Multi Trait Selection with Restriction for Cutup Carcass Value in Broiler Chicken: Genetic Relatedness of Lines Involved Based on Randomly Amplified Polymorphic DNA

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ABSTRACT : Five broiler chicken lines, namely HC, BPB2, CPB2, PB2 and UM1, involving in a selection program and differing in selection intensity and genetic background, were screened for randomly amplified polymorphic DNA (RAPD) polymorphism using 10 selected decamer primers. Nine primers amplified the genomic DNA, generating 200 to 2,500 bp and all detected polymorphism between lines. Out of 74 bands scored using these primers, 34 (50.0%) were found to be polymorphic. The number of polymorphic loci ranged from 3 to 6 with an average of 4.33. Lines differed considerably for within-population genetic similarity estimated by band frequency (WS = 93.55 to 99.25). Between-line genetic similarity estimates based on band sharing as well as on band frequency ranged from 71.35 to 86.45 and from 73.38 to 87.68, respectively. Lines HC and PB2 were the most closely related to the other, while BPB2 and CPB2 appeared to be more distant from each other. The between-line genetic distance based on both band sharing and band frequency revealed the similar trends as for Between-line genetic similarity. Based on BS and BF criteria, BPB2 and CPB2 as well as PB2 and UM1 lines can be merged to launch a new genetic group for further progress in biometrical objectives. A phylogenetic tree, derived using Nei's coefficient of similarity revealed the different pattern of genetic distance between lines. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 11 : 1535-1541)***

Key Words : Broiler Chicken, Selected Lines, Cutup Carcass, Genetic Relatedness, RAPD Markers

INTRODUCTION

Amplification of genomic DNA using at least one short oligonucleotide primer, in low stringency conditions, results in multiple amplification products from loci distributed throughout the genome (Williams et al., 1990, Welsh and McClelland 1990). This observation led to the development of a class of molecular typing methods generally known as the arbitrary primer amplification methods, which depending on the specific conditions of amplification or product separation and detection, they are termed RAPD (randomly amplified polymorphic DNA; Williams et al. 1990), AP-PCR (arbitrarily primed PCR; Welsh and McClelland 1990), or DAF (DNA amplification fingerprinting; Caetano-Anolles et al., 1991).

RAPD technique is a prevailing (Williams et al., 1990) tool for identification of populations (Shivaraman et al., 2001; Sharma and Singh, 2001; Seifi, et al., 2004; Yoon and Kim, 2004) and detection of genetic variability of inbred lines (Plotsky et al., 1995; Sharma and Singh, 2001), among lines selected for economic purposes (Bossak et al., 2001; Sharma et al., 2001) and among different species (Smith et al., 1996). The RAPD technique can also be used for analysis of linkage between RAPD markers and quantitative trait loci (QTL) (Tercic et al., 1998). The RAPD procedure is relatively simple, fast, non-radio active detection, inexpensive, and without requirements for target DNA sequence information.

In the present study, an attempt has been made to estimate the genetic relatedness between five broiler lines being maintained at Department of Poultry Science, UAS, Bangalore. The lines were separately subjected to multitrait selection to enhance the cut up carcass value through increase breast and thighs portions. The objective of the study was to understand the rationality of pooling the genotypes in the subsequent generations using DNA differences in population level, based on RAPD technique. Data generated herein will verify an application for the effectiveness of RAPD in detecting polymorphism between diverged lines of chicken and may help in sensible pooling of groups to continue the selection programme in pertinent track.

MATERIALS AND METHODS

Lines and management

An attempt is proceeding to improve the breast and thigh portions in broilers utilizing restricted selection index methodology at the Department of Poultry Science, UAS, Bangalore. The project involves five lines namely PB2, HC

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Received December 28, 2004; Accepted May 11, 2005

(male lines received from project directorate on poultry, Hydarabad), UM1 (developed at Department of Poultry Science, UAS, Bangalore) BPB2 (male line) and CPB2.

