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RESEARCH PAPER

Genetic relationship between generations of chickens using RAPD-PCR

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A B S T R A C T:

This search includes (180 male and 180 female) from the three generations (G) grandparent, (P) parent, and (B) broiler generations of commercial chicken (Ross). Blood samples were collected in 3 ml tubes with EDTA, genomic DNA extracted by Promega's Wizard Genomic DNA Purification Kit. The Nano Drop® spectrophotometer has been used to analyze DNA purity and concentration and it's varied from (1.7 to 1.9). RAPD-PCR was performed using 18 primers from the GenScript USA. The PCR reaction was made up of a 25 l reaction mixture. 40 cycles of DNA denaturation at 94°C for one minute, annealing according to instructions for each primer, extended at 72°C for one minute, and final extension at 72°C for five minutes were performed after an initial phase of DNA denaturation at 94°C for five minutes. 2% agarose gel electrophoresis with a 1x TBE buffer. In order to see the enhanced pattern, a UV transi was utilized. The OPA-07 Primer produced the most (73) bands among all generations used. All of the Primers combined to generate a total of (442) bands, and there were 48 bands that were polymorphic. The primer OPQ-12 had the highest percentage of polymorphisms, (23.53), Primer OPA-07 has the widest molecular weight range (100-1500 bp). The highest genetic similarity is observed 0.813 between (P♀) and (B♂).The dendrogram consists of two clusters, one of which includes (B♀, P♀, B♂) and the other includes (G♂, G♀, P♂), where it is clear that the genetic distance is very high between (G♀ and P♀), while it is less between (B♂ and P♀) and also between (G♂ and G♀) while the distance is intermediate between (B♂ and B♀) and also between (G♂ and P♂). The current study's objective was to use RAPD-PCR to estimate the genetic distance and genetic relationship between chicken generations.

KEY WORDS: Generation; Chicken; relationship; Ross; PCR .
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1. INTRODUCTION:

The precise hybrid breeds utilized here (referred to as "Ross" and "Cobb") are very much the same as those used all over the world. Nearly all of Iraq's meat chickens are derived from two major international poultry genetics companies: Aviagen and Cobb. These genetics companies have strong selective breeding programs and are able to make significant improvements to the genetic potential of their breeds at each generation. Their success depends on the size of their breeding operations and the consequent number of birds, flocks, and choices they can maintain. The breeding flocks maintain the use of the traditional selection breeding methods to achieve what we refer to as the "nucleus" breeding flocks. Grandparents (G) are used to create Parents (P) in the Grandparent (G) generation, and then these Parents are mated to produce fertile eggs, which hatch into the last generation of meat chickens, which is used for meat consumption every year. Over the past 20 years, various types of molecular markers have been become accessible for analyzing genetic variation within and between animal populations. Key participants in animal genetics and breeding programs are these molecular markers that are showing polymorphisms at the DNA level. The most popular markers is RAPD, which enables a quick and affordable assay for determining the genetic diversity of various species. Kjolnerwt al., (2004) have been applied RAPD markers in a wide range of domains, such as gene mapping, population genetics, molecular evolutionary genetics, plant and animal breeding. The effectiveness of RAPD molecular markers in detecting polymorphism in

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several poultry species was discussed by (Salem et al., 2005). Using a total of 60 randomly selected primers, the RAPD investigations assessed genetic polymorphism and relatedness within and among four breeds of chicken and two populations of turkey (Smith et al., 1996). In at least one population, 70% of the primers tested amplified patterns containing at least one polymorphism fragment. The use of RAPD genetic markers to identify polymorphism in several species of poultry was described by (Salem et al., 2005). The RAPD research evaluated genetic polymorphism and relatedness within and among four breeds of chicken and two populations of turkey using a total of 60 randomly chosen primers (Smith et al., 1996). 70% of the examined primers amplified patterns in at least one group that contained at least one polymorphism fragment. The RAPD-PCR has been employed repeatedly in chicken populations to study similarity or variance (Abdulrazaq and Suliaman.,2016). In order to identify polymorphism across five different varieties of chicken the White Leghorn, Rhode Island Red, Red Cornish, White Plymouth Rock, and a native breed called Kadaknath 50 RAPD markers were utilized (Sharma et al., 2001). Determining the genetic similarities inside and between two distinct colored local guinea fowl needed to use 18 primers for RAPD amplification (Haddad., 2020) ; out of the fifty randomly selected primers, twelve produced unique polymorphic RAPD profiles; about 25% of the 96 amplified fragments showed polymorphism. Five local strains of Egyptian chicken that have been chosen to produce eggs and meat have been compared genetically using the RAPD approachBased on six RAPD primers, the genetic closeness of the strains chosen to generate eggs ranged from 72.4% to 85.4%, but the genetic similarity of the two strains selected to produce meat was 86.9% (Ali et al., 2003). A breeder flock of native birds was examined for genetic diversity using a total of twenty arbitrary primers (Rahimi et al., 2005) ; Of the 140 bands that were evaluated, 45 and 55 percent, respectively, were categorized as polymorphic and monophormic. Typically, there were 4 to 16 bands per primer, with lengths varying from 200 to 2100 bp. The average genetic similarity and genetic variance among members of the population were 0.89 and 0.11, respectively. The current study's objective was to use RAPD-PCR to estimate the genetic distance and genetic relationship between chicken generations.

