Research Journal of Biological Sciences 4 (6): 734-737, 2009
ISSN: 1815-8846

Genetic Analysis in Tali Goats Based on 13 Microsatellite Markers

[1]R. Sadeghi, [2]B. Mahmoudi, [2]M.S. Babayev, [2]Y.Rameshknia and [3]M. Daliri
[1]Laboratory of Veterinary, Meshkinshahr, Iran
[2]Department of Genetic, Baku State University, Baku, Azerbaijan
[3]Department of Animal Science,
Genetic Engineering and Biotechnology Institute, Tehran, Iran

Abstract: In this study, the genetic variation in Tali goats were investigated using 13 microsatellite markers (LSCV36, TGLA122, MAF64, oarFCB304, oarJMP23, oarAE133, BM121, BM4621, ILSTS005, ILSTS022, ILSTS029, ILSTS033, ILSTS34) all of 13 loci were amplified successfully. The objectives of this study were to assess the genetic variability among Tali goat breed. The genetic characterizations of this genetic resource are essential to conservation and breeding programs. Blood sample were collected from spreading location of this breed. Genetic variation taking into account all loci had been estimated on the base expected the unbiased average of Heterozygosity (H_e). Furthermore, other criteria of genetic variation including PIC values had calculated in this study. This research was showed that microsatellite technique is a useful tool for evaluation of genetic variation among of domesticated animals.

Key words: Tali goat, microsatellite markers, genetic variation, polymorphism, Iran

INTRODUCTION

There are 20 million goats in Iran that product a variety of products for example: cashmere, mohair, milk and meat products (Esmaeelkhanian *et al*., 2007). About 3.8% from 550 million head goats of word are in Iran. Furthermore, archeology and phylogenetic evidences had proved the origin of from Mesopotamia area and west Zagros in Iran (Mahmoudi *et al*., 2009). Since, the genetic resources required for the future are difficult to predict 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 estimation of the genetic variability within the breed and populations 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. Molecular markers have been shown to be an efficient tool in the quantification of genetic diversity of various populations (Saitaekova *et al*., 1999). Development of molecular biological techniques has created new of molecular biological has created new possibilities for selection strategies and genetic improvement of livestock (Notter, 1999). Discovery of the polymerase chain reaction had a major impact on the research of eukaryotic genome and contributed to the development and application of various DNA markers.

Microsatelite genetic markers are called Short Tandem Repeats (STRs) or Simple Sequence Repeat (SSR) are lengthy sequences 1-6 base pair and they have been distributed in whole all genome. Nowadays, these loci are used in the level of wide for diversity determination and genetic distance on the goats of the world (Saitaekova *et al*., 1999). Instability of microsatellites loci have made an exceptional phenomenon for genetic and evolution studies.

MATERIALS AND METHODS

The blood samples were collected from the 45 animals by puncturing the jugular vein in the vacutainer tubes having EDTA as blood anticoagulant were cool. Then bleeding were transferred them to laboratory (in an ice-cooled box, where they were kept under -20°C in a deep freezer until DNA isolation) and DNA genomic was extracted by salting out metod (Miller *et al*., 1988). We use both spectrophotometry and agarose gel (0.8%) for DNA quality definition.

Corresponding Author: Reza Sadeghi, Laboratory of Veterinary, Meshkinshahr, Iran

Table 1: Microsatellite markers, their sequences, type of repeat, size rang and location

Locus	Primer sequence	Type of repeat	Size range	Chromosome No.
BM121	TGGCATTGTGAAAAGAAGTAAAA			
	CTAGCACTATCTGGCAAGCA	$(TC)_{18}$	165-185	16
BM4621	CAAATTGACTTATCCTTGGCTG			
	TGTAACATATGGGCTGCATC	$(CA)_{19}$	106-148	6
ILSTS005	GGAAGCAATGAAATCTATAGCC			
	TGTTCTGTGAGTTTGTAAGC	$(TC)_{19}$	174-190	10
ILSTS022	AGTCTGAAGGCCTGAGAACC			
	CTTACAGTCCTTGGGGTTGC	$(GT)_{21}$	186-202	Ann
ILSTS029	TGTTTTGATGGAACACAGCC			
	TGGATTTAGACCAGGGTTGG	$(CA)_{19}$	148-191	3
ILSTS033	TATTAGAGTGGCTCAGTGCC			
	ATGCAGACAGTTTTAGAGGG	$(CA)_{12}$	151-187	12
ILSTS34	AAGGGTCTAAGTCCACTGGC			
	GACCTGGTTTAGCAGAGAGC	$(GT)_{19}$	153-185	5
LSCV36	GCACACACATACACAGAGATGCG			
	AAAGAGGAAAGGGTTATGTCTGGA	$(CA)_{16}$	524	19
MAF64	AATAGACCATTCAGAGAAACGTTGAC			
	CTCATCGAATCAGACAAAAGGTAGG	$(TG)_{17}$	121-125	1
oarAE133	AGCCAGTAGGCCCTCACCAGG			
	CCAACCATTGGCAGCGGGAGTGTGG	$(TG)_{21}$	152	Ann
oarFCB304	CCCTAGGAGCTTTCAATAAAGAATCGG			
	CGCTGCTGTCAACTGGGTCAGGG	$(CT)_{11}(CA)_{15}$	119-169	Ann
oarJMP23	GTATCTTGGGAGCCTGTGGTTTTATC			
	GTCCCAGATGGGAATTGTCTCCAC	-	-	27
TGLA122	AATCACATGGCAAATAAGTACATAC			
	CCCTCCTCCAGGTAAATCAGC	$(CA)_{21}$	145	21