Selection for cut up carcass value was practiced each generation from about 250-300 birds within each line by constructing a restricted index involving body weight at six weeks of age, carcass weight, breast weight, thighs weight, low value portions of carcass (neck, wings and back) weight, and a breast index based on breast dimensions. Gene flow among the lines was restricted every generation. Birds from all generations consumed feed and water *ad libitum* up to 6 wk of age and then were feed-restricted according to breeder recommendations. A broiler starter diet was fed from 0 to 4 wk of age (22% CP and 3,000 kcal of ME/kg) and a broiler grower diet from 4 to 6 wk of age (20% CP and 3,000 kcal of ME/kg).

DNA preparation

A sample of 1-2 ml blood was taken via the brachial vein at 15 wk of age from each of 12 birds (6males and 6 females) randomly chosen from each of the five groups. Each blood sample was mixed with 50 µl of 0.5 M EDTA, and frozen at -70°C until analyzed. The DNA was isolated from the aliquots as follows: 25 µl whole blood was thawed at room temperature for 15 min, 500 µl lysis buffer [10 mM Tris-HCI (pH 8.0), 100 mM Nacl, 1 mM EDTA (pH 8.0), 0.5% SDS] and 0.6 U proteinase K were added to the blood. The mixture was incubated for 90 minutes at 56°C and then extracted twice with equal volumes of phenol and chloroform. The DNA was precipitated in 100% ethyl alcohol, rinsed in 70% ethyl alcohol, and dissolved in TE (10 mM Tris, pH 8.0,1 mM EDTA).

DNA amplification and separation

Nine selected primers, described according to the manufacture's recommendation (Bangalre Geni Company), from the sets OPA, OPB and OPG were used. Each primer considered for polymerase chain reaction (PCR) typing was a 10-mer with GC content of 50, 60 or 70 per cent (Table 2). Amplification reactions were carried out in a final volume of 25 µl containing 2.5 mM Tris-Hcl (pH 8.3), 50 mM a KCl, 5.0 mM MgClu 250 µM of each dNTP, 1.0 µM primer, 1 U of AmpliTaq® DNA polymerase, and 75 ng DNA template. Amplification was performed in a COY tempcycler II programmed for 10 min initial denaturation at 96°C, then 42 cycles of 30 s at 96°C, 30 s at 35°C, 45 s at 72°C, and 5 min final extension at 72°C. An aliquot of 15 µl was used for electrophoresis on 1.4% agarose gel at a constant voltage of 80V and visualized by illumination with ultraviolet light after staining with ethidium bromide. A mixture of *Lambda* DNA digested with *Hind III* restriction enzyme of Phi×174 DNA digested with *Hae III* restriction enzyme was used as DNA molecular size marker.

RAPD pattern analysis

Scoring of bands was done utilizing Quantity one (BioRad, USA) software program. By adjusting the determination accuracy of the software only distinct and prominent bands were scored. Comparison of RAPD fingerprints were made only on samples run on the same gel. Genetic relatedness was predicted based on band-sharing frequency (BSF) and band frequency.

The genetic similarity between two birds (BS_{ab}) based upon BS was estimated using the following formula:

$$BS_{ab} = 2b_{ab}/(b_a+b_b)$$

where b_a and b_b are number of bands scored for bird a and b, respectively; b_{ab} is the number of bands common to birds a and b (Lynch, 1990). To estimate BS between birds, band absence or presence within RAPD patterns was scored as zero or one, respectively. The within-population BS frequency was computed as an average of BS_{ab} across all possible comparisons between individuals within a population. The two-way analysis of variance was used to test the significance of within population genetic similarity (SAS Institute, 1998). The BSF between individuals of different populations (B') corrected for within-population similarity was computed according to Lynch (1990) as:

$$B' = 1+B_{xy} - 0.5(B_x+B_y)$$

where B_x and B_y are values of BS for x and y populations, respectively, and B_{xy} is the average of the band sharing estimates of comparisons between birds of populations x and y. The genetic distance (*D*) was determined as *-ln BS*.

