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Analysis of Genetic Diversity in Bangladeshi Chicken using RAPD Markers

[1]M.B.R. Mollah, [1]F.B. Islam, [2]M.S. Islam, [1]M.A. Ali and [3]M.S. Alam
[1]Department of Poultry Science,
[2]Department of Biotechnology,
[3]Department of Fisheries Biology and Genetics,
Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract: Understanding the genetic diversity at molecular level is a prerequisite in developing strategies for effective conservation and utilization of chicken genetic resources. We studied the genetic variation within and between Bangladeshi native (Naked Neck, Frizzle and Non-descriptive indigenous) and exotic (White Leghorn, Rhode Island Red, Commercial layer and broiler) chicken populations by Random Amplified Polymorphic DNA (RAPD). Four out of the 20 random primers exhibited sufficient variability for studied populations. The four primers yielded a total of 39 distinct bands, 25 of which were polymorphic. Estimation of polymorphic loci, intra-population similarity indices and Nei's gene diversity suggested that genetic diversities within a population were high in non-descriptive, Frizzle, Naked Neck, Rhode Island Red and White Leghorn chicken populations compared to the commercial layer and broiler populations. The coefficient of gene differentiation (G_{ST} = 0.34) and gene flow (N_m = 0.98) values reflected a high level of population differences. UPGMA dendrogram segregated the chicken populations in various degree based on their genetic distance. The overall genetic distance among native chicken was relatively low comparison to the exotic populations. The results of present study might have significant impact on the breeding and conservation of native chicken genetic resources in Bangladesh.

Key words: Genetic diversity, native chicken, RAPD markers

INTRODUCTION

Bangladesh has a very rich genetic diversity of native chickens and one of the homelands of the Red Jungle Fowl (*Gallus gallus*), the ancestor of modern domestic chickens. Although, Bangladesh has a rich heritage of native poultry germplasm, recent industrialization of poultry industry is creating threat to indigenous chicken populations because farmers are reluctant to rear low producing native chickens, which strongly supported decisive measures for conserving native genetic resources.

Most Bangladeshi native chickens have a colored appearance, slow growth rate, good taste of meat and low reproductive performance due to broodiness. They have some morphological characters such as naked neck, frizzles, dwarfism etc., which have direct and/or indirect effect on tropical adaptability. The genes that control the naked neck production traits supported better feed efficiency, growth, carcass composition, meat yield and better tolerance to high ambient temperature (Singh *et al*., 2001; Islam and Nishibori, 2009), while The genes governing the egg production trait in frizzles supported the increases of egg production and egg mass and reduces mortality under hot climatic conditions (Garcês *et al*., 2001; Nischal and Sharma, 2002). These two major genes are also believed to confer resistant to diseases. Therefore, the question about the significance of local fowl as genetic pool for future breeding strategies and as a supplier of gene complexes or single gene affecting special traits is still open.

Genetic variability and relatedness among the native and improved breeds/lines of chicken are necessary information required because the genetic variation is considered as the primary biological resource that can be exploited in selective breeding program.

Although, morphological characteristics and production performance variations of some Bangladeshi chickens have been reported (Howlider *et al*., 1995; Islam and Nishibori, 2009), no reports are available based on DNA polymorphism regarding Bangladeshi indigenous chicken. The Random Amplified Polymorphic DNA (RAPD) is a simple and easy method to detect polymorphism based on the amplification of random

Corresponding Author: M.B.R. Mollah, Department of Poultry Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

DNA segments with single primers of arbitrary nucleotide sequence (Williams *et al*., 1990; Welsh and McClelland, 1990; Zhang *et al*., 2002; Mollah *et al*., 2005; Dehghanzadeh *et al*., 2009). This method samples the genome more randomly than conventional methods such as allozyme and RFLP (Lynch and Milligan, 1994; Semenova *et al*., 2002).

