Detection of similarity and genetic distance between Iraqi chicken varieties and different standard strains

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Abstract

Forty-eight wing vein blood samples were collected from different locations of poultry rearing farms and back yard chickens of Nineveh governorate from the of local and exotic chicken. The chicken divided into twelve groups four birds each according to colors and phenotype for the local and exotic chicken respectively. Blood DNA was extracted and amplified by thermocycler apparatus and the electrophoresis was done using 1.2% agarose gel for DNA bands exhibiting. The results showed high genetic similarity within the local chickens ranged between 0.78- 0.96 at an average of 0.88, while it ranged between 0.73- 0.86 at an average of 0.78 in exotic breeds. The degree of similarity between Iraqi and exotic breeds was 0.74-0.88 at average of 0.80. The calculated average of differences among each of Iraqi and exotic chickens and in between were 0.12, 0.22 and 0.20, respectively. However, the genetic distance within the local chicken, exotic breed and in between them was 0.128, 0.24 and 0.21 respectively. The study concluded that the genetic similarity was higher within local chicken groups than those of exotic breeds.

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الكشف عن التشابه والبعد الوراثي بين أنواع الدجاج العراقي والسلالات القياسية الأجنبية

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الخلاصة

جمعت ٤٨ عينة دم من الوريد الجناحي من مناطق مختلفة شملت مزارع الدواجن ودجاج التربية المنزلية في محافظة نينوى. قسمت العينات الى اثنتا عشر مجموعة اعتمادا على الشكل المظهري واللون للدجاج المحلي والأجنبي، احتوت كل مجموعة على أربعة طيور. استخلص الحامض النووي الرايبوزي منقوص الأوكسجين (الدنا) وتم تضخيمه باستخدام جهاز المبلمر الحراري، وتم إظهار حزم الدنا بالترحيل على هلام الأكاروز بتركيز ١,٢%. أظهرت النتائج وجود تشابه وراثي عالي ضمن أنواع الدجاج المحلي والتي تراوحت بين ٠,٧٨ - ٠,٩٦ بمتوسط ٠,٨٨ في حين تراوحت بين ٠,٧٣ - ٠,٨٦ بمتوسط ٠,٧٨ في السلالات الأجنبية، وبلغت نسبة التشابه بين الدجاج المحلي والأجنبي ٠,٧٤-٠,٨٨ بمتوسط ٠,٨٠. وكان متوسط الاختلاف بين مجاميع الدجاج المحلي والمجاميع الأجنبية بين ٠,١٢، ٠,٢٢، ٠,٢٠ على التوالي، وبلغ البعد الوراثي بين المجاميع المحلية والأجنبية وفيما بينهما ٠,١٢٨، ٠,٢٤ و ٠,٢١ على التوالي. خلصت الدراسة إلى أن التشابه الوراثي داخل المجاميع المحلية اعلى من التشابه بين مجاميع السلالات الأجنبية.

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Introduction

Over the last twenty-five years, molecular markers have been utilized to distinguish the different species of chicken. Before the biochemical and molecular markers were identified, the chicken species and varieties were characterized by phenotypic and quantitative traits value (1).

Molecular genetics has enabled opportunities to develop animal breeding programs by direct selection of genomic regions that serve economic traits (2).

Finding out the polymerase chain reaction (PCR) had a great effect on the research eukaryotic genome and shared in the development and enforcement of various DNA markers (3).

The randomly amplified polymorphic DNA (RAPD) technique was described first by (4,5), is a rapid and effective procedure that can be used to produce genotype specific banding patterns. Polymorphism of RAPD fragments is detected as a band's presence or absence and may result from deletion, insertion or differences in the nucleotide sequences in or between priming regions (6).

RAPD is an easy, quick and relatively low-cost screening that uses short oligonucleotide primers of arbitrary sequences to magnify anonymous fragments of genomic DNA (7).

Genetic information gives a unified approach for the variation within and between populations, which is affected by neither the environmental conditions nor the developmental stages of organisms.

