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Relatedness Among Three Native Geese in Erbil Governorate Using Hematological and Molecular Methods

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ABSTRACT: ***The objective of present study was to determine genetic diversity among three geese color types using RAPD markers and hematological parameters. The overall mean, of the live weights , Hemoglobin, Hetrophil %, Lymphocyte %, Monocyte % and H/L ratio were 3.006 (kg), 14.64, 36.896, 49.896, 2.233 and 0.736 respectively. The breed, sex and interaction between them have significant effect on live body weight, Hemoglobin and Monocyte %. Ten primers were used and six out of them were selected based on their number of bands (NB) and polymorphic characteristics. A total of 309 bands observed, ranged from 30 in primer OPB-07 to 54 bands in OPA-20. Five unique bands were found only in white goose, whereas the highest unique band was obtained in primer OPB-01 locus. Overall genetic distance among native geese arrived 64.122 and phylogenetic dendrograms showed that 3 clusters, the first cluster content only white geese (Male and Female) breed, the second one cluster is included piebald geese breed (Male and female) and third one was including gray geese (Male and female) breed. It was concluded that the white geese was closer to piebald geese than to the gray geese breed. The high genetic distance (64.122%) and variation in phenotypic value such as live weight (2.375 to 3.600 kg/bird) for three native geese indicates that these native geese have a good amount of genetic resources to made genetically improvement in further and it means the three goose samples are independent breeds.***

Keywords: Geese, Hematology, RAPD-PCR, Genetic Distance.

1. INTRODUCTION

Geese bird is important poultry commodity for Asian villagers. In Asian the aims of breeding geese country is not only to meat or eggs but also to use them as guard birds and to help control and limited the growth of non wonted grass and weeds. The genetic capacity of Asian geese (Egg or meat production) is generally accepted as less when compared with that in Europe which has modern geese breeds [1]. Even the goose production technology in Asian is not well developed; in Asia goose production is increasingly popular and has become accepted as the type of water bird production [1].

The increased for meat production from geese breeding return to as it is very appropriate for organic bird production and supporting to rural developments [2-5].Live body weight was different among geese breeds were ranged from 3 to 7 kg [6-9].

Assessing genetic variability within or among breeds is essential for genetic diversity among the animal populations. The complete population structure helps to plan strategies for conservation and development of a breed [10]. Genetic variation is the raw material for the breeders, who utilize domestic animal species to people's needs. Furthermore, the increasing genetic data on geese bird using different genetic markers will help to understand the evolutionary history of geese. In addition, it will help to refine the definition of breed [11 and 12]. Recently, molecular markers, revealing polymorphism at the molecular level, has play an essential goals in poultry breeding studies. The random amplification polymorphism DNA (RAPD) marker has been widely used, due to its easy utilization by simple PCR, followed by a denaturing gel electrophoresis for number of fragments and fragments size determination [13].

RAPD markers are adopted as a powerful molecular finger-printing technique which allows distinction even between closely related genotypes. The aim of study is to identify the genetic polymorphism for native geese in Iraq using PCR-RAPD technique.

2. METHODS AND MATERIALS

2.1 Geese samples, DNA extraction and hematological tests:

This study was conducted on three local geese breeds (Fig. 1) Whitse breed, piebald breed and gray breed (15 males and 15 females for each color). A total of 90 geese (12 ± 1 month) blood samples (3 ml/bird) were collected (Figure 1) from jugular vein into 5 ml vacutainer tubes containing the EDTA for DNA extractions (DNA was extraction from the blood sample using QIAamp® DNA Blood Mini Kit ,QIAGEN GmbH Qiagenstr.1 40724 Hilden Germany) and for hematological parameters (Haemoglobin concentration (HB), Packed cell volume (PCV), Erythrocyte Sedimentation Rate (ESR) and Differential Leucocyte Count estimated according to [14-17] , respectively. All laboratory work was done in the biotechnology laboratory at the Department of Animal Resources, College of Agriculture, and

Salahaddin University-Erbil. The quantity and quality of DNA were determined by Nanodrop spectrophotometer and 1% agarose gel electrophoresis, respectively but for PCR products we used 1.5% agarose gel to determent the RAPD bands.

2.2 RAPD primers

In the present study, a total of ten RAPD primers (Table, 1) which were obtained from CinnaGen Inc.; (Iran) were used. Six of them amplified and gives clear band.

