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Molecular Investigation of Microsatellite Markers in 6 Iranian Native Goat Populations

[1]B. Mahmoudi, [1]M.Sh. Babayev, [2]M. Daliri, [3]M. Nejadgashti and [4]S. Esmaeelkhanian
[1]Department of Genetic, Baku State University, Baku, Azerbaijan
[2]Department of Animal Science, Genetic Engineering and Biotechnology Instituted, Tehran, Iran
[3]Department of Animal Science, Faculty of Agriculture, Islamic Azad University of Karaj, Karaj, Iran
[4]Department of Biotechnology, Animal Science Research Institution of Iran, Karaj, Iran

Abstract: In this study the genetic variation among 6 Iranian native goats population was investigated using 10 microsatellite markers (LSCV38, LSCV41, LSCV36, TGLA122 MAF64, oarFCB304, oarJMP23, oarAE133, BM121 and BM4621) every 10 loci were amplified successfully but the data resulting from the LSCV38 locus was ignored because of a large number of Null allele during the analysis steps. The rest nine loci in all populations were 100% polymorphic. Lori (LOR), Raeeni (RAN), Najdi (NAJ) and Korki Jonoob Khorasan (KAJ) populations did not present no deviation of Hardy-Weinberg Equilibrium (HWE). Morkhoz (MOR) and Tali (TAL) population presented no deviation from HWE in locus-population components ($p<0.005$) but considering all populations did not observe deviation from HWE ($p<0.005$). MOR and NAJ population had the highest and the lowest genetic variation within population 0.847 and 0.725, respectively. Unbiased average Heterozygosity (H_e) was expected by Nei. TAL and NAJ population had the lowest genetic distance (0.2728) and the highest genetic distances considering above criteria were for LOR and TAL population (0.7448). Phylogeny tree based on UPGMA method with D_S criterion has been put Iranian goat population in 2 great clusters. Assignment test, considering the analysis nine loci, assigning individual 2 population verify high confidence.

Key words: Iranian native goat, microsatellite markers, genetic variation, genetic distance

INTRODUCTION

Microsatelite genetic markers are called Short Tandem Repeats (STRs) or simple sequence repeat 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. Instability of microsatellites loci have made an exceptional phenomenon for genetic and evolution studies. The goat are the first animal which has been domesticated by human. Because of the high ability against the lack of forage and their high endurance against environmentally hard conditions are called poor man's cow. They play an important role in the rural socials economic. The distribution of wide in the world from Siberian cold locals until African hot deserts is validation their high adaptability with environment hard conditions. There are 3.8% from 550 milion head goat world in Iran. Furthermore, archeology and phylogenetic evidences had proved the origin of goats from Mesopotamia areas and west Zagros in Iran (Bruford *et al*., 2003).

In this study, were estimated hetorzygosity and other criteria polymorphism and genetic distance and genetic variation between six Iranian population native goats using microsatelite markers. The present study is the first research on Iranian native goats using microsatellite markers.

MATERIALS AND METHODS

All in all 159 samples was collecetd from wide areas of these goat from Iran (Fig. 1). Sample size and wide area as follows:

Twenty five samples Markhoz population (MAR) from Kordsatan and Kermanshah provinces, 18 samples Lori population (LOR) from Lorstan province, 18 samples Najdi population (NAJ) from Khozestan province. Thirty samples Tali population (TAL) from Hormozgan province, 30 samples Raeeni population (RAN) from Kerman province and 35 samples Korki Jonob Khorasan population (JAK) from Khorasan province then bleeding were transfered them to the labrotoary and DNA genomic was extracted by salting out method (Miller *et al*., 1988). We use both spectrophotometry and agarose gel (0.8%) for DNA quality definition.

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

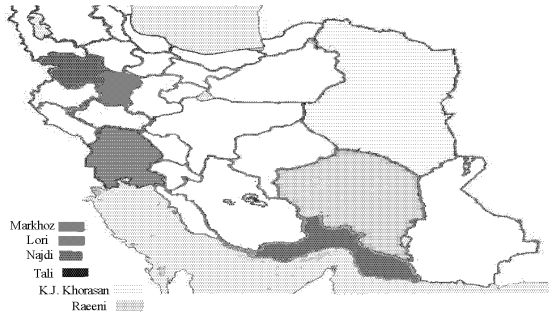

Fig. 1: The spreading regions of 6 Iranian goats populations

Table 1: PCR reaction conditions for all loci exceptional TGLA122, oarAE133 and oarJMP23 loci

PCR process	Temperature (°C)	Time
Denaturation	95	2.5 min
Denaturation	95	30 sec
Anealing	-	30 sec
Extension	72	30 sec
Final extension	72	2.5 min
Maintenance	4	-

PCR reactions: In this study was used 10 microsatellite primer pairs including MAF64,BM4621, BM121, LSCV41, LSCV38, LSCV36, TGLA122, oarJMP23, oarFCB304 and oarAE133 made in Tib Mol Biol company.

