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GENETIC DIVERSITY OF DOMESTIC AND COMMERCIAL TURKEY BREEDS: ASSESSMENT USING RAPD-PCR TECHNIQUE

YOUSIF M.S. AL-BARZINJI[1*] and ARAM O. FATAH[2]

[1]Animal Resources Department, College of Agriculture, University of Salahaddin-Erbil
[2]Animal Wealth & Veterinary, Ministry of Agriculture & Water Resources-Kurdistan Region
[*]Email: Yousif.noori@su.edu.krd; Y_Barzengy@yahoo.com

ABSTRACT

This study was undertaken on 50 local (Black, Red and Narragansett) and 26 commercial turkey breeds (Black, White and Red). Out of twenty random primers used, twelve were able to amplify and showed bands. The total fragment number over all the turkey samples was 324 with size range of fragments from 220 to 2800 bp. Polymorphic fragments and unique bands in all breeds were 60 and 18, respectively. The Nei's gene diversity for overall breeds averaged 0.44. The genetic distance among all turkey breeds ranged from 0.144 to 0.575. The higher genetic distance in local breeds was 0.667 between both (Black and Red) and (Black and Narragansett). On the other hand, the lowest distance was 0.269 between the Narragansett and Red breeds. The overall dendrograms showed three main clusters, the first cluster branch consisted of (local and commercial) black breeds; the second cluster included (local and commercial) Red breeds, while the third cluster included local Narragansett and commercial White breeds. These results showed that the three local turkey breeds are independent to one another.

Key words: Turkey breeds; RAPD Marker; Genetic Diversity

INTRODUCTION

The turkey (*Meleagris gallopavo*, MGA) is the second largest contributor to the world's poultry meat production after chicken (Aslam, 2012). Turkey is the second largest contributor to the world's poultry meat production after chicken. The turkey bird is easy to rise, does not require any special attention, is hardy and is less prone to diseases as compared to chicken (TNAU, 2011).

In 2009, turkey represented 5.8% of the world poultry meat production. The world-wide turkey population has rapidly grown due to increased commercial farming. Global turkey stocks tripled from 178 million in 1970 to over 548 million in 2009. Over the same time period, the production volume increased more than fivefold from 1.2 to 5.3 million tons (Aslam, 2012). Identification and characterization of breeds are important for identification of genetic resources which seeks to prioritize breeds for conservation and development. Assessing genetic variability within and among populations, allelic variation, gene diversity, relationship and genetic distance are also essential for analyzing the complete population structure (Diez-Tascon *et al.,* 2000). The study of the structure and function of genes at the molecular level in a breeding population can help to determine the similarities of the genetic material carried by populations and the genetic variation they possess. Several techniques have been developed to estimate the genetic variation (polymorphism) and genetic relationship among animals, breeds and populations, which includes the RAPD-PCR method (Buduram, 2004). In a study conducted by Ameen, (2013), the total fragment number (TFN) was 395 fragments from five RAPD primers in local turkey breeds in Kurdistan (Black, Red and White).

The objective of this study was to investigate the genetic diversity among local and commercial turkey breeds using the RAPD-PCR.

* To whom correspondence should be addressed.

