

Full Length Research Paper

Measurement of genetic parameters within and between breeder flocks of Arian broiler lines using randomly amplified polymorphic DNA (RAPD) markers

Milad Nikkhoo, Hadi Sayyahzadeh, Ghodrat Rahimi-Mianji, Mozhdeh Nikkhoo, Farnaz Faezi, and Minoos Khamesian*

Laboratory for Molecular Genetics and Animal Biotechnology, Department of Animal Science, Faculty of Animal Science and Fisheries, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.

Accepted 29 March, 2010

The present study was carried out in an attempt to detect the genetic variation within and between four populations of commercial broiler lines using RAPD markers. Thirteen out of twenty random markers which were amplified and generating stable and reproducible bands were selected for genotyping of birds in each broiler lines. The average number of diagnostic bands per primer was 8.08 with an average number of 6.96 polymorphic bands across all breed groups. The highest (94%) and the lowest (73.5%) percentage of polymorphic bands were found within sampled birds of Arian C and D lines, respectively. Among the studied broiler lines, the highest genetic uniformity was found in line A, while the greatest within population genetic diversity was found in line C. Estimation of polymorphic loci, Nei's gene diversity and intra-population uniformity indices suggested that genetic diversities within Arian commercial lines is high. The four strains of broiler lines clustered into two main groups using UPGMA procedure. In the first main group, the closest genetic distance was found between A and B strains, which were first clustered together and then with birds of C strain. The second main group includes birds from D strain. The mean coefficient of gene differentiation ($G_{st} = 0.368$) value reflected a high level of population differences. In total, sixty three RAPD bands were found to be strain dependent specific. Therefore, in order to design new diagnostic primers more effective in genetic discrimination among studied lines, unique bands should be cloned and sequenced.

Key words: Genetic variation, broiler lines, polymorphism, random amplified polymorphic DNA markers.

INTRODUCTION

During the last two decades, different classes of molecular markers have become available for evaluation of genetic

diversity within and between different livestock populations. These molecular markers that are revealing polymorphisms at the DNA level are key players in animal genetics and breeding programs. Among these markers, RAPD is most widely used because it allows a rapid and inexpensive assay for assessment of genetic diversity in different species. It has been discussed that although amplified fragments length polymorphism (AFLP) analysis is superior in terms of efficiency, RAPD markers may still be used as reliable markers in small low-tech laboratories (Kjolner et al., 2004). It also has the advantage that no prior knowledge of the genome under research is necessary (Fischer et al., 2000). Due to the technical simplicity and speed of RAPD methodology, it has been successfully used for the generation of genetic similarities and phylogenetic analysis in various organisms (Zahid et al., 2009). A major drawback of RAPD markers in

*Corresponding author. E-mail: rahimimianji@yahoo.com. Tel: +98 151 3822565. Fax: +98 151 382277.

Abbreviations: AFLP, Amplified fragments length polymorphism; BS, band sharing; BF, band frequency; PCR, polymerase chain reaction; GS, genetic similarity; GD, genetic dissimilarity; UPGMA, unweighted pair group method for arithmetic mean; NTB, number of total bands; NPB, number of polymorph bands; NMB, number of monomorph bands; PLM, percentage of polymorph bands; Na, observed number of alleles per locus; Ne, effective number of alleles per locus; h, Nei's genetic diversity; I, Shannon's information index; H_t, total genotype diversity among; H_s, within populations diversity.

population genetic studies of outbreeding organisms is that they are dominant. Thus, allele frequency estimates for such loci are necessarily less accurate than those obtained with codominant markers such as allozymes and RFLPs (Bardakci, 2001). It has been suggested that two to ten times more individuals need to be sampled for dominant markers to achieve the same degree of statistical power as codominant markers such as allozymes and RFLPs (Lynch and Milligan, 1994). RAPD markers have found a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding.