The within line genetic similarity (WF_i) was estimated using the following equation:

$$WF_i = 1/N(\sum_{i=1}^{N} V_i)$$

where V_i is the proportion of individuals possessing the i^{th} band across all the individuals and N is the total number of bands scored. The genetic similarity between two lines was obtained from the following formula:

$$BF_{ij} = 1/N(\sum_{i=1}^{N}(2V_{i1}V_{i2})/[(V_{i1})^2 + (V_{i2})^2]$$

where V_{i1} and V_{i2} are the frequency of occurrence of the ith band in lines 1 and 2, respectively and *N* is the total number of bands scored. The genetic distance (BF_{ij}) was estimated as *-ln* (BF_{ij}).

Intended to clustering the genotypes, Unweighted Pair Group Average Method (UPGAM) of analysis was

Table 1. Number of selection rounds, average effective population size, average number of progenies and intensity of selection in different lines

Variables	Lines				
	HC	CPB2	BPB2	PB2	UM1
Number of generations	4	4	4	4	4
Av. effective population size	70	84	70	175	147
Av. number of progeny/generation	243	259	247	183	249
Av. intensity of selection (*i*)	1.49	1.49	1.49	1.49	1.49
RGR in EBY (as % of CW, 42 d)[1]	0.366[a]	0.200[b]	0.358[a]	0.350[a]	0.233[b]
RGR in BW (42 d)[2]	48.3[b]	36.0[c]	59.0[a]	49.0[b]	18.7[d]

[1] Realized genetic response per generation in breast weight (42 d).
[2] Realized genetic response per generation in body weight (42 d).

Table 2. The sequence, percentage of CG content, total number of bands, polymorphic bands and their size ranges from the random primers used

No.	Code	Sequence	GC (%)	Number of fragments		Size range of fragments (bp)	
				Total	Polymorphic	Min.	Max.
1	BG-6	5'-CTG AGA CGG A-3'	60	8	4	200	1,300
2	OPA-6	5'-GGT CCC TGA C-3'	70	7	3	200	1,300
3	OPA-16	5'-AGC CAG CGA A-3'	60	11	6	300	1,700
4	OPB-19	5'-ACC CCC GAA G-3'	70	8	4	200	1,900
5	OPC-2	5'-GTG AGG CGT C-3'	70	9	5	200	2,100
6	OPC-3	5'-GGG GGT CTT T-3'	60	7	4	300	1,500
7	OPG-7	5'-GAA CCT GCG G-3'	70	7	5	200	2,500
8	OPP-14	5'-CCA GCC GAA C-3'	70	10	4	200	1,300
9	OPP-17	5'-TGA CCC GCC T-3'	70	10	4	200	1,200
	Overall			78	39	200	2,500

Table 3. The number of amplified fragments (Mean±SEM) scored from randomly amplified polymorphic DNA patterns of nine primers that each amplified at least one polymorphic fragment in each of the five lines

	Lines (n±SEM)				
	HC	CPB2	BPB2	PB2	UM1
BG-6	5.00±0.79	4.43±0.62	4.00±0.62	4.09±0.65	4.64±0.62
OPA-6	4.45±0.71	3.92±0.69	4.06±0.65	4.38±0.58	4.73±0.60
OPA-16	6.00±0.35	6.50±0.62	6.25±0.54	5.60±0.54	5.40±0.58
OPB-19	3.83±0.55	4.17±0.34	4.18±0.46	4.25±0.48	3.60±0.37
OPC-2	5.45±0.47	5.36±0.36	4.45±0.25	5.00±0.26	5.00±0.37
OPC-3	4.50±0.29	4.84±0.34	5.25±0.45	4.33±0.50	4.55±0.34
OPG-7	4.48±0.25	4.57±0.25	4.92±0.26	5.17±0.27	4.40±0.34
OPP-14	4.92±0.42	4.50±0.29	5.36±0.34	5.18±0.33	5.40±0.52
OPP-17	5.58±0.58	4.60±0.50	4.73±0.41	4.50±0.56	4.38±0.42

performed based on RAPD data, with the matrices of Nei coefficients (Nei, 1978) of similarity using *Statistica* software. A dendrogram was constructed to show the phylogenetic relationships among five lines concerned.

