Determine The Genetic Variation of Phenotypic Groups of Quail (Different With Feathers Color ) Using The Chain Polymerase Reaction Technique (PCR)

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Abstract

The main objective of this research was to specify the hereditary variation associated with the plumage color in three local genotypes of Japanese quail which bred in two geographical locations. The birds distributed on six treatments with five birds for each depending on the feather color and geographical locations. DNA extraction was executed from the blood samples of each treatment and amplified by thermo cycler apparatus and the electrophoresis was done using 1.5% agarose gel for DNA bands exhibiting. Genotype influence has been shown that the black color quail B1 of the agricultural research station in the city of Mosul showed maximum genetic similarity with the Black quail B2 of Tikrit University with a value of 0.9549, the highest genetic similarity between different colors found between B1 and W2 that amounted to 0.9391 based on the similarity index (band sharing). While, the least genetic similarity observed between B2 and W1, which went down to 0.8468. Genetic difference values among studied quail groups showed that the groups B2 and W1 showed higher genetic variation, whereas the least genetic difference found between B1 and B2 groups. The average of dissimilarities for each group with all others varied between the values 0.1203 - 0.0851. The present work prove that the effectiveness of RAPD markers in knowing the similarity and specify the inherited relationship within the quail varieties.

Keywords : Genetic variation , RAPD-PCR, Polymorphism, Quail .

1.Introduction

Inheriting variance was estimating using several types of data, including qualitative and quantitative traits, nuclear DNA, proteins and mitochondrial DNA. Thirty years ago, researchers in the field of breeding relied on determining the genetic variation between chicken lines, strains, species on the phenotype and quantitative characteristics values. By the beginning of 1990s of the last century, modern techniques were used in molecular genetics instead of traditional studies. So, the revolution that occurred in molecular biology and the analysis of genetic material of living organisms established the basis for a new stage of molecular indicators that rely on DNA to reveal the genetic variation between and within poultry strains. In this time, several molecular marker techniques have been updated to determine genetic associations within animal species, one of those indicators is Random amplified polymorphic DNA (RAPD) as a molecular techniques available to describe the variation at the DNA level. RAPD technology has the advantage that it does not require to know the nucleotide synthesis of the genetic material to be studied and it is useful for studying populations with small and large numbers of individuals. As well as, RAPD is an easy, quick and relatively lower-cost screening that uses short nucleotide primers of arbitrary sequences to magnify anonymous fragments of genomic DNA [1]. Furthermore, RAPD has several unique advantages such as they do not require the prior knowledge of target sequence, need only small amount of DNA and are simple, fast and less costly [2]. The first how used PCR marker system in genetic analysis was [3,4], showed high level of polymorphism. RAPD polymorphisms are known as the having or not having of particular fragments between individual animals after gel electrophoresis, it not having of a specific fragment is due to sequence variation in one or both of the priming sites, preventing primer annealing and subsequent polymerization. RAPD markers were effective to know polymorphism and genetic variation between quail strains [5,6]. RAPD and microsatellite markers were used to improving broiler performance [9]. Both RAPD and ISSR molecular techniques were successful in determine genotype-specific markers characterizing 10 individuals of quail, the performance and molecular genetic analysis used in this research successfully discrimination between the two genotypes of quail, brown and white colures, males and females [7]. RAPD markers were used to discrimination polymorphism among five quail populations i.e. Japanese, Fawn, Dhakaya, White and Rosetta. Six out of 17 random primers has been checked resulted distinct polymorphic RAPD profiles [8]. [9], who using RAPD-PCR to study the
similarities and genetic dimension between the veins of local Iraqi chickens and different standard foreign strains showed that there are high genetic similarity within the local chickens at an average of 0.88, whereas it was at an average of 0.78 in foreign strains. The degree of similarity between Iraqi and foreign strains was at average of 0.80. The calculated average of differences among each of Iraqi and foreign chickens and in between were 0.12, 0.22 and 0.20, respectively.

2. Material and Methods

This study targeted the detection of the genetic distance or similarity between three phenotypic groups of different local quail with feather color (white W, black B and brown R) that were bred in two geographical locations, the first in the agricultural research station in Rashidiya / Mosul while the second in the fields of Tikrit University so that we have six groups as: W1: white Rashidiya, W2 white Tikrit, B1 black Rashidiya, B2 black Tikrit, R1 brown Rashidiya and the last one is R2 black Tikrit.

2.1. Blood Samples

Blood samples were collected individually from each of the 5 birds for each genotype. The blood collection process was carried out by slaughtering the birds and collecting 5 ml of blood from each bird. The samples were stored in test tubes containing an EDTA and were transported in a cooled box to the laboratory where they were placed in the freezer at a temperature of (- 20 °C) until the time of extraction. The degree of purity and concentration of DNA samples were checked by the Nano Drop® spectrophotometer. Equal amounts of DNA from birds per treatment were mixed to represent the DNA for each treatment.

