

## EVALUATION OF GENETIC VARIABILITY BETWEEN THREE LINES OF CHICKENS BASED ON RAPD-PCR AND 18S rRNA GENE SEQUENCING IN ERBIL (IRAQI KURDISTAN REGION)

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### ABSTRACT

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The current study was carried out to evaluate and identify the genetic variation between Local Black chicken lines with two commercial Layer (Isa Brown) and Broiler (Rose) breeds using RAPD markers and sequencing approach of 18s rRNA gene. From the result of the RAPD marker, all primers used were produced 152 scorable bands ranging from 2 to 9 with the size ranging from 320 to 2990 bp with percentage polymorphic loci 64.86% among chicken populations. The highest amplified fragments by primer OPC-11 and lowest by OPAA-03 were 24 and 8, respectively. The mean of the observed number of alleles (na), effective number of alleles (ne), gene diversity (h), Shannon's information index (I) for all loci found to be 1.6486, 1.5189, 0.2883 and 0.4129, respectively. The existence of a high level of polymorphisms and targeted (74) loci throughout all chicken populations/primers indicate sufficient genetic distance and more genetic variability among chicken populations using RAPD-PCR techniques. Result of blasted sequences of 18srRNA gene of local chicken has GenBank accession number MT808178 and MT808179 by BLAST tool against Gallus gallus, it showed the highest identity 95.74% and 94.88% for data of first and second part, respectively. The overall dendrograms clustered showed that the local chicken was closer to the commercial layer than to the broiler chicken lines. Therefore, it suggests that improving the local Black chicken line according to the layer breeding program to collect genetically invaluable genetic resources.

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## INTRODUCTION

Domestic chicken is the most widespread species among domestic animals (Meydan *et al.*, 2016). According to (Eriksson J. *et al.*, 2008), the current chicken breeds are based on DNA data come from *Gallus sonneratii*. In contrast, *Gallus gallus gallus* is a monophyletic origin for domestic breeds (Akishinonomiya *et al.*, 1994 and 1996), and all domestic chicken breeds may have arisen from solo domestication in Thailand and nearby regions. Local Iraqi chickens are well

suited to the local environmental conditions (-5°C to 50°C), high disease resistance, and are raised throughout the country for meat and egg production and the phenotypic differentiation was used to define the genetic diversity of native chickens. Also, based on their feather color, five distinct genetic lines of local chicken, namely Black, Brown, Barred, White, and White Naked, were derived from indigenous foundation populations, which were a sample collected from all over the country. Since 1986, efforts have been made to purify and conserve Iraqi indigenous chickens (Al-Rawi and Al-Athari, 2002 and Alameri *et al.*, 2019). Compared to commercial chickens, local chicken breeds have a good flavor of meat and eggs. They are known to be proficient mothers, feed competence, good foragers, and slightest care is required for growth. They considered as the national genetic resources and the key factor in constructing renewable agriculture (Ibrahim *et al.*, 2015).

Molecular DNA markers provide valuable information on genetic variability, serve to characterize breed identity between or within species, and make measuring relatedness between populations more credible (Tixier-Boichard *et al.*, 2009). Several forms of DNA molecular markers, such as RAPD, AFLP, SNP, and SSR, have been developed as a result of the rapid development of modern biotechnology (Helal and Ahmed, 2018). Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) assays have been used to investigate genetic diversity among different strains and varieties of chicken which had a significant influence on the conservation and breeding of local chicken genetic resources (El-Gendy *et al.*, 2005; Ghanem *et al.*, 2012 and Ahmed and Adel, 2015). On the other hand, gene/whole-genome sequencing, one of the most powerful current techniques, has been used to describe biodiversity studies in poultry and farm animals. Meanwhile, it can detect all variants in the genome, making it the most straightforward approach and providing more complete information on genetic variability among different chicken breeds (Farrag *et al.*, 2010 and Yang *et al.*, 2013). *Gallus gallus* assembly has been used as a reference genome for assembling sequenced genomes of other birds. Individual chicken Ribosomal Ribonucleic Acid (18S rRNA and 28S rRNA) gene fragments are accessible in the GenBank database (Dyomin *et al.*, 2016). In poultry, the 18s rRNA genes investigated in biodiversity analysis are common. The 18s rRNA gene sequence can be used as a reference tree to compare to other 18s rRNA gene sequences from different chicken breeds in different regions that are accessible in the National Center for Biotechnology Information (NCBI) database (GenBank), and to construct a phylogenetic tree from them (Hadziavdic *et al.*, 2014 and Leonard *et al.*, 2016).

