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PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Evaluation of Genetic Variability and Distances among Five Iranian Native Chicken Populations using RAPD Markers

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Abstract: Genetic variability was studied on five Iranian native chicken populations using Random Amplified Polymorphism DNA (RAPD) markers. The purpose of this study was for the analysis of variation within and between Iranian native chicken populations and for the reconstruction of a phylogenetic tree for these populations using the RAPD marker assay. The populations surveyed were from five provinces including Mazandaran (MZD), Isfahan (ISF), Yazd (YZD), Fars (FRS) and West Azerbaijan (WAZ). On the base of results of this study, the FRS and MZD populations had the highest genetic distance (0.182) and the FRS and ISF populations the lowest one (0.066). The YZD and MZD populations had the highest (0.208) and lowest (0.156) within-population genetic diversity. The phylogenetic tree was reconstructed on UPGMA method and showed two main separated groups. The ISF and FRS populations were first clustered into one group and, then, were clustered into a larger group with YZD and WAZ. Another consists MZD population was clustered separately from this group. This study showed that RAPD technique is an useful tool for evaluation of genetic variation among domesticated animals.

Key words: Genetic distance, genetic variability, Iranian native chickens

INTRODUCTION

Native livestock and poultry breeds are a national capital in developing countries and their conservation and utilization are very important for economy of these countries. Iran is characterized by variable climate conditions because of the country's geographic location and extent. Iranian native poultry is very diverse, resulting from the long-term adaptation to local environments through natural selection and plays very important role in economic sustainability of both small family-based farmers and large-scale production of meat and egg in Iran (Shahbazi *et al.*, 2007).

Recent developments in DNA polymorphism analysis suggest that molecular genetic variability is a more powerful criterion for examining genomic variation among individuals, families, populations and various taxonomic units. Advantages of analyzing genetic polymorphisms at

the DNA level are manifold (Cavalli-Sforza, 1998). The DNA sequences contain more information than protein sequences do and provide much greater genetic polymorphisms. Automation has simplified DNA analysis, which requires many markers to study population variation.

Random Amplified Polymorphic DNA (RAPD) approach that do not require previous DNA sequence information has been widely used to study genetic variability in populations including poultry (Salem *et al.*, 2005). The RAPD technique is a simple and easy method to detect polymorphisms based on the amplification of random DNA segments with single primer of arbitrary nucleotide sequence (Williams *et al.*, 1990; Welsh and McClelland, 1990). This technique is also faster and less expensive than other kinds of DNA fragments analysis and can be used for very small quantity of DNA. It is suitable for work on anonymous genomes as compared

to other types of DNA fragment analysis (RFLP and microsatellite) and can detect polymorphisms in any kind of sequences (Perez *et al.*, 1998). This method also samples the genome more randomly than conventional methods such as allozyme and RFLP (Lynch and Miligan, 1994). However, the RAPD method has certain limitations (Romanov and Weigend, 2001; Salem *et al.*, 2005).

Recently, comparative analysis of allozyme, RAPD and microsatellite polymorphism in Chinese chickens, including five native populations, two fast-growing broiler lines and one layer line, showed that the genetic distances among all populations measured by three methods were different (Zhang *et al.*, 2002a). Thereby, genetic distances from RAPD showed a closer relationship between Chinese native chickens and broiler and layer chickens. Using the same three marker systems, genetic diversity of Chinese native chicken breeds was investigated in comparison with imported broiler and layer breeds (Zhang *et al.*, 2002b). On the contrary, the data obtained from the RAPD analysis indicated that gene diversity within a population was large in Chinese native chickens, intermediate in broilers and low in layers and that there were small differences between Chinese native chickens and both broilers and layers. In another study, the genetic lineage of four ubiquitous port-city (Haimen) chicken populations in China has been explored using RAPD and microsatellite markers (Olowofeso, 2005). The results suggested that these chickens are likely to have originated from the same source.

A detailed genetic study of native chicken populations in these and other studies is a prerequisite for integrating native genetic resources into the poultry sector. Genetic variation of local populations, compared with other chicken populations in a country, needs further investigation in order to identify populations of particular merit. Phylogenetic relationship of native chicken populations requires a better understanding, as they may also be a good source of research material now and in the near future.