The effectiveness of RAPD molecular markers in detecting polymorphism in different poultry species has been reported by Salem et al. (2005). A total of 60 random primers were used in the RAPD analyses to evaluate genetic polymorphism and relatedness within and among four chicken breeds and two turkey populations (Smith et al., 1996). Seventy percent of primers tested amplified patterns with at least one polymorphic fragment in one or more of the populations. Genetic variability has been detected within and between-strain in White Leghorn population by using 50 RAPD primers, where only 12 primers detected polymorphism between the strains (Singh and Sharma, 2002). Genetic similarity estimates between strains, based on band sharing (BS) as well as on band frequency (BF) ranged from 0.756 to 0.958 and from 0.830 to 0.996, respectively. Genetic diversity of Chinese native chicken breeds was analyzed using RAPD polymorphism. Commercial broiler and layer breeds were also included in the analysis. The data obtained from RAPD 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 (Zhang et al., 2002). Fifty RAPD markers were used to detect polymorphism among five breeds of chicken including White Leghorn, Rhodes Island Red, Red Cornish, White Plymouth Rock and a native breed Kadaknath (Sharma et al., 2001). Twelve of the fifty random primers screened yielded distinct polymorphic RAPD profiles. Of the total 96 fragments amplified, about 25% showed polymorphism. RAPD technique was applied to detect genetic similarity between five local chicken strains that have been selected for eggs and meat production in Egypt. Based on six RAPD primers, the genetic similarity between the egg-producing strains ranged from 72.4 to 85.4%, while the genetic similarity between the two chicken strains selected for meat production was 86.9% (Ali et al., 2003). A total of twenty arbitrary primers were used to evaluate the genetic variation in a breeder flock of native fowls (Rahimi et al., 2005). From a total of 140 scored bands, 45 and 55% were described as polymorphic and monophormic, respectively. The average number of bands per primer varied from 4 to 16 and with the sizes varying from 200 to 2100 bp in length. The average genetic similarity

and genetic variance between individuals within the population were 0.89 and 0.11, respectively. The present study was undertaken to assess genetic diversity among breeder flock of commercial Arian broiler lines with twenty 10-mer arbitrary primers using the polymerase chain reaction (PCR).

MATERIALS AND METHODS

Brief history of experimental population

The farms of Arian broiler strain located in the north of Iran (Babol-Kenar Line Breeding Center, Babol, Iran) was established in 1990 with the objective of genetic improvements in Arian breeding stock. The activity of the company was initiated with a breeder flock containing four lines (A, B, C and D) that has been imported from the Netherlands. Genetic improvement is done by selecting the best cocks and hens as parents of the next generations. Parents of each generation are selected among pedigreed and performance recorded birds produced each generation. The company work constantly to produce genetic improvements in their breeding stock and typically use a system of four-way crossing to produce the grant parents of the birds which are raised as broiler parent stock. They selected and developed A and B lines to produce the male parent line with emphasis on growth performance and body conformation (White Cornish origin), while at the same time developing two C and D lines as female parent line with emphasis on reproductive performance (White Plymouth Rocks origin), which are in turn mated to provide the broiler grandparents stock. The Arian breeding company sells crossbred grandparents stock to the vertically integrated poultry producers, independent hatcheries and others who produce the hatching eggs for parent stock breeding companies.

Sample collection and DNA isolation

To estimate RAPD variations within and between four Arian broiler lines, a total number of 200 individuals were sampled. Venous blood samples were collected from 50 birds of both sexes of each line into 5 ml tubes containing 5 mM ethylenediaminetetraacetic acid (EDTA) as anticoagulant agent. The collected blood samples were transferred to the laboratory using cooling chain and stored at -20°C until used for assay. Genomic DNA was isolated by standard salting-out procedure described by Miller et al. (1988). The quantity and quality of the extracted DNA was determined by spectrophotometer and agarose gel electrophoresis, respectively. DNA samples were adjusted to a concentration of 50 ng/μl and exactly 0.5 μl of the DNA samples were used as template for polymerase chain reaction.

RAPD-PCR condition and electrophoresis

Ethanol-precipitated DNA sample extracted from each individual was used as a template in RAPD procedures. The 20 different decamer oligonucleotides RAPD markers were used for genotyping of birds in this study (Table 1). Genomic DNA was amplified by PCR and each 25-μl reaction tube consisted of DNA (25 ng), primers (10 pmol each), dNTP (200 μM each), 10× buffer (10 mM Tris, 50 mM KCl, 0.1% gelatin, pH. 8.4), MgCl₂ (4 mM) and *Taq* DNA polymerase (1 U) with the following profile: initial denaturation of 5 min at 94°C; 40 cycles of 1 min at 94°C, 1 min at 43°C and 1 min at 72°C with a final elongation of 5 min at 72°C. The PCR products were resolved by electrophoresis through 1.5% agarose gel and visualized by ethidium bromide staining. Band patterns

Table 1. Details of primer sequences for 20 RAPD markers employed for genetic characterization in breeder flocks of Arian broiler lines.

