International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2736-1756 Vol. 18 (7), pp. 001-005, July, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Assessing Genetic Relationships and Population Differentiation in Karakul Sheep Using Microsatellite Markers

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Accepted 22 March, 2024

In this study, the genetic variation in Karakul sheep was investigated using 15 microsatellite markers (MCMA2, BMS460, BM1815, OARCP26, OARFCB20, MAF64, OARAE129, BMS332, LSCV38, BM6444, BMS995, MCMA26, BMS678 and OARCP49) and all fifteen loci were amplified successfully. Genomic DNA was extracted from 120 blood samples, using modified salting-out method. Tests of genotype frequencies for deviation from the Hardy-Weinberg equilibrium (HWE) were performed at each locus and revealed significant departure from HWE (P < 0.001) due to heterozygote excess. Parameters of variability such as effective number of alleles and gene diversities corroborated with the high level of variation frequently displayed by microsatellite markers. The fifteen tested loci were all polymorphic. Furthermore, other criteria of genetic variation including polymorphism information content (PIC) values and Shanon information index were calculated in this study. Results showed that, high level of genetic diversity was observed in Karakul breed and this breed was not at risk for conservation concept. This research has also shown that microsatellite technique is a useful tool for evaluation of genetic variation among domesticated animals.

Key words: Karakul sheep, microsatellite markers, genetic variation, polymorphism. Hardy-Weinberg equilibrium (HWE).

INTRODUCTION

The genetic polymorphism and diversity found in the domestic breeds allow farmers to develop new characteristics in response to changes in environment or market conditions. So, the importance of increasing, maintaining and conserving the genetic diversity in these animals for the future has been recognized. There are 54 million sheep in Iran that has variety of products. There are three well-known breeds of sheep for pelt production (Zandi, Gray-Shiraz and Karakul). This breed is very resistant to harsh condition and raised mostly under the semi nomadic system of management. Karakul breed is mainly found in the plain of Sarakhs in the province of Khorasan neighboring Turkmenistan. The number of the breed in the area of distribution is estimated to be 0.3 million. In the past, it was famous for the soft fur and raised for pelt production. At present, the newborn pelt is not raised due to the market demand for this product and increasing demand of the red meat (Monem et al., 2004). This study used microsatellite markers because they are powerful tools for tracking alleles through a population and for estimating genetic variability and inbreeding (Zajc et al., 1997).

The purpose of this study was to reveal the genetic polymorphisms of Karakul pelt sheep by examining the microsatellite DNA and to provide some theoretical basic for conservation and improvement of this sheep.

MATERIALS AND METHODS

Blood samples were collected from 120 sheep of both sexes, by puncturing the jugular vein with the vacutainer. DNA genomic was extracted by modified salting-out method (Miller et al., 1988). DNA quality definition was determined using both spectrophotometry and agarose gel (0.8%). Fifteen microsatellite markers were selected in respect of polymorphisms, a non-linkage criterion for syntenic loci and criterion of location on different chromosomes (Table 1). All PCR reaction were continued with the following component: 1X

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Table 1. Characteristics of the microsatellites under investigation.

Name	Primer sequence	Chromosome no.	Accession no.	Allelic range	Annealing Temperature (°C)	References
MCMA2	TCACCCAACAATCATGAAAC / TTAAATCGAGTGTGAATGGG	13	AF098773	157-201	52	Maddox et al., 2000
MCM63	CCCAATTTGGCAACAGCTACG / ATTGGCCTCTCTCTGATGCAC	9	L37889	120-168	55	Smith et al., 1995
BMS460	TGCCCCATAGTGTAGTGCTC / GCCAGCAGAGAATTGTAGCA	3	G18836	120-148	58	Maddox et al., 2000
BM1815	AGAGGATGATGGCCTCCTG / CAAGGAGACAAGTCAAGTTCCC	20	G18389	Not available	55	Bishop et al., 1994
OARCP26	GGCCTAACAGAATTCAGATGATGTTGC / GTCACCATACTGACGGCTGGTTCC	4	U15698	120-170	55	Ede et al., 1995
OARFCB20	AAATGTGTTTAAGATTCCATACAGTG / GGAAAACCCCCATATATACCTATAC	2	L20004	83-123	55	Buchanan et al., 1993
OARAE129	AATCCAGTGTGTGAAAGACTAATCCAG / GTAGATCAAGATATAGAATATTTTTCAACACC	5	L11051	133-159	52	Penty et al., 1993
MAF64	AATAGACCATTCAGAGAAACGTTGAC / CTCATGGAATCAGACAAAAGGTAGG	1	M62993	109-141	63	Swarbrick et al., 1991
BMS332	GACAAAACCCTTTTAGCACAGG / AATTGCATGGAAAGTTCTCAGC	22	G18841	127-157	57	Maddox et al., 2000
LSCV38	GTTGCAAAGAGCTGGACGTG / CTGGATGGCAAAGTGATTCAG	12	G40990	102-122	54	Maddox et al., 2000
BM6444	CTCTGGGTACAACACTGAGTCC / TAGAGAGTTTCCCTGTCCATCC	2	G18444	128-165	55	Bishop et al., 1994
BMS995	AATTCTTCCAACCTCCAGTGC / ACTTTTCAAGCAGGGCTCAC	13	G18766	121-147	58	Maddox et al., 2000

