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Genetic Relationships Among Six Iranian Goat Populations Based on Random Amplified Polymorphic DNA Markers

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Abstract: The objectives of this study were to assess the genetic variability among six Iranian goat breeds including: Markhoz (MR), Korki of South Khorasan (KK), Black Lori (BL), Najdi (NJ), Korki of Raeini (KR), Tali (TL) and to evaluate the genetic relationships between populations using RAPD markers. The genetic characterization of these genetic resources is essential to conservation and breeding programs. Blood sample (50 individuals per breeds) were collected from spreading location of these breeds. DNA extraction was carried out by Salting-Out method. Initially, a total of 16 ten and eleven-nucleotide arbitrary primers were used but 10 of 16 primers revealed a pattern with scorable amplified bands. From a total number of 115 scored bands 62 (53.9%) and 53 (46.1%) were described as polymorphic and monomorphic, respectively. The average number of bands per primer 11/5 and with sizes varying from 220 to 2310 bp in length. Nei's genetic distances varied between 0.081 and 0.227 in the populations. The phylogenetic tree was reconstructed on Neighbor-Joining method and showed two main separated groups. One includes KK, TL, KR in a branch and then NJ. Another consists BL and MR. This research was showed that RAPD technique is an useful tool for evaluation of genetic variation among of domesticated animals.

Key words: Iraninan goats, genetic variation, genetic distance, RAPD marker

INTRODUCTION

People began to domesticate wild goats at least 10,000 years ago in the Zagros Mountains of western Iran, according to a new study (Javavnrouh, 2002). Raising goats can be a valuable part of a sustainable farm. Integrating livestock into a farm system can increase its economic and environmental health and diversity, thereby making important contributions to the farm's sustainability. Goats can be incorporated into existing grazing operations with sheep and cattle and they can also be used to control weeds and brush to help make use of a pasture's diversity (www.wikipedia.org). There are 20 million goats in Iran that produce a variety of products for example: Korki of South Khorasan and Korki of Raeini breeds for cashmere, Markhoz breed for mohair, Tali, Najdi and Black Lori breeds for milk and meat products (Tavakolian, 2000).

As a result of the shortage of effective conservation, some goat populations, such as the Markhoz (MR) have decreased rapidly in number of sires and population sizes. Since the genetic resources required for the future are difficult to predict, selection for conserving these populations with unique evolutionary history has to be taken into account and breeds should be chosen in order to cover the widest range of genetic variability (Li *et al*., 2002).

The results obtained based on the study of the differences and similarities between the populations as well as the estimation of the genetic variability within the breeds and populations will help in the choice of animals to be used as donors in ex situ conservation, assuring that the germplasm bank will contain the maximum genetic variability which exists in the populations, avoiding duplication of samples (Barker, 1994). Molecular markers have been shown to be an efficient tool in the quantification of genetic diversity of various populations (Saitaekova *et al*., 1999; Barker *et al*., 2001). Development of molecular biological techniques has created new possibilities for selection strategies and genetic improvement of livestock (Notter, 1998). Discovery of the polymerase chain reaction (PCR) had a major impact on the research of eukaryotic genome and contributed to the development and application of various DNA markers (Bardakci, 2001).

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Many methods have been developed over the past two decades that allow detection of polymorphism at the DNA level. The randomly amplified polymorphic DNA (RAPD) technique, described first by Welsh and McClelland (1990) and Williams *et al.* (1990), is a quick and effective method that can be applied to generate genotype specific banding patterns. Polymorphism of RAPD fragments is detected as a band's presence or absence and may result from deletion, insertion or differences in the nucleotide sequences in or between priming regions (Clark and Lanigan, 1993). RAPD is a simple, fast and comparatively low cost assay that uses short oligonucleotide primers of arbitrary sequences to amplify anonymous fragments of genomic DNA (Stepniak *et al.*, 2002) and no prior knowledge of the genome under investigation is necessary to perform the assay (Bowditch *et al.*, 1993). Due to those features, the RAPD analysis has found many uses in different fields of study in both plants and animals. The evolution of goat breeds has been shaped by man over many generations. The local climates, diseases, nutritional environments, selections for different objectives and genetic drifts have contributed to the evolution of diverse goat breeds (Oliveira *et al.*, 2005). Some of populations, such as the Markhoz goat, have decreased rapidly in number of sires and population sizes. Since the genetic resources required for the future are difficult to predict, selection for conserving these populations with unique evolutionary history has to be taken into account and breeds should be chosen in order to cover the widest range of genetic variability (Javanrouh, 2002).

