Genetic Diversity of Syrian local chicken Populations using RAPD- PCR Markers

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Abstract:
The research was conducted on local Syrian chicken populations (black and brown) that were subjected to genetic improvement by selection in a previous study, which was group of parents selected from the base population and the offspring generation F1, to evaluate the genetic diversity between populations, using the molecular markers (RAPD-PCR). The importance of the research comes from the lack of local scientific research related to the genetic and molecular characteristics of the Syrian chicken, and its genotypes are mixed randomly and have not been subjected to any kind of genetic improvement, as these differences are important for molecular studies. The results of the study showed that using 12 RAPD-PCR primers, 7 of them were able to selectively amplify regions in the genome of Syrian chickens and showed clear Polymorphism differences between their bands. The molecular markers revealed 32 bands in the studied chickens and had a medium degree of genetic diversity as it amounted by 0.45, the main allelic frequency was 0.60, and the value of the Polymorphism Information Content (PIC) was 0.35. Cluster analysis to construct a genetic phylogenic tree showed that the studied accessions were distributed into three main populations, so that the RAPD-PCR markers were able to show the reflection of the phenotype and genetic improvement processes by selection on the latent genetic content. And as the markers R03 gave the largest number of bands, it reached 8 bands. This research is considered the first one in Syria to study the genetic diversity of both generations of parents and F1 of Syrian local chicken populations.

Key words: local chicken, RAPD-PCR, molecular markers, cluster analysis, genetic diversity.

Introduction

The genetic study of Syrian local chickens is still limited to traditional breeding processes based on productive traits and morphological characterization (Al-Sheikh Amin., 2007), or genetic evaluation of some productive traits based on morphological pattern, studying the associations between phenotype and genotype, calculating the heritability and genetic correlation for the important productive traits (Al-Haj., 2009-2016) Therefore, the Syrian local chicken is a raw material for studying genetic diversity and knowing the genetic association between the members of these populations. As this study will be the building block for future important genetic studies in order to identify the unique characteristic found in Syrian local chickens and develop the strategies to improve their use in management, production and marketing systems. It will also contribute to the collection of all genetic information that can be exploited in future educational processes, by knowing the genetic stock before starting the educational process, in addition to the possibility of following up important traits and accessing future genetic local that can contribute to improving important productive traits. It was found by (Amena et al., 2015) in a study on Saudi local chickens to produce a genetic fingerprint using 12 locals of the RAPD on 25 samples of local chickens that the total number of bands had reached 87, and the proportion of divergent bands was 34.7%, while the percentage of non-variant bands was 65.3%. The results of the phylogenic tree divided the individuals into two main populations with a genetic similarity of 80%. And the genetic distances were low, as the lowest genetic distance was recorded by 0.09. And in a study in Africa to assess the genetic similarity between seven local breeds selected for egg and meat production in Bangladesh. It was found that the value of genetic similarity between local breeds specialized in egg production ranged from 81.3 to 89.3%, while the value of genetic similarity between local breeds specialized in meat production ranged from 69 to 78.7%. The study showed that RAPD local are effective in detecting genetic similarities as well. They provide basic information on the genetic relationships between local breeds (Monira., 2011), she also used the RAPD technology to estimate the genetic differences and similarities, in one of the local breeds, the Alexandrian strain selected for 16 generations to improve the quality of egg production with a local strain as a control, so that such long selection program is expected to reduce the genetic differences in close populations and this will reflect the response to selection; the average percentage of MAPD genetic differences for both the selected strain and the control was 0.41 and 0.66, respectively, while the percentage of genetic similarities recorded for each of them was 0.64 and 0.35, respectively. As for the recorded genetic distance between the two strains, it was 0.41. The study emphasized the importance of RAPD local in assessing genetic differences and variations among local Egyptian chicken populations (Lamiaa et al., 2014). Also, as pointed out by (Dehghanzadeh et al., 2009)) in a study to assess genetic diversity on five populations of Iranian local chickens (MZD), (ISF), (YZD), (FRS), (WAZ) in order to analyze the differences within and among these populations and reconstruct the phylogenic tree between them using local the RAPD. the (FRS) and (MZD) populations recorded the highest values for genetic distance (0.182), while the (FRS) and (ISF) populations recorded the lowest values (0.066). The two populations (YZD) and (MZD) showed the highest values (0.208) for genetic diversity within the populations. The phylogenic tree was reconstructed based on the UPGMA scheme, which separated the chicken populations into two independent populations. Clusters of (ISF) and (FRS) were as first, then clusters of (YZD) and (WAZ) as larger clusters. While the chicken group of (MZD) was grouped separately from the rest. The results of this study showed the importance of RAPD in assessing genetic differences and variations among local chicken populations. In a study on Egyptian local chickens, the RAPD technology was applied to detect the genetic diversity of DNA bundles between both types of normal and mutant chickens, based on the RAPD local. The detection of polymorphic DNA between the two types of chicken was carried out using fifteen oligonucleotide primers. DNA bundles were lost using these primers within a range of 25% to 75%. The data showed that RAPD - local can detect DNA modifications (Bahy.,2013). Xiang et al., (2010) studied the level of genetic differences that analyzed local chicken breed, Bian chicken, and two control populations of Chinese chickens (Jinghai and Youxi), using 29 microsatellite pointer the total number of 166 distinct bands in three lineages, appeared that 32 of these bands (19.3%) were unique and within one lineage. Whereas Bian chicken had the largest number of 15 (46.9%) private bands, followed by Jinghai chicken with 12 private bands (37.5%). The mean data for morphological variations included 0.5168 and the average expected heterozygous frequency 0.5750 in Bian chicken, which was the highest, while those values in Jinghai chicken were 0.4915 and 0.5505, respectively, which were the lowest. The PIC value of Bian, Jinghai and Youxi chickens ranged in the following values (0.51, 0.49 and 0.49), respectively.

