Mesopotamia Journal of Agriculture

https://magrj.mosuljournals.com

RAPD MARKER TO SCREENING GENETIC DIVERSITY OF LOCAL CHICKEN

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Article information
Article history:
Received:12/8/2022
Accepted:9/11/2022
Available:31/12/2022

Keywords:
Chickens, Relatedness, RAPD-PCR, Genetic

DOI:
https://10.33899/magrj.2022.135086.1190

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ABSTRACT

A total of 500 local chickens from different regions of Iraq, which were divided into five groups according to the regions from which they were taken, namely, the East (E), West (W), North (N), South (S), and Central (C) regions of Iraq. The blood samples were collected into tubes containing an EDTA, DNA extraction was carried. Using 20 primers from Gen Script USA, random amplification of polymorphic DNA (RAPD-PCR) was carried out. The reaction of PCR had a final volume of 25 µl. A total of 473 loci ranging between 200 and 1800 bp were amplified. The higher numbers of bands shown in north(N) local chicken group (101) than the other groups. East (E) and South (S) groups phenotypic revealed higher numbers of the polymorphic bands. The Polymorphism percent (%6.2) in East (E). Between the north (N) and west (W) groups, the genetic similarity score was found to be the highest (0.893). dendogram It was clear that the South(S) appeared to be most distant from the other groups whereas the West (W), North (N) were related closely together whose genetic closeness is highest. The main aim of present study is to assess the genetic specificity chickens in Iraq independs on RAPD analysis, To assess phylogenetic relatedness between groups of chickens, is to understand the extent of genetic variation between local chickens groups in Iraq.

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INTRODUCTION

Breed differences have been discovered to express genetic-environment interactions in the ability of birds to produce and reproduce in harsh conditions. As a result, it is possible to maximize the effectiveness of poultry production in less-than-ideal circumstances by using the constant and scientifically based genetic-environment interaction (Abdelhady *et al*., 2022), by locating and taking advantage of genotypes that have been specially suited to certain settings. Due to this, indigenous breeds are currently a major source of scientific concern. The focus of the studies is on the genetic potential that interacts favorably with specific circumstances. However, using molecular tools, it is possible to identify variations in specific DNA regions across members of the same or distinct groups (Tixier-Boichard, 2002), Consequently, rather than assessing phenotype, the assessment is made at the genotypic levels. Because molecular variation is unaffected by environmental influences or phases of organism development, it is preferred to genetic variation that is statistically calculated from phenotypic parameters of the traits (Siegel et al., 1992). In order to demonstrate the evolutionary links and genetic diversity among various populations, it is used, and it is

frequently used to assess the local breeds. Many studies have focused on the local breeds found around the world. Generate genotype-specific banding patterns using RAPD markers. During the past ten years, there has been a remarkable advancement in the identification of strains in poultry using DNA marker technologies. The efficacy of RAPD in detecting polymorphism between chicken populations, their relevance in population research, and their capacity to build genetic linkages between chicken populations were discussed in (Sharma *et al*., 2001). The RAPD-PCR has been employed frequently in the investigation of similarity or variation in the populations of chickens (Abdulrazaq and Suliaman., 2016, Dehghanzadeh, *et, al.*, 2009, Smith, *et al.*, 1996, Sharma, e*t al.*, 2001, Ali, *et al.*, 2003, Semenova, *et al*., 2002), ducks (Haddad, *et al*., 2019). Guinea fowl (Sharma, *et al.*, 1998, Nahashon, *et al*., 2010 and Daham and Sharma, 2007) Currently, this method is used to distinguish between the genetic resources of commercially significant farm animals and birds. The law governing the protection of strains is heavily reliant on this method.

Simple, quick, and reasonably priced is the RAPD test. Phylogenetic investigations, genetic mapping, population and pedigree analysis, and genotype identification have all quickly adopted it as their preferred method. The aim of present study to assess the genetic specificity chickens Iraq an depends on RAPD analysis, to assess phylogenetic relatedness between groups of chickens, to understand the extent of genetic variation between local chicken groups in Iraq.