MATERIALS AND METHODS

A total of fifty blood samples were collected from both male nad female local turkey breeds (Black, Red and Narragansett) and 26 of commercial (imported from France) turkey breeds (Black, White and Red) in Erbil governorate, Kurdistan region, Iraq. DNA was extracted the blood sample of each bird using a Geneaid Genomic DNA Mini Kit. The DNA extracts of the blood samples belong to turkey birds of the same breed were pooled together into a single DNA sample. The quantity of DNA was measured using the Nanodrop spectrophotometer (Thermo, NanoDrop 1000, UK). The quality of DNA was determined on a 1% agarose gel electrophoresis. Twenty RAPD primers were initially applied and twelve of them successfully amplified and showed bands (Table 1). Genetic parameters of total fragment numbers, size range of fragments, polymorphic fragment numbers, Nei's gene diversity, Nei's genetic identity, genetic distance and phylogenetic tree construction were calculated using the version 3.3, Genepop genetics software (Raymond and Rousset, 1995). Amplifications were performed using a thermal Cycler (Applied Biosystems® Veriti® 96-Well Thermal Cycler, country) with the final reaction volume of 25 µL. Each reaction volume contained: 8 µL of Green Master Mix (25 units/mL Taq polymerase, each dNTPs is 200 µM and MgCl2 is 1.5 mM), 3 µL of RAPD primer (197.13 µM – 599.26 µM), 4 µL (40 ng) of DNA Template and 10 µL of Dnase free water. In present study many protocols for PCR conditions were used but four of them give clear bands. The 1st one for Primer (OPQ-03): programmed for 35 cycles of denaturation at 95°C for 1 min, annealing at 32.5°C for 0.30 min and extension at 72°C for 1 min. An initial denaturation step of 1 min at 95°C and a final extension step of 7 min at 72°C were included in the first and last cycles, respectively. The 2nd protocol for (OPA-14 and OPG-05) used the above program with annealing temperature replaced to 35°C and the 3rd protocol for Primers (OPM-06 and OPM-20) annealing temperature was set at 36°C and 4th protocol for primers (OPM-05, OPP-04 and OPS-01) annealing temperature was 37°C and 5th protocol for primers (OPA-04 and OPB-01) used an annealing temperature of 41°C. The amplification products were run on agarose gel (1.5%) stained with ethidium bromide in an electrophoresis tank contained Tris-borate EDTA buffer. Upon electrophoresis at (How long is the run and voltage), the gel was visualized under UV transillumination.

Table 1. Name, sequence and GC % of indict the primers that amplified and showed bands

No.	Primer Name	Sequence (5' – 3')	GC Content %
1	OPA-04	AATCGGGCTG	60
2	OPA-14	TCTGTGCTGG	60
3	OPB-07	GGTGACGCAG	70
4	OPB-19	ACCCCCGAAG	70
5	OPG-03	GAGCCCTCCA	70
6	OPG-05	CTGAGACGGA	60
7	OPA-20	GTTGCGATCC	60
8	OPB-01	GTTTCGCTCC	60
9	OPB-12	CCTTGACGCA	60
10	OPC-02	GTGAGGCGTC	70
11	OPC-13	AAGCCTCGTC	60
12	OPM-05	GGGAACGTGT	60
13	OPM-06	CTGGGCAACT	60
14	OPM-20	AGGTCTTGGG	60
15	OPN-16	AAGCGACCTG	60
16	OPP-04	GTGTCTCAGG	60
17	OPQ-03	GGTCACCTCA	60
18	OPQ-07	CCCCGATGGT	70
19	OPS-01	CTACTGCGCT	60
20	10 MER	AACGCGCAAC	60

RESULTS AND DISCUSSION

Out of the twenty random primers, twelve were amplified and showed bands. Ten of the twelve primers were polymorphisms and two primers were monomorphism in the local and commercial breeds (Fig. 1).

The TFN for the 12 primes was 324 fragments, ranged from 15 fragments in OPA-14 to 35 fragments in OPP-04, OPA-04 and OPG-05. The size of fragments ranged from 220 to 2800 bp. Similar results were reported by Smith *et al.* (2005) in five turkey breeds where TFN were 24 and Chassin-Noria *et al.* (2005) in six populations of Mexican turkey where TFN were 18. The size of fragments ranged from 220 to 2800 bp in black, red (local), black, red and white (commercial). On the other hand, it ranged from 220 to 2200 bp in Narragansett local breed. These results were in agreement with Smith *et al.* (1996) who reported two turkey populations were the size range of fragments was (250- 2500) bp, Chassin-Noria *et al.* (2005) in six populations of Mexican turkey where the size range of fragments was 220 – 1700 bp.

