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Full Length Research Paper

Genetic characterization of two Sudanese goat breeds (*Capra hircus*) using RAPD molecular markers

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Seven primers of randomly amplified polymorphic DNA (RAPD) were selected to study the genetic variations among 14 individuals of goat (*Capra hircus*) from two domestic Sudanese goat breeds (Nilotic and Nubian). The test generated 59 entirely repeatable RAPD fragment bands and the statistical analysis showed 55 polymorphic bands among the 14 individuals. The genetic distances among the population range from 8 to 72%. The highest dissimilarity coefficient was between individuals within the Nilotic breeds while there was a comparatively low degree of differentiation among the Nubian population. The constructed UPGMA dendrogram of the coefficient of similarity showed that the Nubian clustered together while the individuals from the Nilotic form 4 groups. It was clearly seen that the link between the individual of the Nilotic is quite weak and some of them linked to the Nubian. The results of the study offer useful information about some Sudanese goat breeds.

Key words: *Capra hircus*, RAPD marker, conservation genetics, Sudanese goats.

INTRODUCTION

Goat (*Capra hircus*) is one of the smallest domesticated ruminants which are managed for the production of milk, meat, wool and leather particularly in arid, semitropical or mountainous countries (Morand, 2004). Despite major changes in agriculture due to industrial mergers and technological advances, goats are of considerable economic importance particularly for the poor farming system in Africa (McMillan and Brock, 2005). In Sudan goats are estimated to be about 42.5 million head which is a very large population compared to other African countries (Yousif and Fadl El-Moula, 2006). This population composed of four major local breeds, Nubian, Desert, Nilotic and the Dwarf, distributed throughout the country (Wilson, 1991).

The Nubian (common breed that is distributed in north area of the latitudes12^{o}) and the Nilotic (distributed all over the south area of the latitudes 12^{o}) are well known to have the most considerable economic value and represent important genetic resource that must be conserved (Baker, 1992). These breeds, as well as the other naturalized Sudanese goat breeds, suffer genetic losses due to the indiscriminate miscegenation with other breeds raised in the region. This miscegenation was caused by the deficiency of reproductive control and the disorienttated introduction of exotic breeds. Therefore essential conservation programs based on the analysis of the genetic variability must be intensified to assist in the rational monitoring of future animal improvement, as well as in the preservation and conservation of animal germplasm.

In this study we utilized the random amplified polymorphic DNA (RAPD) molecular markers, which proved to be an efficient tool in the quantification of genetic diversity of various populations (Rahman et al., 2006; Barker et al., 2001) between and within two Sudanese goat breeds.

MATERIALS AND METHODS

Sample collection and DNA extraction

The blood samples were collected from randomly selected pure adult Nubian and Nilotic goat breeds. Samples from the Nubian goats were obtained from the Department of Animal Production of the College of Agricultural Studies, Sudan University, while those for the Nilotic goats were obtained from the College of Natural Resources and Environmental Studies, Juba University. At least 5