2.2. Extraction of DNA

Following the manufacturer instructions, DNA was extracted from blood of quail using PrimePrep Genomic DNA Extraction kit from blood Cat. No. K-2000 (GeNet Bio, Korea). The extracted DNA samples were kept at -20 °C until further assay.

2.3. Polymerase chain reaction (PCR)

Random amplified polymorphic DNA-PCR (RAPD-PCR) was used for the molecular characterization of quail using different primers (Table 1). The primers were obtained from (IDT, USA). Briefly, the PCR reaction was prepared in 20 µl final volume containing 10 µl of 2X Master Mix (GeNet Bio, Korea), 2 µl of the primer, 6 µl of PCR grade water and 2 µl of extracted DNA. The PCR reaction was performed by T100 Thermocycler (BioRad, USA) and the program consist of initial denaturation step at 95°C for 10 min then followed by 40 cycles with 95°C for 1 min for DNA denaturation, 34°C for 1 min for primer annealing and 72°C for 2 min for primer extension. Final extension was at 72°C for 5 min. After that the reactions were cooled at 4°C. The amplified products were separated using electrophoresis in 1.5% agarose gel (Promega, USA), and 5 µl of each PCR product was loaded into the well of agarose gel. The electrophoresis was carried out using 1X TBE buffer (GeNetBio, Korea) at 80 V for 1 hour using power supply (BioRad, USA). A 100 bp DNA marker, 4 µl (Promega, USA) was used as standard molecular weight marker. The gel was examined using gel documentation system (Gel Doc EZ Gel Documentation System, BioRad, USA).

2.4. Molecular genetic 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 (Sharma etal.,2001): 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 (Nei M, and Li WH.,1979): GD = -ln(S). The phylogenetic tree was obtained on the base of genetic similarity using the SPSS Program (classify procedure).

3. Results

A total of 9 decamer primers used among different quail color types and breeding locations, to amplify the genomic DNA, sixty-eight bands successfully generated as polymorphic and monomorphic bands, represented 45 and 23 respectively. The percentage of polymorphism was 66 (table 1). Polymorphism levels differed from one primer to the other. Discriminatory ability was the higher in the primers OPE19, OPA10, OPL7 which recognized 11, 10, 9 different loci respectively, while the primers OPL8, OPB2 exhibit the lowest power of discrimination 4,3 loci, Consecutively.
Table 1. Genetic polymorphism analysis based on the primers used for RAPD-PCR amplification.

| Primer   | Sequence (5' to 3') | Tm | Polymorphic bands | Monomorphic bands | Total Bands | Percentage Polymorphism | Ref. |
|----------|---------------------|----|-------------------|-------------------|-------------|--------------------------|------|
| OPC-08-K | TGGACCGGTG          | 39 | 3                 | 1                 | 4           | 75                       | [5]  |
| OPC-08-K | AGGCGGAAC           | 39 | 7                 | 2                 | 9           | 77                       |      |
| OPC-08-K | ACCACCCACC          | 39 | 7                 | 1                 | 8           | 87                       |      |
| OPL-07-K | AGGTAGCGCAT         | 32 | 4                 | 6                 | 10          | 40                       |      |
| OPL-07-K | ACCACCCACC          | 39 | 7                 | 1                 | 8           | 87                       |      |
| OPL-07-K | AGGTAGCGCAT         | 36 | 6                 | 1                 | 7           | 86                       |      |
| OPL-07-K | ACCACCCACC          | 39 | 7                 | 1                 | 8           | 87                       |      |
| OPL-07-K | AGGTAGCGCAT         | 36 | 6                 | 2                 | 8           | 75                       |      |
| OPE-06   | AGATGCAGCC          | 36 | 8                 | 1                 | 3           | 100                      | [6]  |
| OPE-06   | AGATGCAGCC          | 36 | 5                 | 3                 | 8           | 62                       |      |
| OPE-06   | AGATGCAGCC          | 36 | 4                 | 7                 | 11          | 36                       |      |
| OPE-06   | AGATGCAGCC          | 36 | 4                 | 7                 | 11          | 36                       |      |
| OPE-06   | AGATGCAGCC          | 36 | 4                 | 7                 | 11          | 36                       |      |
| OPE-06   | AGATGCAGCC          | 36 | 4                 | 7                 | 11          | 36                       |      |
| OPE-06   | AGATGCAGCC          | 36 | 4                 | 7                 | 11          | 36                       |      |
| Total    |                     | 45 | 23                | 68                |             |                          |      |

Among quail varieties hereditary similarity values derived from band sharing between the different pairwise of quail groups offered in Table 2. Depending on the similarity guide (band sharing), the black color quail B1 of the agricultural research station in the city of Mosul showed the highest hereditary similarity with the Black quail B2 of Tikrit University with a value of 0.9549, while the highest genetic similarity between different colors found between B1 and W2 that amounted to 0.9391. In contrast, the less genetic similarity observed between B2 and W1, which went down to 0.8468. All six groups organized in a narrow range at a high level of similarity fluctuated from 0.8799 to 0.9148, despite the contrast of geographic location, separate rearing system, and plumage color.

