

Estimation of Genetic Diversity in Four Genetic Groups of Local Kurdish Chicken Using RAPD-PCR

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Abstract

The current study conducted to detect the genetic diversity between four genetic groups of Kurdish local chicken using RAPD-PCR technique. Ten random markers used to amplified DNA were selected for genotyping the four lines. One hundred twenty four polymorphic bands were amplified, the bands size ranged between (2500 and 100 bp). The primer (OPA-05, OPA-18) produced maximum number of polymorphic bands, while primers named (OPA-16) produced minimum number of polymorphic bands. The (WNFS) genetic group showed the highest number of amplified fragments (55) for both male and female (30), (25) respectively. While (BBN) genetic group showed the lowest number of amplified fragments (20) for both male and female (7), (13) respectively. According to the results obtained from the current study, it can be conclude that the four genetic groups differ genetically. Moreover the results will help the breeders to study new selection strategies between the four genetic groups.

Keywords: Kurdish Chicken, Molecular Genetics, Characterization, RAPD.

تقدير التنوع الوراثي في اربع مجموعات وراثية من الدجاج الكوردي المحلي باستخدام تقنية التضخيم العشوائي للحامض النووي متعدد الاشكال

الخلاصة

أجريت الدراسة الحالية للكشف عن التنوع الوراثي بين أربع مجموعات وراثية من الدجاج المحلي الكوردي باستخدام تقنية التضخيم العشوائي للحامض النووي متعدد الاشكال). تم اختيار عشر بادئات عشوائية تستخدم لتضخيم الحامض النووي من اجل التنميط الوراثي للخطوط الاربعة. تم تضخيم مائة و اربعة و عشرين حزمة متعددة الاشكال، و تراوح حجم الحزم بين (100 الى 2500 زوج قاعدي). أنتج البادئات (OPA18, OPA-05) أكبر عدد من الحزم متعددة الاشكال، بينما أنتجت البادئ المسماة (OPA-16) أقل عدد من النطاقات متعددة الاشكال. أظهرت المجموعة الوراثية (الابيض عديم ريش السائق) أكبر عدد من الحزم المتضخمة (55) لكل من الذكور و الاناث (30) ، (25) على التوالي. بينما أظهرت المجموعة الوراثية (الاسود ذو الرقبة البنية) أقل عدد من الحزم المبكرة (20) لكل من الذكور و الاناث (7)، (13) على التوالي. وفقا للنتائج التي تم الحصول عليها من الدراسة الحالية، يمكن الاستنتاج ان المجموعات الوراثية الاربعة تختلف وراثيا. علاوة على ذلك ستمساعد النتائج المرين على دراسة استراتيجيات الاختيار الجديدة بين المجموعات الوراثية الاربعة.

Introduction

The evolution of genetic science during the past decades, and knowledge of the impact of environment factors contribute to the emergence of new adapted poultry strains. Several countries have categorized their livestock (1, 2, 3) in order to satisfy their genetic characteristics and know what genes they have. Therefore the progress of genetic science especially by development of molecular techniques, it has been possible to make selection (4), Identification (5), characterization (6), and genetic improvement (7) of livestock.

Iraq is one of the countries that have wide varieties of livestock animals. Accordingly a project was launched several years ago to characterize the local chicken in the north (Kurdistan region). Many traits were evaluated such as egg trait (8, 9, 10, 11, 12, 13, 14, 15), carcass traits (16, 17, 18, 19, 20), characterization (21), reproduction (22), production (9, 23, 24). More over (25) studied the polymorphism of genetic lines by using RAPD markers. The method named Randomly Amplified polymorphic DNA (RAPD) was used to characterize several organisms (26) genome based on whole genom (27), and chromosome level (28).

Up to date, there is no research on the genetic convergence between these lines, which stated as the main objective of this experiment between the production lines in the province of Kurdistan region.

Materials and methods

Chickens lines:

The current study was carried out in July 2019. Blood samples were collected from a total of (52) local chickens bred at the farm of animal production department, college of Agriculture,

University of Salahaddin Erbil, and at animal production department, college of Agriculture, University of Sulaimani, in association with the ministry of Agriculture in KGR-Iraq. The chickens were representing Black (B=12), Black with brawn neck (BBN=18), white with shank feather (WSF=12), and white non-feathering shank (WNFS= 10) as it shown in Table 1.

Blood samples collection and DNA extraction:

At least 2.5 ml of fresh blood sample was withdrawn from wing vein from each of the chicken in EDTA tube, (23) gauge needle. The blood was gently mixed with anticoagulant, and kept on ice. Subsequently the blood samples were transported to the laboratory and stored at – 20 °C until the isolation of genomic DNA.