Therefore, the present study was performed to characterize the differences among Bangladeshi native chicken and imported exotic breeds/strains using RAPD markers.

MATERIALS AND METHODS

Sample collection: The sample collection and laboratory work of this study was conducted in two phases in between July 2002 and January 2006. The first phase of this experiment was conducted to study the efficiency of RAPD marker for generating polymorphism in different chicken populations (Mollah *et al*., 2005) and the second phase was conducted to analyze genetic diversity among different indigenous and exotic chickens in Bangladesh. For this experiment, five males and five females were randomly sampled from each of the following chicken populations: Naked Neck (NN), Frizzle (FZ), Non-Descriptive indigenous (ND), White Leghorn (WL), Rhode Island Red (RIR) and Commercial Layer (CL) and broiler (CB) strains those were kept in the Department of Poultry Science Farm, Bangladesh Agricultural University, Mymensingh. The characteristics of each population are summarized in Table 1. The laboratory work was performed in the Department of Fisheries Biology and Genetics Laboratory and Central Laboratory of Bangladesh Agricultural University, Mymensingh.

Blood collection and genomic DNA extraction: Blood was collected and prepared for DNA isolation by using the procedure suggested by Hoelzel (1992). Genomic DNA was extracted from blood cell following the phenol and chloroform method. In brief, approximately 10 µL of previously separated blood cells were taken in a microfuge tube containing 450 µL of extraction buffer (100 mM Tris.HCl, pH = 8.0, 10 mM EDTA and 250 mM NaCl and 1% SDS). After adding 25 µL of proteinase K (20 mg mL^{-1}) the mixture was incubated at 37°C overnight for digestion. DNA was purified by successive extraction with phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1), respectively. DNA was precipitated first using 0.6 volume of isopropanol, peletted by centrifugation, then resuspended in TE buffer (10 mM Tris. HCl, 1 mM EDTA, pH = 8.0). DNA was reprecipitated by adding two volumes of ethanol in the presence of 0.3 M sodium acetate and peletted by centrifugation. The pellets were then washed with 70% ethanol, air-dried and finally resuspended in an appropriate volume of TE buffer. DNA quality was checked by electrophoresis in a minigel and quantified using a spectrophotometer (Spectronic Genesys, Thermo Electron Corporation).

Primer selection: Initially, twenty decamer primers of random sequence (Kit A, Operon Technologies, Inc., Alameda, California, USA) were screened on a sub sample of two randomly chosen chickens from each population, to test their suitability for amplifying chicken RAPD that could be accurately scored. Primers were evaluated on the basis of resolution of bands, repeatability of markers and potential to differentiate populations (polymorphism). Finally, four primers (Table 2) exhibiting sufficient variability for population analysis were selected unbiasly for the analysis of the whole sample set of the seven populations.

PCR amplification and electrophoresis: The amplification conditions were based on Williams *et al.* (1990) with some modifications. The PCR reactions were carried out on each DNA sample in a 10 µL reaction mix containing 1 µL of 10x PCR buffer, 2 µL of 10 µM primer, 1 µL of 250 µM dNTPs

Table 1: Description of chicken populations used in this study

Populations	Abbreviation	Source	Features
Naked Neck	NN	Dept. of Poultry Science, Bangladesh Agricultural University, Mymensingh	A medium-sized strain bred as a closed population; has naked neck gene, various plumage patterns and increased adaptability in hot humid environment
Frizzle	FZ	Same as above	A medium-sized strain with frizzled feathers, bred as a closed population; has frizzle gene
Non-descriptive indigenous	ND	A village near Bangladesh Agricultural University, Mymensingh	A stock of primitive, unselected rural backyard fowls, has small body size and various phenotypic characters
White Leghorn	WL	Dept. of Poultry Science, Bangladesh Agricultural University, Mymensingh	Egg type breed, imported around 50 years ago and now kept as closed population
Rhode Island Red	RIR	Government Poultry Farm, Mirpur, Dhaka	Imported dual-purpose breed, kept as random bred population
Commercial Layer	CL	Local hatchery	A commercial brown egg layer
Commercial Broiler	CB	Same as above	A commercial broiler strain