Local chickens are key genetic resources for future breeding programs as well as a source of research material (Romanov and Weigend, 2001). Native chicken populations, for preservation from extinction and improvement, need to be characterized at the molecular genetic level (Meydan *et al.*, 2016). The purpose of this study was to evaluate and identify genetic variance between a local black chicken line and commercial Broiler and Layer breeds in attempt to improve the local chicken for egg or meat production, as well as to characterize the local black chicken line genetically.

## MATERIALS AND METHODS

### Experimental Populations and Blood collection

The present study conducted at the College of Agricultural Engineering Sciences, Salahaddin University- Erbil, Kurdistan Region, Iraq, in cooperation with the Animal Science & Plant Protection Departments / Directorate of Agricultural Research- Erbil, Ministry of Agriculture and Water Resources, Kurdistan Region, Iraq. For this study, Local Black (LB) chicken line of both sexes (8) eight males and (8) eight females used were breeds that have been maintained at the Animal Science Department, Directorate of Agricultural Research- Erbil and one egg-type layer breed Isa Brown (IB) of both sexes (5) five males and (10) ten females from Shimal Company and one meat-type breed white Broiler Rose, (BR) of both sexes (10) ten males and (10) ten females from Eyfan Feed company was chosen. For each of these varieties, One mL of venous whole blood from the wing vein, from the total (51) fifty-one individual chickens of both sexes were collected into 2.0 mL tubes containing 5 mM EDTA and transported on ice and subsequently stored at  $-20^{\circ}\text{C}$ .

### DNA Extraction

Genomic DNA was extracted from the whole blood by using a DNA extraction Kit (QIAGEN) according to the manufacturer's instructions with the minor modification of the amount of sample. The quantity and quality of isolated DNA were determined using a (Nanodrop 1000 Thermo Scientific/ Labtech.). The individual extracted DNA obtained was then pooled according to their line and breeds.

### PCRs Condition and program

For the RAPD-PCR amplification, the 11 ten-nucleotide decamer primers were tested based on these previous studies; {(OPA-10, OPB-07, OPB-08) Ibrahim *et al.* (2015), Saudi Arabia; (OPA-20) Mollah *et a.* (2009) Bangladesh; (OPAA-03, OPAA-07) Omarbly *et al.* (2013) India; (OPC-01, OPC-11) Rahimi *et al.* (2005) Iran; (OPU-09) Olowofeso *et al.* (2006) China; and (OPA-05, OPZ-11) Al-Atiyat. (2010) Jordan, with the GC % contain 60 to 70}. For the 18s rRNA gene-PCR amplification, the specific primers were designed using the software Primer3 (<https://primer3plus.com/cgi-bin/dev/primer3plus.cgi>). To get amplification of 18s rRNA gene fragment in the genomic DNA, the newly own designed primers in all three populations were used (F: CGGCTACCTGGTTGATCCTG and R: CGCCGGTCCAAGAATTTTCAC, F: CGTATTGTGCCGCTAGAGGT and R: TGATCCTTCGGCAGGTTTCAC) for part 1 and 2, respectively and all the primers were synthesized from (Macrogen, Korea). PCRs were performed in a final volume of (20  $\mu\text{l}$  containing 40 ng/ $\mu\text{l}$  and 25  $\mu\text{l}$  containing (80-100) ng/ $\mu\text{l}$  of template DNA), 10 pmol/ $\mu\text{l}$  of each primer also for (forward & reverse) primers, 2X reaction buffer, 1 unit of Prime *Taq* DNA polymerase, 0.5 mM each of dNTPs, 4 mM of  $\text{MgCl}_2$ , and loading dye (GeNet Bio, Korea). PCR amplification was performed in a thermocycler (PCR max Alpha, UK), with an initial denaturation step at  $95^{\circ}\text{C}$  for 300 s followed by 35 cycles of 40 s at  $95^{\circ}\text{C}$ , 50 s at ( $34^{\circ}\text{C}$  and  $60.5^{\circ}\text{C}$ ), (120 s and 85 s) at  $72^{\circ}\text{C}$  and a final extension step at  $72^{\circ}\text{C}$  for 600 s, for the RAPD-PCR and the 18s rRNA gene-PCR amplification, respectively.