RESULTS AND DISCUSSION

Primary information including number of selection rounds (generations), average effective size, average progeny per generation and selection intensity (equal for all lines) along with realized genetic response in body weight and breast weight pertinent to lines considered are provided in the Table 1.

Number of bands

All the nine primers used revealed patterns with distinct, reproducible and scorable amplified fragments (Table 2). The mean number of within line scorable bands from primers varied from 3.83 to 6.00, 3.92 to 6.50, 4.06 to 6.25, 4.09 to 5.60 and 3.60 to 5.40 in HC, BPB2, CPB2, PB2 and UM1 lines, respectively (Table 3). No clear pattern of association between a particular primer with a line could be pointed out. However, the chicken lines seem to differ for the average number of bands per primer. Earlier researchers also showed that the number of bands amplified differed with the primers (Plotsky et al., 1995; Smith et al., 1996; Sharma et al., 1998; Shivaraman et al., 2001). Frequency of the fragments appeared and scored in terms of their relevant

Table 4. Line wise frequency of fragments scored as per corresponding base pair pooled over nine primers

Base pair	Lines[3]					TBN[1]
	A	B	C	D	E	
200	86	86	88	76	82	418
300	146	140	160	148	112	706
400	142	162	142	132	140	718
500	134	134	152	138	122	680
600	134	134	140	120	108	636
700	102	108	92	92	94	488
800	74	76	84	72	46	352
900	60	80	66	68	62	336
1,000	62	82	60	60	32	296
1,100	22	40	32	24	22	140
1,200	10	18	10	8	10	56
1,300	6	6	4	6	10	32
1,500	2	0	2	2	2	8
1,700	2	0	8	4	0	14
1,900	2	2	0	0	0	4
2,000	0	2	0	0	0	2
2,100	2	2	0	0	0	4
2,200	0	0	0	2	0	2
2,400	0	0	4	0	0	4
2,500	1	0	0	0	0	1
Total	987	1,072	1,044	952	842	4,897
WF[2]	96.7	99.25	94.9	94.2	93.5	

[1] Total band number. [2] Within line genetic similarity.
[3] A: HC, B: CPB2, C: BPB2, D: PB2 and E: UM1.

base pair size for each line is given in the Table 4. No clear association between frequency of a fragment and a particular line was found. The order for higher frequencies were found as 400>300>500>600>700>200>800>900> 1,000>1,100 bp. The results of a two-way analysis of variance conducted to bear out the significant differences among within line genetic similarities based on band sharing frequency revealed a high significant influence of primer (as a fixed effect in the model) on band sharing frequency (Table 5).

Polymorphic bands

A total of 78 bands were amplified by the 9 primers and 39 polymorphic bands (50.0%) were produced (Table 2). The total number of the bands ranged from 7 (for OPA-6, OPC-3, and OPG-7) to 11 (for OPA-16). The polymorphic bands ranged from 3 (for OPA-6) to 6 (for OPA-16). The amplified DNA fragments ranged in molecular weight from 200 to 2,500 bp. The primer OPA-16 produced the maximum scorable as well as polymorphic amplified DNA fragments. In contrast, the oligonucleotide OPA-6 generated the minimum scorable (along with OPC-3 and OPG-7) and polymorphic fragments (Tables 2 and 3). No primer produced complex patterns and difficult to interpret. The proportion of the primers capable of detecting the polymorphism among the lines evaluated depends upon the genetic background of the lines, genetic distance between them and complexity of the genome. In present study, all primers could detect polymorphism between chicken lines examined. Earlier reports showed 4 to13 per cent (Smith at al., 1996), 18 per cent (Shivaraman et al., 2001), 24 per cent (Singh and Sharma, 2002), and 40 per cent (Ahlawat et al., 2004) proportion of polymorphic primers. The main reason for 100 per cent polymorphic primers in this study could be the careful selection of primers as per results of the previously published reports and lack of association between primers and lines involved or the PCR procedure followed. The present and earlier studies indicate that using

Table 5. Two-way analysis of variance for effect of line and primer on variation in band frequency

SOV	DF	SS	MS	F-value	Pr>F	Result
Line	4	42.29	10.57	0.44	0.7814	NS
Primer	8	1,234.7	154.3	6.37	0.0001	***
Error	32	775.5	24.2			

NS: Non significant (p>0.05), *** p<0.0001.

