). The DNA analyses was amplified in a T Gradient thermo cycler. At 95°C for 5 min initial denaturation double-stranded DNA was carried. This was followed by 40 cycles: 95°C for 1 min, 42°C for 1 min, 72°C for 2 min. At 75°C for 5 min the complementary strands were synthesized.
The contained of PCR reaction include: DNA 30 ng, primer 10 µM, 1x PCR buffer GoTaq® Green Master Mix, 1x, MgCl2 3 mM, Each: (dATP, dCTP, dGTP dTTP) (400µM), The volume of total reaction was 25 µl. The Gene Ruler ^{TM} 100bp DNA Ladder marker (100 – 1500 bp). In all samples 2 µl of Blue / Orange loading day was added to 10 µl of the product. The 100V power supply was performed was. Electrophoresis long was about 90 min. The products of PCR were tested with electrophoresis on 2% agarose gel in 1X TBE buffer, stained with ethidium bromide (promega, USA). The pattern was amplified by ultraviolet light and photographed.

1.4. STATISTICAL ANALYSIS

The F is genetic similarity between the white guinea birds and black guinea birds. It was computed by used the (bands) fragments amplified in PCR reaction, the formula of Nei and Li (1979) was applied: F=2x Nxy / (Nx + Ny). Data recording and statistical analysis RAPD patterns were recorded because of (1) or absence (0). The polymorphism of each primer was Calculated by use the formula: polymorphism = (Np / Nt) × 100, NP = # polymorphic forms of random primer Nt = total number of sample primer domains (Bowditch et al., 1993).The numerical data were analyses statistically. Excel software were using to calculate the arithmetic means of the analyses traits.

2. RESULTS AND DISCUSSION

The technique of RAPD was used in the present study to assess relatedness of genetic

among guinea birds line. The samples containing eighteen genotypes chosen randomly were tested. Reaction of PCR performed with fourteen selected primers in all of guinea birds of both white and black colors (Table 1). The bands number common to both of white color line and black color line was determined from the band obtained from electrophoresis (Figure 1).

Table 1. Sequences of the primers used GC content

Primer Name	Sequence 5' to 3'	%GC content
OPA-07	GAAACGGGTG	60%
OPA-10	GTGATCGCAG	60%
OPA-03	AGTCAGCCAC	60%
OPA-06	GGTCCCTGAC	70%
OPA-12	TCGGCGATAG	60%
OPA-14	TCTGTGCTGG	60%
OPA-15	TTCCGAACCC	60%
OPA-19	CAAACGTCGG	60%
OPA-20	GTTGCGATCC	60%
OPQ-10	GCTAACCGA	60%
OPA-04	AATCGGGCTG	60%
OPQ-01	GGGACGATGG	70%
OPQ-12	TCTCCGCAAC	60%
OPQ-15	GACGCTTCA	60%

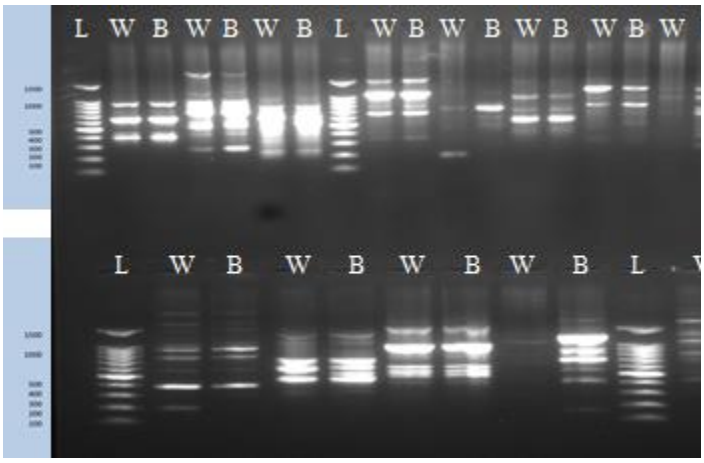

Figure 1. Electrophoretic pattern of genomic DNA amplification in white and Black guinea fowl using OPA-07; OPA-10; OPA-03;OPA-06; OPA-12; OPA-14;OPA-15; OPA-19; OPA-20; OPQ-10;OPA-04; OPQ-01;OPQ-12; OPQ-15 primer.

Table (2) is contain the PCR product from both white color guinea fowl line and black color guinea fowl line. The higher numbers of band shown in Black guinea fowl lines (47) then the white guinea fowl lines of total (92) bands. The polymorphism level detected between the both white and black phenotypes varied. The polymorphic bands showed higher number in black phenotype and the percent of Polymorphism (%6.2). A total of 206 different fragments (bands) are produced, out of them 36 bands were polymorphic, 36 Mono band, 170 Monomorphic band. It is higher than, Al_Jallad, et al. (2012), study 125 different fragments (bands) a total, out of them the polymorphic bands were 93 bands. The number of bands amplified varied from 7 (OPQ-15) to 53 (OPA-03) per Primer. The primer OPA-10 detect highest number of polymorphic bands 12 polymorphic band, while detected 1 polymorphic band with (OPA-07), and there is a lowest number of band. In this study it was found an average number 17.48 of bands, polymorphic bands per primer. The highest range of the molecular weight was (200 - 1500 bp) for the primer OPA-12, and was over in less primer OPA-14 which is (400 – 500 bp) for each primer are given in ,Table (3). The size difference average from 325 to 1325 bp reported by Fadhil, et al. (2016).

