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Genetic Diversity Assessing of Indian Chicken Breeds and Commercial Broiler Strains Using Genetic Markers

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Abstract

Breed characterization requires determination of genetic variation which can be effectively measured within populations. Two Indian chicken breeds (Gramapriya and Kadaknath) and one commercial broiler strain (meat type) were examined genetically using genetic markers system. RAPD analysis revealed the high-level polymorphisms among the breeds thereby indicating their genetic variations. Phylogenetic consensus tree indicated the negligible genetic distance between the selected breeds. Among the 18 blood samples were selected, eight samples were sequenced which revealed multiple SNPs by using PCR for SNP marker M1 and M24. Further, sequencing analysis detected that SNP in *OBR* gene is associated with fat deposition. This study reveals the need of understanding the genetic diversity at molecular levels for effective conservation and utilization of chicken breeds in India.

Keywords

SNP, RAPD, PCR-RFLP, Sequencing, Gramapriya, Kadaknath, Broiler

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INTRODUCTION:

Chicken was domesticated by the Aryan people in the Indus Valley civilization during 2500-2000 B.C. (West and Zhou, 1989; Crawford, 1995). Chickens play very significant socio-cultural and economic roles in Indian societies. Despite the importance, little is known about its genetic diversity regarding the different types and local population varieties. India has 15 different breeds of chicken that have been documented primarily based on morphometric attributes (Acharya and Bhat, 1984). Indigenous chicken breeds are typically reared in extensive systems and are characterized by a higher resistance to disease. The demand for meat products

from these indigenous breeds has increased due to their nutritious and healthy image.

Breed utilization, genetic improvement, and industry consolidation are predicted to have major impacts on the genetic composition of commercial chickens. Consequently, the question arises as to whether sufficient genetic diversity remains within industry stocks to address future needs (Muir et al., 2008).Knowledge-based phenotypic and genotypic data is essential for the characterization of indigenous animal genetic resources providing needed information for effective conservation of useful gene pool against future uncertainties in the face of current global challenges such as population growth and rising consumer demands challenges. Recently, molecular approaches have been more commonly used to obtain less ambiguous results than classical approaches.In the process of evaluating genetic diversity between different chicken breeds, modern molecular tools are of special interest (Groenen et al., 2000; Osman et al., 2006).DNA markers are widely used for genomic mapping, identification of genes controlling commercial traits and analysis of consequences of long term selection and inbreeding (Geldermann and Ellendorff, 1990; Darvasi, 1998; Heyen*et al*., 1999; Buitkamp*et al*., 2005).

DNA-based genetic markers, microsatellites (Short Tandem Repeats (STR) are widely used in gene marker studies due to their co-dominant, highly polymorphic nature, dense distribution in the genome and easy genotyping.The very first DNA markers used were restriction fragment length polymorphisms (RFLP) based on its sequence dissimilarity at restriction enzyme sites (Botstein et al. 1980). RAPD polymorphisms result from DNA sequence variation at primer binding sites and from DNA length differences between primer binding sites. The subsequent generation of markers was single nucleotide polymorphisms (SNP). Single nucleotide polymorphisms are the most recent contribution to studying DNA sequence variation. In the chicken genome, SNP have been identified with a frequency of 1 SNP per 225 bp, which is 5 times as many as in humans (Vignal et al., 2002). The SNP markers are alreadyused to identify disease resistance genes in chickens (Emaraand Kim, 2003; Malek and Lamont, 2003). This study was aimed to determine the genetic variations among Indian chicken breeds and commercial broiler strains using RFLP, RAPD and SNP methods.

METHODOLOGY:

Sample collection

Eighteen blood samples comprised of randomly selected unrelated commercial breeding line, one meat-type white broiler chicken and two Indian local breeds (Gramapriya and Kadaknath) were collected from the wing vein in EDTA 2.0 mL tubes. The samples were transported on ice and subsequently stored at –20°C. DNA from blood samples was exacted using the Spin column kit method and the isolated DNA was quantified in UV-VIS spectrophotomer (Vivaspec Biophoto meter, Germany).

RAPD Amplification

RAPD reaction was performed as per the method suggested by Williams *et al*., (1990) with minor modifications in the annealing temperature of the PCR profile.

