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SHORT COMMUNICATION

Microsatellite Markers based Genetic Diversity Analysis in Damani and Nachi Goat Breeds of Pakistan

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ABSTRACT

The genetic variation in two indigenous Pakistani goat breeds (Damani and Nachi) was studied with 9 microsatellite markers in order to determine the genetic diversity between them. A total number of 50 non relative individuals of Damani (25) and Nachi (25) were sampled to explore genetic polymorphisms and relationship between these two important goat breeds. Result revealed considerable level of genetic diversity in both breeds and a total number of 53 alleles were identified with mean of 3.2 in Damani and 4.6 in Nachi. The genetic diversity in both breeds ranged from 0.51(Nachi) to 0.73 (Damani). High level of genetic differentiation (F_{ST} = 0.20) and low level of gene flow (Nm=0.95) found could be due to their divergent or expanded geographical locations. Heterozygote across two populations (F_{IT}) was found to be 0.15. The mean Polymorphic Information Content (PIC) was 0.70 ranging from 0.54 (MAF33) to 0.83 (ILSTS011), revealed the high level of polymorphism for studied microsatellite markers set in this study. The measures of genetic variation revealed that there is good scope for effective improvement, conservation and designing national breeding policies for goat breeds in future.

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INTRODUCTION

It is rightly claimed that the small ruminants in Pakistan offer a higher potential for milk, meat and wool production. Goats are important components of Animal Genetic Resources (AnGR) of Pakistan and play an important role in the economy of poor farmers. Pakistan has 63.1 million heads of goat population and stands third largest goat producing country in the world (Khan *et al.,* 2008). We have 30 documented goat breeds in Pakistan distributed in all types of geographical regions of the country. The understanding of genetic structure, variation and relationships among different breeds is essential for designing suitable breeding policies to conserve and utilize the available rich goat genetic resource of Pakistan using latest tools of molecular characterization. This depends on the knowledge of their genetic structure based on molecular markers like microsatellites.

Microsatellites, also called short tandem repeats (STR) are among the preferred method of genetic assessment due to their abundance, high polymorphism, low mutation rate, ease of amplification through PCR and small product size (Boyce *et al.,* 1996). The use of microsatellites for genetic characterization of goat (Sulaba *et al.,* 2012) is now accepted worldwide. This study was designed for the genetic characterization of two important goat breeds (Damani and Nachi) to assess their genetic diversity, differentiation and genetic relationships between them.

MATERIALS AND METHODS

Blood samples (10 mL) were collected from jugular vein of 25 animals each of Damani (Khyber Pakhtunkhwa Province) and Nachi (Punjab Province) goat breeds in tubes containing 200 µL EDTA. These unrelated animals were selected from different government livestock farms and breeding areas. Inorganic method was used for genomic DNA extraction. A set of 9 microsatellites (recommended by International Society for Animal Genetics ISAG) as detailed in Table 1 were optimized for PCR amplification through Bio-Rad thermocycler

following the standard method of PCR. The products were run on 12% non-denaturing polyacrylamide gel in 1X TAE buffer at 120 volts for 7 hours. The results of polyacrylamide gel electrophoresis were analyzed by the relative flow method. Statistical analysis for calculation of genetic variability measures such as observed number of alleles (*Na*) and expected number of alleles (*Ne*), observed heterozygosity (*Ho*) and expected heterozygosity (*He*), Shannon's index (*I*) were estimated using POPGENE version 1.31. The amount of inbreeding within population (F_{IS}), among population (F_{IT}), genetic differentiation among population (F_{ST}) and gene flow (*Nm*) were calculated by using GENEPOP version 4.0. Polymorphic Information Content (PIC) was estimated with POWER STAT software.