MATERIALS AND METHODS

Sampling and DNA extraction: Genetic variability was studied for five chicken populations including Mazandaran (MZD), Isfahan (ISF), Yazd (YZD), Fars (FRS) and West Azerbaijan (WAZ). This research project were done in Iran during 2007. The detailed population description is given elsewhere (Shahbazi *et al.*, 2007) and briefly summarized in Table 1. Thirty individuals were sampled randomly from each of populations. The DNA was extracted by optimization of salting out method (Miller *et al.*, 1988).

RAPD-PCR assay: Twenty three primers were tested in the preliminary RAPD analysis and the following 10 primers were eventually used for the assay: OPU-13, OPU-14, OPN-16, OPX-3, OPX-13, OPX-20, OPL-20, OPT-17, OPT-20 and OPG-18.

Amplification reactions were carried out in a final volume of 25 µL containing 10-20 ng of template DNA, 0.6 µM of each primer, 4 mM of MgCl₂, 2 mM of dNTPs, 1 U of Taq polymerase and 10X PCR buffer. Samples were covered with mineral oil and amplified using a DNA thermal cycler and the following amplification conditions: denaturation at 94°C for 3 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 34°C for 1 min and extension at 72°C for 3 min and a final elongation step at 72°C for 5 min.

Amplified products were resolved by size separation using electrophoresis on 1.5% agarose gel containing 0.5 µg mL⁻¹ ethidium bromide. Band patterns were photographed under UV light.

Statistical analysis: Following the statistical analysis procedures described by Bártfai *et al.* (2003), RAPD patterns were visually analyzed and scored from photographs. To analyse and compare the RAPD profiles, we only selected distinct, well-separated bands and

Table 1: Description of the chicken populations used in this study

Population	Source location, province	Distribution	Specific features
MZD	Sari, Mazandaran	Northern Iran (Mazandaran, Golestan and Semnan provinces)	Similar to the Naked Neck breed (NA*NA), with diverse plumage colors, red face and light (ID*ID) or dark (ID*N) feet and shanks
ISF	Isfahan, Isfahan	Central Iran (Chahar Mahall and Bakhtiari and Isfahan provinces)	Black feather color is (MC1R*E), red face, single comb (R*N) and white or brown eggshell color
YZD	Yazd, Yazd	Central Iran (Yazd province)	Tassel (CR*CR), muffs and beard (MB*MB) and diverse plumage colors
FRS	Shiraz, Fars	Southern Iran (Fars province)	Varying plumage colors, single comb (R*N) and light feet and shanks (ID*ID)
WAZ	Orumieh, West Azerbaijan	Northwestern Iran (West and East Azerbaijan provinces)	Various plumage colors, mostly red and brown (the gold gene, S*N), single comb (R*N), light feet and shanks (ID*ID)

The specific gene symbols are given in parentheses

determined the genotypes by recording the presence (1) or absence (0) of these bands. Weak and unresolved groups of bands were neglected.

For the analysis of the RAPD data, we assumed that: (1) markers from different loci did not migrate to the same position on the gel, (2) each band represented the dominant genotype at the locus, while lack of the same band in another individual corresponded to the alternative homozygous recessive genotype in the Hardy-Weinberg equilibrium. 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:

$$BSF_{x,y} = 2N_{x,y} / (N_x + N_y)$$

where, $N_{x,y}$ 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 $BSF_{x,y}$ across all comparisons between individuals. Within population genetic variance (σ^2G) and index of uniformity per band (U) were determined according to the equations:

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

where, V_i is the frequency of the i^{th} band and N is number of bands scored within a population (Lynch and Milligan, 1994; Bártfai *et al.*, 2003).

The dendrograms from the RAPD band frequencies were constructed by means of the Unweighted Pair-Group Method using Arithmetic Average (UPGMA), Nei's (1978) unbiased measures of genetic identity and genetic distance and the POPGENE software version 1.31 software (Yeh *et al.*, 1997).

RESULTS

Ten of twenty three random primers screened yielded distinct polymorphic RAPD profiles in five populations, with a total of 130 fragments amplified and 86 bands showed polymorphism. Figure 1 exemplifies polymorphic band patterns generated by three primers in three populations.