Table-1: description of RAPD primers used in the study

Primer	Sequence (5'→3')
OPAA-01	AGACGGCTCC
OPAA-02	GAGACCAGAC
OPAA-03	TTAGCGCCCC
OPAA-04	AGGACTGCTC
OPAA-05	GGCTTTAGCC
OPAA-06	GTGGGTGCCA
OPAA-07	CTACGCTCAC
OPAA-08	TCCGCAGTAG
OPAA-09	AGATGGGCAG
OPAA-10	TGGTCGGGTG

Amplification was performed in an automated thermal cycler (G-storm, Gradient, UK). Different temperature profiles and cycle repeats were tested for optimizing the PCR. For RAPD amplification, the initial temperature of 94°C for 3 mins, denaturation temperature of 94°Cfor 1 min with annealing and synthesis temperature of 37°Cand 72°C for 1 min was found optimum. The final extension was set as 72°C for 10 mins.Data analysis was done by unweighted pair group method using arithmetic averages (UPGMA) analysis of Dice similarity coefficient using the computer program NTSYSpc 2.0

SNP analysis:

The specific primers were designed using Primer3 Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/) and the designed

oligonucleotides were synthesized in Sigma Corporation USA.

PCR primers for SNP amplification

Marker No	SNP	Variations	Primer (5'→3')	Annealing T (°C)	Product length (bp)	Position of SNP	Genetic marker	Restriction enzyme
M1	rs15197960	G/T	TGCAACACAAGATGCTTTCC CATGGATGCTTTCAGCTTCA	56	595	131	ACW0388	*Dra*1
M24	rs14915286	A/G	TAGCTTTGGCATCCTCACCT AGAAATGTGGATGGGAGCAC	60	522	264	RBsts1	*Alu*1

Restriction fragment length polymorphism (RFLP)

Amplified specific product using M1 and M24 primers (~550bp) was obtained by PCR and analyzed by restriction digestion with restriction endonucleases. M1 primer product was digested with *Dra*1 and M24 primer product was digested with *Alu*I. Restriction was carried out in 20µl volume containing 5µl PCR product, 10X buffer 2µl, BSA 0.2µl, 2 U restriction enzyme 0.5µl, sterile nuclease free water, 12.3 µl. All the reaction mixtures were incubated at 55ºC in water bath for 6 hours except, *Dra*I which was incubated at 65ºC for 4 hours. Separation of digested products in 2% agarose gel was performed. Interpretation of restriction patterns was based on manual visual scoring the positions of the fragments of molecular size ≥100 bp.

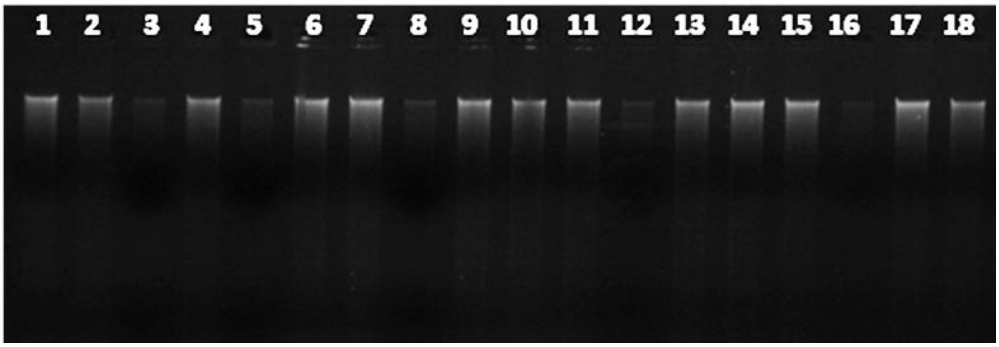
Sequencing

For sequencing the amplifications with ~0.55 kb were analyzed by electrophoresis and were eluted from the gel using gel elution kit (Biolone, USA) according to manufacturer's instructions. The cycle sequencing reaction was performed with 50ng of purified PCR product using ABI PRISM Big Dye Terminators v1.1 cycle sequencing kit (Applied Biosystems Foster city, CA, USA) according to the manufacturer's instruction employing the same primers used for PCR amplification at Eurofins, Bangalore. The purified product was sequenced bi-directionally in order to obtain maximum coverage of the gene on ABI PRISM Genetic Analyzer. The sequences were subjected to nucleotide similarity comparison, and the sequences were placed in GenBank, accessed through National Center for Biotechnology Information (NCBI). The program used for comparison was Basic local alignment search tools (BLAST 2.0; http://www.ncbi.nlm.nih.gov/ blast), allowing comparison with sequences present in the GenBank, DDBJ and EMBL databases. Only the highest-scored BLAST result was considered for phylotype identification, with 98% minimum similarity.

