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Genetic Diversity among Six Breeds of Indian Goat Using RAPD Markers

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Abstract: The genetic variations were studied by RAPD technology among six breeds of Indian goats viz. Barbari, Black Bengal, Jamnapari, Marwari, Sirohi and Jhakrana. Out of 40 primers screened using DNA samples of six goat breeds, only 10 primers generated reproducible and distinct RAPD profile. Only distinct and prominent bands were scored. Estimates of between breed's similarity as indicated by genetic identity and genetic distance ranged between 0.7156 to 0.9506 and 0.0506 to 0.3347, respectively. From dendrogram, it has been observed that Marwari- Sirohi breeds have closer relationship forming a distinct cluster whereas Black Bengal and Barbari were distinctly different from each other.

Key words: Goat breed, polymorphism, genetic identity, genetic distance, dendrogram

INTRODUCTION

Goat is the most prolific ruminant among all domesticated ruminant under tropical and subtropical conditions. It is resourceful and efficient ruminant producing meat, milk, skin and hair. India has the second largest goat population of 170 million heads, which represents 21% of the world's population (807 million, FAO, 2005). The Indian subcontinent contains 20 well-characterized goat breeds, which vary in their genetic potential for the production of milk, meat and fiber; disease resistance; heat tolerance and fecundity (Joshi *et al*., 2004). There is distinct zonal distribution of goats for meat and milk or both. Important milch breeds like Jamnapari, Jhakrana and Barbari are found in northwestern zone, dual-purpose breeds are found in dry southern zone and high prolific meat breeds like Black Bengal are found in northeastern zone. The detailed knowledge on genetic variation within and among different breeds is very important for understanding and developing endogenous economic traits of breeds and for optimizing breeding strategies and regulating germplasm conservation (Yeo *et al*., 2000). 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). The present study was undertaken to study genetic variations among six breeds of Indian goat using RAPD technique. This technique has achieved a great deal of acceptance due to its simplicity, readability directly on gel, low cost investment and requirement of little amount of DNA (Williams *et al*., 1993). This is also highly informative without prior knowledge of sequence information.

MATERIALS AND METHODS

The investigation was carried out on 144 unrelated animals belonging to six breeds of goat viz., Barbari, Black Bengal, Jamnapari, Marwari, Sirohi and Jhakrana. Marwari, Sirohi and Black Bengal are important meat breeds while Jhakrana, Jamnapari and Barbari are important milch breeds. Black Bengal breed is known for high prolificacy and fine quality meat while Jamnapari is the biggest and most majestic milk breed.

RAPD study was carried out in three different sets, each set comprising of 8 animals of each breed making a total of 48 animals for each set. All the parameters of amplifications and electrophoresis were kept similar for all three sets to find out the repeatability of the technique. Blood samples were collected at random from 24 animals of both sexes of each of six breeds. Black Bengal and Jhakrana breeds were maintained in their native breeding tracts in Rajasthan and West Bengal states respectively. Samples for Barbari, Jamnapari and Sirohi breeds were collected from organized herd of Central Institute for Research on Goats (CIRG) Makhdoom, Mathura (Uttar Pardesh) and for Marwari from Central Arid Zone Research Institute (CAZRI), Jodhpur (Rajasthan).

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.

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Table 1: List of RAPD primers

Primer No.	Sequence	Length (bp)
2	5' CCGCGCCGGT 3'	10
4	5' CAGCCTCGGC 3'	10
7	5' ACGTCGAGCA 3'	10
11	5' GCACTGAGTA 3'	10
14	5' GCTGCTCGAG 3'	10
15	5' GCTAGCTACG 3'	10
19	5' TCGCGAGCTG 3'	10
21	5' GTGACGTAGG 3'	10
23	5' GTCCACACGG 3'	10
25	5' TTAGCGCCCC 3'	10

Spectrophotometeric method. Quality of the DNA was checked by the ratio between OD260 and OD280 and also by 0.8% agarose gel electrophoresis.

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 polymorphic and used in the subsequent study. The detail of the sequence is given in Table 1.

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. Amplification profile consisited 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 ϕX174 *Hinf*1 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 (x100) with Leica Camera (Germany) for documentation and further analysis.

Statistical analysis of band patterns: Only distinct and prominent bands were scored. The presence and absence of a band was recorded as 1 and 0, respectively. The genetic similarity and genetic distance was calculated on the basis of Nei's (1972) method of genetic identity and genetic distance respectively using Pop Gene program (Population Genetic Analysis) version 1.31 (Yeh *et al*., 1999). Dendrogram based on distance matrices of genetic distances (Nei's, 1972) between all pair of operational taxonomic units (OTUs) i.e., breeds were constructed using Pop Gene program. This program is an adoption of Neighbor procedure of Phylip version 3.5 (Felsenstein, 1995).