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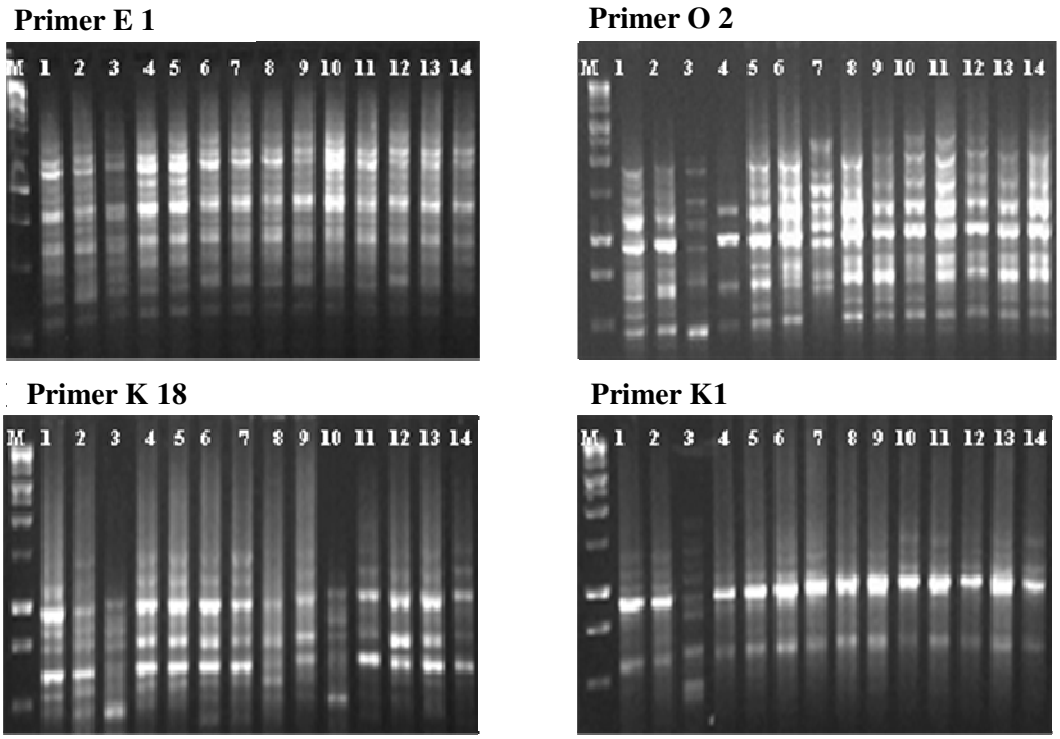

Figure 1. DNA polymorphisms detected among Sudanese goat breeds using different universal primers. M: Standard marker; lanes 1-7 are Nilotic; lanes 8 -14 are Nubian.

ml blood sample were drawn from a vein in the neck of each goat and collected in EDTA vacutainers (Becton Dickinson). The blood was gently mixed with anticoagulant and kept at -20°C.

DNA extraction was carried out by the method of Miller et al. (1988) as follows: To an aliquot of 100 µl blood (after thawing), 700 µl of lyses buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8·0, 0·5% SDS) and 60 µg of proteinase K (20 mg/ml) were added. The mixture was vortexed and incubated at 37°C overnight. DNA was extracted by equal volumes of phenol-chloroform (1:1) and chloroform-isoamylalcohol (24:1). DNA was precipitated by adding 2 volumes of chilled ethanol in the presence of a high concentration of salts (10% 5 M ammonium cetate). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA).

PCR amplifications were performed following the procedure of Micheli et al. (1994). Seven random primers were used in this study (Table 1). The PCR reaction was carried out in 25 µl volumes of reaction mixtures containing 2.5 µl of 10X Taq buffer, 1.5 µl $MgCl_2$ (50 mM) , 2.5 µl dNTPs (2 mM/µl), 2 µl random primer (10 pmol/ µl), 0.5 µl Taq DNA polymerase (5U/µl) and 1 µl of the extracted DNA (10 ng). The mixture was made up to 25 µl by addition of sterilized distilled water. After purification, the resultant DNA was quantified using spectrophotometer, and its integrity was determined after agarose gel electrophoresis (Sambrook and Maniatis, 1989). RAPD/PCR reactions were initiated using an Applied Biometra thermal cycler which was programmed as follows: An initial denaturation step at 94°C for 2 min followed by 45 cycles with 94°C for 30 s for DNA denaturation, annealing as mentioned with each primer, extension at 72ºC for 30 s and final extension at 72°C for 10 min were carried out. The samples were cooled at 4°C. The amplified DNA fragments were separated on 1.4% agarose gel and stained with ethidium bromide. The amplified pattern was visualized on a gel documentation system.

RAPD analysis

PCR products were scored across the lanes as variables. The presence of a band of amplified DNA was scored as (1) and absence as (0). The genetic dissimilarity matrix among genotypes was estimated according to Nei and Leis' (1979). Coefficient of similarity trees were produced by clustering the similarity data with the un-weighted pair group method using statistical software package STATISTCA- SPSS (Stat Soft Inc.). The similarity coefficient was used to construct a dendrogram by the un-weighted pair group method with arithmetic averages (UPGMA) (Rohlf, 1993).

RESULT AND DISCUSSION

RAPD analysis

To ensure that the amplified DNA bands originated from genomic DNA and not primer artifacts, negative control was carried out for each primer/breed combination. No amplification was detected in control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions. Seven out of fifteen primers (46.6%) were successfully amplified polymorphic bands between the two breeds studied. The amplified PCR products of blood DNA showed identical band patterns with similar intensity. However, different PCR patterns were obtained between Nilotic and Nubian breeds (Figure 1).