MATERIALS AND METHODS

In this research, blood samples were collected from local chicken groups from different regions of Iraq, which was divided into five groups according to the regions from which they was taken, namely, the East (E), West (W), North (N), South (S), and Central (C) regions of Iraq. The total number of birds is 500 so that 100 birds per group. Birds' blood samples containing about 3 ml were taken from the wing near the elbow joint and placed in tubes containing an EDTA solution. DNA was then extracted using the procedure described in (Sharma *et al.*, 2016). The Nano Drop® spectrometer was used to measure the concentration of DNA and its relative purity; the purity of the DNA samples ranged from 1.8 to 1.9. The samples were diluted to 30 ng/l for RAPD-PCR usage. using 20 primers from Gen Script USA for the RAPD were among the (20) primers that produced results for the discovery of genomic DNA complementary sites (Table 1). The DNA analysis was amplified in a thermal cycler with a T gradient. Initial denaturation of double-stranded DNA occurred at 95°C for 5 minutes. 40 cycles of 95°C for 1 minute, 42°C for 1 minute, and 72°C for 2 minutes were then performed. The complementary strands were created at 75°C for 5 minutes. The following components are included in the PCR reaction: DNA (30 ng), primer (10 M), 1x GoTaq® Green Master Mix PCR buffer, 1x, MgCl2 (3 mM), Each: (dATP, dCTP, dGTP, dTTP) (400 M), The entire reaction volume was 25 l. The 100 bp DNA ladder marker for The Gene Ruler TM (100 – 1500 bp). 10 l of the product were mixed with 2 l of Blue / Orange loading day in each sample. The 100V power supply operation was completed. The electrophoresis took around 90 minutes. Electrophoresis on 2% agarose gel in 1X TBE buffer with ethidium bromide staining was used to test the PCR products (promega, USA). The pattern was taken after being magnified by UV light. On a UV trans illuminator the amplified pattern was visualized and photographed. The analytical statistics of Because of (1) or lack, data recording RAPD patterns were recorded (0). The formula polymorphism = (Np / Nt) 100, NP = # polymorphic forms of random

primer, was used to calculate the polymorphism of each primer. Nt is the entire collection of sample primer domains (Bowditch *et al.*, 1993). Statistics were used to analyze the numerical data. In all available pair-wise comparisons of individuals between groups, similarity values were calculated using the approach of (Nei and Li .,1979). The dendrogram was produced when the similarity matrix was subjected to cluster analysis using the unweighted pair group for arithmetic mean (UPGMA) cluster analysis technique. Using PAST version 1.34, a dendrogram of genetic distance was created. (Hammer *et al.*, 2001) It was made using closest neighbor technology.

Table (1) : Primers' sequences and GC content

Primers name	5' to 3 Sequence	Content %GC
OPA – 10	GTGATCGCAG	%60
OPA – 07	GAAACGGGTG	%60
OPA – 03	AGTCAGCCAC	%60
OPA – 12	TCGGCGATAG	%60
OPQ – 14	GGAGTGGACA	%60
OPA – 05	AGGGGTCTTG	%60
OPA – 11	CAATCGCCGT	%60
OPA – 09	GGGTAACGCC	%70
OPQ – 11	TGTGCCCGAA	%60
OPQ – 10	GGCTAACCGA	%60

RESULTS AND DISCUSSION

In the current study, the RAPD-PCR approach was employed to evaluate the genetic relatedness of regional chickens groups. Twenty randomly selected genotypes from the samples were examined. Reaction of PCR using 10 chosen primers across all local chicken groupings (Table 1). The bands acquired through electrophoresis were used to determine the number of bands shared by local chicken groups (Figure 1).