Genomic DNA was isolated using a commercial kit, AccuPrep® Genomic DNA Extraction kit (Bioneer, South Korea) with slight modifications. [20 µl of proteinase K, total volume 200 µl of 20 µl whole blood and 180 µl of PBS, and 200 µl of Binding buffer (GC)] were added to 1.5-µl Eppendorf tube, the samples mix immediately by vortex mixer and then incubated at 60 °C for 10 min in boiling water bath. After incubation, 100 µl isopropanol was added and gently pipetted for a few min, then samples spin down at 10.000 rpm for 1 min. The cleared lysates were transferred into binding column tube (2 ml) then centrifuged at 12.000 rpm for 2 min and then the binding column tube transferred to new (2 ml) tube.

The samples were concentrated and the purity of the extracted DNA examined using Nanodrop (Nano-Drop2000, Delaware USA) based on 260 and 280 nm absorbance and agarose gel electrophoresis analysis (29).

RAPD-PCR condition and electrophoresis:

Table (2) was shown the 10 different decamer oligonucleotides RAPD markers, which were

used for genotyping the four lines. The genomic DNA for the four lines was amplified by PCR with following profile: initial denaturation of 5 min at 94 °C; 40 cycles of 1 min at 94 °C, 1 min at 43 °C and 1 min at 72 °C with final elongation of 5 min at 72 °C. The PCR products were resolved by electrophoresis through 1.5% agarose gel and visualized by Ethidium bromide staining (30).

The RAPD bands were scored visually from the Ethidium bromide staining agarose gel. The analysis was based on the generated data from the detection of polymorphic fragments. The genotypes were analyzed in the form of binary variable by scoring the presence as (1) and absence (0). The genetic distance and genetic identity among the genetic groups in both males and females were calculated using Nei equations (31) by using (pop gene) software

Results and discussion

Table (2) was shown all of primers that were used with their information. A total of 10 primers were screened for RAPD analysis. One hundred twenty four polymorphic bands were amplified, the bands size ranged between 100 bp and 2500 bp. The primer (OPA-05, OPA-18) produced maximum number of polymorphic bands, while primers named (OPA-6) produced minimum number of polymorphic bands. The detected polymorphisms were varied among the four genetic groups (black, black brown neck, white shank feather, and white non-feathering shank). The (WNFS) genetic group showed the highest number of amplified fragments (55) for both male and female (30, 25) respectively. While (BBN) genetic group showed the lowest number of amplified fragments (20) for both male and female (7, 13) respectively as we showed in (Table 3).

Table (4) shows the N_a and N_e , h , I for the two groups of male and female Kurdish local chicken was analyzed using ten RAPD markers.

The genetic distance in male groups found highest ($D=1.2040$) between B and WSF and lowest ($D=0.1054$) between (B) and (WNFS). While in female groups it was found that the highest genetic distance ($D=0.9163$) was observed between (WSF) and (WNFS) and the lowest ($D=0.2231$) was between (BBN) and (WNFS). In addition, the males genetic identity was higher ($I=0.900$) between (B) and (WNFS), and lower ($I=0.3000$) was between both (B), and (WSF), and between (BBN) and (WSF). In females genetic identity the highest ($I=0.8000$) was between (BBN) and (WNFS) and the lowest ($I=0.4000$) was between (WSF) and (WNFS) (Table 5). Dendrogram was constructed to show the phylogenetic relationships among the four lines Figure 1 was shown the male phylogenetic relationship that indicates the (WSF) genetic group to be the most distance from the other genetic groups, while B and BBN were closely related. As for female phylogenetic relationship (Figure 2) indicate that (WNFS) line recorded most distance from the other genetic groups, while (BBN) and (WSF) were closely related.

It is obvious that the project of breeding local chicken in Kurdistan region government-Iraq was started 15 years ago. The four mapped genetic groups (B, BBN, WSF, WNFS) were bred and selected for 12 generation, in the directorate of agricultural research in Erbil, moreover it is selected according to the feathering color and appearance of shank feather (12). The results of genetic distance in male genetic groups of this study supporting the findings of (21) who recorded that there was no significant difference between genetic groups (B) and (WNFS) in their egg length, which both inherited by roosters (32). Moreover the (WSF) genetic group was higher in their egg length while the lower recorded for the (BBN) genetic group. Furthermore the genetic distance of the hen genetic groups was linked to the breadth traits, which already inherited by hen. According to the correlation results found by (21), there is

no significant difference between genetic groups (BBN) and (WSF), moreover the (WNFS) genetic group was the higher and the (B) genetic group was the lowest in egg breadth trait. (33) used the RAPD marker in their study to characterize Five Egyptian indigenous chicken strains and they found that RAPD marker is a valuable tool to evaluate genetic diversity of chicken.