Table 1. The sequence of primers used and their polymorphic bands.

Primer	Sequence of the primer	Total number of bands	Number of polymorphic bands	Polymorphism (%)
E1	CCCAAGGTCC	7	6	85.7
K1	CATTCGAGCC	6	6	100
O2	CCAGCCGAAC	15	15	100
K13	GGTTGTACCC	6	6	100
O17	GTGTCTCAGG	6	6	100
K18	GAGCGTCGA	10	9	90
O19	ACAACGCCEC	9	7	77.8
Total		59	55	
Average		8.4	7.9	93.4

RAPD analysis

The obtained results indicated that the seven primers show at least one consistent polymorphic band. The selected primers generated distinctive products in the range of 1.584 - 5.148 Kbp. Total of 59 amplified fragments were distinguished across the selected primers and the statistical analysis showed 55 polymorphic bands among the 14 individuals with an average of 7.9 polymorphic bands per primer. The maximum numbers of fragment bands were produced by the primer O1 (15) with 100% polymorphism while the minimum numbers of fragments were produced by the primer K1, K13 and O13 (6) with 100% polymorphism Pattern of RAPD fragments produced by the seven primers (E1, K1, O2, K13, O17, and K18 O19) are shown in Table 1.

Gene diversity

The pair-wise mean genetic distance value ranged from (above diagonal) 10 to 72%. The greater percentage of variation (72%) was observed inside the population of Nubian followed by two high values (64 and 62%) and both of them between individuals from the two different goat breeds, Nilotic and Nubian (Table 2). The dissimilarity within the same group is small in general and most likely to be less in the Nilotic rather than within the Nubian. The high variations within and between the group were observed with the sample 3 and these variations could be attributed to different genetic characters that may be introduced through hybridization since the herd was originally collected from different areas. Variation between the two breeds is almost high.

According to the dendrogram and cluster analysis (Figure 2), we found two main groups: one was the Nilotic and the other was the Nubian. Despite the sample collection based on the location, it was very obvious that there is a link between some of the Nilotic and the Nubian. Most of the Nilotic were clustered in one group except sample 3 and 6 which were clustered in a separate group that closely linked to the Nubian. The apparent difference in the clustering of the individuals of the Nilotic breed could be attributed to migration of individuals between the populations. This could be an indication of gene flow due to hybridization between the two types of goats and it is very common practice in animal production (Oliveira et al., 2005).

Natural gene flow could also be attributed to the intermingling during the movement of the nomads since they are moving through long distances in Sudan. The Nilotic breed comprised more than one distinct cluster group and that indicates the hetrozygsity of Nilotic goats. Such high level of genetic differentiation within the Nilotic population leads to the speculation that they might belong to different breeds despite their having the same name and similar morphological traits. In contrast to Nilotic, clustering of all the Nubian in one group indicated that all the individuals of the Nubian breed were more closely related and characterized with high genetic similarities. The explanation is that the Nubian breeds existed in remote areas with a small population size and there was less gene exchange with other populations of the goat.

The results of this study contribute to the knowledge of the genetic structure of the Sudanese indigenous goat populations, especially many of the small populations varying on the potential threat of extinction or even being effectively lost with the rapid destruction of the ecological environment. Conservation of genetic diversity should be considered by breeders, in the interest of the long term future of the Sudanese breeds. Since the genetic characterization, contrary to phenotypic characterization, is totally free of environmental influences (Shadma et al., 2008), it is fundamental to use genetic methods of evaluation to maintain the maximum variability as possible in conservation (Aggarwal et al., 2007). Therefore molecular markers can aid estimating the genetic variability of species, breeds and populations, as well as decisions related to selection of breeds/ populations to be conserved (Zhang et al., 2006). Thus the techniques for the analysis of the genetic variability are an essential ingredient for the programs of rational conservation and improvement, since they must be based on the combination of the phenotypic and genetic data (Kumar et al.,

Table 2. Matrix of RAPD dissimilarity among 14 individuals of two Sudanese goat breeds (*Capra hircus*) based on Nei and Lei coefficients.

