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MOLECULAR-PHENOTYPIC ANALYSIS AND EFFICIENCY OF CROSSING ON MEAT PRODUCTION IN LOCAL CHICKEN STRAINS

O. M. Aly[1], M. M. Ahmed[2,3], S. M. Abdel-Rahman[2]

[1]El-Sabhiah Poultry Research Station, Alexandria, Animal Production Research Institute, Dokki, Cairo, Egypt
[2]Nucleic Acid Research Department, Genetic Engineering & Biotechnology Research Institute, Mubarak City for Scientific Research and Technology Applications, Alexandria, Egypt
[3]Current address: Department of Biological Sciences, Faculty of Science, King Abdul-Aziz University, Kingdom of Saudi Arabia
Corresponding author: salahmaa@yahoo.com
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Abstract: DNA was extracted from blood of developed local stains of chicken; Bandara, Gimmizah, and their crossing (Bandara x Gimmizah and Gimmizah x Bandara). RAPD-PCR technique was applied to detect genetic similarity as a band sharing (BS) among chicken hybrids and their parents using nine short oligonucleiotides primers. The genetic similarity as BS-values was calculated and ranged from 68 to 91%. Bandara male with Gimmizah female appeared positive heterosis at 8 wks of age. However, evidence from this study indicated that crossing of the developed local hens is capable of rapidly improving the body weight of their progeny during the marketing age. This study reflected the ability of RAPD-PCR for establishing the association between genetic similarity as a band sharing and heterosis (hybrid vigor).

Key words: chicken, crosses, heterosis, RAPD-PCR, genetic similarity

Introduction

Heterosis is a practical importance in the poultry industry *(Fairfull, 1990, Zhang et al., 2004)*. The level of heterosis depends on the similarity or difference between the parental populations.

For many years, agriculture has been taken advantage of hybrid, as opposed to purebred, animal and plants to improve performance. With the possible exception of dairy cattle, commercially used livestock are generally produced by crossing breeds, strains or lines selected to various degrees for performance in economically important trait *(Gavora et al., 1996)*. It has been proposed that the evaluation of the genetic similarity of populations by DNA fingerprinting (DFP)

may provide a method of predicting their combining ability because band sharing (BS) of DFP between individuals or populations is negatively correlated with their genetic distance *(Hillel 1991, Hillel et al. 1992, Haberfeld et al., 1996)*.

The development of molecular techniques has been created new possibilities for the selection and genetic improvement of livestock. The discovery of the PCR had a major impact on the research of eukaryotic genomes and contributed to the development and application of various DNA markers *(Marle-Koster and Nel, 2003)*.

In this work, we studied the genetic similarity between two developed local strains of chicken and their hybrids using RAPD-PCR markers. The main purpose of this study was to employ RAPD markers technology analysis to supply the information of genetic similarity among these hybrids and their parents. This information was used to evaluate the association between genetic similarity and heterosis for some economical trials in offspring from Bandara and Gimmizah local strains of chicken and their crosses that reported in another study to *Abou El-Ella et al. (2005)*.

Materials and Methods

Chicken strains. Two local strains of chickens (Bandara and Gimmizah) were used in reciprocal crosses to produce two pure-strains and two strain-crosses (i.e. Bandara x Gimmizah and Gimmizah x Bandara). A total of 10 males and 120 females were used and natural mating was used in the family pen (1 male per 12 females). For molecular analysis at 16 weeks of age, blood samples from five males and five females of each breeding group randomly into heparinized syringes via cardiac puncture were taken and then stored at -20°C until DNA extraction.

Body weight trait. Body weight was recorded at hatch, 4 and 8 weeks of age, the data was statistically analyzed to test the significance of the different between means of genotypes *(SAS, 1994)*. Duncans multiple range test was used to compare every two means of the different studied traits *(Steel and Torrie, 1960)*. Average degree of heterosis (ADH %) based on the mid-parents (MP) was estimated according to equation given by *Sinha and Khanna (1975)* as follows:

$$(\text{ADH }\%) = \frac{F_1 - MP}{MP} X\,100$$

Where: F_1 is for Mean of cross and MP is for Mid-parents.