## **Electrophoresis**

Extracted genomic DNA was run on 1 % agarose gel with addition of Red safe dye (GeNet Bio, Korea) and staining gels were visualized under UV-transilluminator and photographed. But for The RAPD-PCR products and 18s rRNA gene PCR products were run on 1.5% and 2% agarose gel respectively.

## **Genetic Data Analysis:**

### **RAPD-PCR Data Analysis**

For the RAPD-PCR amplification, the gel image based on PCR product bands were scored by PyElph (version. 2.5) program (Pavel and Vasile, 2012) based on their absence (0) or presence (1) with the molecular weight of the bands based on the marker. The scores obtained were then pooled for constructing a single data matrix, which was used for estimating the proportion of polymorphic loci, Nei's (1987) the mean of Observed number of alleles ( $n_a$ ), The effective number of alleles ( $n_e$ ), Gene diversity ( $h$ ), and Shannon's Information Index ( $I$ ), Total genotype diversity among populations ( $H_t$ ), Coefficient of gene differentiation ( $G_{ST}$ ), Nei's (1978) unbiased Genetic distance and Genetic Identity, construction of dendrogram and cluster analysis algorithm by unweight Pair Group Method of Arithmetic Means (UPGMA) among populations. All statistical analysis was carried out by using PopGene32 (version 1.32), a computer program (Yeh. *et al.*, 2000).

### **18s rRNA gene Data Analysis**

The 18s rRNA gene-PCR amplification of all the three populations was sequenced from (Macrogen, Korea). The nucleotide sequences of local black chicken line blasted against all the sequences available in the NCBI by the Basic Local Alignment Search Tool (BLAST), (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to find out the pairwise similarity, nucleotides changed and differences between the 18s rRNA gene of local black chicken line and others available in the NCBI. Also, the 18s rRNA gene- sequences of the three chicken lines and sequence of 18s ribosomal RNA gene of *Gallus gallus* (Sequence ID: MG967540.1) available in NCBI were submitted to a Multiple Sequence Alignment-MUSCLE (Multiple Sequence Comparison by Log- Expectation) Version (3.8) Tools (<https://www.ebi.ac.uk/Tools/msa/muscle/>) for aligning sequences and dendrogram construction (Madeira *et al.*, 2019). Also, the 18s rRNA gene- sequence data of local black chicken line submitted to GenBank to get accession number.

## **RESULTS AND DISCUSSION**

In the study, after genomic DNA was extracted, the quantity and quality of isolated DNA were (95.40 to 115.38) ng/ $\mu$ l and (1.88 to 1.95) A260/280 respectively. With the successful of minor modification of the amount of sample, high purity and sufficient amount of genomic DNA is extracted.

### **RAPD-PCR Data Results**

All eleven primers used in the present study were produced different fragment band patterns with a varied number of scorable bands, (Figure 1 and 2). The gel image of the RAPD-PCR products submitted to PyElph (V2.5) program, the characteristics of the fragments produced through populations by the primers were summarized in (Table 1). All primers generated a total of 152 cleared bands

through all three populations. The number of bands varied from 2 to 9 with the size ranged in molecular weight approximately from 320 to 2990 bp. The highest amplified fragments by primer OPC-11 and lowest by OPAA-03 were 24 and 8, respectively, recorded. The total number of amplified fragments per chicken populations by all the primers; white Broiler Rose (BR), layer breed Isa Brown (IB), and Native Local Black (LB) found to be 47, 51 and 54 bands, respectively. The differences in both the number and size of the amplified fragments indicate genetic diversity among the chicken populations.

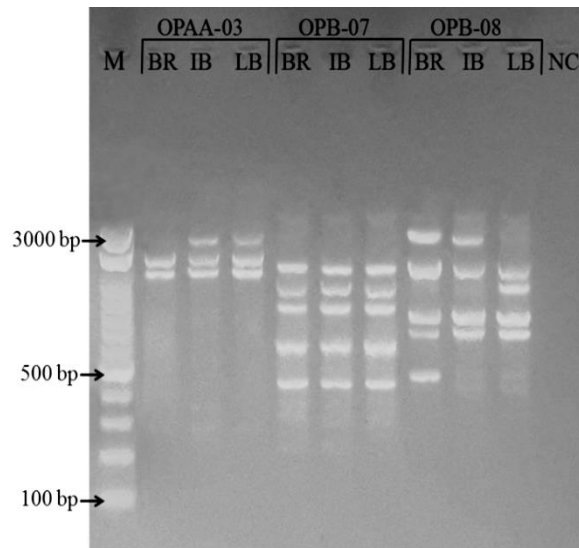


Figure (1): RAPD-PCR fragments of different chicken populations using OPAA3, OPB-07, and OPB-08 primers (M-100 bp DNA Ladder (OneMARK), BR: Broiler Rose, IB: Isa Brown and LB: Local Black Samples, NC- negative control)