Primer	Nucleotide sequence (5'- 3')	Primer	Nucleotide sequence (5'- 3')
OPA01	TCACGATGCA	OPA11	AACGCGTCGA
OPA02	TCTCGATGAA	OPA12	TTCGAGCCAG
OPA03	CGGCCCTGT	OPA13	GAACGGACTC
OPA04	TGGTCACTGT	OPA14	GTGAGGCGTC
OPA05	GGA CTGGAGT	OPA15	GTTGCCAGCC
OPA06	TGGACCGGTG	OPA16	AAAGCTGCGG
OPA07	GGACCCAACC	OPA17	TGAGTGGGTG
OPA08	GGGCTAGGGT	OPA18	TTCCAGGAT
OPA09	GAAACGGGTG	OPA19	AAGCCTCGTC
OPA10	GACCGCTTGT	OPA20	CGCGGCCATA

were photographed under ultraviolet light. The alleles were scored manually from the photographed gel and the genotypes of each individual bird at the different polymorphic loci were recorded by direct counting.

Statistical analysis

RAPD banding patterns were scored visually from ethidium bromide staining agarose gel. For the analysis and comparison of the patterns, a set of distinct, well-separated bands were selected. The genotypes were analyzed in the form of binary variables by recording the presence (1) or absence (0) of these bands only, neglecting other (weak and unresolved groups of) bands. Each locus can be treated as a two-allele system, with only one of the allele per locus being amplifiable by the PCR. We also assumed that marker alleles from different loci do not comigrate to the same position on a gel and that populations are under the Hardy-Weinberg equilibrium (Lynch and Milligan, 1994). Genetic similarity (GS) between individuals *i* and *j* was estimated according to the formula given by Nei and Li (1979):

$$GS_{ij} = \frac{2N_{ij}}{(N_i + N_j)}$$

Where, N_{ij} is the number of bands common in individuals *i* and *j* and N_i and N_j are the total number of bands in individuals *i* and *j*, respectively, with regard to all assay units. Thus, GS reflects the proportion of bands shared between two individuals and ranges from 0 (no common bands) to 1 (all bands identical). Genetic dissimilarity (GD) was calculated as:

$$GD = 1 - GS$$

Nei's unbiased genetic distance was calculated among all populations with all markers, including monomorphic markers. The Nei's unbiased genetic distance is an accurate estimate of the number of gene differences per locus when populations are small. The following indices were used to quantify the amount of genetic diversity within each population examined: number of total bands (NTB), number of polymorph bands (NPB), number of monomorph bands (NMB), percentage of polymorph bands (PLM), observed number of alleles per locus (N_a), effective number of alleles per locus (N_e), Nei's genetic diversity (*h*) and Shannon's information index (*I*). Total genotype diversity among (H_t) and within populations diversity (H_s)

were calculated by applying the G-test to allele frequencies at all loci (Nei, 1978). The similarity matrix was subjected to cluster analysis by unweighted pair group method for arithmetic mean (UPGMA) cluster analysis algorithm and a dendrogram was generated. All statistical analysis was carried out with POPGENE (Francis et al., 1999), computer package (Version 1.31).

RESULTS

Two hundred genomic DNA samples were selected randomly among the individuals of 4 breeder flocks of Arian broiler lines. These templates were then amplified with a total of 20 random primers (Table 1). Four RAPD markers (OPA01, OPA02, OPA03 and OPA04) did not amplify and no band resulted from these markers. Three markers (OPA08, OPA18 and OPA19) did not produce stable bands and was not included in data set for further analyses. Thirteen out of twenty primers (OPA05, OPA06, OPA07, OPA09, OPA10, OPA11, OPA12, OPA13, OPA14, OPA15, OPA16, OPA17 and OPA20) which were amplified and generating stable and easy to score and reproducible bands were selected for genotyping of all individuals from four broiler line populations (Figures 1 and 2). The number of RAPD fragments generated per primer ranged from 3 fragments for primer OPA06 to a maximum of 14 fragments for both primers OPA09 and OPA16. The average number of bands per primer was 8 and the fragment size ranged from 200 to 2500 bp. The total number of bands scored within RAPD profiles amplified by these 13 primers and the number of polymorphic and monomorphic bands amplified per primer within lines are presented in Tables 2 - 5. A total of 101 bands were detected in breeder flock of line A, with an average number of bands and average number of polymorphic bands per primer of 7.775 and 6.85, respectively. The number of polymorphic bands ranged from 4 (OPA06) to a maximum of 13 (OPA16), with an average of 66.66% of polymorphism per primer in line A (Table 2). Of the total 97 amplified bands in breeder flock of line B, 76 were polymorphic, with an average number