Table 2. Genetic similarity values among the 6th quail groups.

| Quail groups | W1  | W2  | B1  | B2  | R1  | R2  |
|--------------|-----|-----|-----|-----|-----|-----|
| W1           | 1   |     |     |     |     |     |
| W2           | 0.9071 | 1   |     |     |     |     |
| B1           | 0.8571 | 0.9391 | 1   |     |     |     |
| B2           | 0.8468 | 0.8771 | 0.9549 | 1   |     |     |
| R1           | 0.8947 | 0.8547 | 0.8947 | 0.9026 | 1   |     |
| R2           | 0.8928 | 0.9217 | 0.9285 | 0.9009 | 0.9247 | 1   |
| Average      | 0.8799 | 0.8999 | 0.9148 | 0.8964 | 0.8943 | 0.9137 |

The data from Table (3) show the number of amplified monomorphic amplicons, which were transported to genetic difference values among studied quail groups, pair-wise comparison between quail flock samples reveals to participation the groups B2 and W1 in the higher genetic variation, while the less genetic difference found between B1 and B2 groups. The average of dissimilarities for each group with all others varied between the values 0.1203 - 0.0851.

Table 3. Genetic difference values among the 6th quail groups .

| Quail groups | W1  | W2  | B1  | B2  | R1  | R2  |
|--------------|-----|-----|-----|-----|-----|-----|
| W1           | 0   |     |     |     |     |     |
| W2           | 0.0929 | 0   |     |     |     |     |
| B1           | 0.1429 | 0.0609 | 0   |     |     |     |
| B2           | 0.1532 | 0.1229 | 0.0451 | 0  |     |     |
| R1           | 0.1053 | 0.1453 | 0.1053 | 0.0947 | 0  |     |
| R2           | 0.1072 | 0.0783 | 0.0715 | 0.0991 | 0.0753 | 0   |
| Average      | 0.1203 | 0.1006 | 0.0851 | 0.1053 | 0.1057 | 0.0862 |

The results from Table (4) include data represents the genetic distance, which estimated through the percentage of polymorphic and monomorphic amplicons. The closer genetic distance was noticed between B1 and B2 (0.0461), whereas the further genetic distance found between B2 and W1 with 0.1662 value. The overall average genetic distance for every single group varied between 0.1284 – 0.0897.
Table 4. Genetic distance values among the 6th quail groups.

| Quail groups | W1   | W2   | B1     | B2     | R1     | R2     |
|--------------|------|------|--------|--------|--------|--------|
| W1           | 0    | 0.0975 | 0     | 0      | 0      | 0      |
| W2           | 0.0975 | 0    | 0.0628 | 0      | 0      | 0      |
| B1           | 0.1542 | 0.0628 | 0    | 0.0461 | 0      | 0      |
| B2           | 0.1662 | 0.1311 | 0.0461 | 0    | 0.1024 | 0      |
| R1           | 0.1112 | 0.1570 | 0.1112 | 0.1024 | 0    | 0      |
| R2           | 0.1133 | 0.0815 | 0.0741 | 0.1043 | 0.0782 | 0      |
| Average      | 0.1284 | 0.1060 | 0.0897 | 0.1100 | 0.1120 | 0.0903 |

Figure 1. A dendrogram of phylogenetic relationships was conducted based on the average genetic distance, the dendrogram arranged the studied quail groups under two clusters, the first one includes the groups B1 and R2 that have lesser genetic distance 0.0897, 0.0903 respectively. The second cluster connected the groups B2, R1, W2 of genetic distance amounted 0.1100, 0.1120, 1160 successively. The last group W1 has the highest genetic distance valued 0.1284, correlated to the second cluster, which has the nearest values.

Figure 2: show scorable polymorphisms in banding patterns among the 6 groups by the primers OPE-06 and OPE-19. Clarify a part of genetic variation among the studied groups in 6 genetic loci included three and seven of polymorphism bands, respectively.

Figure 2. RAPD profile of six local quail genotypes amplified with two different RAPD primers.
Discussion

The present work confirms the efficiency of RAPD markers in figure out the similarity and estimate the genetic relationship between the quail species. The elevated frequency of revealed fragments happens, due to the binding of the random primers to different genomic regions or, to several copies of one gene segment [10]. The various quail groups showed maximum genetic polymorphism, these used primers could detect the genetic parameters between studied groups, which help in choosing the quail-birds specific markers in future studies. Also, some of these primers possibly linked to the quantitative traits of quail birds, because of the high average of similarity levels estimated among the bird's groups (0.899) has corresponded to the same measured economic phenotypic characterization like body weight and egg production (unpublished data) among the quail groups despite the difference of geographical raising location and color, which didn't show a significant effects because all of these bird groups belong to the same strain, and they were not subject to any selection practice. As for feather color, it is undergoing a very limited number of pigmentation genes that do not significantly influence the whole genome structure, that's assured by the obtained clusters and sub-clusters, which include birds different in raising locations and color in each. Further work with a large number of specific primers and sequencing experiments besides intensive selection are required to develop this quail strains.

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