Table 2: Parameters of primers used in RAPD analysis

Primer	Sequences (5' - 3')	(G+C)%	Total scorable bands	Polymorphic bands
OPA12	TCGGCGATAG	60	9	6
OPA16	AGCCAGCGAA	60	9	6
OPA18	AGGTGACCGT	60	10	7
OPA20	GTTGCGATCC	60	11	6
Total			39	25

(Takara, Japan), 1 unit of Taq DNA polymerase (Takara, Japan) and 75 ng of genomic DNA and a suitable amount of sterile deionized water. DNA amplification was performed in an oil-free thermal cycler (Master Cycler Gradient, Eppendorf). Amplification profile consisted of 3 min initial denaturation at 94°C followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 34°C and 2 min extension at 72°C. After the last cycle, a final step of 7 min at 72°C was added to allow complete extension of all amplified fragments.

The amplified product from each sample was separated electrophoretically on 1% agarose gel containing ethidium bromide in 1×TAE buffer at constant voltage of 120 V for 1½ h. Lamda DNA-EcoT 14 I digest and/or 100 bp ladder used as DNA molecular size marker were run alongside the RAPD reactions on each gel. DNA bands were visualized on UV-transilluminator and photographed by a polaroid camera (Gel Cam Polaroid camera, Sigma-Aldrich Corp).

Genetic data analysis: The PCR amplified bands were scored visually by two independent persons on the basis of their presence (1) or absence (0). The scores obtained were then pooled for constructing a single data matrix, which was used for estimating the proportion of polymorphic loci, Nei's (1973) gene diversity (h), gene flow (N_m), coefficient of gene differentiation (G_{ST}), Nei's (1978) unbiased genetic distance (D). Significant test and construction of a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations with 1000 simulated samples were carried out by using POPGENE (version 1.31) (Yeh *et al*., 1999) computer program. Band sharing based intra-population similarity indices (S_i) were calculated for all possible comparisons according to the following formula:

$$\text{Similarity index (Si)} = 2N_{AB}/(N_A + N_B)$$

where, N_{AB} is the total number of RAPD band shared by individuals A and B. N_A and N_B are the numbers of fragments scored for each individual, respectively (Lynch, 1990).

RESULTS

Out of the twenty primers four retained for RAPD analysis produced different fragment patterns with varied

Table 3: Total number and percentage of polymorphic loci, gene diversity and intra-population similarity indices (S_i) of seven populations of chicken

Populations	No. of polymorphic loci	Polymorphic loci (%)	Gene diversity	Similarity (%)
NN	19	48.72	0.21	82.25
FZ	18	46.15	0.20	83.39
ND	20	51.28	0.22	79.68
WL	13	33.33	0.12	85.29
RIR	19	48.72	0.16	82.45
CL	12	30.77	0.13	90.03
CB	12	30.77	0.12	86.88
Overall	25	64.10	0.25	-

number of bands. The primers yielded a total of 39 distinct bands (RAPD markers), 25 (64.10%) of which were considered as polymorphic (either occurring in or absent in less than 95% individuals). The characteristics of the fragments generated by these four primers are summarized in Table 2. Primer OPA20 produced more numerous fragments than the other three primers. Examples of varying degree of polymorphism generated with the four primers are shown in Fig. 1a-d.

The number of polymorphic loci, percentage of polymorphic loci, gene diversity and intra-population similarity indices of seven populations of chicken are shown in Table 3. The overall number of polymorphic loci, percentage of polymorphic loci and gene diversity were 25, 64.10% and 0.25, respectively.