DNA extraction. To an aliquot of 50µl blood (after thawing), 700 µl of lyses buffer (10 mM Tris-HCl, 100mM NaCl, 1 mM EDTA, pH 8.0, 0.5% SDS) and 60 µg of proteinase K (20 mg/ml) were added. The mixture was vortexed and incubated at 37°C overnight. DNA was extracted using phenol-chloroform-

isoamylalcohol (25:24:1) and choloroform-isoamylalcohol (24:1) in equal volumes. DNA was precipitated by adding 2 volumes of chilled ethanol in the presence of a high concentration of salts (0.1 by volume of 3M sodium acetate). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA) *(Sharma et al., 2000)*.

PCR and Gel electrophoresis. PCR was performed in a reaction volume of 25 µl using 25 ng of DNA of each sample, 25 pmol of each primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 0.2 mM dNTPs and 5 unit/ µl Taq DNA polymerase (Fanzyme). Thermal cycling (Perkin Elmer 9700 and Mastercycler gradient) was carried out by initial denaturation at 94°C for 2 min, followed by 45 cycles each at 94°C for 30s, annealing temperature at 28-54 for 30s (Table 1), polymerization temperature at 72°C for 30s and final extension at 72°C for 10 min., then the samples were held at 4°C. The amplified DNA fragments were separated on 2% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

Table 1. List of the random primers, their nucleotide sequence, GC content and annealing temperatures.

Primers	Primer sequence 5' → 3'	G+C content (%)	Annealing temperature ºC /time (s)
1	GGC ACT GAG G	70	45/30
2	GAA TGC GAC G	60	42/30
3	ATG ACG TTG A	40	45/30
4	GAA ACG GGT GGT GAT CGC AG	60	52/30
5	AGG CCC CTG T	70	28/30
6	ATG CCC CTG T	60	28/30
7	AAA GCT GCG G	60	28/30
8	ACC GCC GAA G	70	28/30
9	GGT GAC GCA GGG GTA ACG CC	70	54/30

Scoring and analysis of RAPD patterns. The DNA bands were scored for their presence (1) or absence (0) in the RAPD profile of the chicken hybrids and their parents. The index of similarity between each two strains was calculated using the formula: Bab = 2 Nab/ (Na + Nb), where Nab is the number of common fragments observed in individuals a and b breeds, and Na and Nb are the total number of fragments scored in a and b, respectively *(lynch, 1990)*. The band sharing (BS) values were calculated for each primer separately and the average for all primers was carried out with each comparison. Dendrogram was constructed using the average linkage between groups *(Sneath and Sokal, 1973)*. The generated data matrixes were used for calculation of similarity matrix for all primers according to Jaccard's coefficients *(Jaccard, 1908)*.

Results and Discussion

All nine primers were successfully amplified on DNA from blood samples of the four genotypes. With respect to body weight at the different ages, the higher means of body weight at 4 of age were observed for the parental strain, Bandara (209.6 g) and BXG cross (211.2 g). Great significant differences were found among the four different genotypes chicks of Gimmizah parent and both Bandara x Gimmizah and Gimmizah x Bandara crosses at 8 weeks of age compared to the pure Bandara chicks (Table 2). Genotype had no significant effect at hatch weight, where body weight at hatch depends mainly on egg size.

Average degree of heterosis percentages (ADH %) for body weight are shown in Table 3. The cross of (BXG) showed negative heterosis percentage for body weight at 4 weeks of age (-7.4%), the percentages at hatch and 8 weeks of age were 0.3 and 2.9, respectively, while the reciprocal cross (GXB) have positive heterosis percentages, their averages were 0.5 at hatch and 13.1% at 8 weeks of age. These results are in agreement with *Farghaly et al. (2002)* and *Nawar et al., (2004)*. Results of this study indicted that using Gimmizah as males improved body weight of their progeny during the marketing age (8 wks). The findings in this study agreed with those cited by *Shebl, et al., (1990) and Mandour et al. (1992)* where birds of crosses surpassed pure ones at different ages. The genetic similarity as BS values ranged from 68 to 91%.

Table 2. Means and ± standard errors of body weight (g) at different ages for pure strains and their crosses.

Genotypes	At hatch	4 wks	8 wks
Pure strain:			
Bandara (B)	36.9±3.5	209.6±40.6[b]	413.5±102.7[b]
Gimmizah (G)	36.6±2.8	246.5±42.9[a]	508.9±97.8[a]
Crosses:			
(B x G)	36.9±3.3	211.2±46.1[b]	474.5±92.3[a]
(GxB)	37.0±3.2	234.0±47.5[a]	521.4±48.9[a]

Means within each column having different letters are differed significantly ($p < 0.05$).

