Evaluation of genetic variability in a breeder flock of native chicken based on randomly amplified polymorphic DNA markers

Godrat Rahimi[1]*, Alireza Khanahmadi[1], Ardeshir Nejati-Javaremi[2], Saeid Smailkhanian[3]

[1]Laboratory for Molecular Genetics and Animal Biotechnology, Department of Animal Science, Sari College of Agricultural Science, Mazandaran University, Sari, I.R. Iran [2]Department of Animal Science, College of Agricultural Science, Tehran University, Karaj, I.R. Iran [3]Research Center of Jehad-e-Kesahavarzi, Esfahan, I.R. Iran

Abstract

A study was undertaken to evaluate the genetic variation in the 10th generation of a breeder flock of native breed selected for high egg and meat production in native fowls breeding station, Mazandaran, Iran. Venous blood samples were collected from 100 birds of both sexes. The RAPD-PCR technique was applied to generate a DNA fingerprint of individuals. Initially, a total of 20 ten-nucleotide arbitrary primers were used but 14 of 20 primers revealed a pattern with scorable amplified bands. From a total number of 140 scored bands 63 (45%) and 77 (55%) were described as polymorphic and monophormic respectively. The average number of bands per primer varied from 4 to 16 and with sizes varying from 200 to 2100 bp in length. The estimated band sharing frequency varied from 0.79 to 0.96 between individuals. The average genetic similarity and genetic variance between individuals within the population were 0.89 and 0.11, respectively. The existence of high levels of polymorphism after 10th generation of selection may indicate the accuracy of used the selection program and also the large enough effective population size in this breeding flock. It could be concluded that RAPD markers are effective in detecting genetic similarities and genetic variances among individuals in poultry breeder flocks.

Keywords: *RAPD markers; Genetic variability; Native fowl*

INTRODUCTION

Development of molecular biological techniques has created new possibilities for selection strategies and genetic improvement of livestock. Discovery of the polymerase chain reaction (PCR) had a major impact on the research of eukaryotic genome and contributed to the development and application of various DNA markers. Many methods have been developed over the past two decades that allow detection of polymorphism at the DNA level. The randomly amplified polymorphic DNA (RAPD) technique, described first by Welsh and McClelland (1990) and Williams *et al*. (1990), is a quick and effective method that can be applied to generate genotype specific banding patterns. Polymorphism of RAPD fragments is detected as a band's presence or absence and may result from deletion, insertion or differences in the nucleotide sequences in or between priming regions (Clark and Lanigan, 1993). RAPD is a simple, fast and comparatively low cost assay that uses short oligonucleotide primers of arbitrary sequences to amplify anonymous fragments of genomic DNA (Stepniak *et al*., 2002), and no prior knowledge of the genome under investigation is necessary to perform the assay (Bowditch *et al*., 1993). Due to those features, the RAPD analysis has found many uses in different fields of study in both plants and animals. The RAPD analysis has been used extensively for genome mapping in chickens (Levin *et al*., 1993, 1994; Cheng *et al*., 1995), genetic diversity and parentage analysis in chickens and turkeys (Smith *et al*., 1996), genetic characterization of highly inbred chicken lines (Potsky *et al*., 1995), identification of specific markers (Pyreddy 1993; Zhang *et al*., 1995) and estimation of genetic diversity in inter and intra populations of poultry breeds (Sharma *et al*., 2001).

Correspondence to:* **Godrat Rahimi, Ph.D.
Tel: +98 151 3822571; Fax: + 98 151 3822577
E-mail: rahimimianji@yahoo.com

The RAPD technique was applied to detect genetic similarity between five local chicken strains that have been selected for eggs and meat production. Based on six primers used in this study, the genetic similarity between the chicken strains selected for egg and meat production varied from 0.78 to 0.87 respectively (Ahmed *et al*., 2003). The technique was also used to estimate the genetic distance among pure lines of meat and egg type chickens (Rahsan and Guldehen, 2002). In this study, the maximum genetic distance of 44% was observed between the meat dam line and layer sire line and the average genetic similarity between the lines varied from 0.64 to 0.85.

