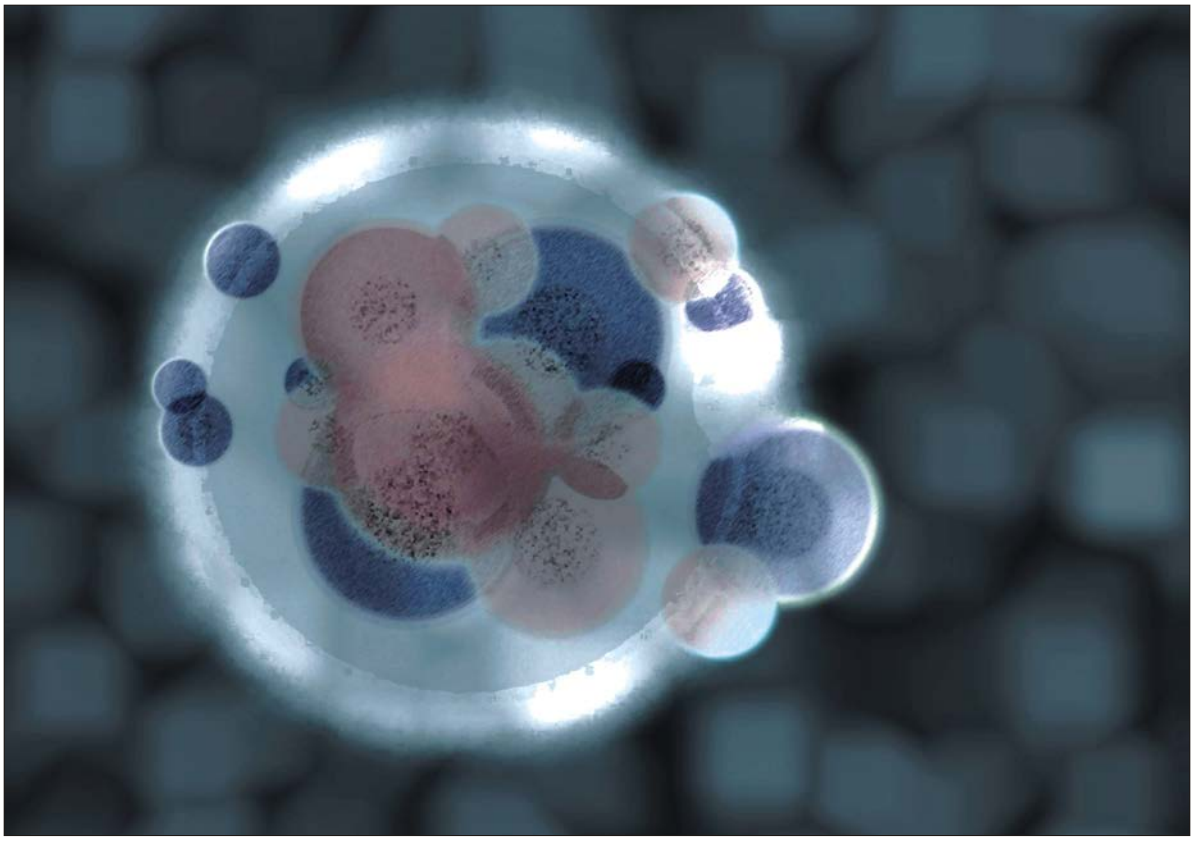

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Molecular Genetic Variations in Indian Goats

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Abstract: In the present investigation, RAPD-PCR was employed to study genetic variations with in six breeds of Indian goat viz. Barbari, Black Bengal, Jamnapari, Marwari, Sirohi and Jhakrana breeds. Gene diversity is an appropriate measure of genetic variability with in a population. The polymorphism found within breeds varied between primers and breeds. Only small proportion (40.93%) of the total bands amplified from 10 selected primers were polymorphic. The information generated from the polymorphic patterns revealed by all the 10 primers was used to find out gene diversity using Pop Gene program (Population Genetic Analysis). The highest gene diversity was found in Black Bengal (0.2378±0.207) while the lowest was found in Sirohi (0.0992±0.174). Higher gene diversity might have been contributed by introduction of out breed population and random mating among individuals. The low genetic variability might be due to the fact that samples were collected from farm and there was more probability for selection rather than random mating thus accounting for low variation with in the breed.