Conclusions

According to the results obtained from the current study, it can be conclude that the four lines differ genetically. Moreover the results will help the breeders to study new selection strategies between the four genetic groups. Further studies are needs to provide the genetic map of these genetic groups based on microsatellite markers.

Table 1: the information of sample collected

| Line | Erbil | | Sulaymaniyah | | Total |
|-------|-------|--------|--------------|--------|-------|
| | Male | Female | Male | Female | |
| B | 2 | 4 | 2 | 4 | 12 |
| BBN | 4 | 5 | 4 | 5 | 18 |
| WSF | 2 | 4 | 2 | 4 | 12 |
| WNFS | 2 | 3 | 2 | 3 | 10 |
| Total | 10 | 16 | 10 | 16 | 52 |

Table 2: List of RAPD markers

| Primer codes | Primer sequence | (G+C)% | Size range of Fragments (bp) |
|--------------|---------------------|--------|------------------------------|
| OPA-03 | 5'-AGT CAG CCA C-3' | 60.0 | 160 - 1300 |
| OPA-04 | 5'-AAT CGG GCT G-3' | 60.0 | 600 – 1200 |
| OPA-05 | 5'-AGG GGT CTT G-3' | 60.0 | 500 – 3000 |
| OPA-12 | 5'-TCG GCG ATA G-3' | 60.0 | 450 - 2800 |
| OPA-16 | 5'-AGC CAG CGA A-3' | 60.0 | 300 – 1700 |
| OPA-18 | 5'-AGG TGA CCG T-3' | 60.0 | 130 - 1500 |
| OPA-19 | 5'-CAA ACG TCG G-3' | 60.0 | 550 – 2300 |
| OPA-20 | 5'-GTT GCG ATC C-3' | 60.0 | 400 - 1600 |
| OPB-07 | 5'-GGT GAC GCA G-3' | 70.0 | 450 - 1400 |

| | | | |
|--------|---------------------|------|------------|
| OPC-02 | 5'-GTG AGG CGT C-3' | 70.0 | 200 - 2100 |
|--------|---------------------|------|------------|

Table 3: Number of bands amplified in each primer for the four genetic groups

| Primer codes | B | | BBN | | WSF | | WNFS | | Total* |
|--------------|------|--------|------|--------|------|--------|------|--------|--------|
| | Male | Female | Male | Female | Male | Female | Male | Female | |
| OPA-03 | 0 | 3 | 0 | 0 | 3 | 1 | 4 | 3 | 14 |
| OPA-04 | 0 | 0 | 0 | 0 | 4 | 1 | 4 | 4 | 13 |
| OPA-05 | 4 | 0 | 0 | 4 | 0 | 0 | 7 | 5 | 20 |
| OPA-12 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 2 |
| OPA-16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| OPA-18 | 4 | 5 | 1 | 2 | 4 | 0 | 2 | 2 | 20 |
| OPA-19 | 3 | 3 | 1 | 1 | 3 | 4 | 2 | 2 | 19 |
| OPA-20 | 1 | 0 | 0 | 3 | 0 | 0 | 5 | 1 | 10 |
| OPB-07 | 0 | 0 | 5 | 2 | 3 | 3 | 3 | 2 | 18 |
| OPC-02 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 4 | 7 |
| Total ** | 12 | 11 | 7 | 13 | 17 | 9 | 30 | 25 | 124 |

B= Black, BBN= Black brown neck, WSF= White with shank feather, WNFS= White non-feathering shank. * The total of bands amplified for each primer. ** The total of bands amplified for each gender within genetic groups.

Table 4: The observed number of alleles, effective number, and gene diversity of males and females in Kurdish local chicken