Despite large improvements in modern poultry production systems, the back-yard chicken production still has an imperative role in the welfare and nutrition of rural people in Iran as well as many other developing countries. However, information on the genetic characterization of local chicken is scanty and more studies are needed to characterize these populations genetically and to estimate the genetic variability between and within them. Such information could be effectively used to formulate appropriate selection plans. The objective of the present study was to evaluate the genetic variability in a breeder flock of native chicken kept in the Native Fowls Breeding Station, Mazandaran, Iran, based on RAPD-DNA markers.

MATERIALS AND METHODS

DNA Isolation: Venous blood samples were collected from 100 birds of both sexes into 3 ml tubes containing 5 mM EDTA as anticoagulant agent. Genomic DNA was isolated from 1 ml blood aliquots by a standard procedure using phenol/chloroform extraction and ethanol precipitation method (Miller *et al*., 1988). The quantity as well as the quality of the extracted DNA were determined by a spectrophotometric method based on absorbance at 260 and 280 nm respectively.

RAPD-PCR Condition and Electrophoresis: The 20 ten-nucleotide RAPD primers of arbitrary sequence were tested for their potential use in the amplification of scorable bands (Table 1). PCRs were performed in a final volume of 20 µl containing 15 ng of genomic DNA, 200 µM each of dNTPs, 0.5 µM of each primer, 2.5 mM of $MgCl_2$, 1 unit of *Taq* DNA polymerase, and 1X PCR reaction buffer. Negative control (lacking DNA) was set up for each reaction mastermix to check for DNA contamination. Amplification was carried out in a thermocycler (Biometra), with an initial denaturation step at 94°C for 2 min followed by 45 cycles of 1 min at 94°C, 1 min at 43°C, 1 min at 72°C and a final extension step at 72°C for 5 min. The PCR products were run on 1.5% agarose gel and visualized by ethidium bromide staining.

Table 1. Primer sequences used for the amplification of the RAPD loci.

Primer	Sequences (5′-3′)	Primer	Sequences (5′-3′)
1	TCA CGA TGC A	11	AAC GCG TCG A
2	TCT CGA TGA A	12	TTC GAG CCA G
3	CGG CCC CTG T	13	GAA CGG ACT C
4	TGG TCA CTG T	14	GTG AGG CGT C
5	GGA CTG GAG T	15	GTT GCC AGC C
6	TGG ACC GGT G	16	AAA GCT GCG G
7	GGA CCC AAC C	17	TGA GTG GGT G
8	GGG CTA GGG T	18	TTC CCA GGA T
9	GAA ACG GGT G	19	AAG CCT CGT C
10	GAC CGC TTG T	20	CGC GGC CAT A

Statistical Analysis: The RAPD profiles of 100 individuals were characterized as matrices of zeros and ones by scoring bands on agarose gel as their presence (“1”) or absence (“0”). Only the bands that met the criteria of clarity were scored. The band sharing frequency (BSF) was used to estimate the genetic similarity for each primer (Lynch, 1990). The BSF between chickens of x and y was calculated as:

$$BSF_{xy} = \frac{2N_{xy}}{N_x + N_y}$$

Where N_{xy} is the number of common fragments observed in individuals x and y. N_x and N_y are the total number of fragments scored in x and y, respectively. The within population genetic similarity (*WGS*) was computed as an average of BSF_{xy} across all comparisons between individuals. Within population genetic variance (σ^2G) and uniformity of RAPD fingerprinting pattern (*U*) were determined according to the equations:

$$\sigma_G^2 = 1 - WGS \quad , \quad U = \frac{1}{N}\sum_{i=1}^{n} V_i$$

Where Vi is the frequency of the i^{th} band and *N* is the number of bands scored within a population (Lynch and Milligan, 1994).