Key words: Goat breed, RAPD, polymorphism, gene diversity

INTRODUCTION

Local livestock breeds are products of indigenous knowledge and should be regarded as national asset. However, due to indiscriminate breeding within the native stock as well as with exotic breeds, there is a marked decline in the population of unique animals conforming to the true attributes of native breeds. Awareness of the value of genetic resource has stimulated the study of genetic diversity of native breeds. Genetic variations that can be effectively measured within and between populations make the basis of breed characterization (Hetzel and Drinkwater, 1992). Therefore the detailed knowledge on genetic variation within and among different breeds is very important for understanding and developing endogenous economic traits of breeds (Yeo *et al*., 2000) and for optimizing breeding strategies and regulating germplasm conservation.

There is an utmost need for conservation of indigenous goat breeds, one of the prime animal genetic resources of India. It has second largest goat population of 170 million heads, which represents 21% of the world's population (807 million, FAO, 2005).

There are twenty well-defined breeds of goats apart from many non-descript goats (Acharya, 1982; Joshi *et al*., 2004). The breeds are diverse and specific in their performance traits. These have evolved and adapted under varied climatic conditions ranging from cold arctic type climate of Ladakh region (-40°C) to hot humid climate of coastal regions and dry hot climate of Rajasthan (40°C).

Genetic markers provide useful information at different levels: population structure, gene flow, phylogenetic relationship, patterns of historical biogeography and the analysis of parentage and relatedness (Feral, 2002). The development in DNA technologies have made it possible to uncover a large number of genetic polymorphism at the DNA sequence level and to use them as markers for evaluation of the genetic basis for observed phenotypic variability. The DNA markers possess unique genetic properties and methodological advantages that make them more useful and amenable for genetic analysis compared to other genetic markers. The DNA markers provide information on every region of genome at nucleotide level regardless of the level of gene expression and also remain unaffected by environmental and developmental changes. Molecular markers have been shown to be an efficient tool in quantification of genetic diversity of various populations (Saitbekova *et al*., 1999; Barker *et al*., 2001).

RAPD-PCR was employed in this study to find out genetic variations among six breeds of Indian goats. This technique has achieved a great deal of acceptance due to its simplicity, readability directly on gel, low cost investment, requirement of little amount of DNA (William *et al*., 1993). This is also highly informative without prior knowledge of sequence information. RAPD-PCR technique has been successfully applied for differentiating geographically isolated populations or breeds (Bowditch *et al*., 1993), species identification (Kemp and Teale, 1994), establishing genetic relationship

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(Appa Rao *et al*., 1996; Smith *et al*., 1996; Geng *et al*., 2002), estimating genetic diversity in different livestock and poultry species (Ali, 2003; Kumar *et al*., 2003; Bhattacharya *et al*., 2004; Parmar *et al*., 2004; Chen *et al*., 2004; Mollah *et al*., 2005; Oliveira *et al*., 2005), genome mapping (Levin *et al*., 1993), characterization of different livestock breeds (Gwakisa *et al*., 1994; Nagaraja *et al*., 2003; Bhattacharya *et al*., 2003; Sukla, 2003; Jha, 2004), Inbred chicken lines (Plotsky *et al*., 1995; Wei *et al*., 1997) and quail lines (Kumar *et al*., 2000).

MATERIALS AND METHODS

A total of 180 animals of both sexes were selected at random from six breeds of goats viz., Barbari, Black Bengal, Jamnapari, Marwari, Sirohi and Jhakrana breeds maintained at either in their native breeding tracts or organized herd at various places. Barbari and Jamnapari goats are found in Agra, Matura and Aligarh districts of Uttar Pradesh State (Northern part of India). Jamnapari is the biggest and most majestic breed. Barbari is the small sized goat breed. It is mainly meant for milk. Marwari, Sirohi and Jhakrana are native of Rajasthan state (Northwestern part of India). Black Bengal goat is native of West Bengal state (Northeastern part of country). It is known for high prolificacy and fine quality meat.

