



Use of RAPD Markers Technique to Evaluate Genetic Variation in Two Types of Local Ducks

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Abstract: This Study was carried out to assess the genetic variance between two local Iraqi (white and gray) of domestic ducks by RAPD-PCR technique using five random primers. The total bands number that shown by the RAPD primers (APO-08, APOF-09, APO-10, APO-16, APO-18) was 68, 81, 79, 53, 63 for the white local ducks, while it was 58, 69, 66, 61, 62 for the local gray ducks respectively. Additionally, the primers showed 59 shared bands. OPA-08 showed the highest number of the shared bands (22) while APF-09 resulted the lowest (4 bands). The overall number of specific bands in local white ducks (285 bands) was excel the specific bands of gray ducks (257 bands). The total similarity percentage between both lines was 8.87% while individually, APO-08 showed the highest similarity percentage (17.6%) comparing with the lowest similarity percentage (3.50%) that represented by APF-09.

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

Introduction

Ducks are considered as a one of the most important birds raised in the world and the global consumption of ducks birds ranged between (4-5%) of the total poultry products (FAO, 2014). Its consumption has increased rapidly over the past decade as a diet rich in unsaturated fatty acids and therefore is preferred by the consumer (MIFFAF, 2013). In recent times, modern technology in genetics has introduced a number of helpful molecular markers to analyzes the genetic diversity among different types of breeds and species, In

order to preserve as sources of natural wealth in most of the developed countries (Sharma et al., 2015). The RAPD-PCR technique is a type of PCR reaction that randomly amplifying segments of DNA and applied in the study of genetic diversity. The technique also helped in comparing genomic DNA of various living organisms that were evolutionarily separated (Kumar & Gurusubramanian.,2011). Accordingly, due to the low number of studies on local ducks, its characteristics in terms of productive qualities for getting enough

information to build an integrated database around it specially in the genetic variation field and investigating it relates to the productive qualities of the growth and meat production, So this study aimed to compare productivity and genetic variation between white and gray domestic ducks using RAPD- PCR technique.

Materials and Methods:

Thirty blood samples were collected after slaughtering the birds of the local white and gray ducks in sterilized tubes containing EDTA. The G-DNA extraction was performed using DNA extraction kit (Kit name, Company, Country) according to the manufacturer instructions. The quality and quantity of each extracted G-DNA sample was determined by nano-drop device (Name,

Thermo-scientific™, USA) under 260/280 nm wavelength

The PCR reaction mixture was prepared by adding 2 ml in tube of 100 ml, 1.5 ml from the primer and 10 ml from master mix. The volume was completed to 20 ml by adding 6.5 ml from distilled water table (1). DNA fragments were amplified using 5 molecule specific primers as shown in Table (2).

The PCR reaction mixture involved 2 ul of the DNA templet, 1.5 ul of each primer separately, 10 ul of master mix and the volume completed to 20 ul with dd-water. The PCR conditions included 3 min of initiation at 95 °C followed by 40 cycles involved 1 min of denaturation at 95 °C, 1 min of annealing at 32 °C and 1.5 min of extension at 72 °C ended with 7 min at 72 °C as a final extension step.

Table (1): The primers used in the (RAPD-PCR) technique (El-Araby &Saleh , 2016).

	Primer name	Sequence of Primer
1	OPA-08	5'GTGACGTAGG'3
2	OPF-09	5'CCAAGCTTCC'3
3	OPA-10	5'GTGATCGCAG'3
4	OPA-16	5'AGCCAGCGAA'3
5	OPA-18	5'AGGTGACCGT'3

ELECTROPHORESIS

The electrophoresis was performed to investigate the presence of DNA in the blood samples, as well as the polymerase chain reaction and the presence of marker DNA to differentiate the volumes of the PCR reaction on the agarose gel, After the pelvis was prepared and washed, and the comb was attached to one side of the pelvis, the plastic pieces were placed on the edge of the pelvis. The alkalose gel was centrifuged at a concentration of (2%), dissolving (0.5) g of karose in 25 of the 10X TBE solution and placed in a flask And then heated with microwave until the bright color of the mixture was added. After that, a quantity of the