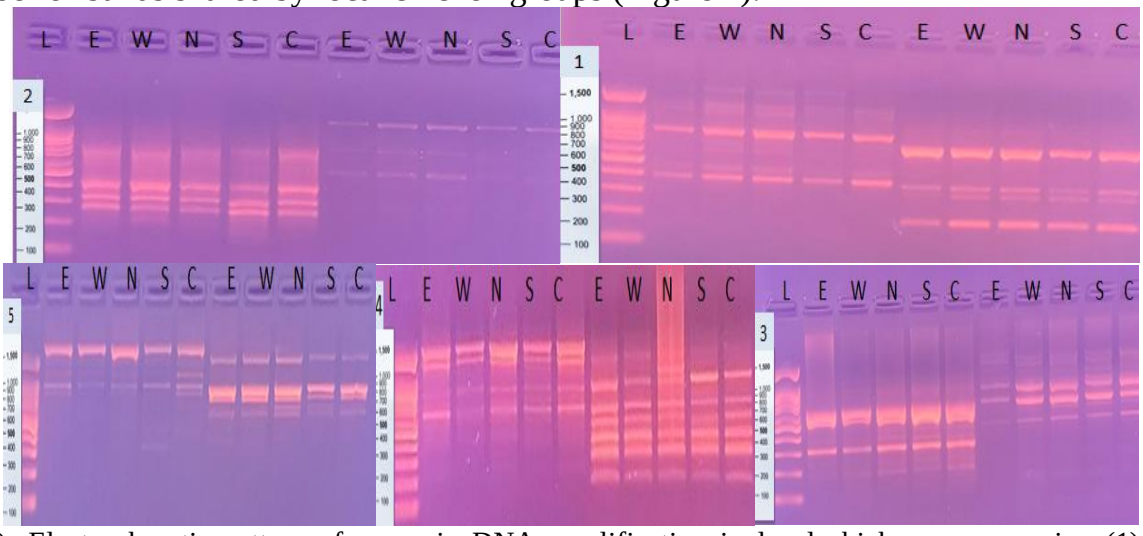

Figure (1): Electrophoretic pattern of genomic DNA amplification in local chicken groups using (1) OPA_10, OPA_07, (2) OPA_03, OPA_12,(3) OPA_14, OPA_05, (4) OPA_11, OPA_09,(5) OPQ_11, OPQ_10 Primer.

Table (2) includes the PCR results from local chickens groups. The higher numbers of bands shown in north(N) local chicken group (101) than the other groups of total (473) bands. The

polymorphism level detected between all local chicken groups variable phenotypes The polymorphic bands were more numerous and had a greater percentage of polymorphism (%6.2) in the east(E) group than in the south(S) group phenotypic. There are 473 distinct pieces (bands) created in all, of which 34 were polymorphic, 73 monomorphic, and 439 monomorphic. It is greater than, (Abdulrazaq, *et al*., 2020) studied a total of 80 distinct fragments (bands), 29 of which were polymorphic. For each Primer, the number of bands amplified ranged from 25 (OPA-12) to 137 (OPQ-14). The primer OPA-10 detects the maximum number of polymorphic bands—six—while (OPA-09), (OPQ-10), and there is a lowest number of bands—detect only one polymorphism band each. In this investigation, an average of 8.74 polymorphic bands per primer were discovered. For the primer OPQ-14, the maximum range of the molecular weight was (200 - 1800 bp), whereas the lowest range was for primer OPA-03, which is (200 - 800 bp) (3). The reported size discrepancy ranges in size from 325 to 1325 bp (Fadhil, *et al*., 2016).

Table (2): Total number of bands from local chicken groups

Local Chicken groups	Total number of bands	polymorphic band	% Polymorphism
East (E)	89	9	10.1
West (W)	94	7	7.4
North (N)	101	8	7.9
South (S)	92	9	9.8
Central (C)	97	8	8.2
All	473	41	8.7

Table (3): Number of bands, polymorphic bands, Mono band, Monomorphic band, Polymorphism %, Size (bp) for different prime

Primer number	Total number of bands	polymorphic band	Mono band	Monomorphic band	% Polymorphism	Size (bp)
OPA-10	37	6	4	31	16.22	300 - 1500
OPA-07	52	3	8	49	5.77	200 - 890
OPA-03	38	3	6	35	7.89	200 -800
OPA-12	25	5	2	20	20.00	500 -1200
OPQ-14	137	5	24	132	3.65	200 -1800
OPA-05	38	4	5	34	10.53	200 - 1500
OPA-11	41	3	6	38	7.32	300 - 1500
OPA-09	48	1	9	47	2.08	200 - 1000
OPQ-11	29	3	4	26	10.34	300 - 1500
OPQ-10	28	1	5	27	3.57	300 - 1500
sum	473	34	73	439	8.74	200-1800

In table 4 The values of Jaccard`s similarity coefficients of RAPD profile generated through 10 primer on 500 chicken accession (5) Groups. The north (N) and west (W) groups shared the most genetic closeness (0.893), whereas the south (S) and west (W) groups shared the least genetic similarity (0.724). This finding agrees with Sharma and Singh's (2002) how used Five strains, G, H, I, J, and C, with different selection histories, were examined for

genetic similarities using randomly amplified polymorphic DNA (RAPD). To ascertain the degree of relatedness among various local chicken populations, phylogenetic relationships and genetic distance were done. Between-strain estimations ranged from (0.820 to 0.969). The RAPD is built upon many methods for examining genetic diversity. The combination of these strategies will improve the genotype diversity screening process.