The numbers of polymorphic loci were higher in native chicken than exotic ones. Among the native chickens, maximum number polymorphic loci were detected in ND (20) followed by NN (19) and FZ (18). In contrary, the degree of polymorphism was relatively low in CL and BL populations. Similarly, the within population gene diversity was relatively high (0.20-0.22) in native chickens, intermediate in RIR (0.16) and low in CL (0.13), CB (0.12) and WL (0.12) (Table 3). The coefficient of gene differentiation and the gene flow estimates considering all populations across all loci were 0.34 and 0.98, respectively.

The intra-population similarity indices (S_i) were relatively high in the exotic chickens (82.45-90.03%) compared to the native ones (79.76-83.39%). Among the exotic populations, CL showed higher genetic similarity (S_i = 0.900) in comparison to the RIR, CB and WL populations while among the native populations, FZ showed higher within-population genetic similarity (S_i = 0.834) than the ND and NN, respectively. The lowest intra-population similarity was observed in the ND population (S_i = 0.797).

Nei's (1978) unbiased genetic distances between populations are presented in Table 4 and the corresponding UPGMA dendrogram in Fig. 2. The values of pair wise comparisons of genetic distance between

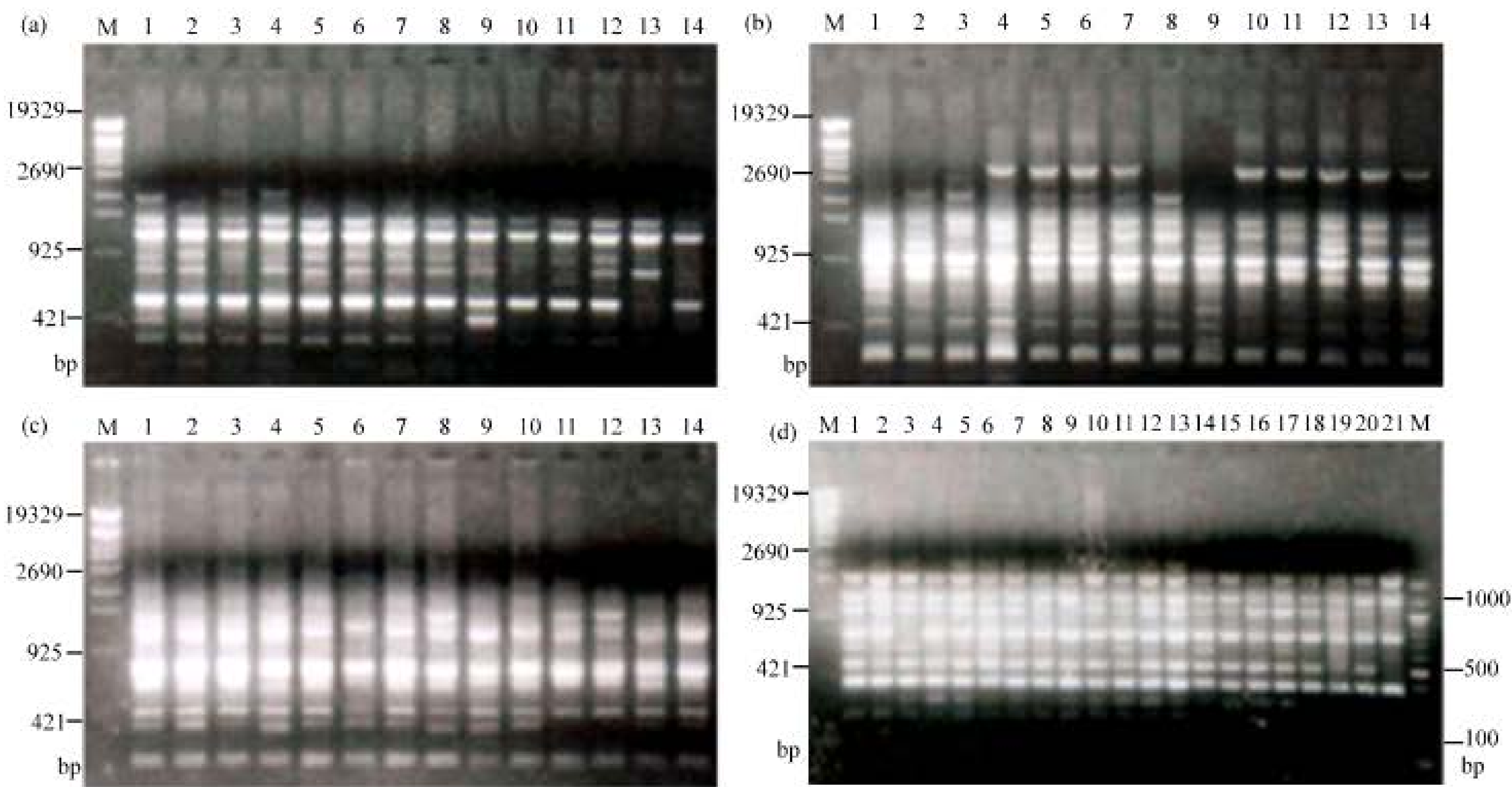