2.MATERIALS AND METHODS

This search includes (180 male and 180 female) from the three generations of commercial chicken (Ross), blood samples were taken. The sampling protocols involved a total of chickens from the (G) grandparent, (P) parent, and (B) broiler generations. Blood samples were drawn and stored at -20°C in 3 ml vials with EDTA. According to the (Beutler, et., al 1990)s instructions, 300 µl of blood had its genomic DNA extracted by Promega's Wizard Genomic DNA Purification Kit. The Nano Drop® spectrophotometer has been used to analyze DNA purity and concentration. The DNA samples' purity varied from 1.7 to 1.9. RAPD PCR, samples were then diluted to 30 ng/ µl. RAPD-PCR was performed using 18 primers from the GenScript USA Series (Table 1); nine of them produced outcomes. The PCR reaction was carried out using 30 ng of genomic DNA, 10 M of each primer, and 25 l of the reaction mixture. The annealing temperatures for the cycle parameter were calculated at Tm based on the GC sequence composition. A 40 cycles of DNA denatured at 94°C for one minute, annealing according to instructions for each primer, extension at 72°C for one minute, and final extension at 72°C for five minutes were performed after an initial phase of DNA denaturation at 94°C for five minutes. The PCR results were examined by electrophoresis on 2% agarose gels in 1x TBE buffer (Promega, USA) stained with ethidium bromide. The magnified pattern was observed using a UV transi, and it was then recorded. The RAPD bands were graded based on whether they were present (1) or not (0). The method for calculating the degree of similarity between any two populations is similarity = 2nxy / nx + ny and genetic distance = 1/(2nxy / nx + ny). Each primer's polymorphism was determined using the following formula: Wear Np equal to the number of polymorphic bands of random primer, and polymorphism% = (Np/Nt) x 100. Nt is equal to the sample primer's overall band count (Bibi, et., al 2009).

3.RESULTUS

The data shown in Table (2) reveals that the OPA-07 Primer produced the most (73) bands among all generations used, whereas the OPQ-12 and OPU-01 Primer generated the fewest bands (34) bands. All of the Primers combined to

generate a total of (442) bands, and there were 48 bands that were polymorphic. When compared to the other primers used in this study, the primer OPQ-12 had the highest percentage of polymorphisms, (23.53), while the primer OPA-17 had the lowest percentage, (4.35). Primer OPA-07 has the widest molecular weight range (100-1500 bp), and Primer OPA-06 has the narrowest range (400 - 1200bp). Table (3) shows the genetic similarity between generations, whether males and females Where the highest genetic similarity is observed 0.813 between (P) females and (B) males, while the lowest genetic similarity is 0.591 between (G) males and B males. In Figure (2), The dendrogram consists of two clusters, one of which includes (B♀, P♀, B♂) and the other includes (G♂, G♀, P♂), where it is clear that the genetic distance is very high between (G♀ and P♀), while it is less between (B♂ and P♀) and also between (G♂ and G♀), while the distance is intermediate between (B♂ and B♀) and also between (G♂ and P♂).