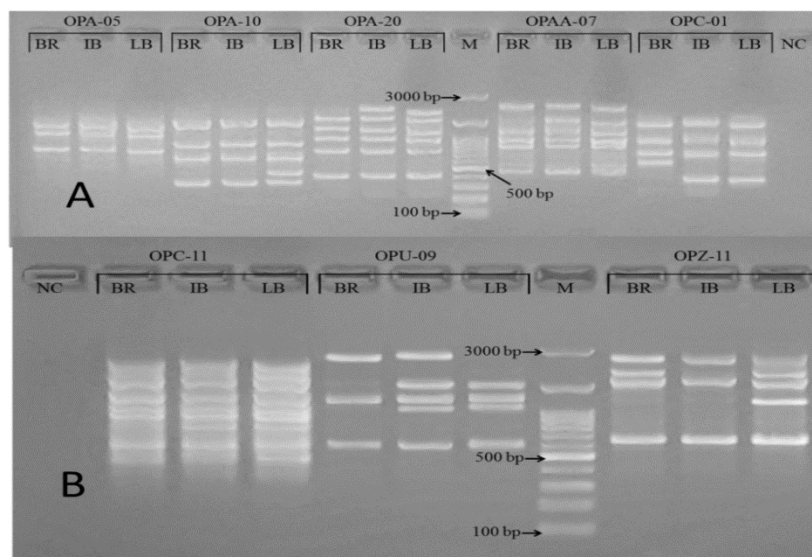


Figure (2): RAPD-PCR fragments of different chicken populations using (A: OPA-05, OPA-10, OPA-20, OPAA-07, and OPC-01), (B: OPC-11, OPU-09, and OPZ-11) primers (M-100 bp DNA Ladder (OneMARK), BR: Broiler Rose, IB: Isa Brown and LB: Local Black Samples, NC- negative control)

Table(1): Characteristics of the fragments produced through populations by RAPD-PCR technique

Name of primer	Chicken Populations			Overall/ primers	
	Broiler Rose (BR)	Isa Brown (IB)	Local Black (LB)		
	No. of bands	No. of bands	No. of bands	Size ranged (bp)	No. of bands
OPAA-03	2	3	3	1320-2940	8
OPB-07	5	5	5	440-1410	15
OPB-08	5	4	4	490-2990	13
OPA-05	3	3	3	780-1460	9
OPA-10	4	4	5	320-1500	13
OPA-20	5	6	6	410-2600	17
OPAA-07	5	6	6	480-2720	17
OPC-01	4	4	4	370-1530	12
OPC-11	7	8	9	490-2680	24
OPU-09	3	5	4	580-2920	12
OPZ-11	4	3	5	660-2880	12
Overall/ populations	47	51	54	320-2990	152

### Genic Variation Statistics Data Results

In this study, from the Summary of Genic Variation Statistics Nei's (1987), (PopGene32); Total (74) loci targeted by all primers throughout all the chicken populations and the mean of the observed number of alleles ( $n_a$ ), The effective number of alleles ( $n_e$ ), Gene diversity ( $h$ ), and Shannon's Information index ( $I$ ) for all the loci among all three populations were found to be 1.6486, 1.5189, 0.2883 and 0.4129, respectively. Nei's Analysis of Gene Diversity in these three Populations was showed the mean value of total genotype diversity among populations ( $H_t$ ) was (0.2883) and the Coefficient of gene differentiation ( $G_{ST}$ ) was (1.000). Among 74 loci which are considered 26 (35.14%) monomorphic and 48 (64.86%) polymorphic, including 28 (58.33%) of unique loci. The RAPD-PCR technique is produced 152 scorable bands with the percentage polymorphic loci 64.86% among the chicken breeds. The result of percentage polymorphic loci shows greater than the result reported by( Rahimi *et al.*, 2005; Olowofeso *et al.*, 2006; Mollah *et al.*, 2009; Ibrahim *et al.*, 2015 and Helal and Ahmed 2018) reported that were 45%, 48.94%, 64.10%, 34.7%, and 34.56%, respectively. The result of the observed number of alleles ( $n_a$ ) was 1.648 shows smaller than the result was reported by (Nikkhoo *et al.*, 2011) who reported that was 2.00 but the result of the observed number of alleles ( $n_a$ ) shows higher than reported by( Helal and Ahmed 2018) who reported that was 1.173. The result of The effective number of alleles ( $n_e$ ) was 1.518 shows greater than the result were reported by (Nikkhoo *et al.*, 2011 and Helal and Ahmed 2018) reported that were 1.473 and 1.238, respectively but lower than reported by (Al-Atiyat, 2010) it was 1.65. The result of Shannon's Information index ( $I$ ) was 0.412 shows smaller than the result were reported by(Al-Atiyat, 2010 and