RESULTS AND DISCUSSION

In the current study the 9 microsatellites were employed on two Pakistani goat breeds Damani and Nachi. A total of 53 alleles were found in both breeds, OarAE101 showed the highest number of alleles per locus (7) in Nachi while MAF33 showed one allele per locus in Damani (Table 2) with mean of 3.2 in Damani and 4.6 in Nachi goat breed. It is generally consider that the amount of genetic variations within an organism described by the number of alleles at each locus provide the measure of genetic variability and have an impact on breeds differentiation within the population (Arora and Bhatia, 2006). The effective numbers of alleles is measure of levelness of alleles, and the mean in both breeds was slightly less than the observed number of alleles and varied from 1.00 (MAF33) to 4.65 (ILSTS011) in Damani with mean values of 2.70 and 2.93 in Damani and Nachi, respectively. The average observed heterozygosity values for all loci were 0.73 and 0.51 in Damani and Nachi, respectively, ranging from 0.00 (MAF33 in Damani and OarVH72 in Nachi) to 1.00 (MAF70, BM1818, ETH152, OarFCB48 in Damani and MAF70 in Nachi). The average observed heterozygosity (0.73) was higher than expected heterozygosity (0.56) in Damani and it was less than expected heterozygosity (0.63) in Nachi. This might be due to the selection pressure or inbreeding within population. The expected heterozygosity is as a good estimator of the genetic variability within population (Kim *et al.*, 2002) revealed variations from 0.00 (MAF33 in Damani) to 0.80 (ILSTS011 in Damani). It is considered that high average expected heterozygosity value of the breed might be accredited to the large number of alleles identified in the tested loci. In the current study, Nachi have almost high number of alleles at all loci and average expected heterozygosity value than Damani. The average observed heterozygosity in Damani and Nachi was comparable with other goat breeds such as Ardi goat breed of Saudi Arabia (0.553; Aljumaah *et al.*, 2012), Albanian Muzhake goat (0.69; Hykaj *et al.*, 2012), Portuguese goat breed (0.63; Bruno-de-sousa *et al.*, 2011) and Gohilwari (0.51; Kumar *et al.*, 2009).

The inbreeding coefficient was measured by Wright's F_{IS} and the mean value for both breeds is given in table 2. The average inbreeding coefficient values were -0.32 and 0.16 in Damani and Nachi respectively. The lowest F_{IS} was observed for loci BM1818 and ETH152 (-1.00) in Damani and the highest value was observed for loci OarVH72 (1.00) in Nachi, while the values closer to zero indicates the low level of inbreeding within the population (Ghazy *et al.*, 2013). The low F_{IT} (0.16) and high F_{ST} (0.20) values showed a considerable divergence between the two studied breeds as expected, that might be due to their distant geographical area and occasional migration between them. Both breeds occur almost at bordering area of two neighboring provinces i.e. Punjab (Nachi: Multan, Sahiwal and Bawalphur) and Khyber Pakhtunkhwa (Damani: Dera Ismael khan and Peshawar) of Pakistan. It was also estimated by low level of gene flow (*Nm* = 0.95) between the two breeds.

The mean Polymorphic Information Content (PIC) was 0.70 ranging from 0.54 (MAF33) to 0.83 (ILSTS011), revealed the high level of polymorphism for studied microsatellite markers set in this study. All the studied microsatellite markers showed PIC value more than 0.5. Nevertheless, high polymorphic values for all markers further sustained the availability for the Linkage mapping, parentage analysis and genetic studies of other goat breeds in the country. The data provided valuable molecular genetic information for further genetic studies, genetic differentiation and forensic applications in future. The findings of this study may facilitate the researchers

Table 1: Microsatellite Markers with their product size range, annealing temperature and chromosomal location

Marker	Primer sequence (5′ - 3′)	Product size	Tm (°C)	Chrom. #
MAF70	F: GCAGGACTCTACGGGGCCTTTGC R: CACGGAGTCACAAAGAGTCAGACC	124-166	62, 59	4
OarAE101	F: TTCTTATAGATGCAACTCAAGCTAGG R: TAAGAAATATATTTGAAAAAACTGTATCTCCC	99-123	54, 54	6
MAF33	F: GATCATCTGAGTGTGAGTATATACAG R: GACTTTGTTTCAATCTATTCCAATTTC	121-141	55, 52	9
OarVH72	F: CTCTAGAGGATCTGGAATGCAAAGCTC R: GGCCTCTCAAGGGGCAAGAGCAGG	121-135	66, 71	25
BM1818	F: AGCTGGGAATATAACCAAAGG R: AGTGCTTTCAAGGTCCATGC	253-284	57, 60	32
ILSTS011	F: GCTTGCTACATGGAAAGTGC R: CTAAAATGCAGAGCCCTACC	167-173	58, 56	24
MM12	F: CAAGACAGGTGTTTCAATCT R: ATCGACTCTGGGGATGATGT	122	58, 50	9
ETH152	F: TACTCGTAGGGCAGGCTGCCTG R: GAGACCTCAGGGTTGGTGACAG	157-169	67, 64	5
OarFCB48	F: GAGTTAGTACAAGGATGACAAGAGGCAC R: GACTCTAGAGGATCGAAAAGAACCAG	143-167	60, 60	17

Table 2: Genetic variation of Damani and Nachi goat breads using selected 9 microsatellite markers