East (E), West (W), North (N), South (S), and Central genotypes of local chicken groups were explored using a UPGMA dendogram based on Nei's genetic distance (C). The dendogram, which was created using genetic distance to highlight the evolutionary links between the regional chicken groupings. It was evident that the West (W) and North (N) were closely linked and had the most genetic similarities, whilst the South (S) appeared to be the most distant from the other groups. The West (W) and the North (N) sharing a close resemblance suggests that all native breeds are roughly comparable and branched from the same evolutionary tree. Based on RAPD data, the dendrogram displayed separations among the studied chicken groups. Utilizing the (Nei and Li , 1979) approach for phylogenetic analysis, generally manufactured two clusters that could be clearly separated based on the RAPD data of the groups of chickens in study (Figure 2). Using the program PAST, simple matching genetic distances were calculated between all local chicken groups (Hammer *et al.*, 2001). Molecular markers have been utilized to differentiate between domestic Gallus gallus strains (Fulton, 2008). The random amplified polymorphism DNA (RAPD) method was used in this work to identify genetic diversity and similarity among local chicken groups. According to a study, RAPD-PCR is successful at identifying polymorphism in local chicken groups and establishing their genetic relationships with one another (Sharma et al., 2001). For the purpose of effectively utilizing the genetic resources of chickens, breeding systems must be developed to detect genetic diversity at the molecular level (Mollah *et al*., 2009). According to the study's findings, the South chicken group has a high degree of genetic diversity, making it a valuable genetic resource for upcoming chicken breeding initiatives. The RAP utilized in this work was considerably polymorphic, showing high variation among groups, making it useful for analysis of population genetic variability, breed characterization, and supporting breeding strategies. The current study's polymorphism results also shown that the primers were successful in determining the genetic specificity between various genotypes.

The homozygous or heterozygous condition of the animal is recorded in these genotypes. As a result, the ability to describe each genotype under examination offers a promising perspective for varietal identification. The preservation of regional breeds of poultry depends significantly on knowledge of the genetic diversity within chicken groups and the relationship between their performance and genetic markers (Wilkinson *et al.*, 2012). A dendrogram created using RAPD data revealed a distinct division between the groups of chickens under study. This may be the result of the fact that continual attempts made over a long period of time to develop these local chickens resulted in these Iraqi chickens being raised for diverse purposes and originating from different structures. The ability to genetically measure the differences between different genotypes is a very helpful tool for genetic improvement (El-Sabrout *et al*., 2014).

Table (4): Jaccard`s similarity coefficients of RAPD profile generated through 10 primer on 500 chicken accession (5 Groups)

Local chicken	East (E)	West (W)	North (N)	South (S)	Central (C)
East (E)	1.000	.812	.810	.724	.788
West (W)	.812	1.000	.893	.771	.752
North (N)	.810	.893	1.000	.821	.817
South (S)	.724	.771	.821	1.000	.835
Central (C)	.788	.752	.817	.835	1.000