In this study was used 13 microsatellites primer pairs including MAF64, BM4621, BM121, LSCV36, TGLA122, oarJMP23, oarFCB304, oarAE133, ILSTS005, ILSTS022, ILSTS029, ILSTS033 and ILSTS34. Most of primers used were independent and belonged to different choromosomes. These loci in prior studies had been amplified on the goat (Maudet *et al*., 2001; Yang *et al*., 1999; Hanrahan *et al*., 1994; Dixit *et al*., 2008). They showed polymorphism in the goat of world. Nine microsatellite markers, their sequences, type of repeat, size rang and their location showed Table 1.

All PCR reactions were continued the following componenet: 200 μM dNTPs, 3.5-6 mM $MgCl_2$, 0.25 μM each of primer, 0.5 unit Taq DNA polymerase, 150 ng DNA. The final volume was 15 μL. Reactions were run on a thermal cycler (Biometra 96 block T-gradient, Germany). in this study, anealing temperature was modified as folowing: MAF64 (62.5°C), BM4621 (58°C), LSCV36 (55°C), oarFCB304 (60.5°C) and BM121 (65.5°C). The rest of PCR process is in accordance with the Table 2.

For oarJMP23 and TGLA122 primers were used PCR programe (Crawford *et al*., 1995), for oarAE133 was used PCR programe (Hanrahan *et al*., 1994) and For ILSTS005, ILSTS022, ILSTS029, ILSTS033 and ILSTS34 primers. The 'touchdown' PCR protocol was used.

The alleles and genotypic frequencies directly were identified from the gel. Hardy-Weinberg Equilibrium (HWE) had been tested based on likelihood ratio for different locus-population combinations and the number of observed and effective alleles by POPGENE software (Yeh *et al*., 1999). Polymorphic Information Content (PIC) were estimated by HET softwar (Ott, 1989).

Table 2: PCR reaction conditions for all loci exceptional TGLA122, oarJMP23 and oarAE133 loci

Stage	PCR process	Temperature (°C)	Time
1	Denaturation	95	2.5 min
2	Denaturation	95	30 sec
3	Anealing	-	30 sec
4	Extension	72	30 sec
5	Final extension	72	2.5 min
6	Maintenance	4	-

Fig. 1: A Tali goat

Characterizations of Tali goats: The Tali goats are medium-sized and mostly are brown or light brown colored. Most animals was Polled. Natural service is method of Breeding for this goat. Its main distribution areas are the coastal region of Hormozgan province along the gulf and in some parts of Boushehre province especially near the towns of Minab, Bandarabbas, Khamir, Bandarlengeh and on Qeshm Island in the Strait of Hormuz. The male and most of female have horns. Height at shoulder is 76 cm in adult male and 68 cm in adult female goat, respectively (Fig. 1).

RESULTS AND DISCUSSION

PCR reactions were successfully done on all thirteen primers. Seven allel in the TGLA122 locus observed in the prior studies on wild goats (*Capra ibex*) but nine allel in Tali goats were observed. in this study that the most numerous of stutter was observed in oarJMP23 locus and the posible explanation for this failure is the perfect of locus and least numerous of stutter was obseved in oarFCB304 locus that the possible explanation for this failure is the interrupt of locus.

For the 13 microsatellites loci analyzed, expected heterozygosity estimates were calculated after Nei (1973), as implement in the POPGENE software to determine genetic variation within the breed. Heterozygosity is defined as the probability that a given individual randomly selected from a population will be heterozygous at a given locus. The observed and effective number of alleles was also calculated using POPGENE software (Kimura and Crow, 1964; Yeh *et al*., 1999). The tests for deviation from Hardy-Weinberg equilibrium were also derived using the exact test of POPGENE.

Number of allele (n), number of allele effective (n_e), expected the unbiased average of Heterozygosity (H_e) and Polymorphic Information Content (PIC) values at locus showed Table 3.

Yang *et al*. (1999) H_e value of oarFCB304 locus estimated 0.854 on Chinese goats but it was 0.543 in Tali goat.

Each 13 loci analysis was 100% polymorphic. Highest number of allele objective was 11 allele for oarJMP23 loci and lowest number of allele objective was 3 allele for oarAE133 loci. Highest and lowest number of allele effective was 9.7 and 2.1 for oarJMP23 and oarFCB304 loci, respectively.

All average the number of allele objective and effective was 7.38 and 4.85, respectively. Highest and lowest PIC value was 0.887 and 0.525 for oarJMP23 and oarFCB304, respectively. The average of PIC value for this population was 0.704, it was between 0.746-0.8 in Chinese goats (Yang *et al*., 1999).