2.3 PCR amplification of RAPD primers

Amplifications were performed using a thermal cycler (MJ RESEARCH-PTC-200 Gradient Peltier Thermal Cycler ® 60- Well) with the final reaction volume of 20 µL. Two µL sample DNA was added to each tube to make the final volume (20 µL). Each reaction contained: 11 µL of Red Master Mix (AMPLIQON A/S Stenhuggervej 22-Germany), 25 Units/mL Taq polymerase, each dNTPs is 200 µM and MgCl2 was 1.5 mM), 2 µL of RAPD primer (197.13 µM–599.26 µM), 2 µL (30 ng) of DNA template and 5 µL of DNase free water. Many protocols were used but only one protocol gives as clearly bands. The primers (OPA-04, OPA-14, OPA-20, OPB-01, OPB-07 and OPB-12): programmed for 35 cycles of denaturation at 95 °C for 1 min, annealing at 37- 40 °C for 1 min and extension at 72 °C for 1.5 min. An initial denaturation step of 5 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 PCR amplification products were run in a 1.5% agarose gel (Staining with Ethidium bromide in Tris-borate EDTA buffer) and visualized under UV transillumination. The control reactions were set up without genomic DNA to avoid any DNA contamination.

2.4 Genotypic and statistic analysis

The RAPD bands were scored as present (1) or absent (0) in each pattern. All genetic parameters in present study were calculated by using Genepop software, version, 3.3 [18].

The PROC GLM (General Linear Model) procedure [19] was used to analyze the data and Employed for evaluation of live weight and blood traits. Strain, sex and interaction between them were fitted in the following model:

$$Yijk = \mu + Ai + Bj + (AB)ij + \varepsilon ijk \quad i = 1,\ldots,3;\ j = 1,\ldots,2;\ k = 1,\ldots,6$$

Where:

Yijk = observation k in level i of factor A (Breed) and level j of factor B (Sex)

µ = the overall mean

Ai = the effect of level i of factor A (1= Gray, 2= White and 3= Piebald)

Gray **White** **Piebald**

Figure 1. Image of geese breeds.

3. RESULTS

3.1 Phenotypic results

This part of results included the performance traits under studies:

3.1.1 Body weight and blood traits

The overall mean, of the live weights and blood traits were presented in Table (2). Mean live weight was 3.006 (kg). The live weight in this study was higher than reported by [20] in same breeds was arriver (2.933 Kg). In the other side's this results was lower than reported on different geese breeds at the same age [6] 5.534, [7] 6.730, [8] 3.972, [21] 3.657, and [9] 3.470 kg.

The higher lives weight ($P \leq 0.001$), were recorded in Piebald (3.225 kg) and White (3.158 kg) geese while lower was observed in Gray (2.637Kg) geese (Table, 2).

Table 1: Name, sequence and percentage content of GC for all used primers

No.	Primer name	Primer sequence 5ʾto 3ʾ	GC%
1	OPA-04	AATCGGGCTG	60
2	OPA-14	TCTGTGCTGG	60
3	OPA-20	GTTGCGATCC	60
4	OPB-01	GTTTCGCTCC	60
5	OPB-07	GGTGACGCAG	70
6	OPB-12	CCTTGACGCA	60

The sex had significant effect on the live weight (Table, 3). Male's geese at 1 year of age produced significantly ($P \leq 0.01$) heavier live weight than female at same age, this result may be due to the male's hormone effect, which testes male product testosterone hormone which have positive role to increasing the body weight. Similar result reported by [20] for significantly of sex in body weight. A table (4) presents the interaction of breed with whereas sexes. Significance interaction were found between breeds and sexes in weight higher value 3.60 recorded in male Piebald and lowest value (2.375 kg) in female gray geese. These results show good variation in live weight for native geese due to phenotypic and genotypic variation between native geese and selection for above traits within native geese can speed up the performance in native geese in Kurdistan.

Table (2) shows the differences among native geese in blood traits. The overall means of Hemoglobin, Hetrophil %, Lymphocyte %, Monocyte % and H/L ratio were 14.64, 36.896, 49.896, 2.233 and 0.736 respectively. The results show significant differences among strains in Hemoglobin and Monocyte% (Table 2). Non significant results obtained in this study for sex

(Table, 3) on blood traits except in Monocyte % the female's geese have higher value than males. As in table (4) the significance interaction between breeds and geese sexes were found in Hemoglobin and Monocyte % were higher hemoglobin value (17.65 g/ 100cm^3) recorded in White male goose.