These studies reflected the effectiveness of RAPD as potential genetic marker. The objective of the current study was to investigate RAPD-DNA markers development to distinguish genetic differences and similarity between and within Iranian six goat populations by estimating genetic distances.

MATERIALS AND METHODS

This research was conducted in Department of Biotechnology at Animal Science Research Institute of Iran (ASRI) during two years from 2000 to 2002.

DNA isolation: Genetic variability among six Iranian goat breeds including: Markhoz (MR), Korki of South Khorasan (KK), Black Lori (BL), Najdi (NJ), Korki of Raeini (KR), Tali (TL) were analyzed. Blood samples (5 mL) from 300 animals (50 individuals per breed) were collected from spreading areas of these goats (Fig. 1). Genomic DNAs were extracted using salting-out method with some modifications (Miller *et al.*, 1988). The quantity as well as the quality of the extracted DNA were determined by a spectrophotometric method based on absorbance at 260 and 280 nm respectively.

RAPD-PCR condition and electrophoresis: The 16 ten eleven-nucleotide RAPD primers of arbitrary sequence were used but 10 of 16 primers revealed a pattern with scorable amplified bands (Table 1). PCRs were performed in a final volume of 20 μL containing 25 ng of genomic DNA, 200 μM each of dNTPs, 0.5 μM of each primer, 3.5 mM of $MgCl_2$, 1 unit of Taq DNApolymerase and 1X PCR reaction buffer. Negative control (lacking DNA) was set up for each reaction mastermix to check for DNA contamination. Amplification was carried out in a thermocycler (Biometra), with an initial denaturation step at 94°C for 5 min followed by 45 cycles of 1 min at 94°C, 1 min at 42°C, 1 min at 72°C and a final extension step at 72°C for 7 min. The PCR products were run on 1.5% agarose gel and visualized by ethidium bromide staining.

Computation and statistical analysis: The RAPD profiles of 300 individuals were characterized as matrices of zeros and ones by scoring bands on agarose gel as their presence (1) or absence (0). Only the bands that met the criteria of clarity were scored. The Band Sharing Frequency (BSF) was used to estimate the genetic similarity for each primer (Lynch, 1990). The BSF between individuals of x and y was calculated as:

$$BSF_{XY} = \frac{2N_{AB}}{N_A + N_B}$$

Where N_{AB} is the number of common fragments observed in individuals A and B. N_A and N_B are the total number of fragments scored in A and B, respectively. The within population genetic similarity (WGS) was computed as an average of BSFxy across all comparisons between individuals.

The between-population genetic similarity (BGS) corrected for WGS, was calculated according to Lynch (1990) as:

$$BGS = 1 + S`_{IJ}\ IJ\ 0.5\ (S_I + S_J)$$

Where $S`_{IJ}$ was the average of the band sharing frequency estimates of the comparisons between goats of population I and J. S_I and S_J were values of WGS for I and

Table 1: Primer sequences used for the amplification of the RAPD loci

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
RAP 1	CAGCCCCTTTC	RAP 6	GAAACACCCC
RAP 2	AATCGGGCTG	RAP 7	TCTCCGCCCT
RAP 3	GGGAGGGTGTT	RAP 8	CCCACACGCA
RAP 4	AGCGTGTCTG	RAP 9	CTCAGGTATGC
RAP 5	TCACGTCCAC	RAP 10	CGAACCTGATC