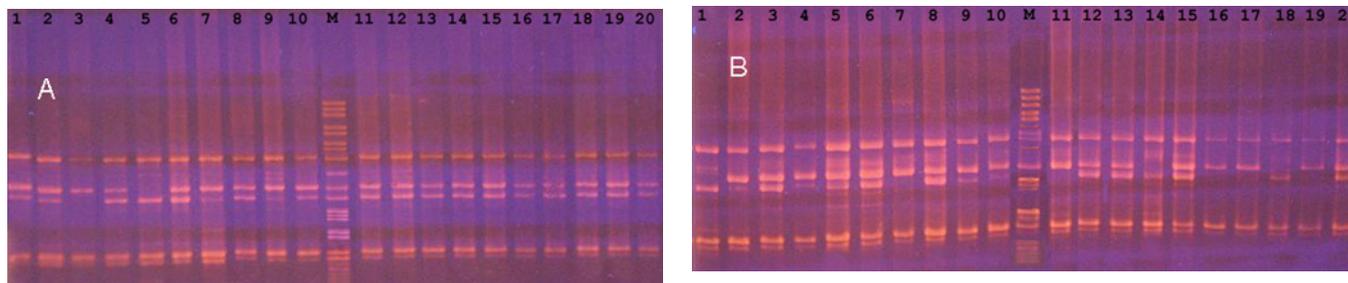


Figure 1. RAPD generated from individual bird of male parent of A and B lines using primer OPAN 14. A: line A, B: line B, lanes 1 - 20 birds from lines A and B, lane M: Molecular weight marker.

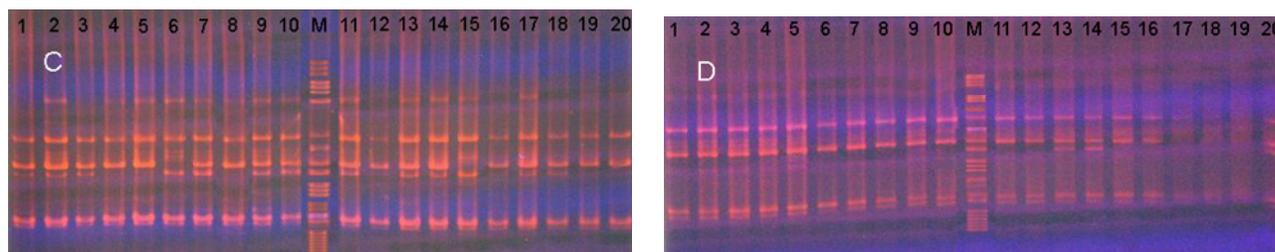


Figure 2. RAPD generated from individual bird of male parent of C and D lines using primer OPAN 14. C: line C, D: line D. Lanes 1 - 20, birds from lines C and D, lane M: Molecular weight marker.

Table 2. The total, polymorphic, monophormic and percentage of polymorphic bands of each RAPD markers obtained from Arian broiler, line A.

Primers	NTB	NPB	NMB	%PLM
OPA05	8	6	2	75
OPA06	3	3	0	100
OPA07	12	11	1	91.66
OPA09	7	7	0	100
OPA10	10	10	0	100
OPA11	4	4	0	100
OPA12	4	3	1	75
OPA13	13	11	2	84.61
OPA14	5	3	2	60
OPA15	8	7	1	87.5
OPA16	14	14	0	100
OPA17	4	3	1	75
OPA20	9	7	2	77.77
Mean	7.77	6.85	0.92	86.66

NTB = Number of total bands; NPB = number of polymorph bands; NMB = number of monomorph bands; %PLM = percentage of polymorphism.

Table 3. The total, polymorphic, monophormic and percentage of polymorphic bands of each RAPD markers obtained from Arian broiler, line B.

