

## Genetic Analysis in Tali Goats Based on 13 Microsatellite Markers

<sup>1</sup>R. Sadeghi, <sup>2</sup>B. Mahmoudi, <sup>2</sup>M.S. Babayev, <sup>2</sup>Y. Rameshknia and <sup>3</sup>M. Daliri

<sup>1</sup>Laboratory of Veterinary, Meshkinshahr, Iran

<sup>2</sup>Department of Genetic, Baku State University, Baku, Azerbaijan

<sup>3</sup>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 (Esmaelkhanian *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.

Microsatellite 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.

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

Locus	Primer sequence	Type of repeat	Size range	Chromosome No.
BM121	IGGCAIIGIGAAAAGAAGTAAAA CTAGCACATATCIGGCAAGCA	TC <sub>n</sub>	165-185	16
BM4621	CAAAIIGACIIATCCIIIGGCIG IGTAACTATIGGGCIGCAIC	CA <sub>n</sub>	106-148	6
ILS IS005	GGAGCAATGAAATCIIATAGCC IGIICIGIGAGIIIGIAAGC	nn <sub>n</sub>	174-190	10
ILS IS022	AGICIGAAGGCCIGAGAACC CTIACAGICCIIGGGGIIIGC	GI <sub>n</sub>	186-202	Ann
ILS IS029	IGI IIIIGAIGGAACACAGCC IGGAI IAGACCAGGGIIGG	CA <sub>n</sub>	148-191	3
ILS IS033	IAT IAGAGIIGGCICAGIGCC ATGCAGACAGI I IAGAGGG	CA <sub>n</sub>	151-187	12
ILS IS34	AAGGGICIAAGICACIGGC GACCIGGII IAGCAGAGAGC	GI <sub>n</sub>	153-185	5
LSCV36	GCACACATACACAGAGATGCG AAAGAGGAAAGGGI IATIGCIGGA	CA <sub>n</sub>	524	19
MAF64	AAIAGACCAIICAGAGAAACGIIIGAC CTCAICGAAICAGACAAAGGIAGG	IG <sub>n</sub>	121-125	1
oarAE133	AGCCAGIAGGCCICACCAGG CCAACCAIIGGCAGCGGAGIGIGG	IG <sub>n</sub>	152	Ann
oarFCB304	CCC IAGGAGCIIICAA IAAAGAAICGG CGCIGCIGICAACIGGGICAGGG	CI <sub>n</sub> , CA <sub>n</sub>	119-169	Ann
oarJMP23	GIAICIIIGGGAGCCIGIGGII IATC GICCCAGAIGGGAATIGICICAC	-	-	27
IGLA122	AAICACAIGGCAAAIAGIACA IAC CCCICCCAGGIAAAICAGC	CA <sub>n</sub>	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 chromosomes. 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 component: 200 µM dNTPs, 3.5-6 mM MgCl<sub>2</sub>, 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, annealing temperature was modified as following: 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 programme (Crawford *et al.*, 1995), for oarAE133 was used PCR programme (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 HE T softwar (Ott, 1989).

Table 2: PCR reaction conditions for all loci exceptional IGLA122, oarJMP23 and oarAE133 loci

Stage	PCR process	Temperature (°C)	Time
1	Denaturation	95	2.5 min
2	Denaturation	95	30 sec
3	Annealing	-	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 Boushehr province especially near the towns of Minab, Bandarabbas, Khamir, Bandarlengh 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 allele in the TGLA122 locus observed in the prior studies on wild goats (*Capra ibex*) but nine allele in Tali goats were observed. In this study that the most numerous of stutter was observed in oarJMP23 locus and the possible explanation for this failure is the perfect of locus and least numerous of stutter was observed 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.

## REFERENCES

- 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 heterologous microsatellite 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. Evol.*, 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>.
- Saitakova, 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>.