For identifying genetic diversity between native White chicken lines and two commercial Broiler (Rose) and Layer (Isa Brown) chicken breeds using RAPD markers and a sequencing technique a study showed that there were a large number of polymorphisms and target sites (71) in all chicken populations, indicating that RAPD-PCR techniques provide sufficient genetic distance and higher genetic diversity among chicken populations. According to both phylogenetic trees, local white chickens had a stronger sense of individuality and were slightly closer to commercial broiler breeds than to laying hens. As a result, it indicates that strengthening the local chicken line requires a broiler breeding program, as well as cross-breeding with other local chicken breeds to obtain important genetically significant new breeds (Dilger et al., 2022).

The importance of the present study is coming from the fact that it represents the first step to identifying the genetic resources of the local chicken and knowing the genetic energies inherent in it in order to design strategies to preserve and benefit from it due to its economic and national importance. To provide breeders with fast and accurate tools that help identify the genetic sources of the desired traits and determine the genetic sites responsible for them, thus enabling the selection and breeding of individuals carrying the desired traits at the molecular level with quick and simple laboratory tests, thus saving a lot of time and effort required for selection by traditional breeding methods.

The aim of the current investigation is to estimate the genetic diversity and molecular variations of the parent and offspring populations of Syrian local chickens using RAPD local.

Material and Methods

I. Blood samples

The blood samples of the study birds were collected from the populations of local Syrian chickens (black and brown, that genetically improved by selection in previous research. About 1 ml of venous entire blood from the wing vein was obtained, and added into 2 ml tubes containing 5 mM of EDTA anti-coagulant (ethylenediaminetetra-acetic acid), transferred using ice, and stored at -20°C.

