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Age Related Variations in Dna Samples of Skin Fibres in Gaot (Capra Hircus) Through RAPD Analysis

Science

KEYWORDS : Random amplified polymorphic DNA, PCR, skin fibres.

*** TRIPATHI M** Mata Gujri Mahila Mahavidyalaya (Auto.) Jabalpur, (M.P.) India. * Corresponding author

ZAIDI B Mata Gujri Mahila Mahavidyalaya (Auto.) Jabalpur, (M.P.) India.

ABSTRACT

The present research was undertaken to observe genetic variations in skin fibers of different age group of Indian goat (Capra hircus) using RAPD technique. In this study, 15 skin samples from ventral region of Capra hircus goat species were taken for the isolation of DNA and processed by random amplified polymorphic DNA (RAPD) assay using random primers (RBA7, RBA8, RBA9, and RAN3). By RAPD assay, several distinct band profiles were obtained in the examination of fetal, neonatal and adult goat skin samples. The results of the present investigation also revealed that the four RAPD primers were able to identify and classify the variations in different samples based on their genomic imprinting. This study showed clear genetic heterogeneity exists among different age groups of goat.

INTRODUCTION

Goats have been raised in various areas of the world for their production of meat, milk skin and fiber as well. This species is well known for its high ability to adaptation in the tropical and subtropical regions and especially in the arid. Present study was undertaken to find out age related genetic variations in skin fibres of Indian goat *(Capra hircus)* using RAPD technique. This technique has achieved a great deal of acceptance due to its simplicity, due to unequal crossing over or VNTR (**Variable Number Tandem Repeat**) loci; it can generate polymorphism or unique pattern of individual samples. The restriction recognition sites giving clear images shows correlation between the genetic map and physical map of a chromosome. The physical changes localized in genetic images may be due to deletions, insertions or nucleotide changes of restriction sites.

The popularity of PCR is primarily due to its apparent simplicity and high probability of success. Unfortunately, because of the need for DNA sequence information, PCR assays are limited in their application. The discovery that PCR with random primers can be used to amplify a set of randomly distributed loci in any genome facilitated the development of genetic markers for a variety of purposes **(**Williams, Welsh 1990**)**. The simplicity and applicability of the RAPD technique have captivated many scientists interest. Perhaps the main reason for the success of RAPD analysis is the gain of a large number of genetic sequences that require small amounts of DNA without the requirement for cloning, sequencing or any other form of the molecular characterization of the genome of the species in question.

Principle of the RAPD Technique

The standard RAPD technology (Williams, 1990) utilizes short synthetic oligonucleotides (10 bases long) of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR. Amplification products are generally separated on agarose gels and stained with ethidium bromide.

Recently, sequence characterized amplified regions (SCARs) analysis of RAPD polymorphisms (Paran, 1993, Bardakci, 1999) showed that one cause of RAPD polymorphisms is chromosomal rearrangements such as insertions/deletions. Therefore, amplification products from the same alleles differ in length and will be detected as presence and absence of bands in the RAPD profile.

MATERIALS AND METHODS

The present study was conducted in 15 indigenous goats (Capra hircus). Genomic DNA was extracted from tissue samples using the phenol-chloroform method Strauss (1995), Sambrook et.al. (1989).

Once collected, samples of the hides were washed with tap and distilled water and dried at 40°C. The samples were then kept at -20°C until processing.

This method involves digest a tissue sample using proteinase K, followed by precipitation of proteins with 5 M NaCl, Extraction with Phenol/Chloroform DNA precipitation with 70% ethanol. DNA is hydrated in Tris-EDTA buffer. This protocol provided good-quality DNA suitable for analysis with molecular markers. This new protocol has potential for use in identifying leather products of these species using molecular markers based on RAPDs.

Amplification of polymorphic regions using RAPD primers by the polymerase chain reaction (PCR)

Amplification reactions were carried out according to the method of Lage et al. (2008) in a final volume of 25 µL, containing dNTP mix (2.5mM each): 1.5µl, Random Primer : 100ng , Taq DNA, Polymerase Assay Buffer A (10X): 1X , Taq DNA Polymerase enzyme : 1.5U, Glass distilled water, and 20ng genomic DNA. Amplification reactions were performed in an MPI® thermal cycler with a program of 45 iterative cycles of 5 min at 94°C, 1 min at 38°C and 2 min at 72°C, with an initial denaturation of 45 sec at 94°C, 45 °C at 1min, 72 °C at 2min and a final extension at 72°C for 10 min.

Performed RAPD reactions with the Genomic DNA provided, using random primer RBA7, RBA8, RBA9, RAN3 under PCR conditions.

Each sample was amplified with each primer at least twice to ensure reproducibility of banding patterns. To avoid contamination of PCR experiments, all the necessary precautions were taken; in particular, the procedures of pre- and post-amplification aliquots were separated and fresh reagents for each experiment were used whenever possible. To test the reliability of the PCR products, controls with no genomic DNA were used routinely.

Analysis of amplified products

PCR products were loaded on to 1.8 % Agarose Gel and resolved by electrophoresis. The gel was subsequently stained with ethidium bromide and viewed under UV-light. Photographs were documented subsequently.