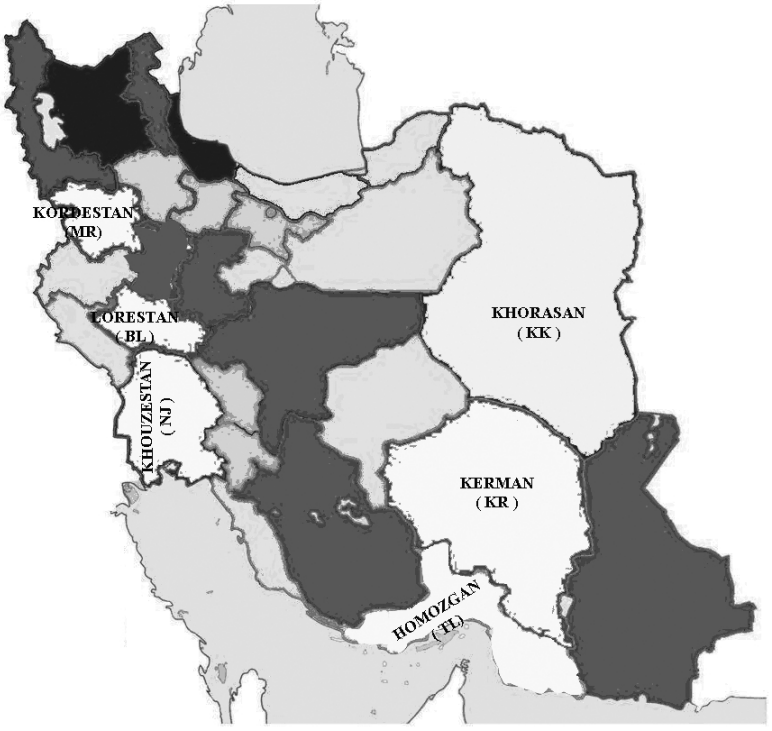

Fig. 1: The spreading regions of six Iranian goat populations

J populations, respectively. $S`_{IJ}$ were used to determine genetic distance (D_{IJ}) according Lynch (1991) as follows:

$$D=Ln\left[S'_{IJ}/(S_I.S_J)^{\frac{1}{2}}\right]$$

This formula is an analogue of Nei (1978) estimator.

Uniformity of RAPD fingerprinting pattern (U) were determined according to the equations:

$$U=\frac{1}{N}\sum_{i=1}^{N} V_i$$

Where V_i is the frequency of the ith band and N is the number of bands scored within a population (Lynch and Milligan, 1994).

Dendrograms were constructed using Neighbor-Joining (NJ) (Saitou and Nei, 1987) by POPTREE software (Takezaki, 2000) with 1000 bootstrap replications and POPGENE (Yeh *et al*., 1999).

RESULTS AND DISCUSSION

In the present study, as a result of an initial RAPD analysis on genomic DNA of goat, 10 out of the 16 primers were chosen for further analysis, on the basis of the presence of diagnostic bands (Fig. 2). Each of these primers amplified on average 7 to 22 bands of sizes varying from 220 to 2310 bp. A total of 115 diagnostic bands were scored within RAPD profiles amplified by these 10 primers. From a total number of 115 scored bands 62 (53.9 and 53 46.1%) were described as polymorphic and monomorphic respectively (Table 2). The average number of bands per primer 11/5 fragments. The average number of polymorphic bands per primer varied from 4 to 9. The highest and the lowest number of polymorhic bands were recognized for primer 2, 4 (70%) and 8 (40.9%), respectively (Table 2). The average uniformity index for RAPD fingerprinting pattern of each primer is calculated and its ranged from 0.657 to 0.714 (Table 3). The lowest

Table 2: Summary of the results of RAPD analysis with 14 arbitrary primers: total number of detected bands (TDB), No. polymorphic bands (NPB) and percentage of polymorphic bands (PB%)

Primer	TDB	NPB	PB (%)	Primer	TDB	NPB	PB (%)
RAP 1	7	4	57.1	RAP 6	15	7	46.6
RAP 2	10	7	70	RAP 7	9	6	66.6
RAP 3	11	7	63.6	RAP 8	22	9	40.9
RAP 4	10	7	70	RAP 9	12	6	50
RAP 5	8	4	50	RAP 10	11	5	45.4

Table 3: Uniformity Index (UI) of RAPD fingerprinting pattern and within-population genetic diversity (WGD) for 6 goat populations

Goat breeds	MR	KK	KR	BL	NJ	TL
UI	0.714	0. 689	0.665	0.701	0.672	0.657
WGD	0.286	0.311	0.335	0.299	0.328	0.343