4.DISCUSSION

The RAPD markers are efficient at identifying genetic similarities between breeds of chicken, and they offer a possible mechanism for investigating inter-breed genetic similarity and establishing genetic relationships (Ali,et al.,2003). These results concur with a study by (Abdulrazaq and Suliaman, 2016), which indicated that when five local chicken populations' RAPD profiles were evaluated, the highest number of polymorphic bands was (23), while the lowest number was (7). The lowest polymorphism percentage was (9.48), while the highest was 19.77. In a related investigation, (Singh and Sharma, 2002) 12 primers used discovered 22% polymorphisms, which they attributed to high genotype homology. The best method currently available for biological individualization is fingerprinting. In hens from the meat and layer pure lines, (VGN and BLGEN., 2002) discovered an average of 9.2 polymorphic bands per primer using RAPD-DNA fingerprinting. An average of 12.77 polymorphic bands per primer were found in the study of (Abdulrazaq and Suliaman, 2016), and a significant degree of genetic similarity was also discovered between the meat chickens and its 0.741 between Ross and Indian. The genetic closeness within the native egg and meat-type strains was 0.79 and 0.89, respectively, in a study that was identical to this one (Ali,et al.,2003). for the population of White Leghorne (21.9%) (Singh and Sharma, 2002). Additionally, it was noted that commercial chickens from various regions shared a significant degree of genetic similarity, according to Ali (2003a). The fact that the birds were bred from chickens raised for meat and eggs may have contributed to their great genetic diversity. This indicates that both the genetic characteristics of Roos broiler chickens' males and females are inherited directly from the P females' parents. As for the ancestor G, it is very close, so that it is located in one cluster, with the parents of the males P. In order to obtain the desired traits, the closeness in the ancestors must be as high as possible. From the current study, we note this high affinity, in order to obtain B sons with high meat characteristics, which were taken from the parents of P females, which are genetically close to the male and female G grandparents. Although the meat chicken generation is completely capable of growing older and having checks of their own, they are not typically utilized for breeding. They aren't employed because, as indicated previously, each new importation brings in a variety of genetic lines, each of which has distinct traits that are sought in the following generation. Then, these lines are crossed to create a new generation that is distinct from the previous one once more. So forth animal production frequently uses crossbreeding. Inbreeding, a term that some people may be more familiar with, is the opposite of this. (Zhang, et al., 2002) reported that a great difference of genetic variation was observed between the broiler and layer chicken breeds. The commercial layers were bred for egg production, whereas the broilers were bred for the production of meat (Shen, et., al 2002) ;(Sharma, et., al 2001). The White Leghorn and White Rock and Rhode Island Red and Barred Plymouth Rock egg-producing strains shared between 81.3 and 89.3% of their genetic makeup. The potential benefits of hybrid vigor increase with parent genetic distance. White Leghorne residents (21.9%) are represented by (Singh and Sharma, 2002). Furthermore, the application of RAPD markers is a practical and effective technique, offering a possible tool for the identification of genetic variation among individuals in flocks of chicken for breeding. A high degree of genetic similarity was also noted among the commercial chickens from various regions (Ali, 2003a). It is evident from this study the genetic characteristics of Roos broiler chickens (both male and female) are directly inherited from the parents of (P female).

Table 1: Nucleotide sequences of selected random primers and (%GC) content ratio.

Primer name	Sequence 5' to 3	%GC content
OPA-01	CAGGCCCTTC	70%
OPA-06	GGTCCCTGAC	70%
OPA-07	GAAACGGGTG	60%
OPA-09	GGGTAACGCC	70%
OPA-17	GACCGCTTGT	60%
OPA-13	CAGCACCCAC	70%
OPQ-12	TCTCCGCAAC	60%
OPU-01	ACGGACGTCA	60%
OPA-19	CAAACGTCGG	60%

Table 2: Primer number, Total number of bands, Mono band, Monomorphic band, % Polymorphism, Size (bp).

Primer number	Total number of bands	polymorphic band	Mono band	Monomorphic band	% Polymorphism	Size (bp)
OPA-01	54	6	1	48	11.11	200 - 1500
OPA-06	39	3	1	36	7.69	400 - 1200
OPA-07	73	4	0	69	5.48	100 -1500
OPA-09	40	7	0	33	17.50	300 -1500
OPA-17	69	3	0	66	4.35	300 -1500
OPA-13	55	7	0	48	12.73	200 - 1500
OPQ-12	34	8	0	26	23.53	400 - 1500
OPU-01	34	6	1	28	17.65	400 - 1500
OPA-19	44	4	0	40	9.09	300 - 1500
SUM	442	48	3	394	12.13	100-1500

Table 3: Genetic similarity of RAPD profile generated through nine primers on chickens

Ross	**1:B♂**	**2:B♀**	**3:P♂**	**4:P♀**	**5:Gp♂**	**6:Gp♀**
1:B♂	**1.000**	.747	.629	.813	.647	.667
2:B♀	.747	**1.000**	.726	.790	.591	.630
3:P♂	.629	.726	**1.000**	.727	.709	.805
4:P♀	.813	.790	.727	**1.000**	.633	.707

5:Gp♂	.647	.591	.709	.633	**1.000**	.810
6:Gp♀	.667	.630	.805	.707	.810	**1.000**