Primers	NTB	NPB	NMB	%PLM
OPA05	10	8	2	80
OPA06	3	2	1	46.66
OPA07	8	6	2	75
OPA09	8	8	0	100
OPA10	7	5	2	71.43
OPA11	6	5	1	83.33
OPA12	4	4	2	50
OPA13	8	7	1	87.50
OPA14	6	4	2	66.66
OPA15	10	7	3	70
OPA16	12	9	3	75
OPA17	6	5	1	83.33
OPA20	9	6	3	66.66
Mean	7.46	5.85	1.77	73.50

NTB = Number of total bands; NPB = number of polymorph bands; NMB = number of monomorph bands; % PLM = percentage of polymorphism.

of bands and average number of polymorphic bands per primer of 7.46 and 5.85, respectively. The number of polymorphic bands ranged from 2 (OPA06) to a maximum of 9 (OPA16), with an average of 37.76% of polymorphism in line B (Table 3). A total of 104 bands were detected in

population of line C, where the average number of bands and average number of polymorphic bands per primer was 8 and 7.54, respectively. The number of polymorphic bands ranged from 4 (OPA06, OPA17) to a maximum of 13 (OPA16), with an average of 94% of polymorphism in

Table 4. The total, polymorphic, monophormic and percentage of polymorphic bands of each RAPD markers obtained from Arian broiler, line C.

Primers	NTB	NPB	NMB	% PLM
OPA05	10	9	1	90
OPA06	5	4	1	80
OPA07	10	9	1	90
OPA09	10	10	0	100
OPA10	6	6	0	100
OPA11	6	6	0	100
OPA12	5	5	0	100
OPA13	6	6	0	100
OPA14	5	5	0	100
OPA15	11	10	1	90.90
OPA16	13	13	0	100
OPA17	5	4	1	80
OPA20	12	11	1	91.66
Mean	8	7.54	0.46	94

NTB = Number of total bands; NPB = number of polymorph bands; NMB = number of monomorph bands; %PLM = percentage of polymorphism.

breeder flock of line C (Table 4). Of the total 118 amplified bands in breeder flock of line D, 99 were polymorphic, with an average number of bands and average number of polymorphic bands per primer of 9.15 and 7.62, respectively. The number of polymorphic bands ranged from 2 (OPA012) to a maximum of 14 (OPA09), with an average of 80.70% of polymorphism in line D (Table 5). Na and Ne, h, l, for all the four populations were analyzed using thirteen RAPD markers and their respective values were found as 2, 1.473, 0.286 and 0.442 (Table 6). The values for H_t were 0.286 while H_s and the mean coefficient of gene differentiation (G_{st}) value was found to be 0.181 and 0.368, respectively (Table 7). The within population genetic uniformity and diversity of RAPD markers for each line are shown in Table 7. Among the studied broiler lines the highest genetic uniformity was found in line A, while the greatest within population genetic diversity was found in line C (Table 7). The Nei (1978) measures of genetic distance and identity between pairs of broiler breeder lines are given in Table 8. It shows that the birds from population of lines A and B had the highest identity (0.856), while the birds from lines A and D populations showed the greatest genetic distance (0.217). In the genetic similarity, dendrogram constructed on the basis of comparative analysis of the total loci obtained with the 13 RAPD primers across the four populations two main groups can be seen (Figure 3). The first main group consisted of two subgroups. The first subgroup contains birds from breeder flocks of line A and B, while the second subgroup of the first main group includes C line. The second main group includes birds from D line.

Table 5. The total, polymorphic, monophormic and percentage of polymorphic bands of each RAPD markers obtained from Arian broiler, line D.

Primers	NTB	NPB	NMB	%PLM
OPA05	9	9	0	100
OPA06	6	5	1	83.33
OPA07	9	7	2	77.77
OPA09	14	14	0	100
OPA10	8	7	1	87.50
OPA11	6	5	1	83.33
OPA12	4	2	2	50
OPA13	13	13	0	100
OPA14	9	5	4	55.55
OPA15	11	9	2	81.81
OPA16	13	12	1	92.30
OPA17	8	5	3	62.50
OPA20	8	6	2	75
Mean	9.07	7.61	1.46	80.70

NTB = Number of total bands; NPB = number of polymorph bands; NMB = number of monomorph bands; %PLM = percentage of polymorphism.