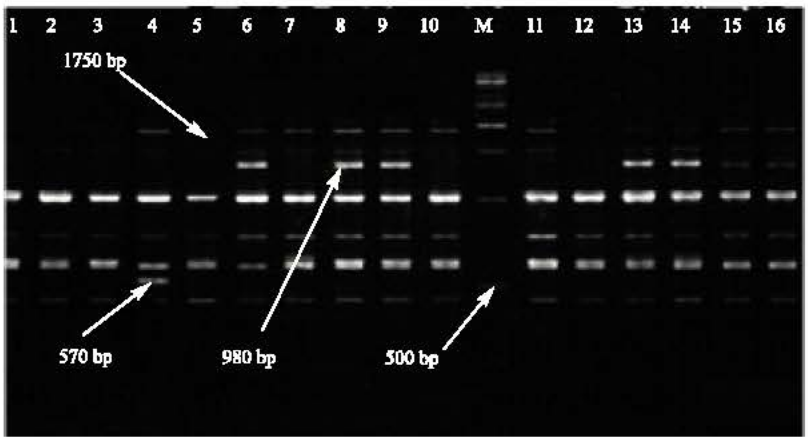

Fig. 2: Amplification products generated by primer RAPD-2. Lane M: DNA marker, Lanes 1-16: specific RAPD fragments from different individuals in MR populations

Table 4: Nei's genetic distances from RAPD data. The standard genetic distances are below the diagonal and between-population genetic similarity (BGS), above the diagonal for 6 goat populations

Goat breeds	MR	KK	KR	BL	NJ	TL
MR	-	0.850	0.857	0.829	0.839	0.830
KK	0.162	-	0.863	0.796	0.813	0.861
KR	0.153	0.146	-	0.855	0.864	0.922
BL	0.186	0.227	0.156	-	0.810	0.813
NJ	0.175	0.206	0.145	0.210	-	0.840
TL	0.185	0.149	0.081	0.206	0.166	-

uniformity index was observed in TL population and highest uniformity index was observed in MR population. These results reflected, restrict distribution and low size population in MR population and it is necessary to do urgent conservational strategies on this population. The between-population genetic similarity (BGS) varied between 0.796 to 0.922. Smith *et al.* (1996) observed BGS in four different populations of poultry breeds varied from 0.87 to 0.98. Probability, lack of breeding programs and different environmental conditions in Iranian goat breeds April 13, 2007 between-population genetic similarity (BGS). The estimates of genetic distances between the populations were calculated to help in the study of genetic relationships and genetic divergence between pairs of populations for standard genetic distances (Dp) (Nei, 1972) (Table 4). The analysis of genetic diversity and relatedness between or within species, populations and individuals is a prerequisite towards effective utilization and protection of animal genetic resources. With DNA being the only basis of genetic differences between distinct organisms, DNA fingerprinting presently is the ultimate method of biological individualization Nei's genetic distances varied between 0.081 and 0.227 in the populations. The lowest genetic variability was observed between KR×TL populations (8.1%) and the greatest variability was BL×KK (22.7%). These results reflect large geographically distant and different environmental

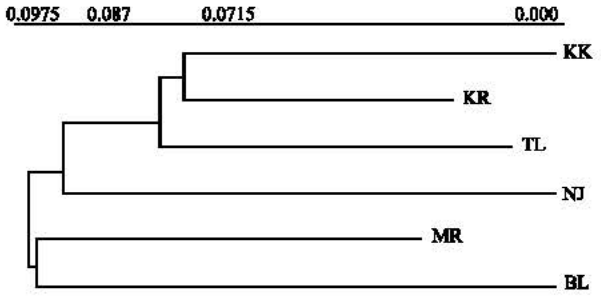

Fig. 3: Dendrograms showing evolutionary relationships among 6 Iranian goats using Neighbor-Joining (NJ) algorithms

condition between BL and KK. The phylogenetic tree was reconstructed on Neighbor-Joining method and showed two main separated groups. One includes KK, TL, KR in a branch and then NJ. Another consists BL and MR (Fig. 3). One possible explanation for this clustering could be short geographic distance between distribution area and similar climates for those are in each cluster. The slightly phenotypic differences among Najdi population with three populations in same cluster are resulted they be in one cluster but NJ with slightly further. Differential climates between two provinces (Lorestan and Kordistan with mountain climate) and their adjacent province (Khouzestan with hot climate) was caused that NJ set in a separate cluster from BL and MR. The RAPD technique is an efficient method for studies of genetic similarity between Iranian goat populations

CONCLUSION

This research showed high variation within and between studied Iranian goat populations based on

RAPD markers. Additionally, the use of RAPD markers represents a useful and efficient method and thus provides a potential tool for detection of genetic variability among Iranian goat populations. Therefore, there is enough genetic variation left to generate further progress in the years ahead

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