These loci in prior studies had been amplified on the goat (Maudet *et al*., 2001; Yang, 1999; Hanrahan *et al*., 1994).

They showed polymorphism in the goat of world. 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 U Taq 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), LSCV38 (54°C), LSCV36 (5°C), oarFCB304 (60.5°C) and BM121 (65.5°C).

The rest of PCR process is in accordance with the Table 1 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 by POPGENE software (Yeh *et al*., 1999). Nei *et al*. (1983) standard genetic distance and D_A genetic distance matrices were calculated by MICROSAT software and dendrograms were constructed using Unweighted Pair-Group Method using an Arithmetic Average (UPGMA) by POPGENE software (Yeh *et al*., 1999) with 1000 bootstrap replication. Unbiased average expected Heterozygosity (He) (Nei, 1978) was calculated by POPTREE software. Polymorphism criteria such as Polymorphic Information Content (PIC) and the number of observed and effective alleles were also estimated by HET (Ott, 1989) and POPGENE software, respectively.

RESULTS AND DISCUSSION

PCR reactions were successfully done on 10 primers. The data resulting from the LSCV38 locus was ignored because of a large number of Null allele during the process of analysis. The possible explanation for this failure is point mutation event at the primer site and the lengthy of locus (O`Connel and Wright, 1997).

TGLA122 locus was amplified the first on domesticated goat. Seven allel in the locus observed in the prior studies on wild goats (capra ibex) but 13 allel in iranian native 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. Figure 2 showing alleles concerning BM4621 marker on Tali population.

Morkhoz (MOR) and Tali (TAL) populations present no deviation of HWE in locus-population component the level of probability ($p<0.005$). Lori (LOR) population in 4 loci (BM121, MAF64, TGLA122, LSCV36) Raeeni (RAN) in 3 loci (TGLA122, BM121, LSCV41) Najdi (NAJ) in 2 loci (LSCV41, BM121) and Korki Jonoob Khorasan (KAJ) don't show the deviation of Hardy-Weinberg Equilibrium (HWE).

Most and least unbiased expected hetrozygosity is for MAR (0.847) and NAJ (0.725), respectively. H_e and PIC values at locus-population combinations, per population showed Table 2.

Yang *et al*. (1999) H_e value of oarFCB304 locus estimated 0.854 on Chinese goats but it was 0.842 in iranain native goat.

We calculated shanon information index by POPGENE software that the most of value is for TAL population (2.3198) in oarJMP23 locus and the least of value is for NAJ population (0.6931) in oarAE133 locus that this problem accordance with number of allele in each loci. Each 9 loci analysis was 100% polymorphic.

Highest a nd lowest number of allele objective was 17 and 7 allele for oarJMP23 and LSCV41 loci with, respectively. Highest and lowest number of allele effective was 13.2and 5.4 for oarJMP23 and oarAE133 loci with, respectively.

Table 2: H_e and PIC values at locus-population combinations, per population

Population	MAR		LOR		NAJ		TAL		RAN		JAK	
locus	H_e	PIC	H_e	PIC	H_e	PIC	H_e	PIC	H_e	PIC	H_e	PIC
MAF64	0.794	0.749	0.775	0.714	0.664	0.689	0.741	0.666	0.734	0.734	0.851	0.818
BM4621	0.894	0.865	0.697	0.622	0.859	0.816	0.884	0.850	0.817	0.817	0.846	0.800
BM121	0.849	0.816	0.846	0.794	0.808	0.753	0.674	0.606	0.888	0.888	0.818	0.778
LSCV36	0.839	0.802	0.797	0.751	0.730	0.654	0.727	0.678	0.921	0.921	0.811	0.788
TGLA122	0.871	0.839	0.876	0.835	0.751	0.686	0.872	0.843	0.839	0.839	0.886	0.859
LSCV41	0.877	0.845	0.797	0.742	0.749	0.740	0.870	0.740	0.889	0.889	0.801	0.755
OarJMP23	0.903	0.889	0.832	0.809	0.909	0.880	0.913	0.887	0.900	0.900	0.889	0.871
OarFCB304	0.884	0.820	0.708	0.618	0.541	0.421	0.543	0.525	0.635	0.635	0.702	0.639
OarAE133	0.756	0.697	0.675	0.582	0.514	0.375	0.624	0.541	0.777	0.777	0.629	0.553

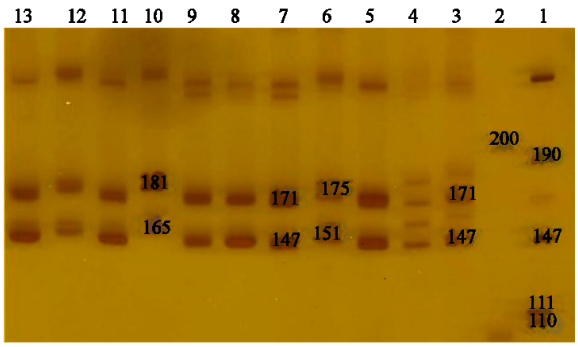

Fig. 2: Polyacrylamide nondenaturing gels (8%) showing alleles concerning BM4621 marker on Tali population.DNA size markers are on wells 1, 2 the alleles and sizes showed in bp