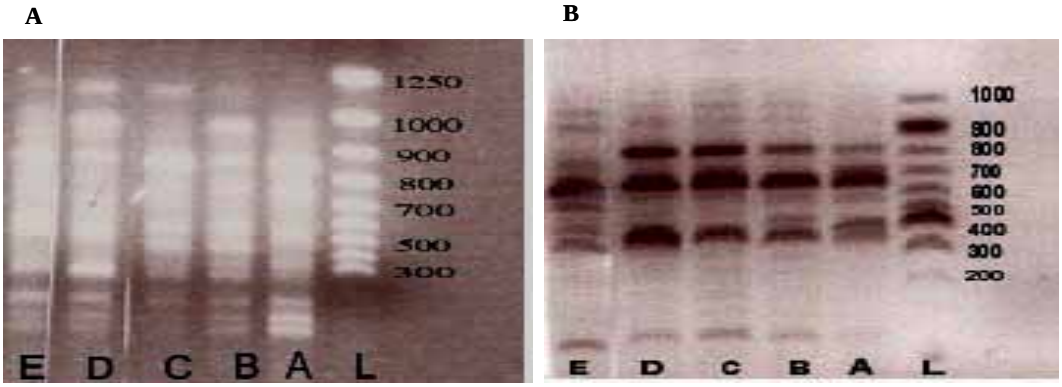

Figure 1. RAPD amplification products generated by primers BG-6 (A) and OPB-19 (B) for each of the five lines; lane A: HC, lane B: CPB2, lane C: BPB2, lane D: PB2, lane E: UM1, Lane L is molecular size marker (mixture of DNA digested with *Hind* III restriction digestion enzyme and *Phi* 174 DNA digested with *Hae* III restriction enzyme).

Table 6. Estimates of within line band sharing frequency from the amplified patterns of each nine selected primers that revealed one or more polymorphic fragments in each line

Primers	Lines (n±SEM)				
	HC	CPB2	BPB2	PB2	UM1
BG-6	94.96	81.25	76.48	76.54	85.40
OPA-6	78.02	71.12	71.10	77.40	83.97
OPA-16	91.42	93.6	98.52	82.29	92.91
OPB-19	75.37	79.44	74.41	74.79	77.08
OPC-2	87.73	92.30	87.55	88.46	85.20
OPC-3	87.50	82.20	87.03	74.47	72.29
OPG-7	81.10	80.34	85.88	95.10	89.01
OPP-14	71.12	81.90	88.51	88.24	79.87
OPP-17	93.60	85.72	82.99	81.62	86.75
Mean±SEM	84.94±2.22	83.77±2.27	82.72±2.76	82.10±2.39	83.61±2.10

Table 7. Within line (diagonal and bold), between lines (above diagonal) genetic diversity and between line genetic distances (below diagonal) among lines, estimated based on band sharing frequency

	Lines				
	HC	CPB2	BPB2	PB2	UM1
HC×HC	84.90[a]	83.56	81.56	86.45	83.24
HC×PB2	18.32	83.77[a]	71.35	74.16	73.70
HB×PB2	20.44	33.75	82.72[b]	82.10	79.61
PB2	14.56	29.85	19.72	82.10[b]	83.61
UM1	18.34	30.51	22.80	17.90	83.61[c]

Table 8. Within line (diagonal and bold), between lines (above diagonal) genetic diversity and between line genetic distances (below diagonal) among lines, estimated based on band frequency

	Lines				
	HC	CPB2	BPB2	PB2	UM1
HC×HC	96.70[ab]	84.76	82.71	87.68	85.24
HC×PB2	16.53	99.25[a]	73.38	75.08	75.73
HB×PB2	18.98	30.95	94.90[b]	84.51	80.87
PB2	13.15	28.67	16.83	94.25[b]	85.61
UM1	15.97	27.79	21.23	15.53	93.55[c]

random primers instead of selected ones, RAPD analysis requires screening of a large number of random primers in order to detect polymorphism, because the amplification from the arbitrary primers depends on the binding sites in the genome. Hence, comparatively large numbers of random primers are required to detect polymorphism to be utilized for genetic analysis.