DISCUSSION

The analysis of genetic variation and relatedness between or within species, populations and individuals is a prerequisite towards effective utilization of molecular DNA markers for the discrimination of genetic resources that are economically important such as poultry and other farm animals. In this work, we compared the applicability of RAPD-PCR technique as genetic markers to characterize the genetic diversity in different breeder flocks of Arian broiler lines (Babol-Kenar Line Breeding Center, Babol, Iran). However, no such reports on genetic diversity using molecular markers were available for Arian broiler lines. In the present study, the mean total number of bands in four populations of A, B, C and D lines was ranged at 101, 107, 104 and 118, respectively. The average number of diagnostic bands per primer was 8.08 with an average number of 6.96 polymorphic bands across all breed groups. The highest and the lowest percentage of polymorphic bands were found within sampled birds of Arian line C (94%) and line D (73.5%), respectively. Some of these bands were scored in a given lines (positive markers), while absent in the other lines (negative markers). In total, sixty three RAPD bands were found to be line specific. The average of estimated polymorphic bands (83.72%) in our study was higher than that reported by Singh and Sharma (2002) in White Leghorn population (21.9%). The RAPD technique was applied to evaluate the genetic variability in a breeder flock of native fowls chicken (Rahimi et al., 2005). Using 20 random decamer primers, only 14 primers detected polymorphic bands. From a total number of 140 scored bands, 63 (45%) and 77 (55%) were described as poly-

Table 6. Summary of genetic parameters estimate in breeder flocks of Arian broiler lines using RAPD markers.

Populations	Na	Ne	h	I
Line A	1.88 ± 0.32	1.53 ± 0.33	0.31 ± 0.15	0.46 ± 0.22
Line B	1.76 ± 0.42	1.45 ± 0.37	0.26 ± 0.18	0.40 ± 0.26
Line C	1.94 ± 0.23	1.60 ± 0.35	0.34 ± 0.16	0.50 ± 0.21
Line D	1.84 ± 0.37	1.52 ± 0.36	0.30 ± 0.18	0.44 ± 0.25
Across lines	2.00 ± 0.000	1.473 ± 0.329	0.286 ± 0.157	0.442 ± 0.200

Na: Observed number of alleles; Ne: effective number of alleles; h: Nei's genetic diversity; I: Shannon's information index.

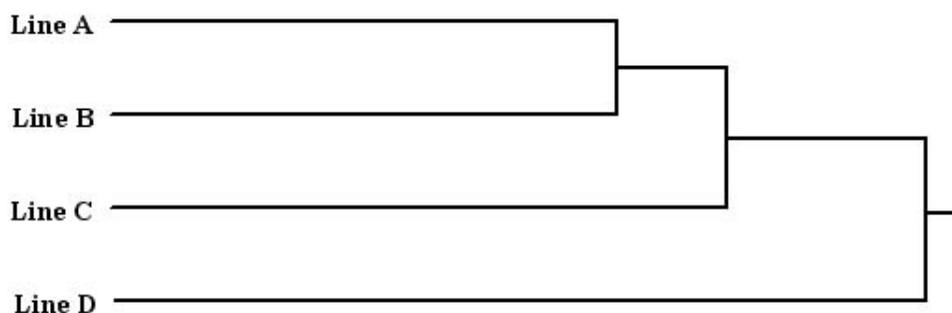
Table 7. Summary analysis of genetic variability across all breeder flocks in Arian broiler lines using RAPD markers.

Parameters	Line A	Line B	Line C	Line C
U	0.6908	0.7358	0.6613	0.7018
WPD	0.3092	0.2642	0.3387	0.2982
Across populations	H _t	H _s	G _{st}	-
	0.286	0.181	0.368	-

U and WPD: Within population genetic uniformity index and diversity; H_t and H_s: total genotype diversity among and within populations; G_s: mean coefficient of gene differentiation.

Table 8. Nei's genetic distance (below diagonal) and genetic identity (above diagonal), between breeder flocks of Arian broiler lines using RAPD markers.