Numbers of amplified fragments varied from four (OPX-20) to 19 (OPN-16 and OPG-18). The greatest number of polymorphic bands detected was 16, which were produced by the primer OPN-16. The primers OPX-20 and OPU-14 produced three bands, being the least

Table 2: Indices of uniformity (per RAPD primer and average) and within-population gene diversity in the Iranian native chicken populations

RAPD primers	Populations				
	MZD	FRS	ISF	YZD	WAZ
OPG-18	0.863	0.905	0.914	0.828	0.891
OPU-13	0.925	0.914	0.929	0.866	0.935
OPN-16	0.814	0.733	0.705	0.670	0.765
OPU-14	0.644	0.655	0.683	0.644	0.706
OPX-03	0.750	0.764	0.712	0.814	0.762
OPL-20	0.921	0.857	0.916	0.878	0.847
OPT-17	0.717	0.743	0.670	0.560	0.487
OPX-13	0.978	0.911	0.813	0.885	0.867
OPX-20	0.941	0.958	0.991	0.984	0.933
OPT-20	0.891	0.936	0.842	0.791	0.915
Average index of uniformity	0.844	0.840	0.820	0.792	0.811
Within-population gene diversity	0.156	0.16	0.18	0.208	0.189

Table 3: Nei's (1978) unbiased measures of genetic distances between the Iranian populations

Populations	MZD	ISF	FRS	YZD	WAZ
MZD	****				
ISF	0.133	****			
FRS	0.1819	0.066	****		
YZD	0.1598	0.1189	0.1366	****	
WAZ	0.1154	0.0781	0.1017	0.09	****

number of polymorphic bands generated by the informative markers. OPX-13 and OPU-13 showed the greatest and lowest percentage of polymorphism across all populations, with 100 and 46.1% polymorphic bands, respectively. Length of the fragments amplified with individual primers varied from 237 (OPT-17) to 3240 (OPN-16) bp. Based on the results of RAPD analysis, five primers were 50% or more polymorphic and the other five primers had this parameter below 50%.

Altogether, average primer polymorphism in a single population ranged between 46.4% (MZD) and 53.5% (YZD). At the same time, the individual primer polymorphism had a much greater variation, from 11.8% (OPU-13 in MZD) to 90% (OPT-17 in WAZ). The YZD and MZD populations had the greatest (0.208) and lowest (0.156) within-population gene diversity, respectively, whereas the average gene diversity in populations was 0.179 (Table 2).

The Nei's (1978) unbiased genetic distance (D) based on polymorphic bands varied between populations. Using the average band-sharing coefficient in a population and in pairwise populations, respectively, the distances calculated ranged from 0.066 (FRS vs. ISF) to 0.182 (MZD vs. FRS) chickens (Table 3).

The phylogenetic tree was reconstructed on Unweighted Pair-Group Method using Arithmetic Average (UPGMA) and showed two main separated