II. Extraction of DNA (genetic material)

- 3µL of blood was taken and added by 500µL of extraction solution (Lysis) to it.
- 10 Mm (PH= 7.5) of (Lysis: 67 Mm Sucorose, 20 Mm Tris cl MgCl2, 0.5% Triton (X100)) was supplemented.
- The samples were centrifuged for 5 minutes at a speed of 14,000 r/sec.
- The resulting precipitate was dissolved with 200 µL of TEN + Pronase (10mM TrisCl, PH=8,1mM EDTA,10mM Nacl,100µg/ml Pronase) and incubated at 37°C for one hour after adding 20µL of Proteinase K (at a concentration of 20mg/ml).
- Centrifugation was carried out for 10 minutes at a speed of 1810 r/sec.
- The supernatant was taken and 700µL of isopropanol was added to it, then centrifuged at 1810 rpm/sec for 10 minutes to form a precipitate of DNA. The supernatant was discarded, the precipitate was dried, and the precipitate was dissolved in 150µL of TE (10mM TrisHCl,1mM EDTA PH=8) according to (Bello et al., 1999).

III. Molecular Marker /RAPD/

The present assay is based on polymerase chain reaction (PCR) using single random short primers consisting of 10 nitrogenous bases each. To complete this study, 12 different random primers were used (Table 1).

Table (1): List of Primers for RAPD-PCR Marker.

Primer	The sequence of the nitrogenous bases	Primer	The sequence of the nitrogenous bases
OP-D08	5’-GTGTGCCCCA-3’	OP-A10	**5’- GTGATCGCAG-3’**
OP-R03	5’-ACACAGAGGG-3’	OP-K07	**5’- AGCGAGCAAG-3’**
OP-L11	5’-ACGATGAGCC-3’	OP-N20	**5’- GTGTGCCCCA-3’**
OP-D05	5’-TGAGCGGACA-3’	OP-N01	**5’- CTCACGTTGG-3’**
OP-D15	5’-CATCCGTGCT-3’	OP- M09	**5’- GTCTTGCGGA-3’**
OP-O09	5’-TCCCACGCAA-3’		
OP-O08	5’-CCTCCAGTGT-3’		

Table (2): Components of the PCR amplification reaction in the RAPD test.

Materials used in the PCR reaction	Amount needed to prepare a sample (µl)
distilled water dH2O	**17.3**
10X PCR buffer	**1.5**
$MgCl_2$25mM	**1**
Dntp 2mM	**0.4**
primer (10 p.mol/µl) (50ng)	**2.5**
Taq DNA polymerase	**0.3**
template DNA (50 ng/µl)	**2**
final size	**25**

IV. Statistical Analysis

PIC Polymorphism Information Content (PIC) was calculated for each molecular markers according to the following equation:

$$PIC = 1-\Sigma ni-1fi2$$

Where: fi is the frequency of the i genotype, n is the sum of the resulting DNA fragments. These values help to determine the strength of discrimination of the molecular site depending on the number of penodes in each site and the value of the relative frequency of penodes in the studied group. The value of the (PIC) ranges between 0-1. If the value of PIC>0.5, then the molecular markers is considered very useful. If the value of PIC>0.50>0.25, then it is acceptable. If the value of PIC<0.25, then it is of little use. The value of the PIC corresponds to the value of the genetic diversity index.

- **Estimating the value of Genetic diversity (GD)**

Also, called Expected Heterozygosity (HE): it was calculated for each molecular markers according to the equation that was developed by (Vos et al.,1995) $HE=1-\Sigma pi2$ Where p is the frequency of all types i in the genotypes or the studied cultivars, and the value of HE ranges between 0-1, 0 (no inhomogeneity), 1 (existence of inhomogeneity, i.e. the presence of a large number of recurrent heterozygous allozoites).