RESULTS:

A total of eighteen blood samples were collected and the pattern of genomic DNA in agarose gel electrophoresis is shown in Fig - 1. Quantification of DNA revealed that sample 6 recorded highest DNA content (2120 ng/µl).

Fig -1: Genomic DNA isolated from chicken blood samples



RAPD analysis:

The three operon primers (OPAA3, OPAA7, & OPAA8) produced polymorphic band patterns, which were clear, scorable and interpretable in all the chicken populations. The OPAA3 primer produced a total of 71 bands across all the chicken populations whereas with individual DNA produced 2 to 5 bands, with size ranged from approximately 400 to 3000 base pairs as showed in (Fig- 2). The OPAA7 primer produced most number of bands 138 among three primers with 4-9 bands per sample, with size ranged from approximately 200 to 3000 base pairs as showed in (Fig-3). The OPAA8 primer produced most number of bands 70 among three primers with 2-5 bands per sample, with size ranged from approximately 400 to 3000 base pairs as shown in Fig-4.

Fig-2: OPAA3 primer

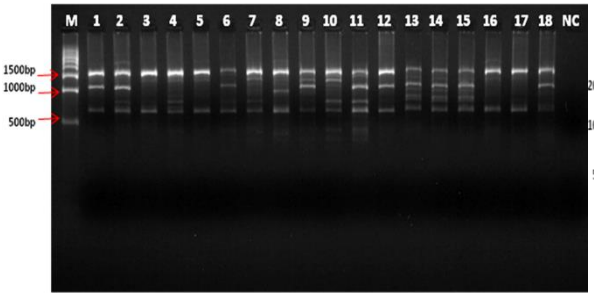


Fig-3: OPAA7 primer

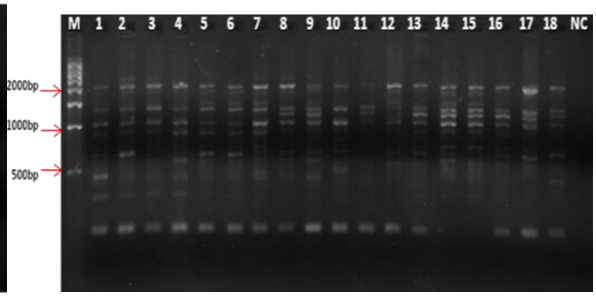
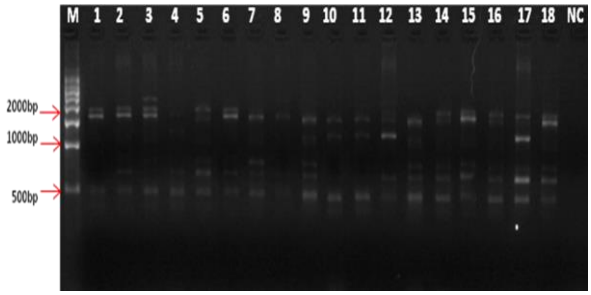


Fig-4: OPAA8 primer



(M-500bp ladder, Lane 1-18 Samples, NC- negative control

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

SNP analysis:

Standardized primers (M1 and M24) were run at optimized conditions. For M1 SNP analysis out of the 18 samples 13 samples showed two bands but 5 samples showed only one band which interpret that these samples had the SNP and created the restriction site hence the product was undigested and showed one band (Fig-5). For M24 SNP analysis out of 18 samples tested 11 samples showed three bands and 7 samples showed two bands which showed presence of the SNP (Fig-6). Thus, the results interpret that these samples share some SNP at investigated positions.