The Tali goats had substantial genetic variation based on their gene diversity and average number of alleles per locus. The average genetic variation (0.726) in Tali goats more than Indian indigenous goats breeds: Barbari, Jamnapari and Sirohi (Ganai and Yadav, 2001).

It also demonstrated that microsatellite genotyping is a useful tool for evaluating variation among important goat populations.

Table 3: n, n_e, H_e and PIC values at locus in Tali goat population

Locus	n	n_e	H_e	PIC
BM121	5	2.9	0.674	0.606
BM4621	9	7.5	0.674	0.606
ILSTS005	9	5.2	0.751	0.745
ILSTS022	8	5.1	0.865	0.815
ILSTS029	7	2.7	0.725	0.692
ILSTS033	9	4.1	0.605	0.537
ILSTS34	8	5.1	0.751	0.745
LSCV36	7	3.5	0.727	0.699
MAF64	5	3.5	0.731	0.702
oarAE133	3	2.6	0.624	0.541
oarFCB304	5	2.1	0.543	0.525
oarJMP23	11	9.7	0.913	0.887
TGLA122	9	6.9	0.872	0.843
Mean	7.38	4.85	0.736	0.704
SD	2.25	2.22	0.113	0.115

CONCLUSION

The result of this study suggests that there is substantial genetic variation and polymorphism across the studied loci in Tali goats. The study suggests scope for its further genetic improvement and to undertake appropriate breeding strategies to avoid inbreeding in the population.

REFRENCES

Crawford, A.M., K.G. Dodds, C.A. Pierson and A.J. Ede *et al*., 1995. An autosomal genetic linkage map of the sheep genome. Genetics, 140: 703-724. PMID: 7498748.

Dixit, S.P., N.K. Verma, S.P.S. Ahlawat, R.A.K. Aggarwal, S. Kumar, R. Chander and K.P. Singh, 2008. Molecular genetic characterization of Kutchi breed of goat. Curr. Sci., 95 (7): 946-952.

Esmaeelkhanian, S., J.A. Aliabad and H. Seyedabadi, 2007. Genetic relationships among six Iranian goat populations based on random amplified polymorphic DNA markers. Pak. J. Biol. Sci., 10 (17): 2955-2959.

Ganai, N.A. and B.R. Yadav, 2001. Genetic variation within and among three Indian breeds of goat using hetrologous microsatelite markers. Anim. Biotechnol. 12: 121-136. PMID: 11808627.

Hanrahan, V., A.J. Ede and A.M. Crawford, 1994. Ovine x-chromosome microsatellite at the oarAE25 and oarAE133 loci. Anim. Gen., 25: 370-375. PMID: 7818183.

Kimura, M. and J.W. Crow, 1964. The number of alleles that can be maintained in a finite population. Genetics, 49: 725-738. PMID: 14156929.

Li, M.H., S.H. Zhao, C. Bian, H.S. Wang, H. Wei, B. Liu, M. Yu, B. Fan, S.L. Chen, M.J. Zhu, S.J. Li, T.A. Xiong and K. Li, 2002. Genetic relationships among twelve Chinese indigenous goat populations based on microsatellite analysis. Gen. Select. Evolu., 34: 729-744. PMID: 12473236.

Mahmoudi, B., S.M. Babayev, M. Daliri, M. Nejadgashti and S. Esmaeelkhanian, 2009. Molecular investigation of microsatellite markers in 6 Iranian native goat populations. J. Anim. Vet. Adv., 8 (3): 420-423.

Maudet, C., G. Luikart and P. Tberlet, 2001. Development of microsatellite multiplexes for wild goats using primers designed from domestic bovidea. Gen. Select. Evolut., 33: 193-203. INIST: 14418935400-01032389. 0110.

Miller, S.A., D.D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucl. Acids Res., 16: 1215-1215. PMID: 3344216.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA, 70: 3321-3323. PMID: 4519626.

Notter, D.R., 1999. The importance of diversity in livestock populations of the future. J. Anim. Sci., 77: 61-69. PMID: 10064028.

Ott, J., 1989. Program HET version 1.10. Utility programs for analysis of genetic linkage. Rockefeller University. New York, USA. ftp://linkage.rockefeller. edu/software/utilities.

Saitaekova, N., C. Gaillard, G. Obexer-Ruff and G. Dolf, 1999. Genetic diversity in Swiss goats breeds based on microsatellite analysis. Anim. Gen., 30: 36-41. PMID: 10050281.

Yang, L., S.H. Zhao, K. Li, Z.Z. Peng and G.W. Montgomery, 1999. Determination of genetic relationships among five indigenous chinese goat breeds with six microsatellite markers. Anim. Gen., 30: 452-455. PMID: 10612235.

Yeh, F.C., R. Yang and T. Boyle, 1999. POPGENE version 3.1, Microsoft Window-based Freeware for Population Genetic Analysis, University of Alberta. Edmonton, AB, Canada. ftp://ftp.microsoft.com/ Softlib/MSLFILES/HPGL.EXE. http://www.Ualberta. ca/~fyeh/popgene.pdf.