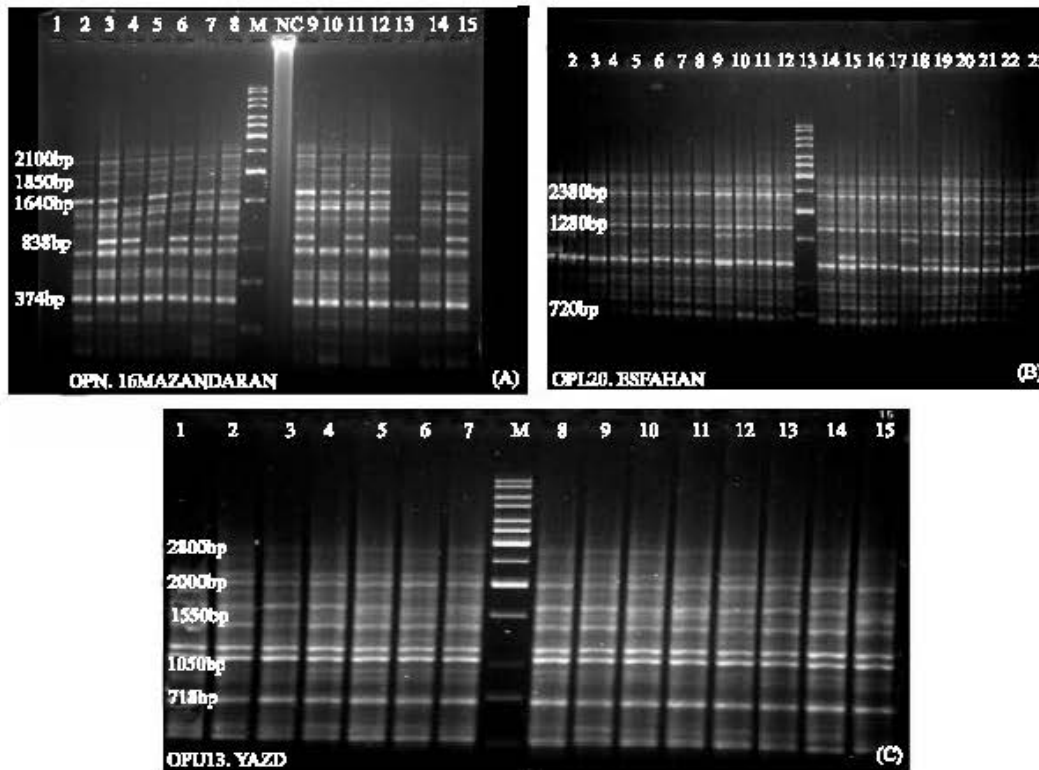


Fig. 1: RAPD band patterns obtained with three selected primers, (A) OPG-18, (B) OPL-20 and (C) OPU-13 (C) in the MZD, ISF and YZD populations, respectively. M: Molecular weight markers (1 Kb ladder)

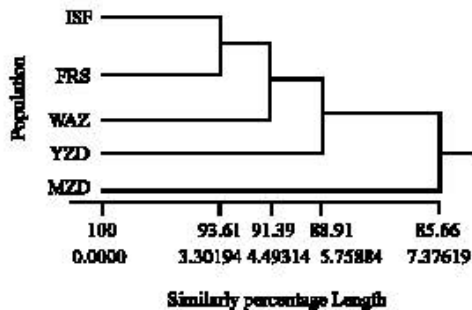


Fig. 2: Dendrogram showing evolutionary relationships among 5 Iranian Native Chicken populations using Unweighted Pair-Group Method using Arithmetic Average (UPGMA) by POPGENE software with 1000 bootstrap replications

groups. The ISF and FRS populations were first clustered into one group and, then, were clustered into a larger group with YZD and WAZ. Another consists MZD population was clustered separately from this group (Fig. 2). The RAPD technique is an efficiency method for studies of genetic similarity between Iranian Native Chicken populations.

DISCUSSION

Genetic variations and phylogenetic relationships between various breeds and stocks are now been examined using type II markers including RAPD markers (Emara and Kim, 2003; Bártfai *et al.*, 2003). These markers have been used to evaluate genetic variation and relatedness within poultry species (Flotsky *et al.*, 1995; Smith *et al.*, 1996; Semenova *et al.*, 1996; Romanov and Weigend, 2001; Sharma *et al.*, 2001; Ali and Ahmed, 2001; Semenova *et al.*, 2002; Zhang *et al.*, 2002a,b; Ali *et al.*, 2003).

Although, specific RAPD markers for each population have not been found in this study, significant differences at several loci were observed. For instance, some loci were monomorphic in certain populations, while being polymorphic in the others. Overall, the results of RAPD analysis showed that within-population gene diversity in five local chicken populations of Iran was very low (16-21%). This was a contrast with the gene diversity estimates previously obtained for the same populations using microsatellite markers (62-79%, Shahbazi *et al.*, 2007), but seems reasonable because

microsatellites have much higher polymorphism and are considered to be more appropriate molecular tools for studying genetic biodiversity and relationships. Similar results were reported by Zhang *et al.* (2002b), when analyzing gene diversity in Chinese chicken populations using RAPD (26%) vs. microsatellite markers (76%).

On the basis of RAPD analysis, the Fars and Mazandaran populations, which are separated by a long distance and natural obstacles including the Alborz and Zagros Mountains, had the highest genetic distance (0.182) as compared to other populations. The populations of two adjacent and climatically similar Fars and Isfahan provinces have the least genetic distance (0.066). Again, these data contradicted with the earlier microsatellite-based study by Shahbazi *et al.* (2007) that demonstrated a different pattern of phylogenetic relationships between these populations and greater genetic distance estimates (0.097-0.696). Yet, our results were consistent with differences in RAPD-vs. microsatellite-based genetic distances and relationships among Chinese local chicken strains (Xi-Uan *et al.*, 1998; Zhang *et al.*, 2002b).

CONCLUSIONS

In abstract, use of the RAPD technique is simple and straightforward in a preliminary assessment of within and between population genetic diversity in local poultry breeds. However, one should be cautious in interpreting data resulted from the RAPD analysis since RAPD-derived phylogenies may contradict phylogenetic relationships obtained with microsatellites. Using a phylogenetic tree plotted with the UPGMA method, the chicken populations were divided into two groups. Group 1 included the ISF, YZD, FRS and WAZ populations and Group 2 the MZD only.

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