Fig-5: RFLP analysis SNP marker M1 using *Dra*1 restriction enzyme

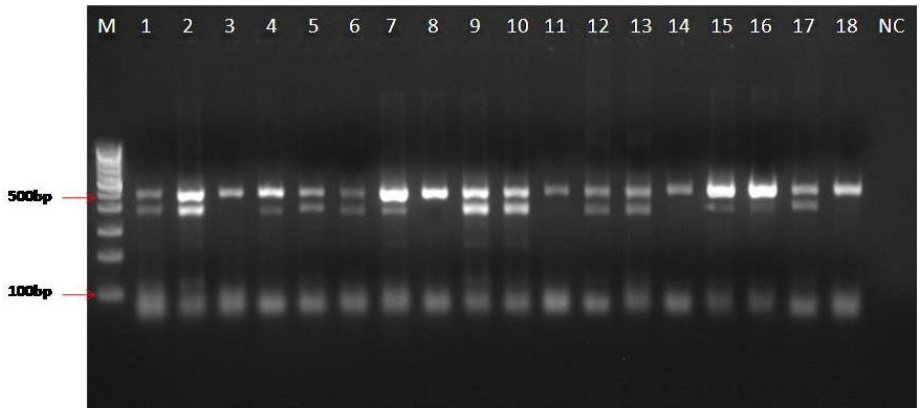
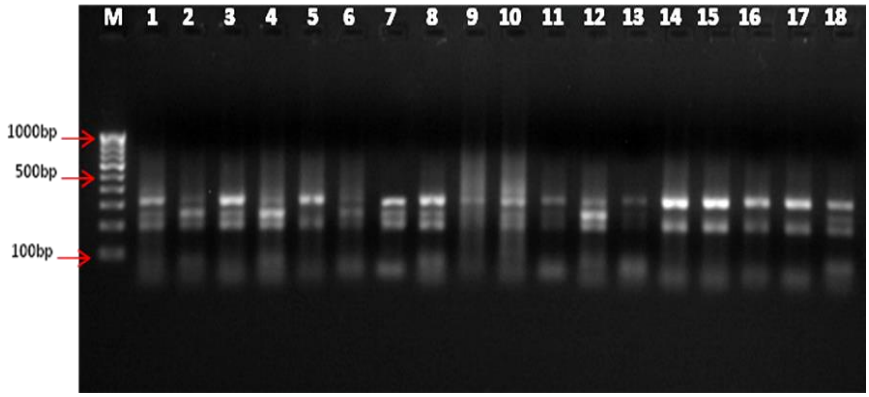


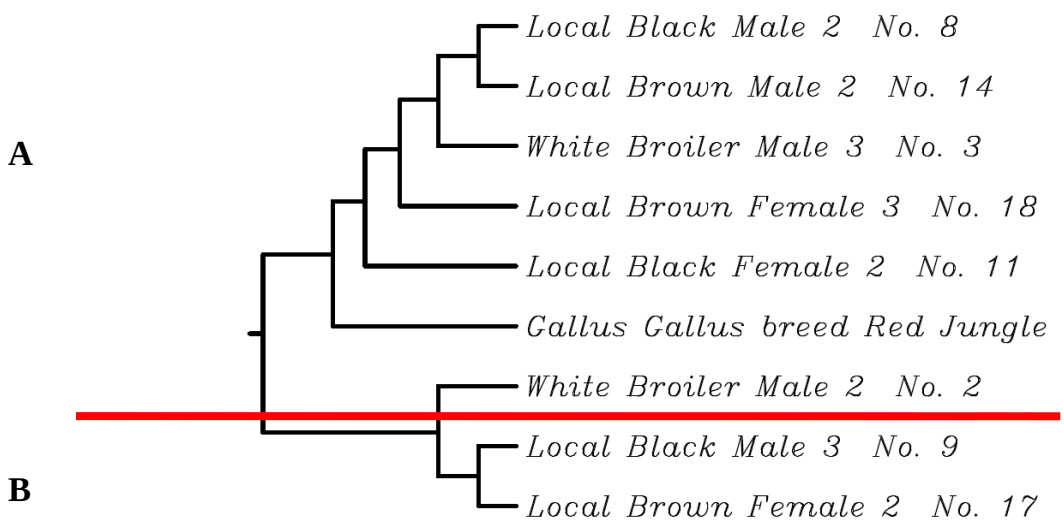
Fig-6: RFLP analysis SNP marker M24 using *Alu*1restriction enzyme



Sequencing analysis

For confirmation of our PCR-RFLP results we performed sequencing for right samples for M1 SNP analysis. The sequencing results of the samples
White Broiler Male 2 (No.2)
(>0513_062_009_CHK2_CHK-M1-A12.ab1),
White Broiler Male 3 (No.3)
(>0513_062_010_CHK3_CHK-M1-B12.ab1),
Local Black Male 2 (No.8)
(>0513_062_011_CHK8_CHK-M1-C12.ab1),
Local Black Male 3 (No.9)
(>0613_002_001_CHK9_CHKM1-H03.ab1),
Local Black Female 2 (No.11)
(>0513_062_012_CHK11_CHK-M1-D12.ab1),
Local Brown Male 2 (No.14)
(>0513_062_013_CHK14_CHK-M1-E12.ab1),
Local Brown Female 2 (No.17)
(>0613_002_002_CHK17_CHKM1-A04.ab1),
Local Brown Female 3 (No.18)
(>0513_062_014_CHK18_CHK-M1-F12.ab1)}
The obtained sequence of M1 allele were further used for multiple sequence alignment in Clustal W software and compared. The results were shown in Fig-7. The local black female 2 No.11 match to red jungle showing their similarity for M1 allele sequence. Other samples formed two clusters showing their similarity. Cluster (A) showed these digested samples and cluster (B) showed these undigested samples.

