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Genetic variability among sheep breeds by random amplified polymorphic DNA-PCR

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Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was employed to assess the genetic variability and phylogenetic relationship among six breeds of sheep, viz., Malpura, Kheri, Chokla, Garole and two crossbreds Avikalin and Bharat Merino. Twenty-four individuals from each breed/crossbred were selected randomly. Initially, 40 primers were screened, of which 16 were found polymorphic and utilized for estimation of genetic variability and phylogenetic relationship among the breeds. The genetic distance was found highest between Malpura and Garole (D=0.1428), and the lowest (D=0.0612) between Avikalin and Chokla. However, the genetic identity was observed highest (I = 0.9406) between Avikalin and Chokla and the lowest (I = 0.8669) between Malpura and Garole. The Kheri sheep was found close with Chokla (D=0.0741), followed by Malpura sheep (D=0.0902) based on genetic distance. The phylogenetic tree also showed that Avikalin and Chokla are more close, whereas Malpura and Garole are distant to each other. The present study suggested that RAPD-PCR can be used successfully for analyzing genetic variation and phylogenetic relationship among breeds of sheep.

Keywords: Dendrogram, genetic distance, genetic identity, RAPD-PCR, sheep breeds

Introduction

In India, there are about 62.6 million sheeps with a vast genetic diversity comprising of 42 breeds. These breeds are widely distributed in varied range of agro-climatic zones of the country facilitating the large genetic diversity. The adaptability of these breeds in different zones represents variation in the gene pool and this variation is the basis for conservation of germplasm of sheep breeds. However, the genetic diversity of breeds of sheep is declining due to the breed substitution/crossbreeding and this warrants serious attention to find out ways to conserve it at the maximum level.

The genetic diversity can be measured by various markers like polymorphisms in the gene product, such as, enzymes, blood groups, serum proteins or antigens; however, these markers do not reveal much polymorphism to differentiate the breeds/species to that extent. Microsatellite DNA markers have been utilized for estimation of genetic variability among exotic breeds of sheep[1-4] as well as in Indian breeds of sheep[5-7]. Similarly, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique has been used for estimation of the genetic variability among the breeds/species, but it has low reproducibility[8]. RAPD markers are the randomly amplified target regions of less functional part of the genome that do not strongly respond to selection on the phenotypic level. Such amplified regions might accumulate more mutations, thereby offering a wider potential in assessing the intra/interbred genetic differentiations. The RAPD-PCR has been used to estimate the genetic variability among livestock species[9-12], sheep breeds[13-16], and goat breeds[11,17]. However, an application of the RAPD technique in the diversity analysis of Indian breeds of sheep is lacking. Therefore, the present study was undertaken to apply RAPD-PCR technique as a tool to estimate the genetic variability and phylogenetic relationship among six breeds of sheep adapted in the semi-arid climate of Rajasthan state comprising North-West part of India.

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Materials and Methods

Twenty-four animals (irrespective of sex) of each breed of sheep, viz., Malpura, Kheri, Chokla and Garole, and two crossbred strains, viz,. Avikalin and Bharat Merino were taken for the study. Approximately 5 mL venous blood was collected from each animal in 15 mL centrifuge tubes containing 350 µL of ACD (citric acid, sodium citrate and dextrose) as anticoagulant. Genomic DNA was isolated using phenol-chloroform extraction method with slight modifications[19] [50 µL of proteinase K (20 mg/mL), 100 µL of SDS (sodium lauryl sulphate) and 2 mL of DNA extraction buffer was added in each sample and kept at 55°C for digestion]. DNA pellet was air-dried and dissolved in 0.1× TE buffer (*p*H 8.0) and stored at 48°C till further use. The quantity of DNA was measured by UV spectrophotometer and quality of DNA was ascertained through agarose gel electrophoresis. The intact DNA showing no smearing in gels was used for RAPD analysis.