Different lines	Line A	Line B	Line C	Line D
Line A	1.00	0.856	0.834	0.805
Line B	0.156	1.00	0.836	0.833
Line C	0.181	0.179	1.00	0.807
Line D	0.217	0.183	0.215	1.00

**Figure 3.** Dendrograms generated using UPGMA analysis, showing relationships between breeder flocks of Arian broiler lines using RAPD markers.

morphic and monophormic, respectively. The lower percentage of polymorphism in breeder flock of native fowls (45%) obtained in previously study (Rahimi et al., 2005) in comparison with the present work (average of 83.7%) may be due to differences in population size

and/or different set of used RAPD markers. The monomorphic bands are constant bands and cannot be used to study diversity while polymorphic bands revealed differences and can be used to examine the relationships among the genotypes (Hadrys et al., 1992). Rahsan and

Guldehen (2002) observed an average number of 9.2 polymorphic bands per primer using RAPD markers between meat and layer pure line of chicken. RAPD markers were used to detect polymorphism among five breeds of chicken selected for early body weight and/or egg production (Sharma et al., 2001). Of the total 96 fragments amplified, about 25% showed polymorphism. Polymorphism in a given population is often due to the existence of genetic variants represented by the number of alleles at a locus and their frequency of distribution in a population. Heterozygosity corresponds to a probability that two alleles taken at random from a population can be distinguished using the marker in question (Gupta et al., 2008). Therefore a convenient quantitative estimate of marker utility and the polymorphism detected can be given in terms of the Nei's genetic diversity, Shannon's information index, coefficient of population differentiation, total genotype diversity among and within populations (Zhao et al., 2006). In the present study, the observed and effective number of alleles, Nei's genetic diversity and Shannon's information index were analyzed using thirteen RAPD markers in four lines of Arian broiler lines and their respective values were found as 2.00, 1.473, 0.286 and 0.442, respectively. The average genetic uniformity index for RAPD fingerprinting pattern in our study ranged from 0.6613 (line C) to 0.7358 (line B), respectively. In previous study, within population, genetic uniformity index was analyzed using RAPD markers and it ranged from 0.63 to 0.93 per primer in a breeder flock of Iranian indigenous chickens (Rahimi et al., 2005). In the present study, the similarity coefficients ranged from 0.805 to 0.856 with maximum similarity of 85.6% as observed between birds of line A and line B and lowest similarity of 80.5% as observed between individuals of line A and line D. Between broiler lines genetic distances estimates, band frequency ranged from 0.156 (between line A and B) to 0.217 (between line A and D). Polymorphisms are expected at reasonable frequencies between distantly related lines, while in more closely related strains, fewer polymorphisms may be detectable with RAPD method (Levin et al., 1994). The obtained results for genetic similarities and genetic distances between lines may confer the selection strategies to produce the male parent lines of A and B which are emphasized on growth performance and body conformation. While, the trends of both genetic similarity and genetic distance between two lines of C and D selected as female parents indicates that the selection pressure in these two lines are not in the same direction. It shows that the selection strategies in line C are more closed with line A and B instead of line D. The results for genetic similarity index agreed with the finding of Mollah et al. (2009) who reported higher genetic similarity (82.45 to 90.03%) at genomic level in indigenous chicken populations of Bangladesh. In the present study, the dendrogram based on similarity coefficients was constructed on the basis of comparative analysis of the total loci obtained with the 13 RAPD markers by using the UPGMA method.

The four broiler lines clustered into two main groups. In the first main group, the closest genetic distance was found between A and B lines, which were first clustered together and then with birds of C line. The second main group includes birds from D line. The higher level of mean coefficient of gene differentiation ($G_{st} = 0.368$) across all loci indicated that sufficient genetic differences among different Arian broiler lines are present.

Conclusion

Based on the results obtained in this study, we can conclude that the molecular RAPD marker represent a useful and efficient method in the detection of polymorphism and proved to be quite powerful in distinguishing different broiler lines. Lower genetic distance among birds of A, B and C lines may reflect the selection strategy proximity applied between them and support the hypothesis of the selection strategy being an important factor influencing the genetic relatedness of populations. More importantly, the analysis of bands shows some line dependent specific bands. Therefore, in order to design new diagnostic primers more effective in genetic discrimination among studied lines, unique bands could be cloned and sequenced. Furthermore, development of these line specific markers may be necessary to search quantitative trait loci within population of Arian broiler lines which differ in production performances. In conclusion, to provide more details about the population structures, further studies are required based on the large number of samples and co-dominant microsatellite marker.

ACKNOWLEDGEMENTS

The authors are thankful to the head of Poultry Support Center, Ministry of Agriculture (Jahad-e-Keshavarzi), Eng. M. R. Molla-Salehi for financial support and Dr. H. Shojaee, the head of Babol- Kenar Line Breeding Center for providing the experimental birds.