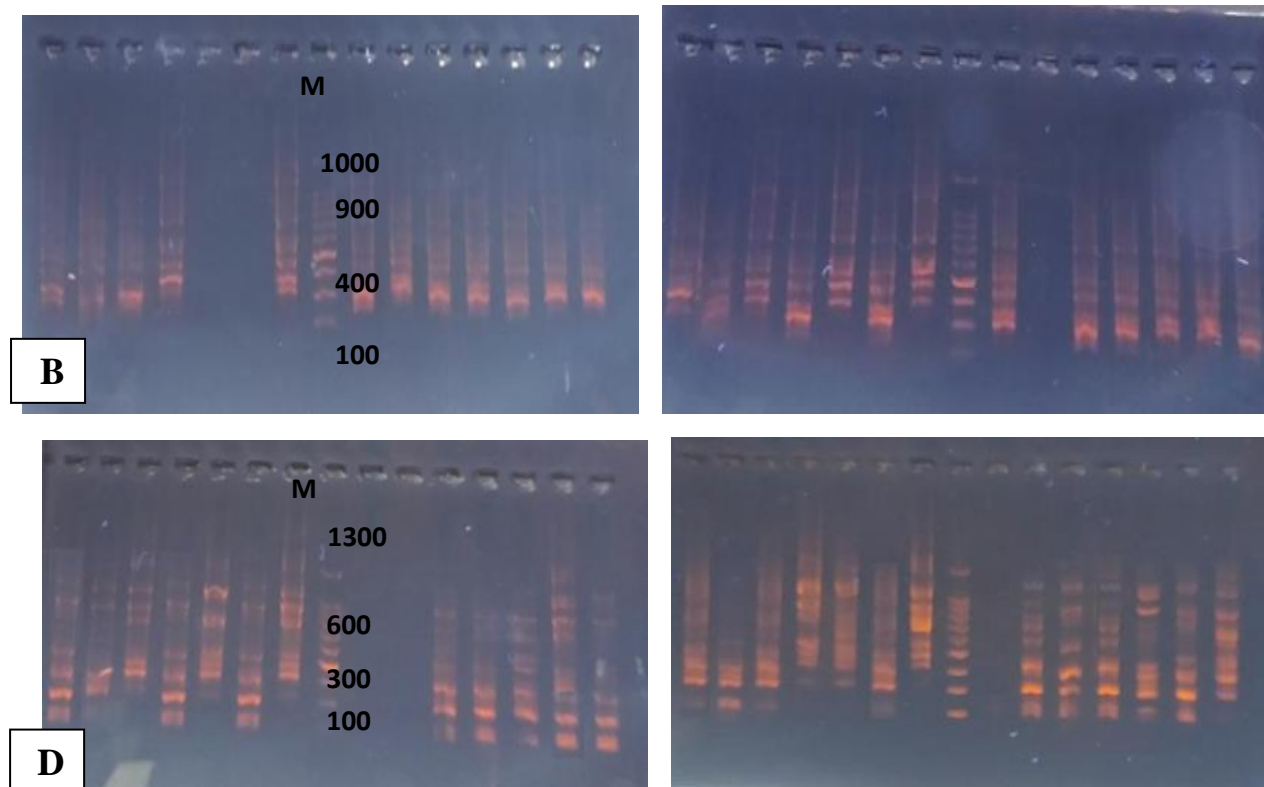
aldehyde bromide dye was added, with the beaker of the beaker in good condition for smoothing the mixture with the mixture. The transfer basin was filled with 400 ml of the solution and the gel was placed in the electrode basin. The samples were placed after mixing with 2 µl of loading dye in the very carefully formed drilling and the electrodes were connected to the power supply. The electrical current is stabilized at 85 mA and the electric relay is operated for 30 min to detect DNA and (50 min) if the polymerase reaction (PCR) is detected, the mixture is left until the blue bromophenol dye from the drill to the side After the completion of the migration process, the results were read by an ultraviolet (UV) device attached to the camera Accurate photographic documentation system imaging

(Photo documentation system) Note to DNA packs interoperability with dye (Ethidium bromide).

The electrophoresis results were analyzed statistically according to Dunning *et al.* (1990) to estimate the similarity index. All the calculations were performed using POP Gene.

Results and Discussion

The RAPD-PCR results revealed that OPA-16 produced the highest number of bands Reaching 81 in white ducks and 69 in gray ducks, While the OPF-09 and OPA-18 showed the lowest number of bands in both white and gray ducks, with 53 and 58 band respectively.



A: Amplification of OPF-09 in local gray ducks, B: Amplification of OPF-09 in local white ducks, C: Amplification of OPA-16 in local gray ducks, D: Amplification of OPA-16 in local white ducks. M: Volumetric guide (Lader).

Total, variant and similar bands produced by primers in local white and gray ducks

Table (2) shows the results of the **variant**, **similar** and total bands. The total number of bands produced by all primers were 344 in white ducks and 316 in grey ducks. Among them, 285 variant bands appeared in white ducks while 257 variant bands presented in gray ducks. These results did not correspond to the results obtained by EL-Araby & Saleh (2016) who used 13 primers these prefixes showed 92 of the total bands, were it was 37 in the rate of 40.2% bands individually. Additionally, there were 59 similar bands

between the two lines that produced by other primers. Our study did not agree with Basha *et al.* (2016) when he studied three breeds of ducks (Sudanese, Muscovi, white pekini) and Egyptian geese breed. So there are 73 bands of special bands and also found 24 special bands.