Figure 1 depicts the amplification products of different sizes, which are easily resolved by electrophoresis and visualized by staining with ethidium bromide. No line-specific fragment was revealed in this study. Line/strain-specific bands were reported by Smith et al. (1996), Tercic et al. (1998), Bossak et al. (2001), Sharma et al. (2001), and Ali et al. (2003). In all of their studies RAPD technique were used to verify the genetic similarity or distance between two distinct breeds such as RIR and LH (Sharma et al., 2001), populations developed for particular productive purposes (Bossak et al., 2001) or diversely selected lines (Tercic et al., 1998; Zhang et al., 1995). The lines concerned in the current study were generally colored birds originated from double -purpose flocks with main selection goal of improving in body weight as well as egg production. Hence, lack of line-oriented amplified fragments was not unexpected.

Within line genetic similarity

Within line band sharing frequency from the amplified patterns of each nine selected primers revealed one or more polymorphism fragments in each line are presented in Table 6. Within line genetic similarity was calculated based on band sharing (Table 7) and band frequency (Table 8). The HC group showed the highest genetic similarity (0.849) followed by CPB2 (0.838), PB2 (0.836), BPB2 (0.827) and UM1 (0.821). Higher within genetic similarity in HC may be due to long term intra population selection history. The PB2 line also has undergone selection for body weight during many generations before being incorporated in the base population of the current study. The genetic similarity values based on band frequency were numerically a little higher than those based on band sharing. A slight change in the rank of lines concerned with respect to within genetic similarity was also observed compared to the above mentioned order. Based on band frequency, the CPB2 group showed the highest rank followed by HC, BPB2, PB2 and UM1. The superiority of UM1 group with regard to higher genetic variability was unexpected since it concerned as a pure line. Such variability could be attributed to relaxed selection on this line for many generations before being involved in the current study. In contrast, the hybrid groups were expected to demonstrate more variability due to hybridization of genomes from two different parental lines. However, the within genetic variability observed was less than UM1 likely due to common selection procedure applied.

The genetic similarity values obtained in this study were in fair conformity with those reports revealing the comparison between lines of a same breed. Singh and Sharma (2002) studied the genetic variability in five leghorn lines. The genetic similarity between lines ranged

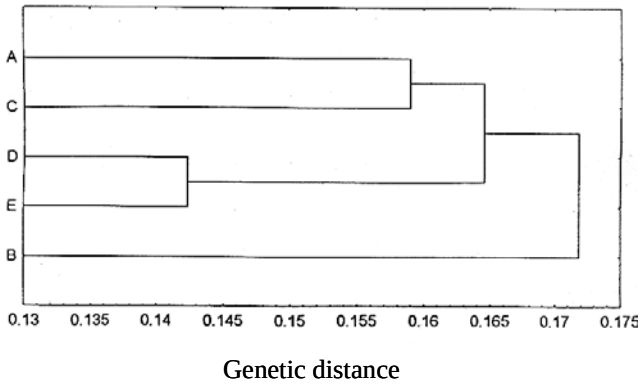

Figure 2. Unweighted Pair Group Method Analysis (UPGMA) polygenetic tree (dendrogram) constructed between A = HC, B = CPB2, C = BPB2, D = PB2, and E = UM1, based on RAPD data.

from 0.836 to 0.968 and 0.820 to 0.969, based on band sharing and band frequency, respectively. Within population (strain/line) variability is a reflection of population structure, selection history and breeding strategies. The present lines have been maintained as closed flocks since their inception in this selection program. Selection is an important factor, which may influence the intra-population genetic variability to a large extent. Hence, the consistently high levels of genetic similarity in all five lines studied could at least to some extent be attributed to the recent selection pressure directed to escalate the breast muscle on all lines.