DNA extraction: Blood was collected (about 10-20 mL) from jugular vein of each animal aseptically in separate sterile glass vacuum tube containing ACD (acid citrate dextrose). The samples were stored at -20°C. DNA was isolated from blood following the protocol of Clamp *et al.* (1993) with some modifications (Shashikanth, 1999). Quantity and quality of DNA was determined by U.V. Spectrophotometeric method. Quality of the DNA was checked by the ratio between OD260 and OD280 and also by 0.8% agarose gel electrophoresis. Out of 180, good quality DNA of 144 animals, 24 from each breed was chosen for genotyping. All the samples were standardized to 30 ng/5 mL.

Primers: A total of 40 random oligonucleotide primers were used for amplification. All the random primers were 10 bp long and with high GC content and were custom synthesized from M/s Bangalore Genei, Bangalore, India. The preliminary screening revealed 10 primers to be informative and used in the subsequent study.

PCR amplification: The PCR reactions were performed in 15 μL volume having 30 ng of genomic DNA, 1.25 mM each of dNTPs, 20 pM of primer, 1.0 U of Taq DNA polymerase and 1.5 μL of 10×Taq DNA polymerase buffer and sterile distilled water to make the final volume. PCR cycling conditions comprised of 5 min initial denaturation at 94°C followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 36°C for 45 sec, extension at 72°C for 1 min and a final extension of 5 min at 72°C. The PCR products were electrophoresed on 1.5% agarose gels containing ethidium bromide. The Ø×174 *Hin*f1 digest of Gibco BRL was used as molecular size marker. RAPD bands were visualized under UV Transilluminator and photographed on Kodak B and W film (×100) with Leica Camera (Germany) for documentation and further analysis.

Statistical analysis of DNA fingerprint pattern: The information generated from the polymorphic patterns revealed by all the 10 primers was used to find out gene diversity between six breeds of goats for assessing the genetic variability with in breeds.

Gene diversity: It is an appropriate measure of the genetic variability with in a population. It is also known as heterozygosity. For any given locus heterozygosity is the probability that two alleles chosen at random from population are different from each other. Gene diversity was calculated by Pop Gene program (Population Genetic Analysis) version 1.31 (Yeh *et al*., 1999) (http://www.ualberta-ca/~fyeh/) based on Nei's 1973 method as mentioned below: -

The gene diversity between ith and jth population as

$$D_{ij} = H_{ij} - (H_i + H_j)/2$$
$$= (J_i + J_i)/2 - J_{ij}$$

Where, $H_i = 1 - J_i$ and $H_{ij} = 1 - J_{ij}$

It is considered a population that is subdivided in to sub populations. Where X_{ik} is the frequency of the Kth allele in the ith sub population. The gene identity in the sub population is given by

$$J_i = \sum_k X^2_{ik}$$

and

$$J_{ij} = \sum_k X_{ik} X_{JK}$$

RESULTS AND DISCUSSION

Ten random polymorphic primers were used after initial screening of 40 primers to determine the RAPD polymorphism in six breeds of goats. The Ø×174 *Hin*f1 digested DNA was used as molecular marker. The RAPD profile of one of these primers is shown in Fig. 1. The proportion of polymorphic bands generated in six breeds has been presented in Table 1.

A total of 239 bands were generated from 10 random primers, which gave amplification in all the six breeds. The

Table 1: Number and percentage of polymorphic bands in six breeds of goat

	No. of polymorphic bands (%)						
Total bands	B	BB	JP	M	S	JH	Overall (%)
239	109 (45.60)	153 (64.02)	87 (36.40)	70 (29.29)	69 (28.87)	99 (41.42)	97.83 (40.93)

Table 2: Gene diversity in six breeds of goat

Gene diversity					
B	BB	JP	M	S	JH
0.1779±0.2114	0.2378±0.2073	0.1342±0.1951	0.0993±0.1728	0.0992±0.1744	0.1488±0.2010

B = Barbari; BB = Black Bengal; JP = Jamnapari; M = Marwari; S = Sirohi; JH = Jhakrana