Nikkhoo *et al.*, 2011) they reported that were 0.58 and 0.442, respectively. Total genotype diversity among populations (Ht) was 0.2883 shows slightly greater than the result was reported by (Nikkhoo *et al.*, 2011) who reported that was 0.286. The result of Gene diversity (h) was 0.288 and Coefficient of gene differentiation ( $G_{ST}$ ) was 1.00 higher than reported by (Mollah *et al.*, 2009 and Nikkhoo *et al.*, 2011) for Gene diversity (h) they reported 0.25 and 0.286 and for Coefficient of gene differentiation ( $G_{ST}$ ) were 0.34 and 0.368, respectively. The result of Gene diversity (h) shows smaller than the result was reported by Mercan and Okumuş, 2015, who reported that was 0.675 compared with commercial layer and broiler populations. In addition, our result shows sufficient knowledge in the setting condition of the RAPD-PCR program and accuracy of the selection primers and the large enough effective population size and also based on the results obtained by the RAPD-PCR technique from the Summary of Genic Variation Statistics Nei's (1987) and Nei's Analysis of Gene Diversity, the existences of the high level of polymorphisms and targeted (74) loci throughout all the chicken populations by all primers may indicate a sufficient genetic distance and more genetic variability among chicken populations in this study.

The result of Nei's unbiased measures of genetic identity and genetic distance shows the highest genetic identity of local black are 0.4730 and 0.4865 and lowest genetic distances are 0.7487 and 0.7205 to Broiler Rose and Isa Brown, respectively in (Table 2). The native black chicken line shows genetically highest similarity 0.4865 and lowest distance 0.7205 with the commercial layer breed. The result from the RAPD marker indicates the genetic distance between the native black chicken line and commercial layer breed agreed with those reported by (Mollah *et al.*, 2009) who is reported that local chicken lines and commercial Broiler have highest genetic distance than the commercial layer. The result showed slightly more identity and less distance of local black to layer breed than the Broiler.

Table (2): Nei's Unbiased Measures of Genetic Identity (above diagonal) and Genetic distance (below diagonal)

Populations	Broiler Rose (BR)	Isa Brown (IB)	Local Black (LB)
Broiler Rose (BR)	****	0.7432	0.4730
Isa Brown (IB)	0.2967	****	0.4865
Local Black (LB)	0.7487	0.7205	****

### Phylogenetic tree based on RAPD marker

The dendrogram based on Nei's (1978) genetic distance was constructed based on a comparative analysis of the total loci obtained with the eleven RAPD markers by using the UPGMA method among populations. The phylogenetic tree shows the three chicken lines clustered into two main groups, (figure 3). In the first main group, the closest genetic distance was found to be 0.15 between both commercial Broiler Rose and Isa Brown lines, which are clustered together. The genetic distance of the second main group includes chickens of local black which is found to be 0.37. This result from constructed dendrogram similar to

the result was reported by( Mercan and Okumuş, 2015) in which the commercial layer and broiler placed in the closed cluster. The dendrograms construct results shows the local black chicken line have smaller genetic distances with the commercial layer breed than the commercial broiler breed.