Vanhala *et al.* (8) and Corzo *et al.* (9) denoted the usefulness of molecular information in the evaluation of the genetic variation and divergence. The Iraqi native chickens consist of different varieties descended from the red jungle fowl which mixed with different standard important strains, it is characterized by low egg production and body weight, but it is known by its resistance to diseases and extreme environmental conditions (10).

The objective of this study was to evaluate the genetic variability in a six breeder groups of the local chicken in Mosul city and the vicinity, which were divided according to the color (white, brown, black, black with white and naked neck, and mixed of colors) compared with New Hampshire, Japanese Bantam (chabo) Leghorn and cochin chicken based on RAPD-DNA markers, to generate genetic information on the local chicken structure for future development strategies.

Materials and methods

Chicken groups

This work consists of twelve deferent groups of local chicken and exotic standard strains as shown below. A total of 48 individuals of four from each type was utilized.

G1: Black G7: Leghorn
G2: Black and White G8: Silkie
G3: White   Local G9
G4: Mixed colors G10: Cochin   Broiler breeder
G5: Naked neck G11: Indian Shamo
G6: Brown G12: New Hampshire

DNA isolation

Blood samples were collected into 5 ml tubes containing EDTA. Genomic DNAs were obtained from blood samples (collected from wing vein) as described by Macrogen

Company Kit. The purity and concentration of DNA samples were checked by the Nano Drop® spectrophotometer. The same quantities of DNA from the birds of each group were mixed to perform the representative DNA.

DNA amplification and electrophoresis condition

Amplification reactions were carried out in a final volume of 20 µl (Table 1).

Table 1: Amplification reactions

Component	Volume	Final concentration
HS Prime *Taq* Premix	10 µl	1X
Primer	3 µl	1 µM
PCR grade water	5 µl	---
DNA (100 ng/µl)	2 µl	2 ng/µl
Total	20 µl	

Amplification was achieved in a thermo cycler programmed for 10 min initial denaturation at 95 ℃, then 40 cycles of 30 s at 95 ℃, 30 s at 34 ℃, 60 s at 72 ℃, and 5 min final extension at 72 ℃.

Agarose gel electrophoresis

1.2% agarose gel (Jena Bioscience, Germany). Loading 6 µl of each PCR product. The electrophoresis was carried out at 3V/cm for 1 hour using power supply MP 300V (Major Science, UK) containing 1X TBE buffer (GeNetBio, Korea). A 1kb bp DNA marker, 4 µl (Promega, USA) was used as a standard molecular weight marker.

Gel documentation

The gel was stained in a 200 ml solution containing ethidium bromide 0.5 µg/ml and subsequently examined under UV light using the gel documentation system (BioDocAnalyze, Germany).

Statistical Analysis

To estimate the number of polymorphic and monomorphic bands. Bands were scored visually on the bases of their presence 1 or absence 0. Genetic similarity (GS) was calculated using the following equation (11): $GS = 2Nab / Na+Nb$.

Were Nab represent the entrant bands between the groups a and b. Na+ Nb the total scrod bands for the same groups. Genetic polymorphism between groups was resolved as $GP = 1 - GS$. Genetic distance was evaluated as (12): $GD = -In(S)$. The phylogenetic tree was obtained on the base of genetic similarity using the SPSS Program (classify procedure).

Results

RAPD-PCR method was followed to study the genetic similarity and genetic distance of Iraqi local chicken and

some imported exotic breeds. Nine random decanucleotide primers of the sequences, GC content and Tm ºC (table 2). A total of 60 loci included forty percentage of polymorphism were obtained.

The table 3, indicates the similarity and the differences between different chicken groups. The results expressed high homogeneity among Iraqi chicken groups which were varied from 0.78 to 0.96 with an average of 0.88, while it ranged from 0.73 to 0.86 with middling 0.78 in the analogous exotic breeds. The similarity between the Iraqi local chicken versus pooled foreign groups was ranged from 0.74 to 0.88 with a median of 0.80.