- **Estimates of genetic similarity**

The data obtained from the RAPD technique were genotyped in a binary array based on the presence or absence of the DNA fragment, and the number (1) for the presence of the fragment and (0) for its absence were given for each model separately, and were recorded in a two-count data matrix. The value of genetic similarity was calculated between each two models; Genetic i and j at each genetic site for each specific molecular markers, according to the following equation that mentioned by (VOS et al., 1995) :

$$GSij = 2Nij\ 1\ (Ni+Nj)$$

Where Nij: is the number of fragments common to genotypes i and j, and Ni, Nj are the sum of fragments in the two genotypes, respectively; for all chromosomal loci studied by AFLP technique. Thus, the value of GSij reflects the proportion of fragments common to two cultivars or genotypes, and its value ranges from 0 (as there are no common fragments) to 1 (as the fragments are similar or homologous between the two genotypes). Cluster Analysis; the data of the similarity matrices obtained by the RAPD technique were converted into measurements of the genetic dimension (D) using the following equation (NEI ., 1979)

: $D=1-GS$

Which it was used to obtain the dendrogram using the UPGMA Unweighted pair Group Method with Arithmetic Averages.

Results and discussion:

The results showed that after screening all accessions by all the primers across all the chicken populations, using RAPD- PCR, there were 32 bands of polymorphism differences, using 7 primers (that only gave results); where the primer: R03 was distinguished by detecting the largest number of bands (8 bands), followed by the primer D15, which revealed (6 bands), then the primer L11, which revealed (5 bands) and finished by the primer O08, which revealed (4 bands), while the primers D08, D05, and O09 showed the least number of bands (3 bands only),as shown in Table 3. The results were stratified based on the presence or absence of a gang. The DNA that indicates the presence or absence of an allelic. The results also showed that the value of the average main allelic frequency for each genotype was 0.60, and the range ranged from 0.84-0.47 for all studied molecular loci. The average genetic diversity for all studied molecular local was 0.45 In local Syrian chickens, after one generation of selection, that is, the RAPD-PCR primers are effective in detecting genetic diversity in local chicken patterns, as these results were valuable in protecting the genetic resources of local chicken patterns in order to devise breeders according to the production destination.

As for the value of the PIC, the average was 0.35 and ranged between 0.54 -0.12, i.e., it is reasonably useful according to this criterion, as the genetic diversity among the studied birds is \ moderate. The importance of RAPD-PCR comes in detecting polymorphisms in the absence of any prior information about the target genome (Table 3).

Table(3): Major-Allele Frequency, Genetic Variation, and PIC Value (the value of the marker's usefulness in detecting morphological variations)

Primer	Principal allelic repeat	Genetic diversity	PIC
OP-D08	0.63	0.44	0.34
OP- D15	0.47	0.62	0.54
OP-L11	0.56	0.49	0.37
OP-D05	0.65	0.44	0.34
OP- R03	0.53	0.50	0.37
OP-O09	0.84	0.17	0.12
OP-O08	0.54	0.49	0.37
Mean	0.60	0.45	0.35

Table 3, summarizes the molecular markers used in the statistical analysis with the number of heterogeneous plots that appeared next to each marker with the range of allele sizes given by the studied the molecular markers.

Table (4): Properties of the fragments produced across populations by the RAPD-PCR

Primer	The number of different pieces	size of bands (nucleotide diplopia) (bp)
OP-D08	3	**100,150,200**
OP- D15	6	**450,500,550,600,650,700,750, 800**
OP-L11	5	**50,100,150,200,250**
OP-D05	3	**50,100,150**
OP- R03	8	**300,350,400,450,500,550,600, 650,700,750,800**
OP-O09	3	**250,400,500**
OP-O08	4	**300,350,400,450**
Overall/ populations	32	**50 -800**
average	4.5	**-**