Table 3. Average degree of heterosis percentages (ADH %) for body weight at different ages in the first generation.

Crosses	(ADH %)		
	At hatch	4 wks	8 wks
(BXG)	0.3	-7.4	2.9
(GXB)	0.5	2.6	13.1

On the other hand, DNA samples of the two parents and the two hybrids produced a RAPD profile. A series of several DNA fragments of the five samples were amplified with all primers. The amplified products were found to be reproducible when reactions were repeated using the same reaction conditions. Data in Figure 1 and Table 4 showed the genetic similarity estimated as band sharing (BS) for all primers used. The genetic similarity (%) ranged from 68 to 91%. RAPD analysis has been used for constructing parsimony tree among the two hybrids and their parents (Figure 2). Closer proximity of both Bandara and Gimmizah to the progeny of Bandara x Gimmizah crosses, while the others were the most difference *(Zhang et al., 2004; Nowzari et al., 2005)*. This method has been used for constructing trees in other organisms such as farm animals: buffalo, cattle, goat and sheep *(Appa Rao et al., 1996; Ali et al., 2003)*.

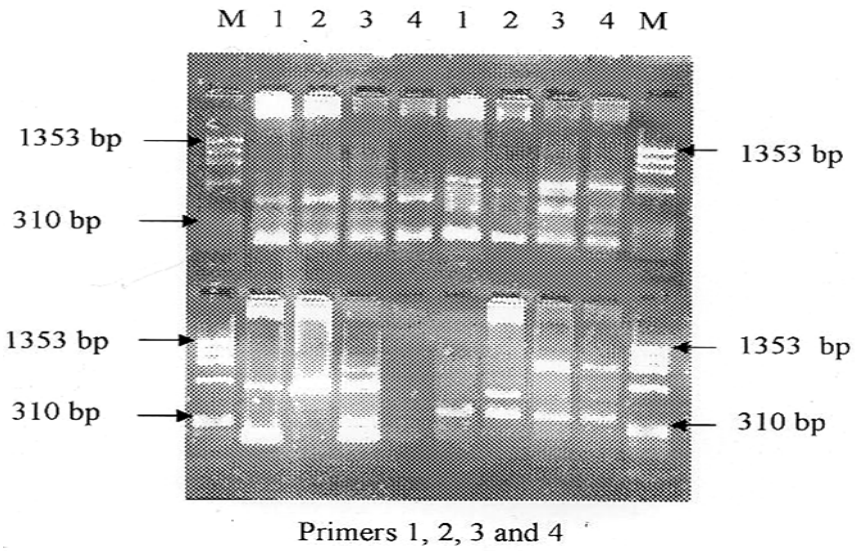

Figure 1. The RAPD amplification products generated by different random primers (1-60: Lane M: DNA marker Lane 1: Bandara, Lane 3 Gimmizah, Lane 3: Bandara X Gimmizah, Lane 4: Gimmizah X Bandara.

Table 4. Genetic similarities estimated as band sharing (BS) for each primer between local chicken hybrids and their parents based on RAPD data.

No. of primer	Comparisons					
	1*x2	1x3	1x4	2x3	2x4	3x4
1	1.00	1.00	1.00	1.00	1.00	1.00
2	0.90	0.70	0.40	0.80	0.50	0.80
3	0.70	0.70	0.70	0.40	0.00	0.40
4	0.40	0.80	0.50	0.30	0.40	0.80
5	0.00	1.00	1.00	0.00	0.00	1.00
6	0.80	1.00	0.40	0.80	0.40	0.40
7	0.00	1.00	0.00	0.00	1.00	0.00
8	1.00	1.00	0.70	1.00	0.70	0.70
9	0.70	1.00	0.70	0.70	1.00	0.70
Mean	0.79	0.91	0.68	0.71	0.71	0.73