No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14*
1	0.00													
2	0.10	0.00												
3	0.43	0.43	0.00											
4	0.56	0.53	0.72	0.00										
5	0.51	0.51	0.61	0.18	0.00									
6	0.49	0.49	0.62	0.24	0.08	0.00								
7	0.50	0.47	0.61	0.25	0.19	0.19	0.00							
8	0.53	0.57	0.57	0.28	0.13	0.16	0.26	0.00						
9	0.53	0.57	0.61	0.28	0.16	0.16	0.26	0.16	0.00					
10	0.48	0.48	0.62	0.32	0.24	0.20	0.27	0.17	0.14	0.00				
11	0.54	0.61	0.54	0.34	0.28	0.27	0.32	0.19	0.19	0.20	0.00			
12	0.48	0.56	0.64	0.27	0.25	0.27	0.29	0.25	0.21	0.23	0.25	0.00		
13	0.51	0.51	0.58	0.21	0.19	0.21	0.25	0.19	0.19	0.20	0.21	0.13	0.00	
14	0.50	0.50	0.57	0.28	0.19	0.22	0.23	0.16	0.23	0.21	0.15	0.21	0.08	0.00

*Individuals 1 to 7 are Nilotic; Individuals 8 to 14 are Nubian.

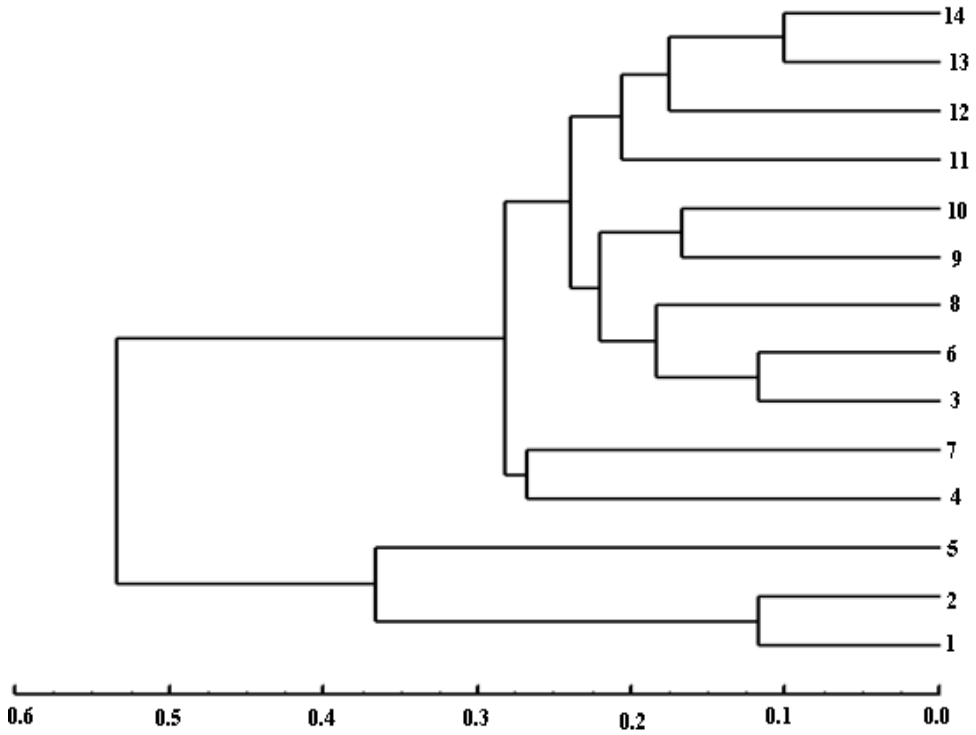

Figure 2. Dendrogram showing relationship among 14 individuals of Sudanese goat breeds generated by UPGMA method based on RAPD analysis (Individuals designated with numbers 1 to 7 are Nilotics while individuals designated with 8 to 14 are Nubian breeds).

2005). In conclusion, the calculation of pair wise genetic similarities indices between individuals may help to select animals possessing the least genetic similarity and might be a valuable tool for selection in the future germplasm collections. The data reported here provide a valuable insight into genetic diversity and genetic relationships between Nilotic and Nubian breeds, which can be used to improve the breeding strategies.

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