Forty decamer RAPD primers (Operon Technologies Inc.) with 60-70% GC content (OPA-1 to OPA-20 and OPB-1 to OPB-20) were selected from literature and screened on pooled DNA of six breeds (24 animals from each breed). Finally, 16 primers were selected based on their distinct polymorphism and produced more than 7 number of bands. The list of primers, their sequence and GC content has been given in Table 1. The PCR reaction mixture was comprised as: 1× PCR buffer [containing $(NH_4)_2SO_4$], 1.5 m*M* $MgCl_2$, 100 µ*M* dNTPs mix, 1 U Taq DNA polymerase (Bangalore Genei), 25 ng of each primer and 25 ng of template DNA in 20 µL reaction volume. The amplification was performed in Thermal Cycler (Biometra, Germany) with the following amplification conditions: initial denaturation at 94°C for 2 min, followed by denaturation at 94°C for 1 min, annealing at 40°C for 1 min, extension at 72°C for 2 min for 40 cycles and final extension at 72°C for 5 min. The 20 µL PCR product was loaded in 1.5% agarose gel and run at 100 V for 4 h. Gel photograph were captured on gel documentation system.

Table 1—List of RAPD primers and their nucleotide sequences

No.	Primer	Primer sequence	G+C content (%)
1	OPA-6	5'-GGTCCCTGAC-3'	70
2	OPA-7	5'-GAAACGGGTG-3'	60
3	OPA-10	5'-GTGATCGCAG-3	60
4	OPA-15	5'-TTCCGAACCC-3'	60
5	OPA-16	5'-AGCCAGCGAA-3'	60
6	OPA-17	5'-GACCGCTTGT-3'	60
7	OPA-18	5'-AGGTGACCGT-3'	60
8	OPA-19	5'-CAAACGTCGG-3'	60
9	OPA-20	5'-GTTGCGATCC-3'	60
10	OPB-1	5'-GTTTCGCTCC-3'	60
11	OPB-3	5'-CATCCCCCTG-3'	70
12	OPB-5	5'-TGCGCCCTTC-3'	70
13	OPB-8	5'-GTCCACACGG-3'	70
14	OPB-17	5'-AGGGAACGAG-3	60
15	OPB-19	5'-ACCCCCGAAG-3'	70
16	OPB-20	5'-GGACCCTTAC-3'	60

Only distinct and prominent bands were scored for estimation of various parameters. The presence and absence of band was recorded as "1" and "0", respectively. The binary coded characters (1, 0) were used for genetic analysis. Statistical analyses were carried out for estimation of genetic distance, identity and phylogenetic relationship among the six breeds studied.

The genetic distances are designed to express the genetic differences between two populations as a single number. If there is no difference, the distances could be set to zero, whereas if the populations have no allele in common at any locus, the distance may be set equal to its maximum value, i.e., 1. The genetic identity is expressed as the genetic similarity between individuals of same or different populations. The genetic distances (D) and genetic identity (I) were calculated by POPGENE software[20] using standard genetic distance and identity equations[21].

The relationship among breeds of sheep was analyzed by generating dendrogram using Nei genetic distances[21] with UPGMA (un-weighed pair group method using arithmetic average) analysis through POPGENE software[20].

Results and Discussion

Sixteen oligonucleotide primers of random sequences (10-mer) were used to amplify the DNA from six breeds/synthetics and a total 889 scorable RAPD bands were obtained. The DNA fragments amplified in six breeds using primer OPB-19 and OPA-7 have been presented in Figs 1 and 2, respectively. The bands varied from 2 to 35 with a size range varying from 200 to 2000 bp length. The variation in band profile was observed within the breed; however, most of the bands were not variable between individuals of the same breed. The majority

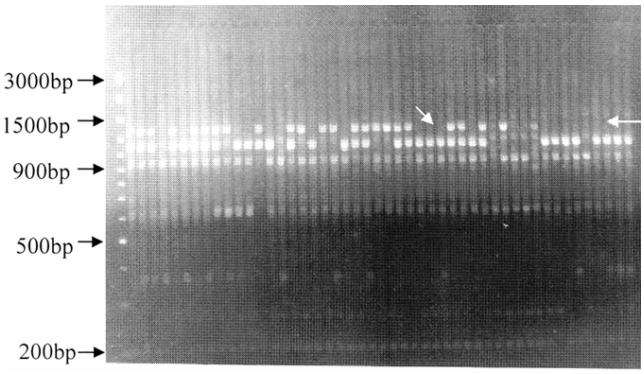

Fig. 1—RAPD profile of six breeds of sheep generated by OPB-19 primer. Lane 1: 100 bp marker, lanes 2-9: Malpura, lanes 10-17: Kheri, lane 18-25: Avikalin, lanes 26-33: Chokla, lanes 34-41: Bharat Merino and lane 42-49: Garole. The difference in the band profile has been shown by arrows (→).

