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Genetic Analysis of Markhoz Goat Based on Microsatellite Markers

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Abstract: In this study, the genetic variation in Markhoz 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 Markhoz 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 showed that microsatellite technique is a useful tool for evaluation of genetic variation among of domesticated animals.

Key words: Markhoz goat, microsatellite markers, genetic variation, polymorphism

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.

In this study, was used 13 microsatellite primer pairs including MAF64, BM4621, BM121, LSCV36, TGLA122, oarJMP23, oarFCB304, oarAE133, ILSTS005, ILSTS022,

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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	$(C)_{18}$	165-185	16
BM4621	CAAATTGACTTATCCTTGGCTG TGTAACATATGGGCTGCATC	$(CA)_{16}$	106-148	6
ILSTS005	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	$(an)_{19}$	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	$(TG)_{11}$	121-125	1
oarAE133	AGCCAGTAGGCCCTCACCAGG CCAACCATTGGCAGCGGGAGTGTGG	$(TG)_{24}$	152	Ann
oarFCB304	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	$(CT)_{11}(CA)_{15}$	119-169	Ann
oarJMP23	GTATCTTGGGAGCCTGTGGTTTATC GTCCCAGATGGGAATTGTCTCCAC	-	-	27
TGLA122	AATCACATGGCAAATAAGTACATAC CCCTCCTCCAGGTAAATCAGC	$(CA)_{21}$	145	21

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	30s
3	Annealing	-	30s
4	Extension	72	30s
5	Final extension	72	2.5 min
6	Maintenance	4	-

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 in 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: MAF 64 (62.5°C), BM 4621 (58°C), LSCV 36 (55°C), oarFCB 304 (60.5°C) and BM 121 (65.5°C). The rest of PCR process is in accordance with the Table 2.

For oarJMP23 and TGLA 122 primers were used PCR programe (Crawford *et al*., 1995), for oarAE 133 was used PCR programe (Hanrahan *et al*., 1994) and For ILSTS005, ILSTS022, ILSTS029, ILSTS033 and ILSTS34 primers. The touchdown PCR protocol was used.

Fig. 1: Markhoz goats in the pasture

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 Markhoz goats: The Markhoz is mainly used for wool, which is sold as mohair. Markhoz was originally kept in the province of Kordestan. The Markhoz goats are medium-sized and Mostly is black, white and chocolate brown colored. Natural service is method of Breeding for this goat. The male and female have horns. Height at shoulder and body weight is 60 cm and 45 kg in adult male and 50 cm and 35 kg 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 Markhoz 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, 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 values (PIC) at locus showed Table 3.

Yang *et al*. (1999) H_e value of oarFCB304 locus estimated 0.854 on Chinese goats but it was 0.884 in Markhoz goat.

Each 13 loci analysis was 100% polymorphic. Highest number of allele objective was 10 allele for oarJMP23 and BM4621 loci and lowest number of allele objective was 4 allele for oarAE133 loci. Highest and lowest number of allele effective was 8.8 and 2.3 for oarJMP23 and ILSTS033 loci with respectively.

All average the number of allele objective and effective was 8.077 and 5.262, respectively. Highest and lowest PIC value was 0.889 and 0.621 for oarJMP 23 and ILSTS 029, respectively. The average of PIC value for this population was 0.767, it was between 0.746-0.8 in Chinese goats (Yang *et al*., 1999).

The Markhoz goats had substantial genetic variation based on their gene diversity and average number of alleles per locus. The average genetic variation (0.814) in Markhoz 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_o, H_e and PIC values at locus in Markhoz goat population

Locus	n	n_e	H_e	PIC
BM121	8	6.0	0.849	0.816
BM4621	10	8.2	0.894	0.865
ILSTS005	9	4.4	0.801	0.765
ILSTS022	7	4.6	0.791	0.743
ILSTS029	9	2.9	0.618	0.621
ILSTS033	8	2.3	0.603	0.630
ILSTS34	8	4.3	0.787	0.736
LSCV36	8	5.7	0.839	0.802
MAF64	6	4.5	0.794	0.749
oarAE133	4	3.9	0.756	0.697
oarFCB304	9	5.9	0.884	0.820
oarJMP23	10	8.8	0.903	0.889
TGLA122	9	6.9	0.871	0.839
Mean	8.077	5.262	0.799	0.767
SD	1.656	1.912	0.096	0.083

CONCLUSION

The result of this study suggests that there is substantial genetic variation and polymorphism across the studied loci in Markhoz 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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