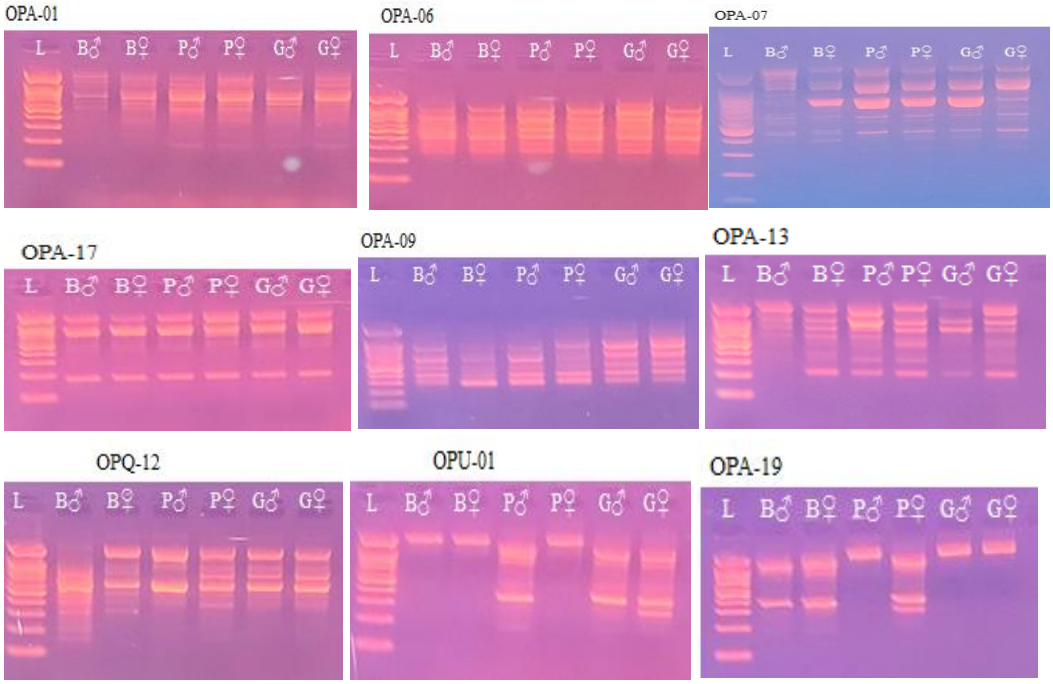

Figure 1: RAPD pattern of Ross chicken's meal and female from three generations G, P, B with nine primers

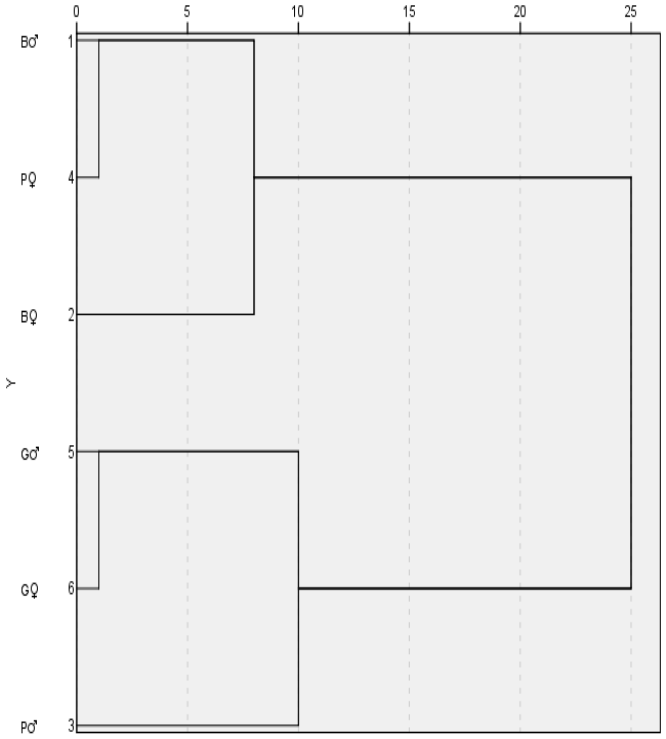

Figure 2:A dendrogram of Ross chicken's meal and female from three generations G, P, B on average linkage cluster analysis RAPD markers

5.CONCLISIONS

The use of RAPD - PCR to clarify the genetic relationship between generations, as genetic similarity between males and females across generations, to ensure obtaining the characteristic of producing meat. And this similarity varies from one generation to

another, where in the generation of the grandparents (G), the genetic affinity was higher compared to the generation of the parents (P), and this is what made the generation of broiler (B) with a high productivity of meat.

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