3.2 Genotypic results

This part of results included the RAPD-PCR amplification and molecular analysis for six RAPD primers in all geese samples under study:

3.2.1 Number (NB) and size of bands (bp)

The total of six primers amplified showed clear bands and were to investigate the genetic variations among the three local geese breeds. All of the six primers were polymorphic over all geese samples (Figures 2). The overall NB for the 6 primes was 309 bands, ranged from 30 in primer OPB-07 to 54 bands in OPA-20 (Table 5). The highest NB found in white Male (57 fragments) and the lowest (46 fragments) was detected in Gray female (Table 5). Results in this study were higher than reported by [22], (2005) were found 102 bands from seven RAPD primers.

As in Table (5) the bands size range over all the native geese, started from 100 bp and ended at 2000 bp. The smallest size of bands was recorded for OPA-04, OPA-14 and OPA-20 (100 bp) in white, piebald and gray , while the highest size bands range was recorded for primer OPB-01 locus (2000 bp) in all males and females native geese . Similar results was reported by [22], (2005) in Polish goose were size range of band ranged from 200 t0 1500 bp.

3.2.2 Number of polymorphic bands (NPB)

The overall % polymorphism band for 309 bands in present study was 14.868 and 17 of 309 bands is polymorphic bands; among the primers the OPA-04 have higher band with higher polymorphic bands was arrived 42.86% (Table 6). Depended on above results, there are possibility to recommended that these loci can be use to define genetic distances among the present native geese. The results were agreed with than reported by [23] in Magpie geese and [22] in Polish goose.

3.2.3 Unique band

Out of six primers one of them gives unique band, the highest numbers was obtained by primer OPB-01 locus, which had 5 bands, all of them were unique (ranged from 250 to 1500 bp) and found in white goose.

3.2.4 Nei's gene diversity and Shannon's information index (I)

The gene diversity/heterozygosity and Shannon's information index in averaged 0.4815 and 0.6743, respectively. These results indicate the diversity among geese's breed are moderately high.

3.2.5 Phylogenetic tree

As in the dendrograms (Figure, 3), the overall genetic distance among native geese arrived 64.122% and three clusters were found, the 1st cluster branch consisted of the white geese (Male and female) breed, the second one cluster is included piebald geese breed (Male and female) and third one was including Gray geese (Male and female) breed.

These results indicated that the gray geese breed is most genetically distant from the white and piebald geese breeds (64.122%), while this cluster indicates a close relationship recorded between Piebald and White was arrived and the results indicated that the Piebald breed was closer to White breed (43.266%) than to the Gray geese breed. Similar results were reported by [23] in Magpie geese and [22] in three line of Polish goose were genetic distance ranged from 23% to 89%.

3.3 Phenotypic and genotypic traits

According to the hematological results in Table (2) and genotypic results in Figure (3) the gray geese's was different from both white and piebald goose, while the last two one is nearly to each other, it means the hematological differences return to the differences in genetic factors and can used it for characterization of bird breeds. On the other side there are high significant differences ($p \leq 0.001$) between white and piebald with gray geese breed for live body weight, also there are higher genetic distance between white and piebald with gray geese was arrived 64.122%.Theses results give us clear idea show that the phenotypic values such as weight and hematological parameters are under genotypic effect and recording of phenotypic values are necessary to obtained better image for diversity study in poultry breeding.

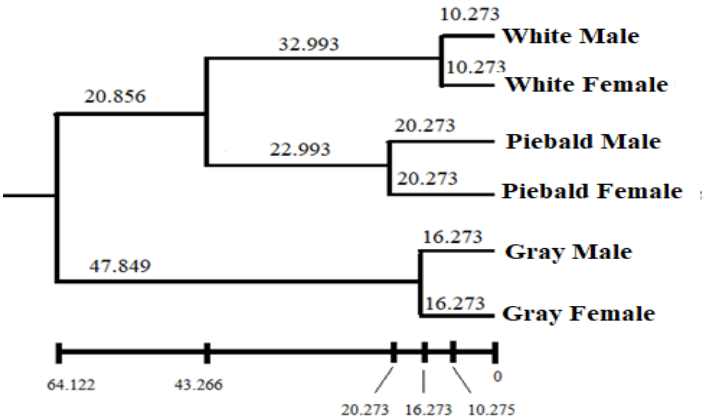

Figure 3. UPGMA dendrograms showing differentiation among geese breeds

Table 6: Total band and percentage polymorphic band.