Table 1. Contd.

MCMA26	TCTCTGCTTTCCAGCCTTATTC / AGAGCTTTTAGGACAGCCACC	18	AF098961	188-212	52	Maddox et al., 2000
BMS678	ACCATCTACTGTGCTATGGCTT / GCAGAAACACAATACTCAGTGC	2	G18734	100-130	54	Gortari et al., 1997
OARCP49	GTGGGGATGAATATTCCTTCATAAGG	17	U15702	85-107	63	Ede et al., 1995

buffer, 200 M dNTPs, 1.5 to 4.5 mM $MgCl_2$, 0.25 M of each Primers, 1 units Taq polymerase and 100 to 200 ng DNA. The final volume was 15 l. Reactions were done with Gradient Master Cycler Ependorf. The cycling protocol was as follow: 5 min denaturing at 95°C followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 52 to 63°C (depending on primers) for 30 s, extension at 72°C for 45 s and the final extension at 72°C for 5 min (Table 2). Amplification products were electrophoresed on 8% denaturing polyacrylamide gels and stained according to rapid silver staining procedure. The gel was photographed using Gel–DocXR (BioRad). Patterns of the different genotypes for each microsatellite locus were analyzed using Gel- Pro analyzer, version 3.1 for windows TM, which determine the allele's sizes in each animal. The observed number of alleles (Na), effective number of alleles (Ne), observed heterozygosity (Ho) and expected heterozygosity (He) were estimated using GenAlEx software, version 6 (Peakall and Smouse, 2006). Test of departure from Hardy-Weinberg equilibrium at each locus were performed using Chi-square and likelihood ratio or G- square test (Hedrick, 2000) using GenAlEx 6 program and POPGENE software version 1.31(Yeh et al., 1999), respectively. Since the maximum amount of heterozygosity is equal to 1, the values of polymorphic markers, like microsatellites, are not sufficiently sensitive to show increase in the variation. For prevalence of this deficiency, we used the Shannon information index (i) . Polymorphism information content (PIC) was calculated using HET software version 1.8 (Ott, 2001). In order to assess the presence of inbreeding within population, the Wright (1978) fixation index (F_{IS}) as a measure of heterozygote deficiency was calculated for Karakul population according to original formulas, and a bootstrap analysis on 1000 replicates was performed to estimate the standard deviation of F_{IS} .

RESULTS

In the present study, fifteen microsatellite loci were used to evaluate the genetic diversity within Karakul sheep breed reared in Iran. PCR reactions were successfully done on all fifteen primers. A total of 121 alleles were detected across the 15 analyzed microsatellite loci. All the loci were polymorphic (2 alleles) (Crawford et al., 1995). Allele frequencies are available from the corresponding authors on request. All loci were deviate from Hardy-Weinberg equilibrium ($p < 0.001$). The number of allele (n_a), number of allele effective (n_e), the observed of heterozygosity (H_o), the expected heterozygosity (H_e), PIC, Shannon information Index (I) and fixation index (F_{IS}) at locus are shown in Table 3. The expected heterozygosity (H_e) ranged from 0.691 (MAF64) to 0.910 (MCMA2) with mean of 0.831 per locus. The number of allele (n) and number of allele effective (n_e), ranged from 4 (MAF64) to 12 (MCMA2 and BMS678) and from 3.2 (MAF64) to 11.2 (MCMA2), respectively.

In the present study, polymorphism information content revealed an average of 0.808 with a range of 0.634 (MAF64) to 0.903 (MCMA2). According to Table 3, Shannon information index ranged from 1.26 at locus MAF64 to 2.45 at locus MCMA2 with mean of 1.93 per locus. Population inbreeding estimate (F_{IS}), was observed to be -0.197 with a range from -0.446 (MAF64) to -0.075 (MCMA2).