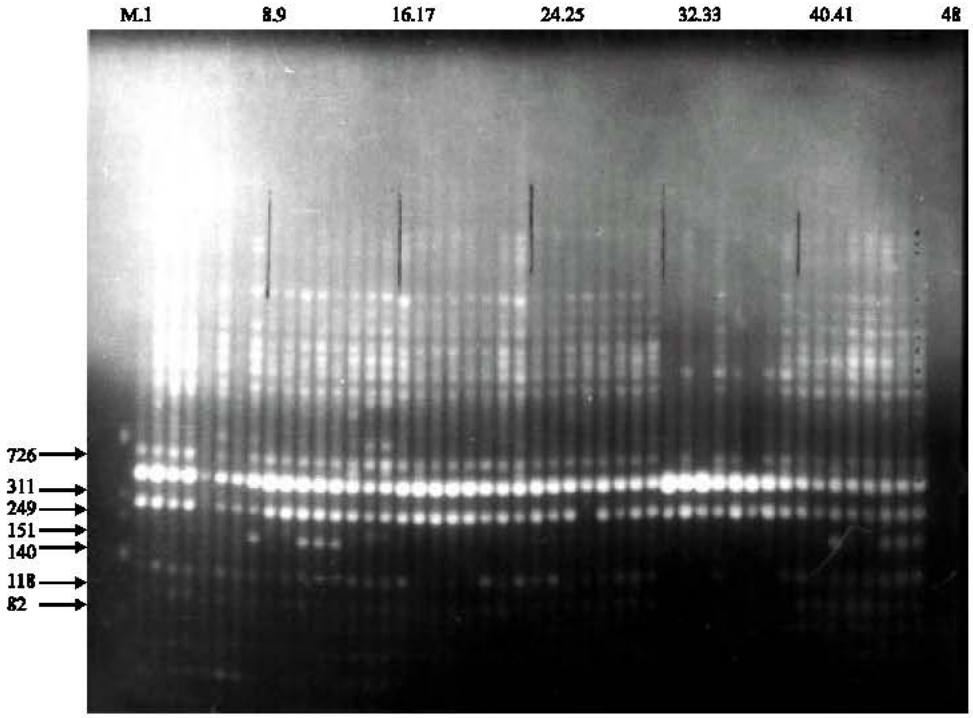

Fig. 1: Polymorphism observed using RAPD primer No. 23 in six breeds of goat. Lane M ϕ X 174 *Hinf*1 Digest, Lanes 1-8 Barbari, 9 to 16. Black Bengal, 17 to 24 Jamnapari, 25 to 32 Marwari, 33 to 40 Sirohi, 41 to 48 Jhakrana

polymorphism found within breeds varied between primers and breeds. In this study only small proportion (40.93%) of the total bands amplified from 10 selected primers were polymorphic. These observations revealed a comparatively low level of genetic diversity between the goat breeds. Similar proportion of polymorphic bands (30-50%) was also reported by earlier workers (Kantanen *et al*., 1995; Wei *et al*., 1997; Anabarason *et al*., 2002).

Gene diversity is an appropriate measure of genetic variability with in a population. Gene diversity was calculated based on Nei's 1973 method. The gene diversity values with in six breeds have been given in Table 2.

The highest Mean heterozygosity was found in Black Bengal (0.2378±0.207) while the lowest was found in Sirohi (0.0992±0.174). These suggested that there was high proportion of heterozygotes genotype in Black Bengal. Higher heterozygosity might have been contributed by introduction of out breed population and random mating among individuals. Low level of heterozygosity with in Sirohi breed indicates low variations or more similarity with in breed. The low genetic variability might be due to the fact that samples were collected from farm and there was more probability for selection rather than random mating thus accounting for low variation with in the breed. Similar range (0.137 to 0.203) of mean heterozygosity values has also been reported by Stephen *et al*. (http\\www.ihhkvl.dk\htm\php\tsapoo \stephen.htm.) while studying RAPD in five ecotypes of sheep. Ganai and Yadav (2001) studied three Indian breeds of goat and revealed average heterozygosity

value of 0.56 using 16 micro satellite markers. This might be due to the fact that micro satellite analysis shows more heterozygosity as compared with RAPD (Zhang *et al*., 2002). The results of this study demonstrate the usefulness of RAPD approach in detecting polymorphism in six breeds of goats. The majority of random primers used gave distinctly reproducible pattern in entire breed studied. However, primer varied in extent of information they generated with some producing highly polymorphic pattern where as other produced less polymorphic products However, approaches employing several DNA marker systems should increase accuracy of genetic studies of these breeds.

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