No.	Primer name	Total Bands	No. of Polymorphic bands	Polymorphic bands (%)
1	OPA-04	21	9	42.86%
2	OPA-14	19	4	21%
3	OPA-20	22	1	4.5%
4	OPB-01	20	0	0.00%
5	OPB-07	12	1	8.33%
6	OPB-12	16	2	12.5%
Mean			4.1	14.868%

4. CONCLUSION

The high genetic distance (64.122%) and variation in phenotypic value such as live weight (2.375 to 3.600 kg/bird) for three native geese indicates that these local geese have a good amount of variation to make genetically improvement in further and it means the three goose samples are independent breeds. The above results can be use by breeders to clarify the mapping of the genetic diversity of the local Iraqi geese and can depend on these results to make mating system or crossing among these native geese or selection within / among native geese to speed up the performance in native geese in Kurdistan. On other side there are similar results in both genetic and phenotypic results it is mean the recording for performance traits are necessary in poultry breeding for got clear image for diversity study in this field of science.

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Table 2: Mean ± SD for effect of geese breeds on weight and blood traits.

Strain	Live weight (kg)	Hemoglobin g/ 100cm^3	Hetrophil %	Lymphocyte %	Monocyte %	H/L ratio
White	3.158 ± 0.35 a	15.97 ± 2.98 a*	35.17 ± 8.09 a	48.17 ± 8.09a	2.14 ± 0.48 b	0.74 ± 0.4 a
Gray	2.637±0.30 b	12.6 ± 1.33 b	38.70 ± 7.4 a	51.70± 7.4 a	2.15 ± 0.29 b	0.74± 0.04 a
Piebald	3.225 ± 0.42 a***	15.35 ± 0.81 a	36.82 ± 2.66 a	49.82± 2.66 a	2.41 ± 0.11 a*	0.73 ±0.01 a
Overall means	3.006	14.640	36.896	49.896	2.233	0.736

Table 3 : Mean ± SD for Effect of geese sex on weight and blood traits.

Sex	Live weight (kg)	Hemoglobin g/ 100cm^3	Hetrophil %	Lymphocyte %	Monocyte %	H/L ratio
Male	3.072 ± 0.31 a*	15.06± 2.26 a	37.56 ± 8.01 a	50.23 ± 8.01 a	2.14 ± 0.40 b	0.735 ± 0.04 a
Female	2.941 ± 0.54 b	14.21 ± 2.52 a	36.56± 4.44 a	49.56 ± 4.44 a	2.33 ± 0.24 a*	0.735 ± 0.02 a

Table 4: Mean ± SD for Interaction between sex and breed on all traits under study.

Strain	Sex	Live weight (kg)	Hemoglobin g/ 100cm^3	Hetrophil %	Lymphocyte %	Monocyte %	H/L ratio
White	Male	3.467 ± 0.05 a	17.65 ± 1.25 a*	52.25 ± 10.5 a	1.7 ± 0.01 d	34.25±10.55 a	0.74 ± 0.05 a
	Female	2.85 ± 0.15 b	14.3 ± 3.5 bc	44.10± 1.6 a	2.58 ± 0.03 a **	31.1 ± 1.6 a	0.70 ± 0.01a
Gray	Male	2.90 ± 0.16 b	12.75 ± 0.75 c	50.70± 11.4 a	2.105 ± 0.24 bc	37.7 ± 11.4 a	0.73 ± 0.06 a
	Female	2.375 ± 0.12 c	12.45 ± 1.95 bc	52.7±2.0 a	2.195 ±0.39 c	39.7 ± 2.0 a	0.75 ± 0.009 a
Piebald	Male	3.60 ± 0.10 a **	15.9 ± 0.7 ab	47.75± 0.15a	2.52 ± 0.01 ab	34.75 ± 0.15 a	0.72 ±0.001 a
	Female	2.85 ± 0.15 b	14.8 ± 0.5 abc	51.9±2.2 a	2.31 ±0.01 abc	38.9 ± 2.20 a	0.74.9 ± 0.01 a

Table 5: Band numbers and bands size range (bp) in native geese

Primer name	Breeds													
	White				Piebald				Gray				Over All	
	Male		Female		Male		Female		Male		Female		Bands	
	No. of band	Size range, bp	No. of band	Size range, bp	No. of band	Size range, bp	No. of band	Size range, bp	No. of band	Size range, bp	No. of band	Size range, bp	No. of band	Size range, bp
OPA-04	13	100-880	13	100-880	16	100-880	16	100-880	16	100-950	16	100-950	90	100-950
OPA-14	10	100-650	10	100-650	8	100-650	10	100-650	4	300-1000	4	100-850	46	100-850
OPA-20	13	110-1250	12	110-1250	6	200-1100	6	200-1100	9	100-1100	8	100-1100	54	100-1250
OPB-01	6	250-1400	6	320-1500	8	130-1700	9	130-1700	7	150-2000	7	150-2000	43	150-2000
OPB-07	8	150-1500	8	150-1500	4	450-1250	4	450-1250	3	300-1250	3	300-1250	30	150-1500
OPB-12	7	110-900	7	110-900	8	120-900	8	120-900	8	130-9000	8	130-900	46	110-900
Total	57	100-1500	56	100-1500	50	100-1700	53	100-1700	47	100-2000	46	100-2000	309	100-2000