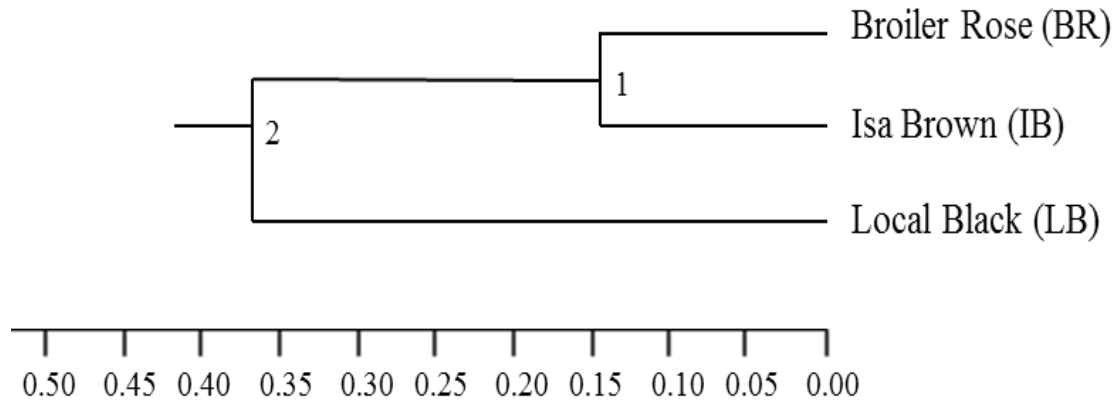


Figure 3. UPGMA Dendrogram Based Nei's (1978) Genetic distance

### 18s rRNA gene Data Results

In the present study, Both parts of the 18s rRNA gene sequence data of local black chicken line submitted to GenBank and published in the Nucleotide-NCBI with GenBank accession number MT808178 and MT808179 for data of the first and the second part respectively. The result of blasted sequences by BLAST tool (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) of 18s ribosomal RNA gene sequence of the local black chicken line (data of the first part) is found the highest query coverage 99% and percent identity 95.74% with *Gallus gallus* reference sequence Accession (GenBank, Sequence ID: MG967540.1, Dyomin A. *et al.*, 2019) from 1873 to 2770 and it is covered 897 nucleotides with the identity 863/904(95%), gaps 13/904(1%), and 0.0 of E-value as shown in (figure 4). Besides, a total of 21 nucleotides are changed in both sequences. Also (data of the second part) is found the highest query coverage 99% and percent identity 94.88% from 2776 to 3300 and it is covered 524 nucleotides with the identity 500/527(95%), gaps 6/527(1%), and 0.0 of E-value (the figure is not present) with a total of 16 nucleotides have been changed in both sequences. The results show high genetic variation in the local black chicken line. The result shows amplification of 1431 bp out of the 1824 bp of original length of 18s rRNA gene from 1842-3664 nucleotides. Missing only 6 bp between first and second amplified parts indicates the successful designed own primers with the determination of high accurate annealing temperature. On the other hand, result shows very good invaluable genetic resources, especially the most A and T nucleotides changed to G and C in the black chicken line, which means, native black chicken line has enough biodiversity and a more stable of the 18s rRNA gene sequence.

### Percent distance matrix result based on the 18s rRNA gene

In this study for Alignment, the sequences of the 18s rRNA gene of all chicken populations and *Gallus gallus* with the reference sequence (GenBank, Sequence ID: MG967540.1) aligned by Multiple Sequence Alignment-



MUSCLE tool, the distance matrix and Phylogram tree are determined based on the data of the first part of the 18s rRNA gene. The result of the percent distance matrix shows the lowest genetic distance between local black and Isa Brown 95.49 (table 3). This result indicates the genetic distance between the native black chicken line and layer breed disagreed with the result reported by( Zhang *et al.*, 2002) who reported that local chicken breeds closer to commercial Broiler than the commercial layer. According to the result obtained from Nei's unbiased measurement of genetic identity and distance of the RAPD marker and percent distance matrix of the 18s rRNA gene sequenced nucleotides, the native black chicken line shows genetically highest similarity and lowest distance with the layer breed than the Broiler breed.

Dendrogram constructed clustered into three main groups (figure 5). In the first main group, the closest genetic distance is found to be 0.026 between Broiler Rose and *Gallus gallus*, which were clustered together. The genetic distance of the second main group includes chickens of Isa Brown which is found to be 0.053, and the genetic distance of local black is 0.22 which is located in the third main group. The dendrogram construct result similar to the result was reported by Zhang *et al.*, 2002, which were a close relationship among broiler breeds. This result shows the local black chicken line has relatively large genetic distances with the commercial broiler breed and *Gallus gallus* than the commercial layer breed. In addition, both dendrograms construct results based on Nei's (1978) genetic distance RAPD marker and of the 18s rRNA gene sequenced nucleotides shows the local black chicken line have relatively small genetic distances with the layer breed than the broiler breed.