The binary genetic similarity matrix calculated based on the Jaccard genetic variance coefficient showed that the genetic kinship relationships between populations and individuals are measured by determining the genetic distance between each two accessions. The symbol (A) refers to the chickens of the selected parents, (B) to the roosters of the selected parents, and (C) to the members of the F1 generation. It was found that the lowest genetic distance was Nei's distance = 0.19, which was recorded between accessions A8 (brown color) and A1 (black color), that is, between two accessions of selected parents. Individuals with continued selection and genetic improvement processes, followed by the value of the genetic distance Nei's distance = 0.21 between the two entries A1 (black color) and C3 (black color), that is, between two entries from the selected parents and F1, after one generation of selection, and the studied individuals are expected to show a higher response for selection after two or several generations. While the largest genetic distance, Nei's distance = 0.79, was recorded between A2 (black color) and C10 (black color), that is, between two accessions of selected parents and F1, followed by the value of Nei's distance = 0.78 between accessions A9 (brown color) and C10 (brown color), Followed by the value of the genetic distance Nei's distance = 0.76 between accessions A6 (brown color) and A4 (brown color), that is, between two accessions of the selected parents. The genetic distance of the naked neck line N of Egyptian local chickens was estimated at Nei's distance = 0.69 from the base population according to (El-Gendy et al., 2008) after one generation of selection, while the genetic distance was 0.83 Nei's distance = after two generations of selection. So that it showed a higher response to selection in the second generation compared to the first generation. Depending on the genetic distances, chickens can be selected and crossed with roosters of the same phenotype (brown or black) with few genetic distances between the parents. We can get generations with a higher level of genetic similarity compared to their parents. Then successive selection is applied for several generations until we get pure strains. from local chickens, then the pure strains are crossed with each other to obtain the phenomenon of hybrid vigor. Table (4) shows the values of the binary genetic dimension matrix based on the Jaccard genetic variance coefficient for all accessions. The results showed through the cluster analysis using the UPGMA pairwise populations method in Figure (1) for the studied chickens, the genetic phylogenic tree, which gave three basic clusters: 1. Where the first cluster branched into several branches that included inputs from some of the selected parental members (B2 rooster and two chickens A3 and A6) with one of the F1 members (C12). 2. The second cluster included entries from some selected parents (B1 rooster and 5 chickens A2, A4, A5, A9 and A10) with individuals from F1 (C1, C2, C5, C6, C7, C8, C9, C10, C11 and C13). 3. The third cluster included entries from individuals from selected parents (A1, A7 and A8 hens) with individuals from F1 (C3 and C4). It is noted among the members of the second cluster group that the cluster was not affected in this group by the phenotypical form of the group members, as the chicken A9 (brown color) of the selected parents was genetically closer to the chicken A10 (black color), which is supposed to be close to its counterparts morphologically (appearance), and this may be due to the great genetic similarity between the entries of this group in terms of common origin, and it is also noted that the bird C7 (black color) of the F1 individuals meets with the bird C5 (brown color), which differs from it in appearance; also, although it be closer to the C8, C10 and C11 birds (black). This overlap between individuals means that morphological symmetry does not always reflect a similar genetic background. This overlap between individuals is normal because all populations of domestic chickens were subject to the same environmental factors and diets but were not subject to long-term breeding and genetic improvement programs. The high differences in the phenotype of the local chicken are a major evidence of the high genetic differences and their genetic component. Genetic characterization based on molecular indicators gives a clear picture of the genetic differences within the studied local chicken populations.