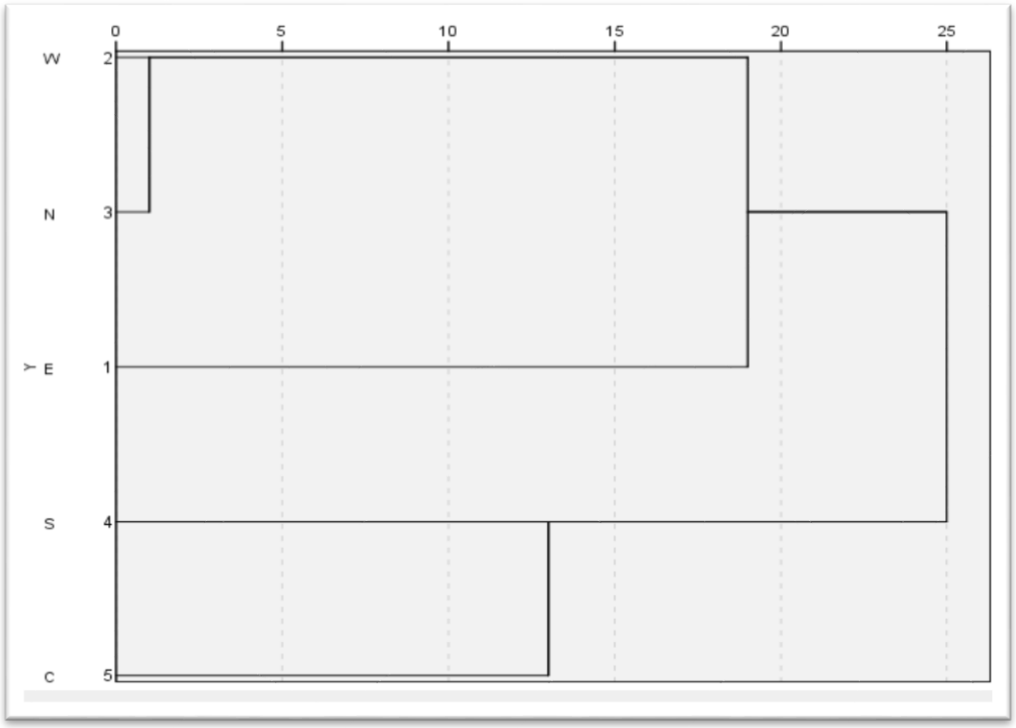

Fig. (2): Dendrogram of 500 chicken (5 Groups) accession generated through 10 primer using Jaccard cluster UPGAM Algorithms.

CONCLUSION

In order to better adapt to environmental changes, comprehend phenotypic variability, and preserve chicken genetic resources that may be used to perfectly direct breeding programs in the future, it is vital to analyze genetic diversity among various chicken groups. The findings of this study are very useful, particularly for scientists who are researching chicken genetics and developing breeding programs to boost the productivity of local chicken in Iraq.

ACKNOWLEDGMENT

Thanks be to God who gave me the determination and perseverance to complete this work, which was carried out on a large scale from north to south.

CONFLICT OF INTEREST

The author support that this research does not conflict with any other research of others.

التنوع الجيني للدجاج المحلي باستخدام RAPD–PCR

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الخلاصة

إجمالي 500 دجاج محلية من مختلف مناطق العراق, تم تقسيمها إلى خمس مجموعات حسب المناطق التي أخذت منها وهي: الشرق (E)، الغرب (W)، الشمال (N) ، الجنوب (S), والمنطقة الوسطى (C) من العراق. تم جمع عينات الدم في أنابيب تحتوي على EDTA ، وتم إجراء استخراج الحمض النووي وتحديد تركيز الحمض النووي ونقاوته النسبية بواسطة مطياف ®Nano Drop ، وتراوحت درجة نقاء عينات الحمض النووي من 1.8 إلى 1.9 ومن ثم إجراء التضخيم العشوائي للحمض النووي متعدد الأشكال (RAPD–PCR) باستخدام 20 بادئة من Gen Script USA. كان لتفاعل PCR حجم نهائي قدره 25 ميكروليتر .ان التضخيم إجمالي 473 موقعًا تتراوح بين 200 و 1800 وزن جزيئي. أعلى عدد الحزم في مجموعة الدجاج المحلية الشمالية (N) (101) ثم المجموعات الأخرى. أظهرت العصابات متعددة الأشكال عددًا أعلى في النمط الظاهري للمجموعة الشرقية (E) والجنوبية (S) ونسبة تعدد الأشكال (6.2٪) في المجموعة الشرقية (E). تم تسجيل أعلى تشابه وراثي (0.893) بين المجموعتين الشمالية (N) والغربية (W)، بناءً على المسافة الجينية التي تم إنشاؤها لإظهار علاقات النشوء والتطور بين مجموعات الدجاج المحلية. كان من الواضح أنمجموعة الجنوب (S) يبدو أكثر بعدًا عن المجموعات الأخرى بينما الغرب (W) والشمال (N) كانا مرتبطين ارتباطًا وثيقًا مع أعلى تشابه وراثي و التطابق الوثيق بين الغرب (غرب) ، شمال (شمال). ان أهداف الدراسة الحالية هي تقييم الخصوصية الوراثية للدجاج الموجود في العراق بناءً على تحليل RAPD–PCR ولتقييم الارتباط الوراثي بين مجموعات الدجاج وفهم مدى الاختلاف الجيني بين مجموعات الدجاج المحلية في العراق.

الكلمات الدالة: الدجاج, الترابط, RAPD – PCR, الوراثة

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