Query	9	TGTCTC-AAGATTACGCCATGCATGTCTAAGTACACACGAGCGGTACAGTGCAAACGT	67
Sbjct	1873	.....A.....A.....-.....-.....-	1929
Query	68	CGAATCGGCTCATTAAATCAGTTATGGTTCCCTTTGGTCGCTCCCTCCCGTTACTTGGAT	127
Sbjct	1930	.....-.....	1988
Query	128	AACTGTGGTAATTCTAGAGCTAATACATGCCGACGAGCGCCGACCTCCGGGGACGCGTGC	187
Sbjct	1989	.....	2048
Query	188	ATTTATCAGACAAAACCAACCCGGGCTCGCCCGCGGCTTTGGTACTCTAGATAACCT	247
Sbjct	2049	.....	2108
Query	248	CGAGCCGATCGCACGCCCCCGTGGCGGCACGACCCATTCGAATGTCTGCCCTATCAACT	307
Sbjct	2109	.....	2168
Query	308	TTCGATGGTACTGTCTGTGCCTACCATGGTGACCACGGGTAACGGGAATCAGGGTTCGA	367
Sbjct	2169	.....	2228
Query	368	TTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGCGCGCAA	427
Sbjct	2229	.....	2288
Query	428	TTACCCACTCCCAGCCCGGGAGGTAGTGACGAAAATAACAATACAGGACTCTTTCGAG	487
Sbjct	2289	.....	2348
Query	488	GCCCTGTAATTGGAATGAGTCCACTTTAAATCCTTTAACGAGGATCCATTGGAGGGCAAG	547
Sbjct	2349	.....	2408
Query	548	TCTGGTGCCAGCAGCCGCGTAATCCAGCTCCAATAGCGTATATTAAAGTTGCTGCAGT	607
Sbjct	2409	.....	2468
Query	608	TAAAAAGCTCGTAGTTGGATCTTGGGATCGAGCTGGCGGTCCGCCGAGGCGAGCTACC	667
Sbjct	2469	.....	2528
Query	668	GCCTGTCCCAGCCCTGTCTCTCGGCGCCCCCTCGATGCTCTTAACTGAGTGTCCCGCGG	727
Sbjct	2529	.....	2588
Query	728	GGCCGAAGCGTTTACTTTGAAAAAATTATAGCGGTCAAAGCACGGTGGGCGGCCGG-AT	786
Sbjct	2589	.....G..T..T.....G..C...C...-...A..	2647
Query	787	ACTCCCCCTAGGAATAATGGGATATGACTCCGCTCTCTTTTTT-TTGGTTTTTCGAAACG	845
Sbjct	2648	.....AG.....A...G.....G...-...A...G.....CG.....	2706
Query	846	GGGACATAAAAAAGAGGGAGGCAAGGGTTTACATA-TGTGCCCTAGAGG-GAA-T-C	901
Sbjct	2707	...C...G..TT.....C...CG...GCA..T..G..T.....G.....T...A..T.	2766
Query	902	TTGG 905	
Sbjct	2767	.... 2770	

Figure (4): Polymorphic sites of the pairwise similarity observed in the 18s rRNA gene sequences of the local black chicken line (Query) and *Gallus gallus* (subject). The dots (.) indicate identity with the reference sequence (GenBank, Sequence ID: MG967540.1, Dyomin A. *et al.*, 2019) by the BLAST tool.

Table (3): Percent distance matrix of the 18s rRNA gene nucleotide sequences

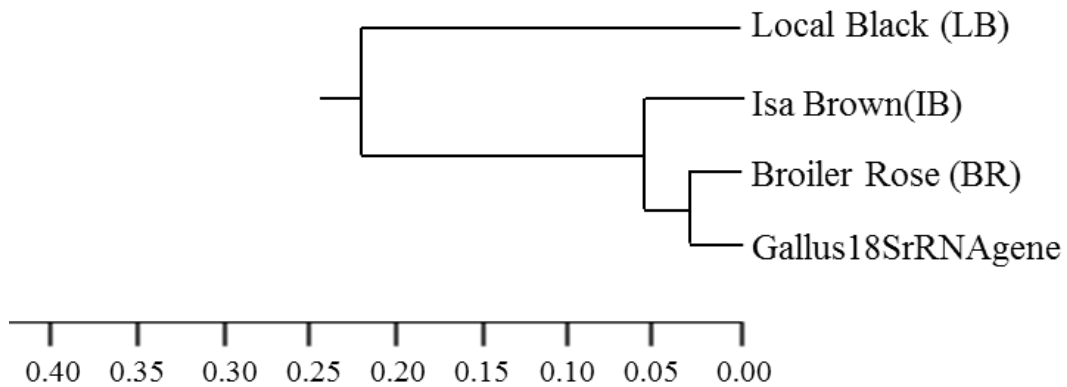
Populations	Local Black (LB)	Isa Brown (IB)	Broiler Rose (BR)
Isa Brown (IB)	95.49		
Broiler Rose (BR)	96.06	99.10	
Gallus gallus	95.95	99.10	99.89