REFERENCES

- Ali BA, Ahmed MMM, Aly OM (2003). Relationship between genetic similarity and some productive traits in local chicken strains. *Afr. J. Biotechnol.* 2(2): 46-47.
- Bardacki F (2001). Random amplified polymorphic DNA (RAPD) markers. *Turk. J. Biol.* 25: 185-196.
- Fischer M, Husi R, Prati D, Peintinger M, Kleunen MV, Schmid B (2000). RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae). *Am. J. Bot.* 87: 1128-1137.
- Francis CY, Rong CY, Boyle T (1999). Popgene Microsoft Windows-based Freeware for Population Genetic Analysis. University of Alberta pp. 1-31.
- Gupta S, Srivastava M, Mishra GP, Naik PK, Chauhan RS, Tiwari SK, Kumar M, Singh R (2008). Analogy of ISSR and RAPD markers for comparative analysis of genetic diversity among different *Jatropha curcas* genotypes. *Afr. J. Biotechnol.* 7(23): 4230-4243.

- Hadrys H, Balick M, Schierwater B (1992). Application of random Amplified polymorphic DNA (RAPD) in molecular ecology. *Molecular Ecol.* 1: 55-60.
- Kjolner S, Sastad SM, Taberlet P, Brochmann C (2004). Amplified fragment *length* polymorphism versus random amplified polymorphic DNA markers: clonal diversity in *Saxifraga cernua*. *Molecular Ecol.* 13: 81-86.
- Levin I, Crittenden LB, Dodgson JB (1994). Mapping DNA polymorphisms using PCR primers derived from the sequence of an avian CRI element. *J. Hered.* 85: 75-78.
- Lynch M, Milligan BG (1994). Analysis of population genetic structure with RAPD markers. *Mol. Ecol.* 3: 91-99.
- Miller SA, Dykes DD, Polesky HF (1988). A simple salting out procedure for extracting DNA from Human nucleated cells. *Nucleic Acid Res.* 16(3): p. 1015.
- Mollah MBR, Islam MS, Ali MA, Alam Ms (2009). Analysis of genetic diversity in Bangladeshi chicken using RAPD markers. *Biotechnology*, 8(4): 462-467
- Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individual. *Genetics*, 89: 583-590.
- Nei M, Li WH (1979). Mathematical modelling for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci.* 76: 5269-5273.
- Rahimi G, Khanahmadi A, Nejati-Javaremi A, Smailkhanian S (2005). Evaluations of genetic variability in a breeder flock of native chicken based on randomly amplified polymorphic DNA markers. *Iran. J. Biotechnol.* 3(4): 231-234.
- Rahsan I, Guldehen B (2002). Estimation of genetic distance in meat and layer pure lines using randomly amplified polymorphic DNA. *Turk. J. Vet. Anim. Sci.* 26: 1117-1120.
- Salem HH, Ali BA, Huang TH, Qin DN (2005). Use of randomly amplified polymorphic DNA (RAPD) markers in poultry research. *Int. J. Poult. Sci.* 4(10): 804-811.
- Sharma D, Appa Rao KB, Singh RV, Totey SM (2001). Genetic diversity among chicken breeds estimated through randomly amplified polymorphic DNA. *Anim. Biotechnol.* 12: 111-120.
- Smith EJ, Jones CP, Bartlett J, Nestor KE (1996). Use of randomly amplified polymorphic DNA markers for the genetic analysis of relatedness and diversity in chickens and turkeys. *Poult. Sci.* 75: 579-584.
- Zahid M, Farah R, Altaf AD, Saima S, Mohammad A, Manzar Q (2009). Genetic diversity analysis of the species of *Gossypium* by using RAPD markers. *Afr. J. Biotechnol.* 8(16): 3691-3697.
- Zhang X, Leung FC, Chan DK, Yang G, Wu C (2002). Genetic diversity of Chinese native chicken breeds based on protein polymorphism, randomly amplified polymorphic DNA, and microsatellite polymorphism. *Poult. Sci.* 81: 1463-1472.
- Zhao WG, Zhang JQ, Wangi YH, Chen TT, Yin Y, Huang, YP, Pan Y, Yang Y (2006). Analysis of genetic diversity in wild populations of Mulberry from western part of northeast China determined by ISSR Markers. *J. Genet. Mol. Biol.* 196-203.