*1: Bandara, 2: Gimmizah, 3: Bandara x Gimmizah 4: Gimmizah x Bandara

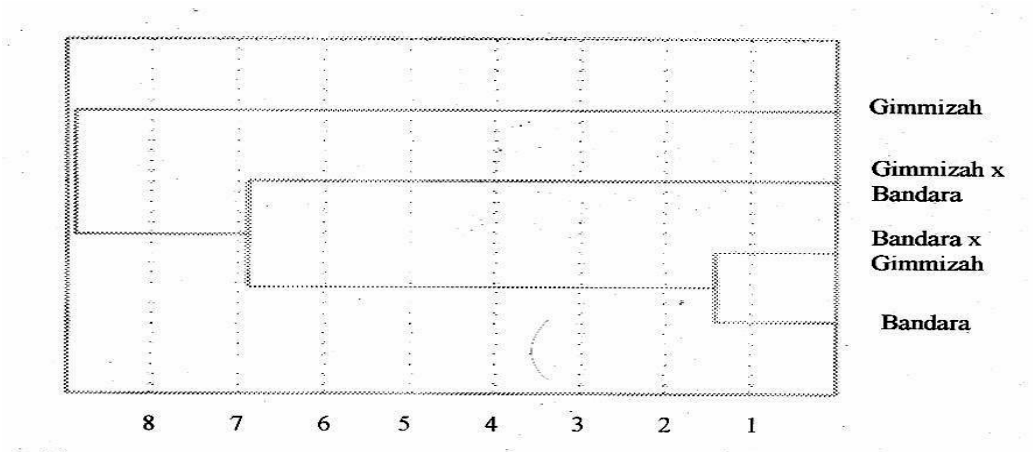

Figure 2. Dendrogram using Average Linkage (Between Groups) based on RAPD data analysis among of the parents chicken and their hybrids.

Our results demonstrated the usefulness of RAPD approach for detecting genetic similarity and/or polymorphisms among local chicken hybrids and their parents. The majority of arbitrary primers used gave distinctly reproducible patterns in all the breeds studies. Thus, the RAPD profile generated for each parents chicken and hybrids can be effectively used as a supporting marker for taxonomic identification, as taxonomic relationship of strains. In taxonomic and molecular systematic, strains relationship markers could be a tool for strains verification and in establishing the status of organisms systematic and its evolution *(Appa Rao et al., 1996)*. The obtained results are in agreement with the findings of several previous reports *(Baradakci and Skibinski 1994, Sharma et al., 2001, Ali*

and Ahmed 2001, Ali et al., 2002, Zhang et al., 2002, Ali et al., 2003). This result supporting such association between DNA fingerprinting similarity and heterosis which has been published before in chickens *(Haberfeld et al., 1996, Gavora et al., 1996)*.

Conclusion

For improvement of meat production in Egyptian stains of chicken, molecular (RAPD-PCR) and phenotypic analysis were used. Where, DNA was extracted from blood of developed local stains of chicken: Bandara, Gimmizah, and their crossing (Bandara x Gimmizah and Gimmizah x Bandara). However, RAPD-PCR technique was applied to detect genetic similarity as a band sharing (BS) between chicken hybrids and their parents using nine short random oligonucleiotides primers. The results showed that the genetic similarity as BS-values was calculated and ranged from 68 to 91%. Where, Bandara male with Gimmizah female appeared positive heterosis at 8 weeks of age. However, evidence from this study indicated that crossing of the developed local hens is capable of rapidly improving the body weight of their progeny during the marketing age. Consequently, this study reflected the ability of RAPD-PCR technique to establish the association between genetic similarity as a band sharing and heterosis (hybrid vigor).

Molekularno-fenotipska analiza efikasnosti ukrštanja na proizvodnju mesa lokalnih linija pilića

O. M. Aly, M. M. Ahmed, S. M. Abdel-Rahman

Rezime

DNK je ekstrahovana iz uzoraka krvi lokalnih linija pilića: Bandara, Gimmizah, i njihovih meleza (Bandara x Gimmizah i Gimmizah x Bandara). RAPD-PCR tehnika je korišćena za otkrivanje genetskih sličnosti, deljenih traka (band sharing - BS) među hibridima-melezima i njihovim roditeljima korišćenjem prajmera devet kratkih oligonukleiotida. Genetska sličnost izražena kao BS vrednosti je izračunata u granicama od 68 do 91%. Bandara muški pilići sa kokicama Gimmizah su pokazali pozitivni heterozis u uzrastu of 8 nedelja. Međutim, dokazi dobijeni u ovom istraživanju ukazuju da se ukrštanjem lokalnih linija može veoma brzo poboljšati telesna masa potomstva tokom komercijalnog uzrasta. Ovo istraživanje održava sposobnost RAPD-PCR za utvrđivanje povezanosti između genetske sličnosti ispoljenu kroz BS vrednosti i heterozis (hibridni vigor).

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