RESULTS AND DISCUSSION

Out of 40 primers screened using DNA samples of 6 goat breeds, only 10 primers generated reproducible and distinct RAPD profile. All the primers detected polymorphic bands among six breeds and RAPD fingerprints ranged from 0.168 to 3.6 kb. The characteristics of amplification profiles generated by primer No. 15 in six breeds of goats are presented in Fig. 1. Estimates of between breed's similarity as indicated by genetic identity and genetic distance ranged between 0.7156 to 0.9506 and 0.0506 to 0.3347, respectively (Table 2). These two measures of genetic relatedness revealed similar trend of relationship among 6 breeds of goats. Similar ranges of genetic identity and genetic distance have also been obtained by other workers (Chen *et al*., 2001; Anabarason *et al*., 2002; Oliveira *et al*., 2005).

The dendrogram was constructed from Nei's genetic distance matrix using Un weighed pair group method using an arithmetic average (UPGMA) method of clustering by Drawgram Program of Phylip package (Fig. 2). From dendrogram it has been observed

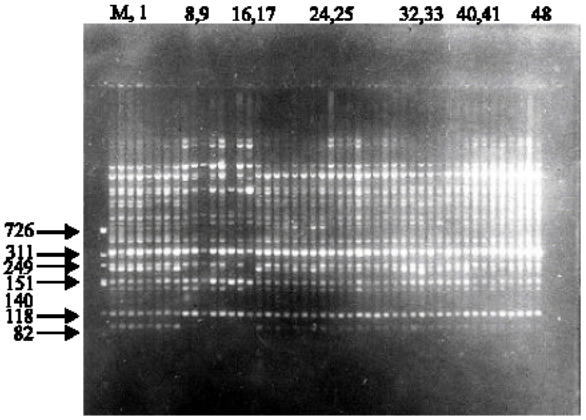

Fig. 1: Polymorphism observed using RAPD primer No. 15 in 6 breeds of goat lane M ϕX174 *Hinf*1 Digest, lane1-8 Barbari, 9-16. Balck bengal, 17-24 Jamnapari, 25-32 Marwari, 33-40 Sirohi, 41-48 Jhakrana

Table 2: Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among six breeds of Indian goats

	B	BB	JP	M	S	JH
B	****	0.7156	0.8151	0.7668	0.7666	0.7514
BB	0.3347	****	0.7983	0.7954	0.7922	0.7950
JP	0.2044	0.2252	****	0.9125	0.9175	0.8799
M	0.2655	0.2289	0.0916	****	0.9506	0.9174
S	0.2658	0.2329	0.0861	0.0506	****	0.9327
JH	0.2858	0.2294	0.1279	0.0862	0.0697	****

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

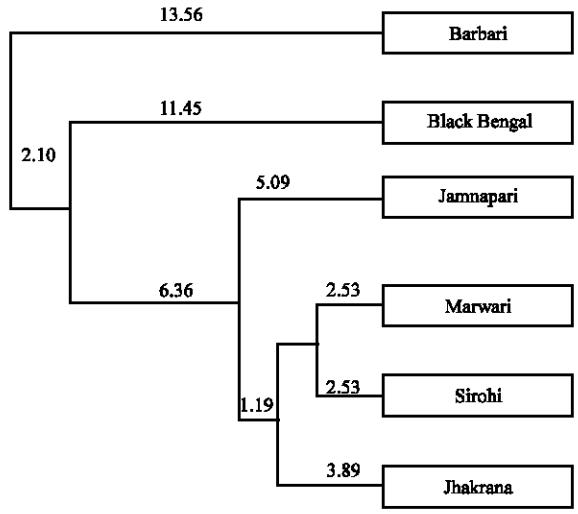

Fig. 2: The phylogenetic tree showing relationship between 6 breeds of goat

that Marwari-Sirohi breeds have the closer relationship forming a distinct cluster whereas Black Bengal and Barbari were distinctly different from each other. Sirohi breed showed maximum genetic similarity and least genetic distance with Marwari. This might be due to the fact that both breeds belong to same zone and also reared for same purpose i.e., for meat production. Barbari breed showed less genetic similarity and maximum genetic distance with Black Bengal again coinciding with their zonal distribution; both breeds are natives of different states falling under different zones and also they are reared for different purposes i.e., Black Bengal is a meat breed while Barbari is reared for milk. Further more, the Barbari breed is supposed to have its origin in East Africa (Jindal, 1984; Ganai and Yadav, 2001; Joshi *et al*., 2004) while all other studied breeds has its origin in Indian subcontinent.

The present study revealed the efficacy of RAPD markers in detecting the polymorphism among the breeds and establishing relationship among them. The technique has been successfully applied for establishing genetic relationship (Appa Rao *et al*., 1996; Geng *et al*., 2002) and estimating genetic diversity in different livestock species and poultry (Ali, 2003; Bhattacharya *et al*., 2004; Parmar *et al*., 2004; Mollah *et al*., 2005).

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