**Phylogram tree result based on the 18s rRNA gene**

Figure 5. UPGMB Phylogram tree Based on the 18s rRNA gene of chicken by Multiple Sequence Alignment-MUSCLE tools.

**CONCLUSION**

There are no attempts to genetically characterization of native chicken breeds except few efforts of the researchers focused on the resulted banding patterns instead of the study on biodiversity at the DNA level. Hence, our study was focused on the compare the genetic parameters of local chicken breeds with commercial layers and broilers, as a first step to set preservation of genetic resources and designing and establish a breeding program to improve their productivity. Genetic improvement needs to be combining the data on the production program with genetic data to better describe the genetic diversity of local chickens and choose the desired breed. Experimental lines may become very extreme within a given breed because genetic make-up and breeding



programs of commercial lines are in the hands of very few international companies and generally kept confidential. The genetic resources of native poultry breeds especially chicken is cause for concern because the cross-breeding with the imported indigenous chickens in neighboring countries are under gradual erosion of the genetic integrity of the stock populations. Thus we suggest improving the native black chicken line must be done through the layer breeding program and cross-breeding with the other native chicken and commercial layer lines to collect genetically invaluable genetic resources and achieve a desirable new strain of native chicken layer.

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## DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest.

### تقييم التباين الوراثي بين ثلاثة خطوط من الدجاج اعتمادا على RAPD-PCR و 18s rRNA والتسلسل الجيني في أربيل (إقليم كردستان العراق)

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<sup>5</sup> قسم وقاية النبات- مديرية البحوث الزراعية أربيل- وزارة الزراعة والموارد المائية، إقليم كردستان، العراق.

### الخلاصة

استهدفت الدراسة الحالية تحديد وتقييم التغيرات الوراثية للدجاج الاسود المحلي مع سلالتين تجاريتين من الدجاج البياض (Isa Brown) وفروج اللحم (Rose) باستخدام المؤشر RAPD وطريقة التسلسل لحين 18s rRNA. اظهرت نتائج المؤشر RAPD، وجميع البادئات المستخدمة في التجربة والتي أنتجت 152 حزمة وتراوحت من 2 إلى 9 حزم مع الحجم الجزيئي تراوح من 320 إلى 2990 bp وان النسبة المئوية للحزم المتباينة 64.86%. بين مجاميع الدجاج. حيث وجد أعلى عدد من الحزم المتباينة في البادئ OPC-11 وأقل الحزم المتباينة في البادئ OPAA-03 كانت 24 و 8 ، على التوالي. ان متوسط عدد الأليلات المشاهدة (na)، والعدد المؤثر للأليلات (ne)، والتنوع الجيني (h)، ودليل معلومات شانون (I)، وجدت لجميع المواقع التي هي 1.6486، 1.5189، 0.2883 و 0.4129 على التوالي. وتبين وجود مستوى عالٍ من التباين واعطت (74) موقعا جينيا في جميع مجاميع الدجاج / البادئات إلى مسافة وراثية وأكبر تباين جيني بين مجموعات الدجاج باستخدام تقنيات RAPD-PCR. نتيجة BLAST التسلسل الجيني 18s rRNA في الدجاج المحلي له MT808178 و MT808179 في البنك الجيني، لـ *Gallus gallus* كما وجد أعلى تشابه بنسبة 95.74% و 94.88%، على التوالي. أظهرت صورة شجرة القرابة أن الدجاج المحلي لديه مسافات وراثية كبيرة نسبياً مع فروج اللحم واقل مسافة وراثية مع الدجاج البياض. لذلك يقترح أن يتم تحسين خط الدجاج المحلي من خلال برنامج تربية البياض للحصول على سلالة جديدة من الدجاج البياض الأصلية المرغوبة.

**الكلمات المفتاحية:** شجرة، التباين الوراثي، دجاج أسود محلي، المؤشرات الجزيئية.

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