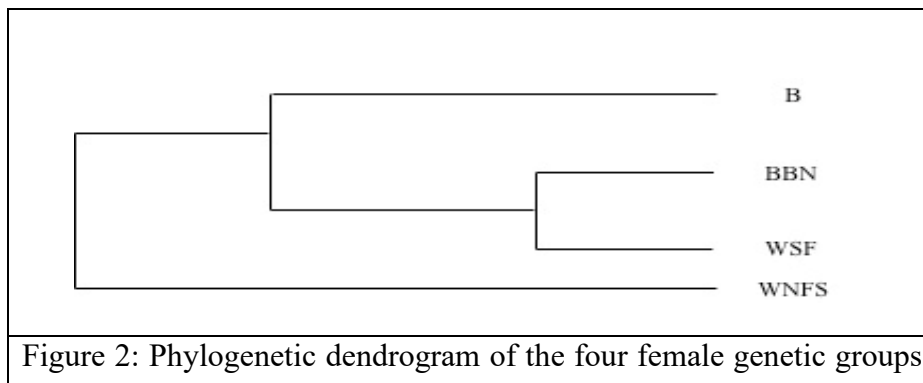
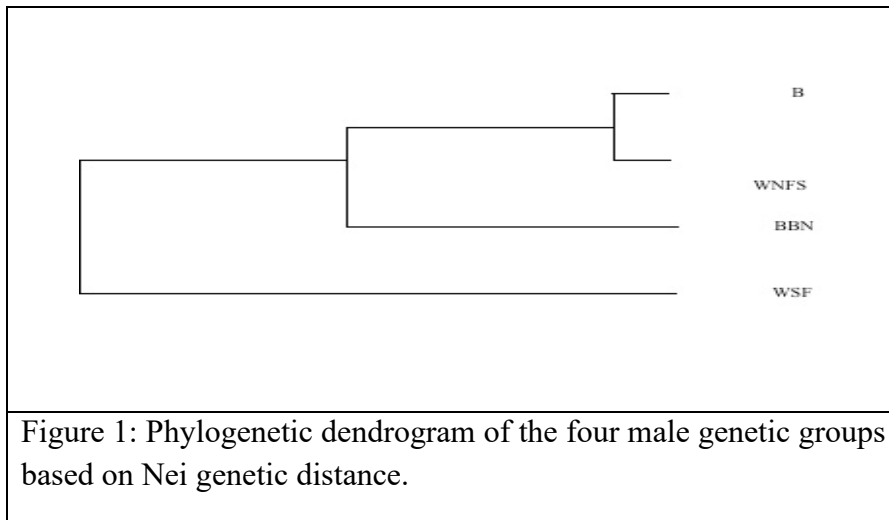
| Locus | Male (N= 4) | | | | Female (N= 4) | | | |
|--------|-------------|--------|--------|--------|---------------|--------|--------|--------|
| | Na | Ne | H | I | Na | Ne | h | I |
| OPA-03 | 2.0000 | 1.6000 | 0.3750 | 0.5623 | 2.0000 | 2.0000 | 0.5000 | 0.6931 |
| OPA-05 | 2.0000 | 2.0000 | 0.5000 | 0.6931 | 2.0000 | 2.0000 | 0.5000 | 0.6931 |
| OPA-12 | 2.0000 | 2.0000 | 0.5000 | 0.6931 | 2.0000 | 1.6000 | 0.3750 | 0.5623 |
| OPA-16 | 2.0000 | 1.6000 | 0.3750 | 0.5623 | 2.0000 | 1.6000 | 0.3750 | 0.5623 |
| OPA-18 | 2.0000 | 2.0000 | 0.5000 | 0.6931 | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| OPA-19 | 2.0000 | 1.6000 | 0.3750 | 0.5623 | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| OPA-20 | 1.0000 | 1.0000 | 0.0000 | 0.0000 | 1.0000 | 1.0000 | 0.0000 | 0.0000 |
| OPA-04 | 2.0000 | 1.6000 | 0.3750 | 0.5623 | 2.0000 | 2.0000 | 0.5000 | 0.6931 |
| OPC-02 | 2.0000 | 1.6000 | 0.3750 | 0.5623 | 2.0000 | 2.0000 | 0.5000 | 0.6931 |
| OPB-07 | 2.0000 | 1.6000 | 0.3750 | 0.5623 | 2.0000 | 1.6000 | 0.3750 | 0.5623 |
| Mean | 1.9000 | 1.6600 | 0.3750 | 0.5453 | 1.7000 | 1.5800 | 0.3125 | 0.4460 |
| S.D. | 0.3162 | 0.2989 | 0.1443 | 0.2013 | 0.4830 | 0.4367 | 0.2224 | 0.3130 |

Na = observed number of alleles; Ne = effective number of alleles; h = gene diversity; I = Shannon’s information index.

Table 5: Nei’s unbiased measures of genetic identity and genetic distance for male and female Kurdish local chicken

| Gender | Genetic groups | B | BBN | WSF | WNFS |
|--------|----------------|--------|--------|--------|--------|
| Male | B | 1 | 0.6000 | 0.3000 | 0.9000 |
| | BBN | 0.5108 | 1 | 0.3000 | 0.5000 |
| | WSF | 1.2040 | 1.2040 | 1 | 0.4000 |
| | WNFS | 0.1054 | 0.6931 | 0.9163 | 1 |
| Female | B | 1 | 0.5000 | 0.5000 | 0.7000 |
| | BBN | 0.6931 | 1 | 0.6000 | 0.8000 |
| | WSF | 0.6931 | 0.5108 | 1 | 0.4000 |
| | WNFS | 0.3567 | 0.2231 | 0.9163 | 1 |

B= Black, BBN= Black brown neck, WSF= White with shank feather, WNFS= White non-feathering shank



based on Nei genetic distance.

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