Locus	Breed	*Na*	*Ne*	*I*	*Ho*	*He*	*Ave. Het.*	*Fis*	*Fit*	*Fst*	*Nm*	PIC
MAF70	Damani	4	2.67	1.12	1.00	0.64	0.68	-0.59	-0.28	0.11	1.95	0.74
	Nachi	5	4.02	1.47	1.00	0.77		-0.33				
OarAE101	Damani	4	3.18	1.22	0.65	0.70	0.72	0.05	0.28	0.14	1.46	0.82
	Nachi	7	4.12	1.61	0.55	0.77		0.26				
MAF33	Damani	1	1.00	0.00	0.00	0.00	0.21	****	0.90	0.63	0.14	0.54
	Nachi	3	1.78	0.70	0.11	0.45		0.74				
OarVH72	Damani	2	1.88	0.66	0.75	0.48	0.60	-0.60	0.44	0.11	1.93	0.61
	Nachi	4	3.76	1.35	0.00	0.75		1.00				
BM1818	Damani	2	2.00	0.69	1.00	0.51	0.51	-1.00	-0.13	0.32	0.52	0.71
	Nachi	3	2.11	0.90	0.72	0.54		-0.37				
ILSTS011	Damani	5	4.65	1.57	0.70	0.80	0.75	0.10	0.19	0.11	1.93	0.83
	Nachi	5	3.66	1.39	0.66	0.74		0.08				
MM12	Damani	3	2.81	1.06	0.50	0.66	0.59	0.22	0.40	0.08	2.63	0.64
	Nachi	5	2.18	1.07	0.27	0.55		0.48				
ETH152	Damani	2	2.00	0.69	1.00	0.51	0.46	-1.00	-0.15	0.31	0.54	0.61
	Nachi	4	1.74	0.76	0.55	0.43		-0.29				
OarFCB48	Damani	6	4.16	1.55	1.00	0.77	0.71	-0.31	-0.09	0.12	1.82	0.81
	Nachi	6	2.79	1.37	0.77	0.68		-0.17				
Mean	Damani	3.2	2.70	0.95	0.73	0.56	0.58	-0.32	0.15	0.20	0.95	0.70
	Nachi	4.6	2.93	1.18	0.51	0.63		0.16				

and breeders to understand the genetic relationships and breed differences for making future conservation and breeding policies for goat breeds in the country.

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REFERENCES

Aljumaah RS, MM Musthafa, MA Al-Shaikh, OM Badri and MF Hussein, 2012. Genetic diversity of Ardi goat based on microsatellite analysis. Afr J Biotechnol, 11: 16539-16545.

Arora R and S Bhatia, 2006. Genetic diversity of Magra sheep from India using microsatellite analysis. Asian-Austr J Anim Sci, 19: 938-942.

Boyce W M, PW Hedrick, NE Muggli-Cockett, S Kalinowski, MC Penedo and RR Ramey, 1996. Genetic variation of major histocompatibilty complex andmicrosatellite loci: a comparison in Bighorn sheep. Genetics, 145: 421-433.

Bruno-de-Sousa C, AM Martinez, C Ginja, F Santos-Silva, MI Carolino and JV Delgado, 2011. Genetic diversity and population structure in Portuguese goat breeds. Livest Sci, 135: 131-139.

Ghazy A, S Mokhtar, M Eid, A Amin, M Elzarei, K Kizaki and K Hashizume, 2013. Genetic diversity and distances of three Egyptian local sheep breeds using microsatellite markers. Res Zool, 3: 1-9.

Hykaj G, A Hoda, Y Bicoku and L Papa, 2012. Genetic variability of Muzhake goat, estimated by microsatellite markers. Livest Res for Rural Develop. 24: 126.

Khan MS, MA Khan and S Mahmood, 2008. Genetic resources and diversity in Pakistani goats. Int J Agric Biol, 10: 227-231.

Kim KS, JS Yeo, JW Lee, JW Kim and CB Choi, 2002. Genetic diversity of goats from Korea and China using microsatellite analysis. Asian-Austr J Anim Sci, 4: 461-465.

Kumar S, SP Dixit, NK Verma, DK Singh, A Pande, S Kumar, R Chander, LB Singh, 2009. Genetic Diversity Analysis of the Gohilwari Breed of Indian Goat (*Capra hircus*) Using Microsatellite Markers. Am J Anim Vet Sci, 4: 49-57.

Sulabda N, NNW Susari, NLGS Heryani and IK Puja, 2012. Genetic Diversity of Gembrong Goat Based on DNA Microsatellite Markers. Global Vet, 9: 113-116.