Between lines genetic similarity/distance

Between-population genetic variation may reflect the different sources of origin of the lines and their subsequent propagation. The lines HC, PB2, and UM1, originated from different sources, were crossed and subjected to similar selection regimes but their response to selection could vary due to their differential reproductive and productive potentials. Hence, some diversity between them is expected.

Between-line genetic similarity/distance estimates derived from band sharing between five lines concerned are presented in Table 7. The corresponding values from band frequency are also given in Table 8. Based on pair-wise similarity index (band sharing) the maximum genetic similarity (or minimum genetic distance) was found among HC and PB2. In contrast, the least amount of genetic relatedness was calculated for CPB2 and BPB2. For other pairs, the values ranged from 73.70 to 83.56 describing high levels of relatedness. The genetic similarity values based on band frequency were utterly of same trend as those derived from band sharing but the values were generally a little higher. The PB2 line as a parental line in the pedigree of CPB2 and BPB2 groups was expected to show more genetic similarity with these lines. However, the values obtained did not fulfil the expectation likely due to considerable change in their genetic make up as a consequence of crossing with HB (a parental line for BPB2) and HC lines as well as long term direct selection for higher body weight and indirect selection for breast yield.

The differences observed between genetic similarity values derived from band sharing and band frequency in this study was in disparity with those from Sharma and Singh (2001) and Shivaraman et al. (2001). As in these studies, the values based on band sharing were utterly higher than those derived from band frequency. In contrast, Singh and Sharma (2002), in agreement with the findings of the current study reported the higher genetic similarity values based on band frequency compared to those from band sharing. The levels of BS revealed by RAPD patterns in the present study were lower than those detected by hybridization with radiolabled minisatellite probes (Dunnington et al., 1991) and RAPD technique (Sharma and Singh, 2001; Singh and Sharma, 2002). The values, however, were fairly concord with those reported by Sharma et al. (2001) and Ahlawat et al. (2004).

Using *Statistica* software, Unweighted Pair Group Average Method (UPGAM) of analysis was performed based on RAPD data, and a dendrogram was constructed to show the phylogenetic relationships among the five lines of consideration (Figure 2). The HC group appeared to be most distant from the other lines whereas BPB2 and CPB2 as well as PB2 and UM1 were closely related. Considering the same paternal line (PB2) in the pedigree of CPB2 and BPB2, the maximum genetic distance between them reveals the substantial impact of selection in diversifying genetic make up of them while segregation of genes afforded decreasing source of raw material for variability generation by generation. Based on these findings, pooling of BPB2 and CPB2 as a separate group in the future generation is suggested. The PB2 and UM1 can also be merged to launch the other line for further progress in biometrical objectives. The HC group is recommended to be kept as a distinctive line.

Therefore, from the present study, it could be concluded that RAPD markers are effective in detecting polymorphism between five lines concerned as HC, CPB2, BPB2, PB2 and UM1, and provide a potential tool for studying inter as well as intra line genetic variability and for establishing genetic relationship. It has been pointed out that there is no considerable genetic variability within lines studied. However, bearing out the specificity and sequence homology of no line specific bands could be found using nine primers of concern and needs further detailed study using more number of primers and hybridization, cloning and sequence experiments.

REFERENCES

Ahlawat, S. P. S., J. Sunder, A. Kundu, R. N. Chatterjee and R. B. Rai. 2004. Use of RAPD-PCR for genetic analysis of Nicobari fowl of Andamans. Br. Poult. Sci. 45:194-200.

Ali, B. A., M. M. M. Ahmed and O. M. Ali. 2003. Relationship between genetic similarity and some productive traits in local chicken lines. African J. Biotech. 2:46-47.

