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The Investigation of Genetic Variation in Taleshi Goat Using Microsatellite Markers

Bizhan Mahmoudi and Majnoun Sh. Babayev
Department of Genetic, Baku State University, Baku, Azerbaijan

Abstract: In this study, the genetic variation in Taleshi goats were investigated using 9 microsatellite markers (LSCV41, LSCV36, TGLA122, MAF64, oarFCB304, oarJMP23, oarAE133, BM121, BM4621) all of nine loci were amplified successfully. The objectives of this study were to assess the genetic variability among Taleshi goat breed. The genetic characterizations of this genetic resource are essential to conservation and breeding programs. Hardy-Weinberg Equilibrium (HWE) had been tested in the level of probability ($p<0.005$). 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 and Shanon information index had calculated in this study. This research was shown that microsatellite technique is a useful tool for evaluation of genetic variation among of domesticated animals.

Key words: Taleshi goat, microsatellite markers, genetic variation, polymorphism, heterozygosity, HWE

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. 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 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 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 48 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.

In this study was used 9 microsatellite primer pairs including MAF64,BM4621, BM121, LSCV41, LSCV36, TGLA122, oarJMP23, oarFCB304 and oarAE133 made in Tib Mol Biol company. 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). They showed polymorphism in the goat of world. Nine microsatellite markers, their sequences, type of repeat, size rang and their location showed in Table 1.

Corresponding Author: Bizhan Mahmoudi, Department of Genetic, Baku State University, Baku, Azerbaijan

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

Locus	Primer sequence	Type of repeat	Size range	Chromosome No.
BM4621	CAAATTGACTTATCCTTGGCTG			
	TGTAACATATGGGCTGCATC	CA_{14}	106-148	6
BM121	TGGCATTGTGAAAAGAAGTAAAA			
	CTAGCACTATCTGGCAAGCA	TC_{18}	165-185	16
LSCV41	-	-	-	27
LSCV36	GCACACACATACACAGAGATGCG			
	AAAGAGGAAAGGGTTATGTCTGGA	CA_{16}	524	19
MAF64	AATAGACCATTCAGAGAAACGTTGAC			
	CTCATCGAATCAGACAAAAGGTAGG	TG_{13}	121-125	1
oarFCB304	CCCTAGGAGCTTTCAATAAAGAATCGG			
	CGCTGCTGTCAACTGGGTCAGGG	CT_{11}, CA_{15}	119-169	Ann
TGLA122	AATCACATGGCAAATAAGTACATAC			
	CCCTCCTCCAGGTAAATCAGC	CA_{21}	145	21
oarJMP23	GTATCTTGGGAGCCTGTGGTTTATC			
	GTCCCAGATGGGAATTGTCTCCAC	-	-	27
oarAE133	AGCCAGTAGGCCCTCACCAGG			
	CCAACCATTGGCAGCGGGAGTGTGG	TG_{24}	152	Ann

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	-

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), LSCV41 (55°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) and for oarAE133 was used PCR programe (Hanrahan *et al*., 1994).

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).

Characterizations of Taleshi goats: Taleshi goats are kept for milk production. They have long hair on the rump and upper thighs. The Taleshi goats are medium sized and mostly is black, white and chocolate brown colored. Natural service is metod of Breeding for this goat. The breed is well adapted to the humid region. The male and most of female have horns. Height at shoulder and body weight is 65 cm and 45 kg in adult male and 61 cm and 40 kg in adult female goat, respectively.

RESULTS AND DISCUSSION

PCR reactions were successfully done on all nine primers. Seven allel in the TGLA122 locus observed in the prior studies on wild goats (*Capra ibex*) but eight allel in Taleshi 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 9 microsatellites loci analyzed, observed and 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), the observed Heterozygosity (H_o) expected the unbiased average of Heterozygosity (H_e) and Polymorphic Information Content (PIC) values at locus shown in Table 3.

Yang *et al*. (1999) H_e value of oarFCB304 locus estimated 0.854 on Chinese goats but it was 0.662 in Taleshi goat.

We calculated shanon information index by POPGENE software that the most of value in oarJMP23 locus and the least of value in oarAE133 locus that this problem accordance with number of allele in each loci. Each 9 loci analysis was 100% polymorphic. Highest and

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

Locus	n	n_e	H_o	H_e	PIC
BM4621	5	2.3705	0.6042	0.5842	0.5352
BM121	7	4.3748	0.5854	0.7799	0.7341
LSCV41	8	4.2355	0.6667	0.7719	0.7348
LSCV36	6	4.1525	0.4043	0.7673	0.7252
MAF64	7	5.2432	0.2645	0.8256	0.8142
oarFCB304	4	2.9610	0.1562	0.6623	0.6615
TGLA122	8	5.1125	0.1429	0.8125	0.8011
oarJMP23	10	3.4311	0.8015	0.7132	0.7105
oarAE133	5	3.9523	0.1335	0.7510	0.7509
Mean	6.7	3.8820	0.4176	0.7408	0.7186
SD	1.87	1.1540	0.2550	0.0767	0.0825

lowest number of allele objective was 10 and 4 allele for oarJMP23 and oarFCB304 loci with, respectively. Highest and lowest number of allele effective was 5.2 and 2.3 for MAF64 and BM4621 loci with, respectively.

All average the number of allele objective and effective was 6.7 and 3.8, respectively. Highest and lowest PIC value was 0.8142 and 0.5352 for MAF64 and BM4621, respectively. The average of PIC value for this population was 0.7186 but it was between 0.746-0.8 in Chinese goats (Yang *et al*., 1999).

The Taleshi goats had substantial genetic variation based on their gene diversity and average number of alleles per locus. The average genetic variation (0.417) in Taleshi goats lesser 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.

CONCLUSION

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

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