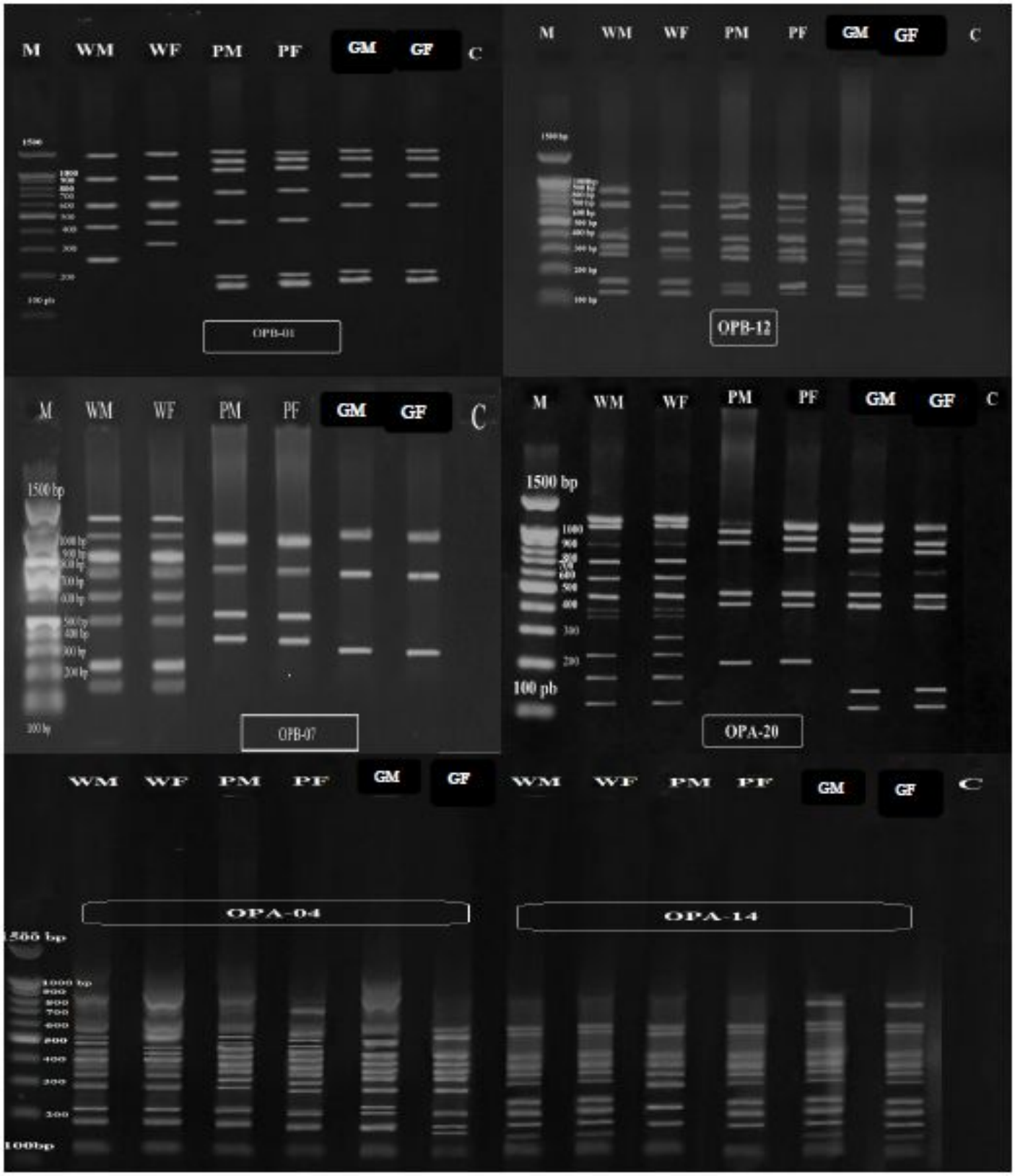

Figure 2: Gel electrophoresis for six RAPD- Primers in geese breeds.

Where: WM White male; WF White female; PM Piebald Male; PF Piebald female; GM Gray male; GF Gray female.