Bossak, N., T. Furukawa, A. Dmitriyev Smirnov and T. Mitsuhashi. 2001. Random amplified polymorphism DNA assay for detecting genetic variations in chicken subjected to long-term selection. IX Plant and Animal Genome Conference. San Diege, CA.

Caetano-Anolles, G., B. J. Bassam and P. M. Gresshoff. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Biotechnology 9:553-557.

Dunnington, E. A., O. Gal, P. B. Siegel, A. Haberfeld, A. Cahaner, U. Lavi, Y. Plotsky and J. Hillel. 1991. Deoxyribonucleic acid fingerprint comparisons between selected populations of chickens. Poult. Sci. 70:463-467.

Lynch, M. 1991. Analysis of population genetics structure by DNA fingerprinting, In: (Ed. T. Burke, G. Dolf, A. J. Jeffreys and R. Wolff) DNA fingerprinting: Approuches and applications, pp. 113-126 (Basel, Swizerland, Birkhauser Verlag AG).

Nei, P. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genet. 89:583-590.

Okimotto, R. and J. Dodgson. 1996. Improved PCR amplification of multiple specific alleles using internally mismatched primers. Biotechniques, 21:20-26.

Plotsky, Y., M. G. Kaisar and S. J. Lamont. 1995. Genetic characterization of highly inbred chicken lines by two DNA methods: DNA fingerprinting and polymerase chain reaction using arbitrary primers. Anim. Genet. 26:163-170.

Saifi, H. W., B. Bhushan, S. Kumar, P. Kumar, B. N. Patra and A. Sharma. 2004. Genetic identity between Bhadawari and Murrah breeds of Indian buffaloes (*Bubalus bubalis*) using RAPD-PCR. Asian-Aust. J. Anim. Sci. 17(5):603-607.

Sharma, D., K. B. Appa Rao, R. V. Singh and S. M. Totey. 1998. Random amplified polymorphic DNA (RAPD) for evaluating genetic relationship among guinea fowl varieties. Genetical analysis: Biomolecular engineering.

Sharma, D., K. B. Appa Rao, R. V. Singh and S. M. Totey. 2001. Genetic diversity among chicken breeds estimated through randomly amplified polymorphisc DNA. Anim. Biothechnol. 12:111-120.

Sharma, D. and R. V. Singh. 2001. Random amplified polymorphic DNA polymorphism in Rhode Island Red chicken. Indian J. Poult. Sci. 36:1-4.

Sharma, A. K., R. K. Singh, S. Kumar and S. Sharma. 2001. Genetic differentiation of selected and control lines of White Leghorn and Rhode Island Red using DNA markers. Indian J. Poult. Sci. 36:5-8.

Shivaraman, G. K., D. Sharma, S. Haunshi and A. K. Sharma. 2001. Randomly amplified polymorphic DNA polymorphism among native and exotic chicken breeds. Indian J. Poult. Sci. 36:141-146.

Singh, R. V. and D. Sharma. 2002. Within and between strain genetic variability in White Leghorn population detected through RAPD markers. Br. Poult. Sci. 43:33-37.

Smith, E. J., C. P. Jones, J. Bartelett and K. E. Nestor. 1996. Use of Randomly Amplified polymorphic DNA markers for the genetic analysis of related ness and diversity in chickens and turkey. Poult. Sci. 75:579-584.

Tercic, D., A. Holcman and P. Dovc. 1998. Three RAPD markers for growth performance in chicken. The *VI* Plant and Animal Genome Conference. San Diege, CA.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18:7213-7218.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey. 1990. DNA Polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 14:4605-4616.

Yoon, J. M. and J. Y. Kim. 2004. Genetic differences within and between populations of Korean Catfish (*S. asotus*) and Bullhead (*P. fulvidraco*) analysed by RAPD-PCR. Asian-Aust. J. Anim. Sci. 17(8):1053-1061.

Zhang, X., G. R. Mcdaniel and J. J. Giambrone. 1995. Random Amplified polymorphic DNA comparisons among broiler lines selected for incidence of tibial dyschondroplasia. Poult. Sci. 74:1253-1258.