Out of the 324 bands, 18 of them were unique bands. The highest unique band were obtained from OPG05 locus, which had 5 unique bands, three of them (700, 1000 and 1300 bp) in local black breed, one (250 bp) in commercial Black breed and one (1100 bp) in commercial White breed. The lowest number of unique band was 1 band in both OPP-04 (450 bp) and OPM-06 (1150 bp) loci. The highest number of unique band found in locus OPA-04 which have 6 bands (500, 600, 700, 1050, 1400 and 1500 bp) all of them recorded for commercial White breed, but the lowest unique bands found in both

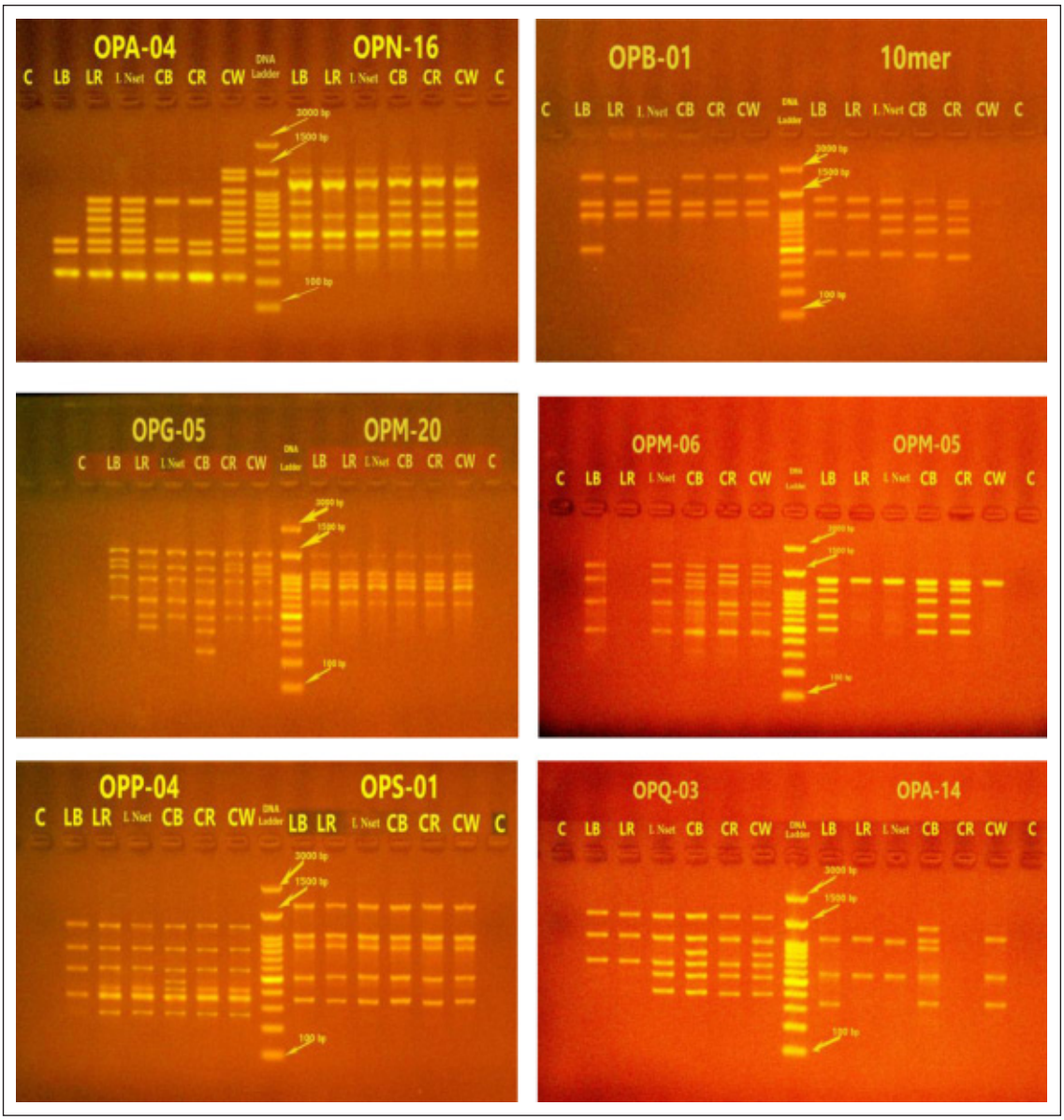

Fig. 1. Gel electrophoresis for twelve RAPD primers in studied turkey breeds.
(C= Negative control, LB= Local Black, LR= Local Red, LNset= Local Narragansett, CB= Commercial Black, CR= Commercial Red, CW= Commercial White).

loci OPP-04 (450 bp) and OPM-06 (1150 bp) in commercial Black breed.