Fig-7: Phylogenetic tree (UPGMA) ofCLUSTAL 2.1 multiple sequence alignment



Note: A-digested sample; B- undigested sample

DISCUSSION:

The chicken is not only a major livestock animal but also an excellent model organism for genetic and evolutionary studies such as for detecting natural and artificial selection under domestication (Burt and White, 2007).Previous studies have also shown that a large amount of genetic variation still exists both within and among the domesticated and commercial chicken breeds despite many generations of selection (Wong et al., 2004, Rubin et al., 2010). PCR-RFLP technique exploits that SNPs are associated with the creation or abolishment of a restriction enzyme recognition site (Narayanan, 1991) and is used for species identification and differentiation (Sankar et al., 2011). Using primers targeting a conserved region in the 12S rRNA gene, followed by restriction enzyme treatment and electrophoretic separation, closely related poultry species could be differentiated (Saini et al., 2007). However, often the ability to differentiate between closely related species requires application of more than one restriction enzyme (Rojas et al., 2009). With three RFLP primers used in this study, differences were observed in the band patterns. The differences in both the number and size of the amplified fragments indicate genomic DNA diversity among the chicken populations. Thus, the RFLP results obtained in the present study conclude that all the varieties have differences in their genomic DNA.

In the SNP analysis for M1 locus, out of the 18 samples 5 samples showed presence of the SNP in the restriction site. Eight samples were selected {five samples (sample No. 3, 8, 11, 14, and 18) based on the undigested bands which presence of the SNP in restriction site and three samples (sample No. 2, 9, and 17 based on the digested bands) from the 18 samples and sequenced. Since we were designed the primer based on the single nucleotide but in the result of sequencing five G allele and 3 three T alleles were observed in the restriction site (TTTAAA) for *Dra* 1 enzyme. The results from the current study detected the SNP in the *OBR* gene is associated with fat deposition. Therefore, detection of these SNP as molecular marker can improve genetic selection programs.

SNP are a new type of DNA polymorphism, mostly bi-allelic, but widely distributed along the chicken genome (Vignal et al., 2002).Jalving et al., (2004) has used SNP for genetic mapping of chicken breeds. In the SNP analysisof OBR gene for M1 and M 24 both alleles were observed. For M1 locus, out of the 18 samples 5 samples showed SNP which created the restriction site. For M24 SNP analysis out of 18 samples tested 11 samples showed three bands and 7 samples showed two bands which showed presence of the SNP. Eight samples were selected from the 18 samples and sequenced for M1 locus. In the sequencing five G allele and 3 three T alleles were observed. Wang et al., (2006) has reported that OBR gene is associated with fat traits in chicken. A SNP was detected in thechicken

OBR gene Exon9 in the previous studies (Gu et al., 2002).

With three primers used in this study, there were real differences in the patterns of fragments produced. The differences in both the number and size of the amplified fragments indicate genomic DNA diversity among the chicken populations. In our results black m1 7 and black m2 8 showed more similarity to broiler chicken samples. Production performance and fitness traits were negatively correlated in chicken (Martin et al., 1990; Pinard et al., 1998). Molecular marker-assisted selection (MAS) may be required and the integration of traditional genetic selection and modern molecular methods may be preferred for breeding chickens in the future (Li et al., 2003). Broiler chickens are known for high fat production hence these two samples can be used for fat production.

CONCLUSION:

The commercial breeding programs of broiler chickens have become more complex and challenging because so many objectives need to be simultaneously considered to reduce production costs, maintain health, and improve product quality. Breeding goals must include increased growth rate, increased breast muscle yield, decreased abdominal fat, maintenance of good development and overall fitness. The relationships of these traits are complex, and some of the traits are very difficult to measure. The results from the current study detected the SNP in the *OBR* gene is associated with fat deposition. Therefore, detection of these SNP as molecular marker in chicken breeds is necessary that can be exploited in selective breeding program.

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