For geese and 15, 12, 22 bands for Muscovi, Sudanese, and white pekini ducks respectively. While in the study of the Muscovi ducks in two different environments, Ogan *et al.* (2014) found that the total number of bands was 59 and 54 for Guinea savannah ducks and Rain forest ducks. While the number of bands

was 19, 16 and the individual bands 40 and 38 individual bands respectively.

Table (2): The special, common and total bands for the local white and grey ducks.

The primers	the local white ducks			the local gray ducks		
	Variant bands	similar bands	Total bands	Variant bands	Similar bands	Total bands
OPA-08	41	22	63	40	22	62
OPF-09	49	4	53	57	4	61
OPA-10	68	11	79	55	11	66
OPA-16	67	14	81	55	14	69
OPA-18	60	8	68	50	8	58
Total	285	59	344	257	59	316

The ratio of similarity and difference within white and grey ducks

Table (3) shows the ratio of similarity and difference (%) within white and grey ducks for each primer. It is clear from the table that the ratio of similarity within any line exceeded 18 % and was higher in gray ducks compared to white ducks as it reached 18.55 and 17.08 %, respectively. The highest ratio of similarity was achieved by primer (OPA-08) for both white and grey ducks, while the lowest ratio of similarity was by (OPF-09) primer for each of them. The percentage of variation was high, with the highest percentage of white ducks 82.91% pared to grey ducks 81.44%. The highest percentage difference was found by

(OPF-09) primer which reached to 92.46% and 93.45% for white and gray ducks, while the primer (OPA-08) showed the lowest percentage of difference for white and grey ducks as it reaching 65.08 and 64.52% respectively. It is clear from the results that the percentage of difference in both of white and grey domestic ducks were close and higher than the ratios of symmetry and this indicates the possibility of preserving them as a source of genetic diversity. This study did not agree with the finding of El-Gendy *et al.* (2005) who found in his study that among five strains the average values of genetic variation within the strains was 0.38 while the genetic similarity was 0.73.

Table (3): The ratio of similarity of white and grey lines for each primer.

The primer	the local white ducks	the local gray ducks
OPA-08	34.92	35.48
OPF-09	7.54	6.55
OPA-10	13.92	16.66
OPA-16	17.28	20.28
OPA-18	11.76	13.79
Average	17.08	18.55

Genetic polymorphism (%)

Table (4) shows the number of polymorphic bands and the genetic polymorphism of local white and grey ducks. Different proportions of the polymorphic produced by each primer to the other. It is noted from the table that the white and grey ducks showed a proportion of

the polymorphism 98.54 and 98.41% respectively. As OPA-16 primer showed the highest percentage of the polymorphic constitute 98.76% and the lowest proportion emerged by OPF-09 primer and reached 98.11% in white ducks and the primer OPA-16 showed the highest percentage of the polymorphic constitute reached to 98.55% while the lowest percentage of the polymorphic constitute by the primer OPA-18 which reaches to 98.27% in grey ducks. Basha *et al.*, (2016) when he studied three breeds of

ducks (Sudanese, Muscovy, white pekini) and Egyptian geese breed by using 19 primers found that amplification process resulted in 189 bands the ratio polymorphic bands were 89.14% As the percentage of genetic variation from ranged 72.22% to 100% while EL-Araby & Saleh, (2016) found that of the polymorphic bands were 55 bands in proportion of the polymorphism 59%. As the primer OPC1 the highest proportion of the different bands 100% produced while the primer OPA-18 produced the lowest proportion of the variant bands 37.5% while each of (Kulikova *et al.*, 2003; Li *et al.*, 2006) noted that the percentage of genetic variation in the wild and local ducks was 80-50%. Whereas, Alyehhodi *et al.* (2010) found the total number of bands in Indian moti ducks has reached 127 bands and the ratio of was 69.8% of them were polymorphic. And the rate of genetic polymorphism was 70% in the belly of Indian Runner (Sankhyan, 2007).

Table (4): The number of single and polymorphic bands and genetic polymorphism (%) for local white and grey duck.

The primer	the local white ducks			the local gray ducks		
	Single band	Polymorphic band	Genetic polymorphism(%)	Single band	Polymorphic band	Genetic polymorphism(%)
OPA-08	62	62	98.41	62	61	98.38
OPF-09	62	52	98.11	62	60	98.36
OPA-10	62	78	98.73	62	65	98.48
OPA-16	62	80	98.76	62	68	98.55
OPA-18	62	67	98.52	62	57	98.27
The total	62	339	98.54	62	311	98.41

Conclusions

The result of this study indicated the presence a clear contrast between the two local ducks, which contributes to the possibility of preserving them as a source of genetic diversity.

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