The highest genetic similarity within Iraqi chicken was found between G1 and G3 (the white and the black chicken groups) which was 0.96, while the lowest genetic similarity detected between G2 and G6 groups (black and white and brown) and was 0.78. On the other hand, the highest degree of similarity between the Iraqi and exotic chicken groups occurred between G3 and G9 and was 0.88, and the lowest degree noticed between G5 and G10 of value 0.75. The calculated average of differences within each of Iraqi and

exotic chickens and in between were 0.12, 0.22 and 0.20, respectively.

Figure 1 shows the pattern of PCR product using the primer opp-17, clarify a part of genetic variation among the studied groups in eleven genetic loci included three of polymorphism bands. Concerning genetic distance which depends on the similarity degree, table (4) showed the genetic distance in calculated groups. The lowest genetic distance was found between G1 and G3 of Iraqi chicken and the farthest genetic distance was existed between the Silkie (Japanese mini chicken) and the Cochin (Huge Asian Chicken). The largest genetic distance among the whole chicken groups in this study was found between G8 and G11 of value 0.31. As for the relationship between Iraqi and foreign chicken groups, the minimum genetic distance found between G3 and G9 was 0.13, and the highest genetic distance represented between G5 and G10 was 0.29. The overall genetic distance within each of the local, standard strains of chicken, and in between was 0.128, 0.24, 0.215, respectively.

Table 2: Genetic polymorphism analysis based on the primers used

Primer	Sequence 5' to 3'	%GC content	Number of loci		Polymer Phism %	Tm ºC
			polymorphic bands	Total bands		
BG-6	CTG AGA CGG A	60	3	8	37.5	32
OPA-6	GGT CCC TGA C	70	3	8	37.5	35
OPA-16	AGC CAG CGA A	60	5	10	50.0	32
OPB-19	ACC CCC GAA G	70	3	7	42.8	35
OPC-2	GTG AGG CGT C	70	2	6	33.3	35
OPC-3	GGG GGT CTT T	60	4	6	66.6	32
OPG-7	GAA CCT GCG G	70	4	8	50.0	35
OPP-14	CCA GCC GAA C	70	4	9	44.4	35
OPP-17	TGA CCC GCC T	70	3	11	27.3	35
Total			24	60	40.0	

Table 3: Genetic similarity and genetic variation values between chicken groups

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
G1	* 1	0.05	0.04	0.09	0.08	0.10	0.14	0.26	0.22	0.26	021	0.18
G2	0.95	1	0.17	0.18	0.18	0.22	0.23	0.19	0.20	0.15	0.15	0.16
G3	0.96	0.83	1	0.11	0.12	0.10	0.13	0.15	0.12	0.18	0.23	0.20
G4	0.91	0.82	0.89	1	0.10	0.17	0.18	0.16	0.17	0.20	0.18	0.12
G5	0.92	0.82	0.88	0.90	1	0.18	0.20	0.14	0.20	0.25	0.17	0.23
G6	0.90	0.78	0.90	0.93	0.82	1	0.24	0.24	0.19	0.14	0.17	0.14
G7	0.86	0.77	0.87	0.82	0.80	0.76	1	0.27	0.18	0.21	0.20	0.25
G8	0.74	o.81	0.85	0.84	0.76	0.75	0.73	1	0.22	0.26	0.27	0.14
G9	0.78	0.80	0.88	0.83	0.80	0.81	0.82	0.78	1	0.22	0.21	0.23
G10	0.74	0.85	0.82	0.80	0.75	0.79	0.79	0.74	0.78	1	0.15	0.22
G11	0.79	0.85	0.77	0.82	0.83	0.83	0.80	0.73	0.79	0.85	1	0.16
G12	0.82	0.84	0.80	0.78	0.77	0.80	0.75	0.86	0.77	0.78	0.84	1

*The numbers above the axis represent the differences and the numbers below the axis represent the similarity