Fig. 1: RAPD banding patterns of different chicken populations using the primers; (a) OPA12, (b) OPA16 and (c) OPA18: Line 1-2 = ISAi757; Line 3-4 = Non-descriptive; Line 5-6 = Shaver 579; Line 7-8 = Fizzle; Line 9-10 = White leghorn; Line 11-12 = Naked neck and Line 13-14 = Rode island red; while primer OPA20 (d): Line 1-3 = ISAi757; Line 4-6 = Non-descriptive; Line 7-9 = Shaver 579; Line 10-12 = Frizzle; Line 13-15 = White Leghorn; Line 16-18 = Naked neck and Line 19-21 = Rhode Island Red. M: Molecular weight marker (Lambda DNA EcoT14 I digest and 100 bp DNA ladder)

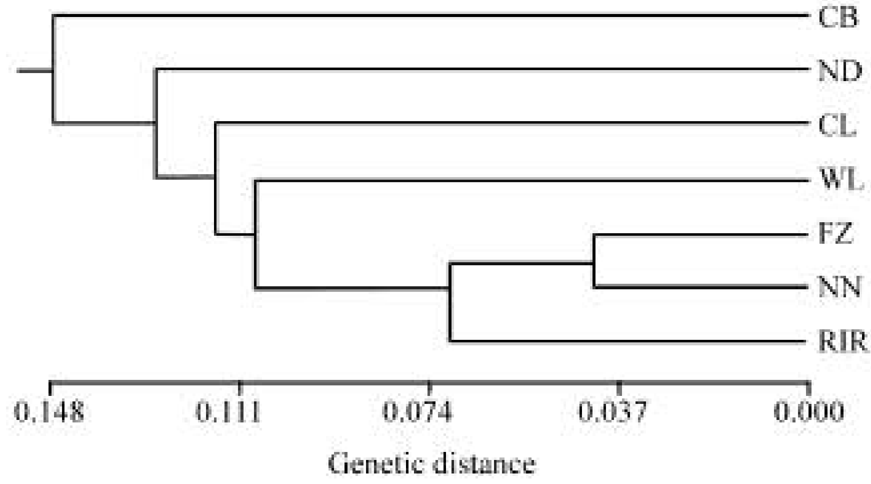

Fig. 2: UPGMA dendrogram based on Nei's (1978) unbiased genetic distance, summarizing the data on differentiation between chicken populations, according to RAPD analysis

Table 4: Nei's (1978) unbiased genetic distance of different population pairs of chicken

Populations	CB	ND	CL	FZ	WL	NN
ND	0.110					
CL	0.134	0.113				
FZ	0.101	0.080	0.108			
WL	0.148	0.110	0.082	0.077		
NN	0.135	0.115	0.097	0.036	0.088	
RIR	0.125	0.121	0.105	0.048	0.108	0.070

populations ranged from 0.036 to 0.148. The overall genetic distance among native chicken was relatively low in comparison to the exotic populations though the ND population showed somewhat higher genetic distance with the rest two native populations. The smallest genetic distance (0.036) was estimated between FZ and NN population pair. Among the exotic populations, CB was separated from WL, CL and RIR populations with relatively high genetic distance (Fig. 2) whereas the genetic distance for WL vs. CL, RIR vs. CL, WL vs. RIR population pairs were marginally low.