All average the number of allele objective and effective was 12.5 and 9.4, respectively. Highest and lowest PIC value was 0.8148 and 0.6684 for MAR and NAJ respectively. But it was between 0.746-0.8 in Chinese goats (Yang *et al*., 1999). Genetic distances matrices then replication 1000 bootstrap had been determined using tow criterions genetic distance D_S and Nei (1978) unbiased.

Genetic distances matrices were showed between 6 Iranian goat populations. Genetic distance matrices based on unbiased Nei (1978) (upper diagonal matrix) and D_S (lower diagonal Genetic distance matrices based on D_A (upper diagonal matrix) and D_S (lower diagonal matrix) using 1000 bootstrap replications using 1000 bootstrap replications (Table 3).

The range of distances D_s had been estimated "between" 0.2728-0.7448. Because low hemozygosity and high hetrozygosity had got resulted big values but lowest genetic distance had got result between TAL and NAJ populations (0.2728) on the base on D_S criteria.

These results were accorded with the origin of 2 populations that both were in Arabia area. Existing 2 populations in sultry and hot areas and having the same morphological characteristics are other evidences this

Table 3: Genetic distance matrices based on D_S (upper diagonal matrix) and D_A (lower diagonal matrix) using 1000 bootstrap replications

	MAR	LOR	NAJ	TAL	RAN	JAK
MAR		0.5125	0.4268	0.4065	0.4848	0.4762
LOR	0.401		0.7139	0.7448	0.6138	0.5638
NAJ	0.323	0.562		0.2728	0.5843	0.7168
TAL	0.469	0. 678	0.610		0.6275	0.6275
RAN	0.362	0.457	0.486	0.539		0.5069
JAK	0.304	0.431	0.540	0.426	0.399	

statement. The highest genetic distance got result between LOR and TAL population (0.7448) this result were accordance with the origin of 2 populations that both were far from each other.

With considering D_S criteria, 2 populations (TAL and NAJ) had highest similarity genetic (0.7272) and 2 populations (TAL and LOR) had lowest similarity genetic (0.2807).

The Fig. 3 shows six Iranian native goat populations in 2 groups:

Group 1 : Markhoz, Tali and Najdi
Group 2 : Korki jonob khorasan, Raeeni and Lori

Phylogeny based on UPGMA method with D_S criterion placed the Iranian goat populations presented in this study in 2 great clusters in the way that MOR and NAJ TAL populations were placed in one great cluster. NAJ and TAL populations were placed in one cluster separately from MOR and the rest tree population were placed in the 2nd great cluster. Placed 2 populations (Tal and Naj) in one cluster are reason on their similarity origin.

In the way that RAN and KAJ were placed in one cluster separately from LOR Assignment test, considering the analytic nine loci, assigning individual to populations carried out with recommendation (Cornuet *et al*., 1999). Fst value calculated 0.127 and hetrozygosity value was >0.6. We assignment test carried out using GENE CLASS software (Piry and Cornuet, 1999). Using Bayesian and frequencies genetic distance methods had been result High accuracy of assigning individuals. The results are summarized in Table 4.

Table 4: The percentage of individuals correctly assigned shows with 3 method frequency, Baysian and Genetic distance that including three procedure as follow: Add one In, Leave one out and With out leave one out (null allele frequencies were set to 0.01, systematically)

						Genetic distance			
	Frequency			Bayesian		D_A		D_S	
Assignment method	Add one In	Leave one out	With out leave one out	Leave one out	With out leave one out	Leave one out	With out leave one out	Leave one out	With out leave one out
Accuracy	98.6%	100%	100%	100%	100%	97.87%	99.29%	99.29%	99.29%

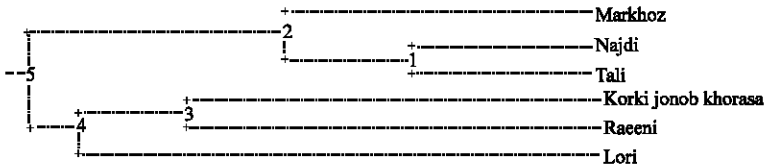

Fig. 3: Pylogenetic tree had been constructe on base genetic distance D_S criteria and using UPGMA method this tree constrcucted the use of 1000 bootstrap replication

CONCLUSION

This research showed high variation within and between studied Iranian goat's populations for 10 microsatellite loci. It also demonstrated that microsatellite genotyping is a useful tool for evaluating variation evolutionary relationships among important goat populations. Microsatellite-based estimates of population relationships were consistent with known demographic history and geographic distances.

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