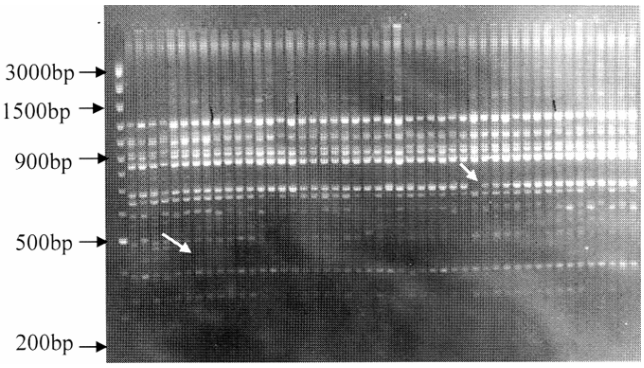

Fig. 2—RAPD profile of six breeds of sheep generated by OPA-7 primer. Lane 1: 100 bp marker, lanes 2-9: Malpura, lanes 10-17: Kheri, lane 18-25: Avikalin, lanes 26-33: Chokla, lanes 34-41: Bharat Merino and lane 42-49: Garole. The difference in the band profile has been shown by arrows (→).

of RAPD primers gave distinctly reproducible bands in all the breeds; some of the primers produced highly polymorphic patterns, but others produced less polymorphic products. The band profile was found similar to the study of Appa Rao *et al.*[9] who reported that the variations may exist between individuals of same species.

The maximum numbers of bands were observed in Garole (13.87 ± 0.26), whereas minimum numbers of bands were recorded in Malpura sheep (13.02 ± 0.24). There was not much difference in the number of bands scored in all breeds using all the primers (Table 2), whereas individual primer showed more variability. Primer OPA-18 gave maximum number of bands (23.1 ± 0.49), while minimum number of bands was recorded with OPA-19 primer (8.04 ± 0.18) in all

Table 2—Average number of bands (± S.E.M.) among breeds of sheep using sixteen primers

Breeds	Av. no. of bands	Min. no. of bands	Max. no. of bands
Malpura	13.02 ± 0.24	2	19
Kheri	13.03 ± 0.26	3	35
Avikalin	13.06 ± 0.26	4	33
Chokla	13.15 ± 0.25	3	32
Bharat Merino	13.63 ± 0.28	5	34
Garole	13.87 ± 0.26	3	34

N=384; Number of observations for each breed

Table 3—Average number of bands (± S.E.M.) per primer

Primer	Av. no. of bands	Primer	Av. no. of bands
OPA-10	13.8 ± 0.40	OPA-7	13.1 ± 0.41
OPA-15	10.1 ± 0.20	OPB-1	16.7 ± 0.41
OPA-16	9.8 ± 0.18	OPB-17	16.0 ± 0.30
OPA-17	12.6 ± 0.57	OPB-19	13.1 ± 0.43
OPA-18	23.1 ± 0.49	OPB-20	12.7 ± 0.30
OPA-19	8.04 ± 0.18	OPB-3	15.5 ± 0.19
OPA-20	9.68 ± 0.19	OPB-5	16.0 ± 0.25
OPA-6	11.2 ± 0.24	OPB-8	12.6 ± 0.29

N=144; Number of observations per primer

the breeds (Table 3). It has been suggested that the sequence of OPA-18 primer may occur frequently in all the breeds and scored maximum number of bands, whereas primer OPA-19 was found less polymorphic within and between breeds. Sharma *et al*[22] found that RAPD technique detects sufficient polymorphism within and between populations. In the present study, an individual primer failed to produce any sex specific or breed specific marker in any of the breed studied. Similarly, Kumar *et al*[16] also did not find any breed specific RAPD marker in Indian breeds of sheep (Madras Red, Mandya, Marwari and Muzaffarnagri).