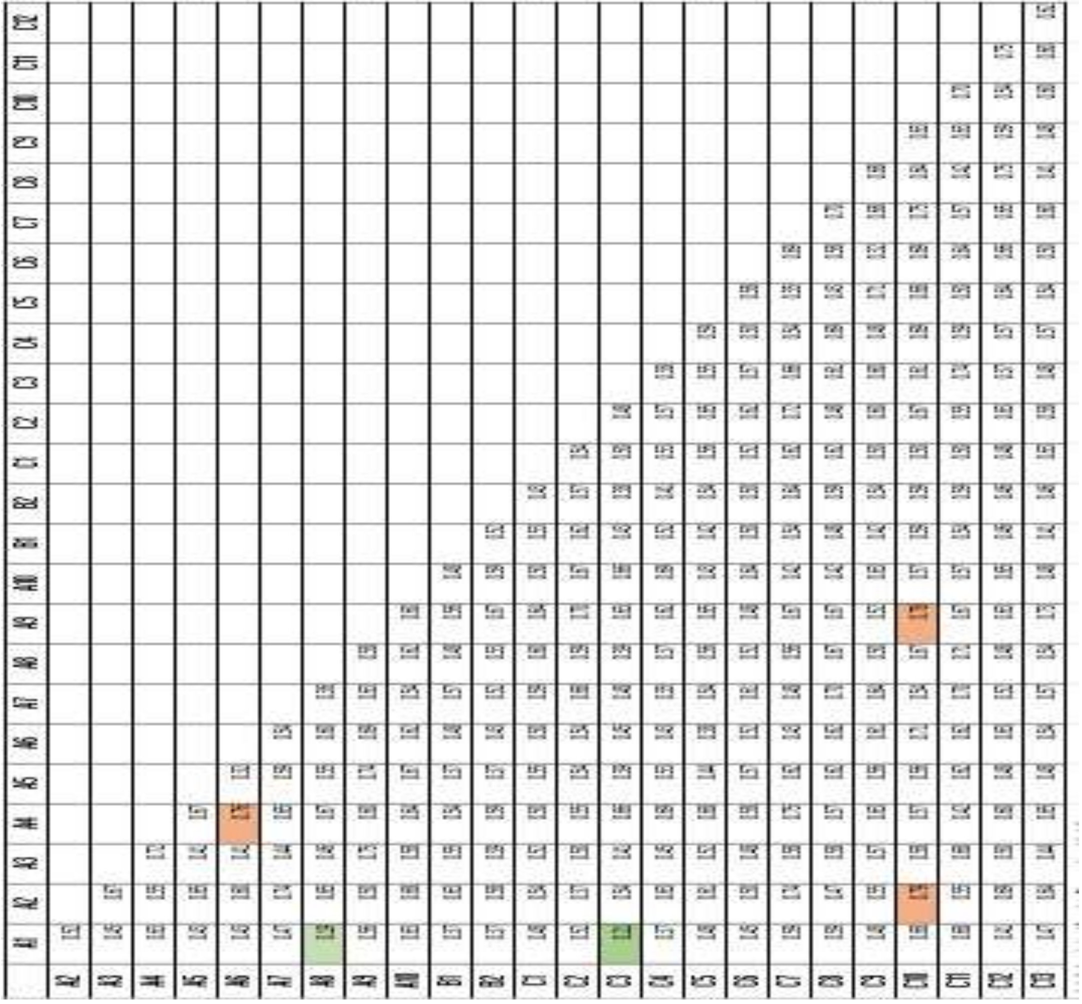
Conclusions:

- Using 7 RAPD-local, revealed 32 bands in the Syrian local chickens, which represented selective regions.
- The molecular index R03 produced the largest number of bands, reaching 8 bands in the Syrian local chickens.
- The average value of PIC, which amounted to 0.35 for the set of local used in the study of Syrian local chicken, proved that the studied local chicken individuals had a medium degree of genetic diversity.
- The average value of the main allelic frequency (genetic similarity) for each gene site was 0.60.
- The mean value of genetic diversity for all studied molecular local was 0.45.
- A study of genetic phylogenic relationships between individuals using the Darwin program showed that there is an overlap between the different studied individuals, indicating that the morphological similarity does not necessarily reflect similarity at the genetic level.

Recommendations:

- Using the molecular markers R03; not O09 molecular index for studying the genetic diversity of Syrian local chickens due to the small number of bands it reversed from the later.
- Using other techniques from other molecular local, such as AFLP local, SSR local, and SNP local, to enrich this type of study.