DISCUSSION

Better understanding of population genetic structure is crucial to develop a sustainable strategy for conservation and effective utilization of low productive livestock species. In recent years, different marker systems have been developed and applied to a range of livestock species. In this study, we used RAPD marker to study the genetic diversity of Bangladeshi indigenous chicken and/also compared with other exotic breeds. Although no specific markers were found to discriminate the studied chicken populations effectively, the RAPD technique disclosed sufficient polymorphism for population analysis. A total of 25 polymorphic loci detected in this study indicated the effectiveness of RAPD technique to study polymorphism and genetic relatedness among the different chicken populations. The average number of bands obtained by individual primer in

this study was ranging from 9 to 11. Since the amplification from these arbitrary sequenced primers depends upon the presence of annealing site on template DNA, different primers are expected to give different number of amplicons. The results agreed well with the findings by Shivaraman *et al*. (2001), Ahlawat *et al*. (2004) and Mollah *et al*. (2005).

In view of intra-population similarity indices (S_i), the proportion of polymorphic loci and Nei's gene diversity (h), illustrated that the Bangladeshi native chicken populations can be considered as genetically more diversified than the exotic chicken populations. Relatively lower genetic variations observed in CL and BL populations compared to native populations might be due to differences in population structure and selection history. Since the exotic populations have a history of long intense artificial selection for either egg number (For example WL, RIR, CL) or body weight (CB), low amount of genetic variability was expected in these populations. Alternatively, as the native chicken populations are not under artificial selection pressure, each of them is considered as large random mating population that increases the heterozygosity/genetic variability in native chicken populations. Like the present study, Smith *et al*. (1996) and Zhang *et al*. (2002) have discovered higher genetic variability at genomic level in native populations in comparison to the exotic ones.

Pair wise genetic distance and homogeneity test were performed to determine the relatedness among different chicken populations. Lower genetic distance among local native chickens reflected the geographical proximity between them and the results supported the hypothesis that the geographical distance is an important factor influencing the genetic relatedness of populations (Wright, 1943). In a survey, Shivaraman *et al*. (2001) and Dehghanzadeh *et al*. (2009) were also observed least genetic distance among native chickens. The higher genetic distance between the imported CB and CL chickens indicated the remote relationship between them. This might be due to the reason that the exotic broilers and layer chickens were bred for different purposes and had different origin. On the other hand, close relationship among different egg type chicken populations might be due to their same breeding purpose. The lower observed genetic distance between the native and exotic population pairs (FZ vs. RIR, FZ vs. WL, NN vs. RIR) indicating that somewhat close relationship between each pair of them. The close genetic relationship among the above native and exotic population might be due to the efforts made over years by Bangladesh Government to improve the local chicken populations by crossing with exotic RIR and WL chicken. Higher level of population differentiation (G_{ST} = 0.34) and low level of gene flow (N_m = 0.98) across all loci indicated that sufficient genetic differences among different chicken populations are present. However, to discuss the detailed population structures, further studies are required dealing with a large number and extensively sampled native chickens from different parts of the country and more RAPD markers. Our future research based on integrating RAPD and microsatellite marker will provide more details about this matter.

In conclusion, RAPD markers found sufficient nuclear DNA level variations among different chicken populations in Bangladesh. The RAPD data presented here might be a good source of information about the diversity of native chicken in Bangladesh.

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