The genetic distance (D) and genetic identity (I) between breeds were calculated using Nei[21] equations through POPGENE software (Table 4). In the present study, an individual primer produced more variable results as compared to results obtained by all primers; hence all primer data were pooled for estimation of genetic parameters. The genetic distance was found highest between Malpura and Garole (D = 0.1428), and the lowest (D = 0.0612) between Avikalin and Chokla. On contrary, the genetic identity was found highest (I = 0.9406) between Avikalin and Chokla and

Table 4—Nei's genetic distance (below diagonal) and genetic identity (above diagonal) among breeds of sheep based on RAPD analysis

Population	Malpura	Kheri	Avikalin	Chokla	Bharat Merino	Garole
Malpura	***	0.9138	0.9030	0.9017	0.8788	0.8669
Kheri	0.0902	***	0.9384	0.9285	0.8985	0.8923
Avikalin	0.1020	0.0635	***	0.9406	0.9141	0.9005
Chokla	0.1035	0.0741	0.0612	***	0.9290	0.9118
Bharat Merino	0.1292	0.1070	0.0899	0.0737	***	0.9183
Garole	0.1428	0.1139	0.1048	0.0923	0.0852	***

the lowest (I = 0.8669) between Malpura and Garole. These results indicated that Avikalin and Chokla are genetically close, whereas Malpura and Garole are more distant. The interbreed variability may be due to a difference in the population architecture and method of selection where samples were collected. In addition to above, both breeds do not have resemblances in terms of body weight, fleece characteristics, reproduction and others traits. Malpura and Garole sheep have been adapted in their respective geographical/climatic conditions, which may cause wider genetic variability among them.

Avikalin, a crossbred between native Malpura and exotic Rambouillet breeds, showed more similarity with Chokla breed. This breed of sheep has been developed as a carpet wool producer. Although, both Chokla and Avikalin are carpet type breeds, but reasons of closeness between these two breeds are difficult to explain. It is assumed that the fragments shared by two closely related individuals of a breed are allelic, which might not be true when comparing different breeds that show greater diversity as the fragments of same size might have derived from non-allelic regions. The variable climatic conditions may accumulate higher genetic variability within or between breeds. Avikalin is derived from Malpura and exotic Rambouillet breed but it has showed more similarity with Chokla, this may be due to sharing of more number of fragments with Chokla. However, it is not always true because the same size fragments might be different in the nucleotide sequence. Kheri is an admixture of various breeds and being adopted by the farmers in the local areas. The study also focuses the genetic relationship of Kheri particularly with the pure breeds. Kheri was found close with Chokla (D = 0.0741), followed by Malpura (D = 0.0902). It is possible that farmers might have used Malpura as dam or sire breed for evolving Kheri in the local areas.

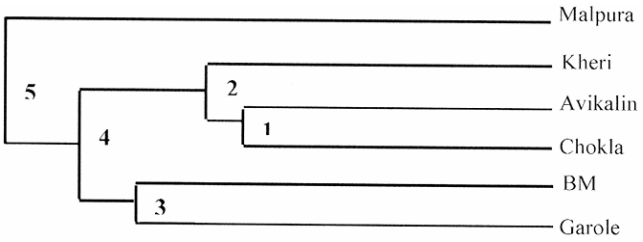

Fig. 3—Phylogenetic relationship among breeds of sheep based on RAPD pooled data from 16 primers using UPGMA analysis through neighbor procedure of Phylip version 3.5 (POPGENE software)

The RAPD data was used for construction of dendrogram among the breeds studied. The phylogenetic relationship among these breeds was revealed through POPGENE software (Fig. 3). The results showed that Avikalin and Chokla are closely related and both of them clustered together, whereas Malpura and Garole are distant to each other and fall in separate clades. The resultant dendrogram also showed close proximity of Kheri to Avikalin, followed by Chokla breed. Kheri might be related to other breeds of sheep of semi-arid region of Rajasthan. A further study is required to include other breeds of semi-arid region of Rajasthan to study the origin of Kheri breed. The present study also supports the finding of Ali[15] that RAPD technique can be used effectively for detection of polymorphism and can establish the relationship among breeds of sheep.

In conclusion, it can be stated that the observations generated using RAPD markers revealed the genetic relationship among six breeds of sheep found in India. The present study suggests that the RAPD-PCR can be used as a tool to estimate the genetic diversity and phylogenetic relationship among breeds of sheep.

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