Table 4: Genetic distance values between chicken groups

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
G1												
G2	0.05											
G3	0.04	0.18										
G4	0.09	0.20	0.12									
G5	0.08	0.20	0.13	0.11								
G6	0.10	0.25	0.11	0.07	0.20							
G7	0.15	0.26	0.14	0.20	0.22	0.27						
G8	0.30	0.21	0.16	0.17	0.27	0.29	0.31					
G9	0.25	0.22	0.13	0.19	0.22	0.21	0.20	0.25				
G10	0.30	0.16	0.20	0.22	0.29	0.23	0.24	0.30	0.25			
G11	0.23	0.16	0.20	0.20	0.19	0.19	0.22	0.31	0.24	0.16		
G12	0.19	0.17	0.22	0.25	0.26	0.22	0.29	0.15	0.26	0.25	0.17	

As in the dendrogram below (Figure 2), the chicken groups were classified into two main clusters, the first one consisted of the six exotic strains, while the second represented the local chicken. Later, each cluster was divided into two sub- cluster branches. The dendrogram explains the relationship between study groups according to the degree of genetic distance.
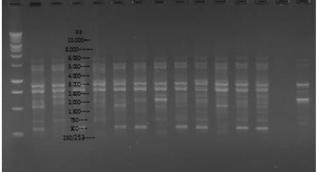

Figure 1: Electropherogram of DNA fragments obtained by PCR technique with opp-17 primer

Discussion

Although Iraqi chickens are characterized by a wide range of external phenotypes (13), low productivity of eggs and meat and other related quantitative traits (10), such characters may be due to their descendants from the same ancestors which were the red jungle fowl (14). Also, these birds did not subject to any selection program, despite their accidental crossbreeding with some imported breeds like Leghorn, New Hamshire, and certain broiler breeder. This information was approved by the obtained genetic distance data and the dendrogram.
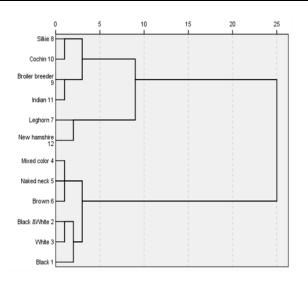

Figure 2: Dendrogram show genetic distance relationships

RAPD-PCR technique is an effective, simple and cheap tool for the determination of genetic diversity (15), which was demonstrated through the gained results that assured the limited differences among the local chicken groups. These findings are expected consequences of random mating for a long time. A total of 60 bands produced by 9 arbitrary primers resulting in different levels of similarity and genetic distance among all studied groups. It was found that the degree of similarity in the groups of Iraqi chickens 0.88 was higher than those of exotic chicken strains 0.80. It is known that Iraqi chickens represent one population, some of which were subjected to hybridization. However, exotic chickens are definite breeds constrained by intensive selection programs for over 50 years, which caused have unique genotype translated to respective traits and ultimately to specific genetic identity (16).

The genetic distance depending entirely on the similarity and was found convergent in Iraqi chickens 0.128. However,

this measurement was substantial in between the exotic chickens 0.24. A significant difference was noticed between the two main groups 0.215. Such difference could be attributed to the aforementioned reasons related to unique and uniform traits of each population, while the whole local chicken did not have any exclusiveness except the disease resistance which was acquired by prolonged environmental adaptation.

According to the attained information, the advantage of similarity and genetic distance should be utilized to improve the local chickens, intensive inbreeding program for several generations accompanied by selection should apply to arise pure genetic structures with partial improvement in production performance (17). Additionally, mixing with the males of egg production breed, continuous selection and performance evaluation should steadily go on.

Conclusion

The study concluded that the genetic similarity was higher within local chicken groups than those of exotic breeds

Acknowledgements

Conflict of interest

On behalf of all co-authors and stating that the submitted manuscript is the authors' original work, has not received prior publication and is not under consideration for publication elsewhere, permission has been received to use any material in the manuscript much as tables, figures etc. or no permissions have necessary to publish the authors' work.

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