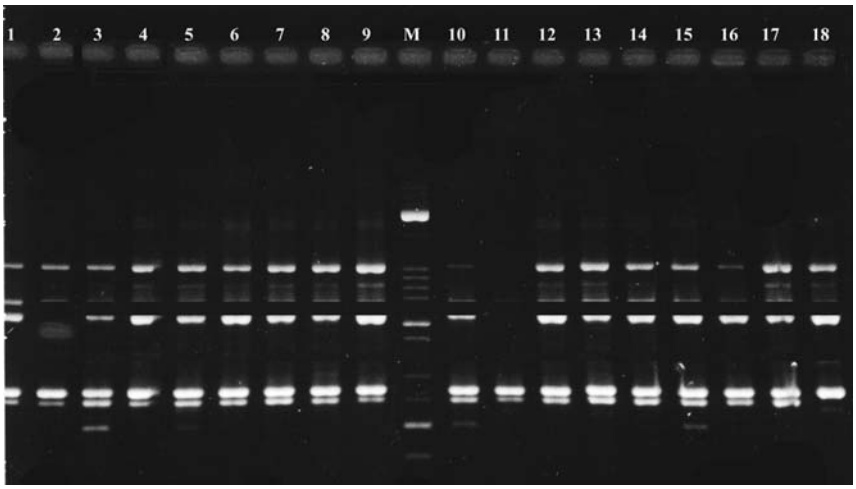

Figure 1. Amplification products generated by primer RAPD-12. Lane M: DNA marker, Lanes 1-18: specific RAPD fragments from different individuals.

RESULTS

In the present study, as a result of an initial RAPD analysis on genomic DNA of chicken, 14 out of the 20 primers were chosen for further analysis, on the basis of the presence of diagnostic bands (Fig. 1).

Each of these primers amplified on average 4 to 16 bands of sizes varying from 200 to 2100 bp. A total of 140 diagnostic bands were scored within RAPD profiles amplified by these 14 primers. The number of polymorphic and monophormic bands amplified per primers are presented in Table 2.

Among 140 scorable bands 63 (45%) were recognized as polymorphic and 77 (55%) as monomorphic bands (Table 2). The average number of polymorphic bands per primer varied from 0 to 9. The highest and the lowest number of polymorhic bands were recognized for primer 9 (72.7%) and 14 as well as 15 (16.7%), respectively (Table 2). The average uniformity index for RAPD fingerprinting pattern of each primer is calculated and its ranged from 0.63 to 0.93 (Table 3).

DISCUSSION

The analysis of genetic diversity and relatedness between or within species, populations and individuals

Table 2. Summary of the results of RAPD analysis with 14 arbitrary primers: total number of detected bands (*TDB*), number of polymorphic bands (*NPB*) and percentage of polymorphic bands (*PB%*).

Primer	*TDB*	*NPB*	*PB%*	Primer	*TDB*	*NPB*	*PB%*
3	16	8	50	11	10	6	60
4	4	0	0	12	9	4	44.4
5	6	2	33.3	14	6	1	16.7
6	11	5	45.4	15	12	2	16.7
8	10	4	40	16	15	9	60
9	11	8	72.7	17	7	2	28.6
10	6	2	33.3	20	11	5	45.5

Table 3. Band sharing frequency (*BSF*) and Uniformity Index (*UI*) of RAPD fingerprinting pattern for 14 arbitrary primers.

Primer	*UI*	*BSF*	Primer	*UI*	*BSF*
3	0.84	0.87	11	0.63	0.79
4	1	1	12	0.82	0.89
5	0.93	0.90	14	0.86	0.94
6	0.69	0.87	15	0.96	0.96
8	0.89	0.93	16	0.84	0.89
9	0.81	0.86	17	0.82	0.92
10	0.79	0.90	20	0.88	0.90

is a prerequisite towards effective utilization and protection of animal genetic resources. With DNA being the only basis of genetic differences between distinct organisms, DNA fingerprinting presently is the ultimate method of biological individualization. Rahsan and Guldehen (2002) observed an average number of 9.2 polymorphic bands per primer using RAPD-DNA fingerprinting between meat and layer pure line of chickens. In this study we found on average number of 4.46 polymorphic bands per primer and the BSF estimated per primer ranged from 79% to 96%. Smith *et al*. (1996) observed similar findings for BSF in four different populations of poultry breeds. The BSF reported by this group in two-turkey populations varied from 77% to 97%. In our study the genetic similarity and genetic variance within the population concerned were estimated as 89 and 11%, respectively. In a similar study involving native egg and meat type strains (Ahmed *et al*., 2003), the genetic similarity within egg and meat type chickens were 0.79 and 0.89, receptively. The estimated polymorhic bands (45%) in the present study was higher than that reported by Singh and Sharma (2002) for the White Leghorne population (21.9%).

Based on the results obtained, the existence of high levels of polymorphism after 10 generations of selection may indicate the accuracy of the used selection program and also the large enough effective population size in this breeding flock. Therefore, there is enough genetic variation left to generate further progress in the years ahead. Additionally, the use of RAPD markers represents a useful and efficient method and thus provides a potential tool for detection of genetic variability among individuals in poultry breeder flocks.

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