DISCUSSION

Over the past decade, numerous studies have been carried out on genetic diversity in domestic livestock (mainly in small ruminants), based on the analysis of microsatellite loci, worldwide (Dalvit et al., 2008; Kusza et al., 2010). The assessment of Karakul breed genetic variability based on the analysis of 15 microsatellite markers indicates that they exhibit high levels of genetic variability due to the extensive production system, low pressure of artificial selection and possibility of random mating. The genetic diversity of the breed was higher when compared with genetic diversity in other Iranian sheep (Zahedi etal., 2007; Molaee et al., 2009; Sharifi sidani etal., 2009) and this breed was not at risk for conservation concept.

In the global test of deviation from HWE, the deviations from the expected value may be due to a variety of causes: Excess of heterozygote individuals than homozygote individuals, migration, high mutation rate in microsatellite and artificial selection in all breeds (Aminafshar et al., 2008). Deviation from HWE at microsatellites loci have also been reported in various studies

Table 2. PCR reaction conditions for all loci.

Stage	PCR process	Temperature (°C)	Time
1	Denaturation	95	5 min
2	Denaturation	95	30 s
3	Annealing	Variable	30 s
4	Extension	72	30 s
5	Final extension	72	45 s
6	Maintenance	4	5 min

Table 3. Population genetic parameter for each microsatellite marker.

Locus	No. of alleles		Heterozygosity		PIC	I	F_{IS}
	Observed (n_a)	Effective (n_e)	Observed (H_o)	Expected (H_e)			
MCMA2	12.00	11.16	0.979	0.910	0.903	2.45	-0.075
MCM63	7.00	5.69	0.978	0.824	0.801	1.83	-0.186
BMS460	6.00	4.56	0.968	0.781	0.748	1.63	-0.240
BM1815	6.00	3.80	0.989	0.737	0.697	1.52	-0.342
OARCP26	6.00	4.79	0.989	0.791	0.763	1.68	-0.250
OARFCB20	6.00	5.17	1.000	0.807	0.779	1.71	-0.240
OARAE129	7.00	5.66	0.989	0.823	0.800	1.82	-0.201
MAF64	4.00	3.24	1.000	0.691	0.634	1.26	-0.446
BMS332	7.00	5.34	0.978	0.813	0.787	1.80	-0.204
LSCV38	8.00	5.95	1.000	0.832	0.812	1.92	-0.202
BM6444	9.00	7.99	1.000	0.875	0.862	2.13	-0.143
BMS995	11.00	9.83	0.969	0.898	0.889	2.34	-0.078
MCMA26	11.00	9.92	1.000	0.899	0.891	2.35	-0.112
BMS678	12.00	9.96	0.989	0.900	0.891	2.39	-0.100
OARCP49	9.00	8.17	1.000	0.878	0.865	2.14	-0.140
Mean	8.07	6.75	0.989	0.831	0.808	1.93	-0.197
SD	2.49	2.53	0.012	0.064	0.078	0.36	0.101

(El Nahas, 2008; Aminafshar et al., 2008; Sharifi sidani et al., 2009). High value of average expected heterozygosity (0.831) within the breed could be attributed to the large allele numbers detected in the tested loci (Kalinwski, 2002). The average direct count of heterozygosity overall loci in the population is more than the expected heterozygosity. Negative F_{IS} values observed in the present study indicate an excess of heterozygotes and so it does not probably encounter problems that results from inbreeding depression. This result may explain the observed high value of direct count of heterozygosity in population and the deviation from HWE which were detected in all loci.

The Karakul sheep has substantial genetic variation based on allele diversity (mean number of alleles per locus, 8.07) and gene diversity (mean expected heterozygosity, 0.831). The high values of various diversity measures obtained in the present study for Karakul were in accordance with that of other domestic sheep breeds (Arora et al., 2008; Esmail Khanian et al., 2007 and Banabazi et al., 2007). It also demonstrated that microsatellite genotyping is a useful tool for evaluating variation among important sheep populations.

ACKNOWLEDGEMENTS

The current study was supported by The Animal Science Research Institute, Karaj, Iran and Broujerd Branch, Islamic Azad University, Broujerd, Iran. We thank Mr Salari, for his help in the laboratory. However, we are grateful to all the staff of Animal Science Research Institute who helped us in this study.

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