The overall polymorphic fragments was 60 were obtained out of 324 TFN from 12 primers. The highest PFN found at locus OPG-05 (12 bands), whereas the lowest PFN found at locus OPN-16 (1 band). These results were in range with that reported by Smith *et al.* (2005), Chassin-Noria *et al.* (2005) and Ameen, (2013) in different turkey breeds.

The genetic distance among turkey breeds ranged from 0.144 to 0.575, the lowest genetic distance was recorded between the the local Black breed and commercial Black breed and between local Red and commercial Red breed, at 0.144. On the other hand, the highest genetic distance (0.575) was observed between each the following breeds;local Black x local Red, local Black x local Narragansett, local Narragansett x commercial Black, local Red x commercial Black, commercial Black x commercial Red, local Black x commercial Red, commercial Black x commercial White and between local Black x commercial White. The genetic distance was 0.323 between (local Red x local Narragansett, local Narragansett x commercial Red, commercial Red x commercial White and between local Red x commercial White). Furthermore, the genetic distance between local Narragansett and commercial White was 0.203. These results were higher than that reported by Smith *et al.* (2005) in five turkey strains. Moreover, the result showed that there were various genetic distances among studied turkey breeds.

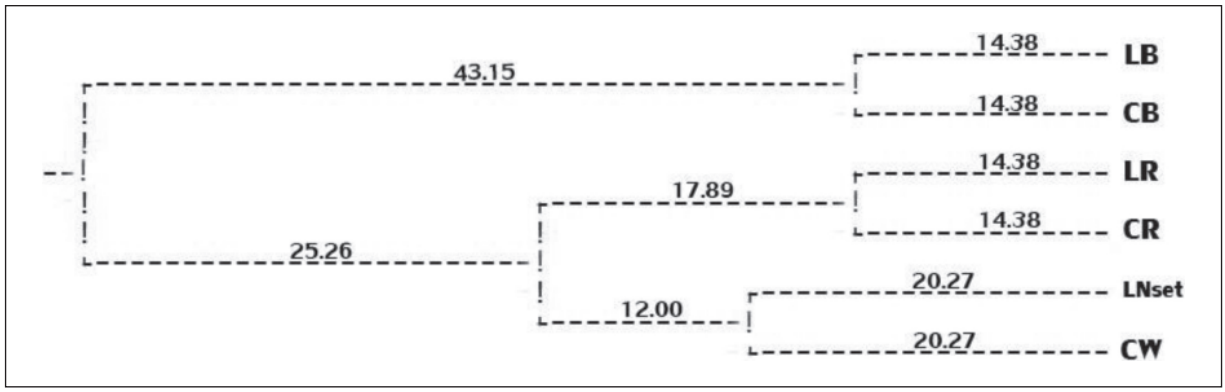

Fig. 2. UPGMA dendogram showing differentiation among the local and commercial turkey breeds.
(C= Negative control, LB= Local Black, LR= Local Red, LNset= Local Narragansett, CB= Commercial Black, CR= Commercial Red, CW= Commercial White).

There are three main clusters, the 1st cluster branch consisted of the local Black breed and commercial Black breed, the 2nd cluster included the local Red breed with commercial Red breed, while the 3rd cluster included the local Narragansett and the commercial White breed. These results showed that there are genetic distances among the three local and commercial breeds, it means that all of them are independent breeds and there are genetic similarities between pair (local with commercial) turkey breeds in each cluster.

CONCLUSIONS

The high distance among the three turkey breeds found in this study indicated that there are high genetic distance among turkey breeds (Black, white, and Red/Narragansett) and moderately genetic distance among local and commercial turkey breeds. The commercial black and red turkeys were genetically near to the local black and red turkeys, respectively.

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