RESULTS & DISCUSSION

The profile of amplified DNA primarily depends on nucleotide sequence homology between the template DNA and oligonucleotide primer at the end of each amplified product. Nucleotide variation between different sets of template DNAs will result in the presence or absence of bands because of changes in the priming sites (see fig.).

This study applied the DNA extraction protocol with 5 M NaCl in combination with proteinase K digestion to the analysis of skin samples obtained by a non-invasive method. This technique was successful in extracting DNA in an adequate quantity as well as moderate purity, necessary conditions for RAPD-PCR analysis (see fig.).

The advantages make this technique a fast, economical and very effective method for DNA extraction. In fact, we did not find reports of any previous study that considered DNA extraction from mammalian skin; similar protocols for DNA extraction have been implemented only in forensic research in humans (White and Densmore, 1992).

We optimized the RAPD-PCR using four markers until we obtained reproducible band patterns of different bands for each samples with each marker after the comparison with the results obtained individually for each sample.

All of the four primers used in this study were successfully amplified with polymorphic bands among the skin samples studied as shown in Figures 1 to 4.

The RAPD technique has also been used for constructing trees in other animals such as buffalo, cattle, goat and sheep (Appa Rao et al., 1996), tilapia fish (Baradakci and Skibinski, 1994), bacteria (EL Hanafy et al., 2007) and date palm (Soliman et al., 2003).

This study reveals that structural variation in genetic imprinting exist among different age groups in goat skin fibers and has demonstrated the usefulness of the RAPD approach for detecting DNA polymorphism in the concerned samples. Jaccard coefficient method used for comparing the similarity and diversity of sample sets.

RAPD amplification products were in the range of 100–1500 base pairs.

All of the reaction products were completely reproducible in 3 DNA samples.

The protocol was efficient in extracting genomic DNA from skin fibres. Analysis of whole genomic DNA in agarose gel and amplified fragments (fig. 1) (250and 700bp), (fig. 2) 200 to 1000bp, (fig. 3) 600 to 1500bp & (fig. 4) 300 to 1100bp by PCR demonstrated that the extracted DNA had high molecular weight, one of the most important aspects for successful amplifications of larger fragments (Borges, 1997).

PHOTOGRAPHS

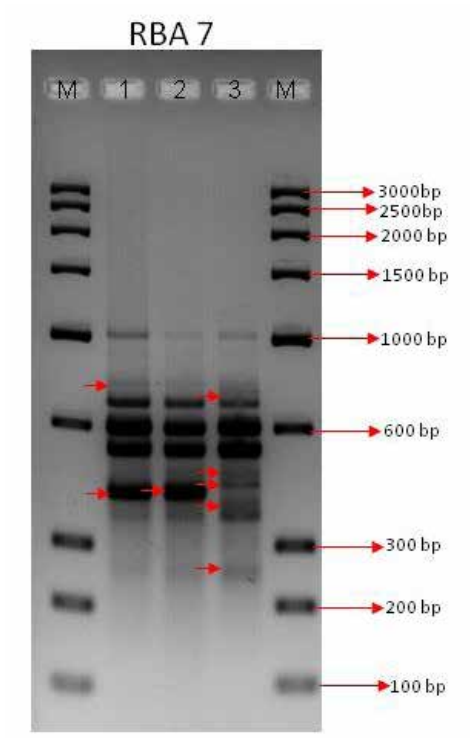

Fig.1. RAPD Profile of three samples generated using 4 RAPD primers
Lane M: Low Range DNA Ruler (Cat# 612652370501730)
Lane 1: RAPD of Ventral Foetus with Primer RBA 7
Lane 2: RAPD of Ventral Neonate with Primer RBA 7
Lane 3: RAPD of Ventral Adult with Primer RBA 7

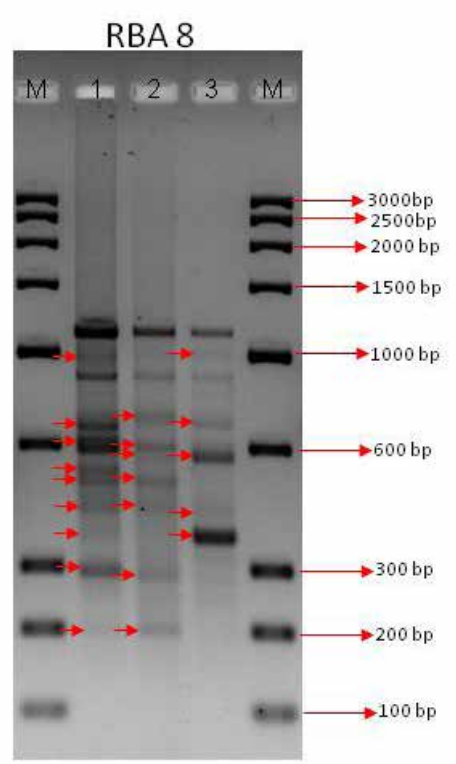

Fig.2. RAPD Profile of three samples generated using 4 RAPD primers
Lane M: Low Range DNA Ruler
Lane 1: RAPD of Ventral Foetus with Primer RBA 8
Lane 2: RAPD of Ventral Neonate with Primer RBA 8
Lane 3: RAPD of Ventral Adult with Primer RBA 8