Table 2. Number of bands for different groups of guinea fowl

guinea fowl	Total number of bands	polymorphic band	% Polymorphism
white guinea fowl	45	2	4.4
Black guinea fowl	47	3	6.4
All	92	5	10.8

The genetic similarity coefficient for analysis different guinea birds line. The F values for white guinea birds and black guinea birds within each primers were the mean genetic similarity coefficients for all of fowls of a given different colors. The average of F was 0.73 for white guinea birds and 0.78 for black guinea birds. It was Strikingly lower that reported by Sharma, et, al., (1998), which was (0.95 – 0.97).Within the primers, the highest coefficient of genetic similarity between the different guinea birds lines was 0.93 in (OPQ-01) were the lowest 0.76 was in (OPA-12). Thin is similar with reported by Bawej,

Table 3. Number of bands, % Polymorphism, Size (bp),Primer efficiency and Discriminatory Value for different prime

Primer number	Total number of band	polymorphic band	Mono band	Monomorphic band	% Polymorphism	Size (bp)
PA-07	15	1	3	14	6.67	500 - 1000
OPA-10	25	12	2	13	48.00	100 - 1400
OPA-03	53	5	10	48	9.43	220 - 1200
OPA-06	15	7	4	8	46.67	100 - 800
OPA-12	35	2	8	33	5.71	200 - 1500
OPA-14	7	1	1	6	14.29	400 - 500
OPA-15	7	1	1	6	14.29	401 - 500
OPA-19	7	1	1	6	14.29	402 - 500
OPA-20	7	1	1	6	14.29	403 - 500
OPQ-10	7	1	1	6	14.29	404 - 500
OPA-04	7	1	1	6	14.29	405 - 500
OPQ-01	7	1	1	6	14.29	406 - 500
OPQ-12	7	1	1	6	14.29	407 - 500
OPQ-15	7	1	1	6	14.29	408 - 500
Sum	206	36	36	170	17.48	0

et al. (2012), who found coefficient of genetic similarity was 1 between both colors white and gray guinea birds, and it is indicative of entire similarities. The high and average genetic similarity was determined as the mean for primers, 0.97 between both colors. But I found 0.76 between white and black colors of guinea birds determined as the mean for primers used, (Table 4).

Table 4. Coefficient of genetic similarity (F) for different groups and primers

	Within groups		
Primer	white guinea fowl	Black guinea fowl	white/Black
OPA-07	0.84	1.00	0.92
OPA-10	0.93	0.82	0.87
OPA-03	0.80	0.85	0.82
OPA-06	0.86	0.92	0.89
OPA-12	0.73	0.80	0.76
OPA-14	1.00	0.82	0.91
OPA-15	0.91	0.78	0.85
OPA-19	0.80	1.00	0.90
OPA-20	0.81	0.92	0.87
OPQ-10	0.94	0.89	0.92

Nahashon, et, al., (2003) when determining the genetic similarity between guinea birds and hens it ranged from 0 - 0.46 and within guinea birds population from 0.89 – 0.98. It is a strong indicative of the lowest genetic similarity between species. In the other hand Sharma, et, al., (1998), ranged the genetic similarity from 0.95 – 0.97 within variety but between variety from 0.99 – 1,they show that genetic variation was lowest between and within varieties, when the evaluated the genetic variation from three (white, lavender and pearl) guinea birds. In the present study the coefficient of similarity, calculated among both white and black guinea birds 0.76 is lowest slightly than that reported by Sharma, et, al., (1998). In the study of Bawej, et al. (2012) study the mean value ranged from 0.92 - 1.0 within primers and 0.65 genetic similarity for white guinea birds and 0.64 for grey guinea birds within the color varieties of guinea fowl. It is know that the same primers with different poultry species may produce various results in various laboratories depending on the status of reaction, which translates into the genetic similarity coefficients. On the other hand, the various primer use in the same species gives various amounts of PCR products, which interpret in to various values of the genetic similarity coefficient.

3. CONCLUSIONS

Through the present study it was confirmed that RAPD is a valuable tool to evaluate genetic relationships. It indicated the effectiveness of RAPD markers in detecting the estimating of similarity and polymorphism between and within guinea birds lines. Genetic similarity evaluating

are useful for conservation of native guinea birds lines as a genetic resource and natural monument.

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