Table (4): Genetic distance between studied chicken access



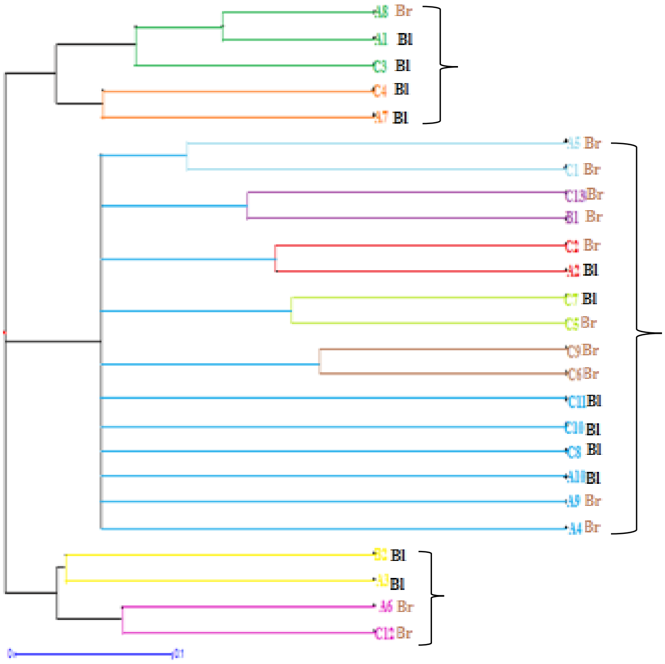


Figure (1): Another form of the Neighbor-joining tree among the Syrian local chicken (jelly accessions studied) by UPGMA method

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التنوع الجيني لمجموعات الدجاج المحلي السوري باستخدام مؤشرات الـ -RAPD PCR

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الملخص

تم إجراء البحث على مجموعات الدجاج السورية المحلية (السوداء والبنية) التي خضعت للتحسين الوراثي عن طريق الانتخاب في دراسة سابقة ، والتي كانت عبارة عن مجموعة من الآباء المنتخبة من قطيع أساس و F1 أفراد الجيل الأول ، لتقييم التنوع الجيني بين المجموعات . باستخدام المؤشر الجزيئي (RAPD- PCR) تأتي أهمية البحث من قلة الأبحاث العلمية المحلية المتعلقة بالصفات والوراثية والجزيئية للدجاج السوري، كما أن تراكيبه الوراثية خليطة ولم تخضع لأي نوع من أنواع التحسين الوراثي فهذه التباينات مهمة للدراسات الجزيئية. وقد أظهرت نتائج الدراسة أن استخدم 12 بادئ RAPD تمكن 7 بوادئ منها من تضخيم مناطق انتقائية في جينوم الدجاج السوري وأظهرت تباينات شكلية واضحة في تعدد الأشكال بين حزمها إذ كشفت هذه المؤشرات عن 32 حزمة في الدجاج المدروس, ووجد درجة متوسطة من التنوع الجيني حيث بلغت 0.45 ، وكان التكرار الأليلي الرئيسي 0.60 ، وبلغت قيمة نفع المؤشر في الكشف عن التباينات الشكلية (PIC) 0.35. أظهر التحليل العنقودي Cluster Analysis لتشييد شجرة القرابة الوراثية توزع المدخلات المدروسة على ثلاثة مجموعات رئيسية بحيث تمكنت مؤشرات الـ -RAPD PCR من إظهار انعكاس الشكل الظاهري وعمليات التحسين الوراثي بالانتخاب على المحتوى الوراثي الكامن. و إذ أعطى المؤشر R03 أكبر عدد من الحزم بلغ 8 حزم. ويعد هذا البحث الأول في سوريا لدراسة التنوع الجيني لكلا الجيلين الآباء والأبناء F1 من الدجاج المحلي السوري.

الكلمات المفتاحية: الدجاج المحلي، المؤشرات الجزيئية، RAPD, التحليل العنقودي، التنوع الجيني.