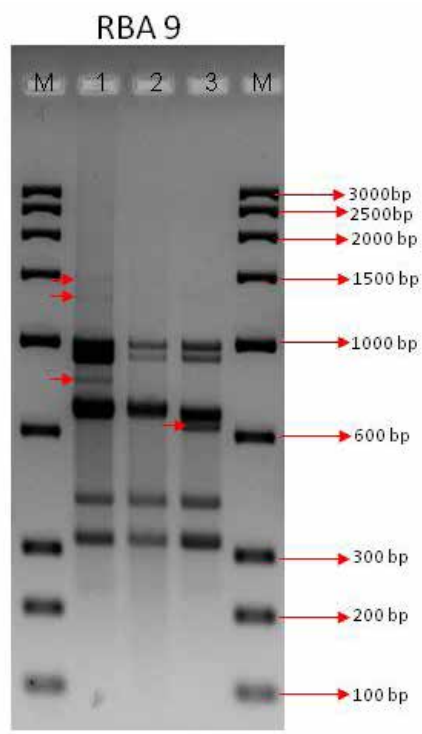

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Fig.3. RAPD Profile of three samples generated using 4 RAPD primers
Lane M: Low Range DNA Ruler
Lane 1: RAPD of Ventral Foetus with Primer RBA 9
Lane 2: RAPD of Ventral Neonate with Primer RBA 9
Lane 3: RAPD of Ventral Adult with Primer RBA 9

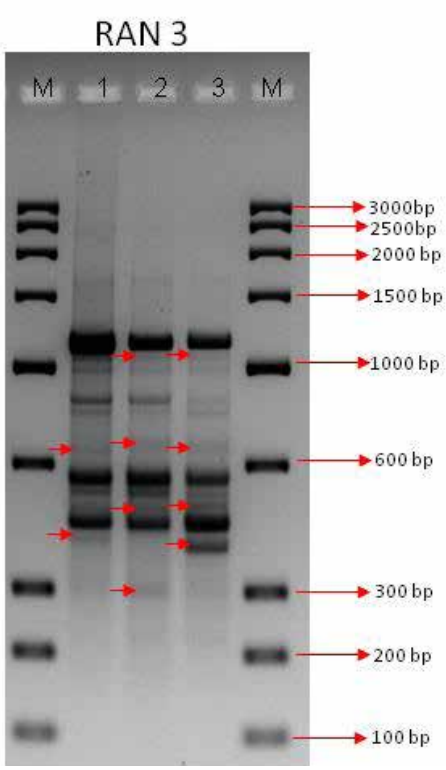

Fig.4. RAPD Profile of three samples generated using 4 RAPD primers
Lane M: Low Range DNA Ruler
Lane 1: RAPD of Ventral Foetus with Primer RAN 3
Lane 2: RAPD of Ventral Neonate with Primer RAN 3
Lane 3: RAPD of Ventral Adult with Primer RAN 3

Note: Red arrow indicates the polymorphic bands

Similarity Matrix computed with Jaccard coefficient:

	Foetus	Neonate	Adult	Foetus R	Neonate R	Adult R
Foetus	1	0.667	0.523	1	0.667	0.523
Neonate		1	0.476	0.667	1	0.476
Adult			1	0.523	0.476	1
Foetus R				1	0.667	0.523
Neonate R					1	0.476
Adult R						1

Distance matrix based on Jaccard coefficient:

	Foetus	Neonate	Adult	Foetus R	Neonate R	Adult R
Foetus	0	0.333	0.477	0	0.333	0.477
Neonate		0	0.524	0.333	0	0.524
Adult			0	0.477	0.524	0
Foetus R				0	0.333	0.477
Neonate R					0	0.524
Adult R						0

Distance matrix based on RMSD coefficient:

	Foetus	Neonate	Adult	Foetus R	Neonate R	Adult R
Foetus	0	0.333	0.477	0	0.333	0.477
Neonate		0	0.524	0.333	0	0.524
Adult			0	0.477	0.524	0
Foetus R				0	0.333	0.477
Neonate R					0	0.524
Adult R						0

Note: Foetus –R, Neonate-R, Adult-R are the repeat of Foetus , Neonate , Adult sample which were considered for the statistical compilation.

Phylogenetic Tree Analysis:

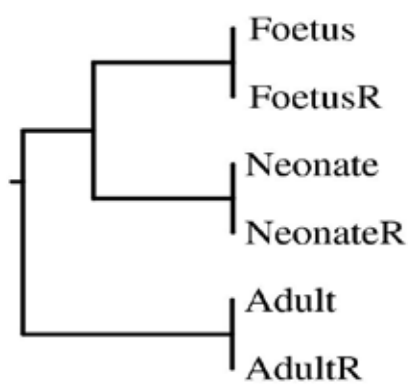

Figure 5. Phylogeny tree showing the relationships skin fibres obtained by RAPD analysis using four different primers.

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