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Molecular and phenometric characterization of Bhakarwali goat breed of India

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ABSTRACT

Bhakarwali is recently registered as 34th goat breed of India. It is distributed in the hilly tracts of Jammu and Kashmir. The breed is distinct with superior qualities such as high temperature resistance and milk, meat and fiber productivity under the low input system. It's characterization at phenotypic level was carried out by surveying the breeding tract and at genotypic level by microsatellite markers. Information on body traits, performance traits and managemental practices were collected by interviewing the goat keepers. All the microsatellite loci selected for diversity analysis were polymorphic and a total of 190 alleles were identified across the 23 microsatellite loci. OMHC1 depicted the highest number of alleles (15) while ILSTS065 had the lowest (2) with 8.26±0.663 mean number of alleles per locus. Expected number of alleles ranged from 1.065 (ILSTS044) to 6.755 (OMHC1) with a mean value of 3.613±0.367 alleles per locus. The observed heterozygosity ranged from 0.063 (ILSTS044) to 0.915 (OMHC1) with a mean of 0.629±0.045. Corresponding values of expected heterozygosity varied between 0.061 (ILSTS044) to 0.852 (OMHC1) with a mean of 0.639±0.043. Heterozygote deficiency was negligible as average F_{IS} value was only 0.002±0.033. Bottleneck was examined using all the three mutations models and was found to be absent. Normal L-shaped curve indicated lack of mode shift in the population. This is the first-hand report on current diversity status of Bhakarwali goat and is expected to be useful in planning conservation and in facilitating their effective use in future breeding programs.

Keywords: Bhakarwali goat, Bottleneck, Diversity, Inbreeding, Jammu and Kashmir, Microsatellite

Goats (*Capra hircus*) are known to be accompanied with human society since the dawn of agriculture. Goat, one of the first domesticated animals is utilized for its milk, meat, fiber and skin throughout the world. Domestication of goat is said to have occurred in Fertile crescent approximately 10,500 years earlier (Paim *et al.* 2019). Goats present in western Asia are thought to be evolved from their wild ancestor bezoar ibex (*Capra aegargus*). Goat is considered as an important livestock species and is referred to as "Poor man's cow" in India and other developing countries (MacHugh and Bradley 2001); being well-adapted to low input agricultural environment. Goats are geographically widespread in India ranging from the high altitude Himalayas to Rajasthan deserts and humid coastal areas (Joshi *et al.* 2004). Indigenous goat population has evolved mainly through adaptation to local agro-ecological conditions and to some extent through artificial selection for different needs (Tantia *et al.* 2018). As per 19th livestock census, goat population in India was 135.17 million in 2012 registering a decline of 3.82% over the 2007 census (19th livestock census, 2012). According to latest records, India has 34 registered goat breeds (www.nbagr.res.in) which describes 41% of indigenous goat population only. Fifty nine percent of total goats are still categorized as non-descript. Hence, there is a need to characterize non-descript populations along with ensuring maintenance and improvement of genetic variability in registered breeds.

Breed characterization is primarily complied into two steps: phenotypic and genotypic. In general, diversity of any population is assessed in three different forms: inter-population diversity (between breeds), intra-population diversity (within breeds), and the interrelationships between populations. Through phenotypic characterization, morphological traits are utilized to identify and document diversity within and between distinct breeds, whereas, genetic diversity is measured by molecular characterization (FAO, 2011). Microsatellite or simple sequence repeats markers (SSR) are most prominent among different options of characterization at genetic level. Advantages of using SSRs over other molecular markers include high heterozygosities, a greater degree of polymorphisms, relative abundance, Mendelian inheritance and simplified analysis. In recent years, different studies have been

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reported for genetic diversity estimation of various goat breeds using microsatellites markers (Mishra *et al*. 2010, Vijh *et al*. 2010, Zaman *et al*. 2013, Singh *et al*. 2015, Raghavendra *et al*. 2017, Tantia *et al.* 2018 and Verma *et al.* 2019).

Present work was carried out to characterize Bhakarwali goat population for its phenotypic traits and assessment of genetic variation using microsatellite genotyping under Network project on Animal Genetic Resources of ICAR-NBAGR, India, in collaboration with Sher-e-Kashmir University of Agricultural Sciences and Technology (SKUAST), Jammu. The Bhakarwali goat population was registered as a breed (INDIA_GOAT_0700_ *BHAKARWALI* _06034) in 2018. Total goat population of Jammu and Kashmir is 20.17 lakhs (19th livestock census, 2012) of which Bhakarwali population is 8.80 lakhs (www.nbagr.res.in). The breed has got its name from nomadic community of Jammu and Kashmir, *Bhakarwal* who are real custodian of these goats along with some other communities. It is also referred as *Kaghan* goat. This study represents the first report on characterization of morphometric and molecular genetic diversity and mutation-drift equilibrium in Bhakarwali goat population.

MATERIALS AND METHODS

Phenotypic characterization: Survey was conducted in Bhakarwali goat's habitat mainly in the hilly tract of Jammu and Kashmir. It comprises of Poonch, Rajouri, Reasi, Udhampur, Jammu, Kathua, Doda, Kishtwar and Ramban district ranging between 32°17′N–33°51′N and 74°08′E-75°54′E (Fig. 1). Information on morphological, socio-economic, management and performance parameters was collected through a standard questionnaire recommended by ICAR-NBAGR (2012). There was uniformity and purity within the population of these goats.

Collection of blood samples: Samples (50) were collected from breeding tract of Bhakarwali goat as per FAO recommendations. Samples were collected randomly to avoid any chance of relatedness. Blood was collected from jugular vein in 10 ml vacutainer tubes having EDTA (Ethylene diamine tetra acetic acid) as anticoagulant. Samples were stored at –20°C until DNA extraction.

DNA extraction, quantification and amplification: Isolation of genomic DNA was performed using phenol-chloroform extraction method. The integrity and quantity of DNA was assessed through 1% agarose gel by direct comparison with a standard marker as well as spectrophotometrically (Nanodrop spectrophotometer). Twenty five FAO (http://dad.fao.org/en/refer/library/guideline/marker.pdf) and ISAG (International Society for Animal Genetics) recommended microsatellite markers for goat were selected for the diversity analysis of Bhakarwali goat population. These were highly polymorphic markers spread across the genome. Forward primer of each marker was 5' labeled with a fluorescent dye (FAM, VIC, NED and PET). PCR amplification was performed in 10 µl reaction volume. Reaction mixture consisted of 10–20 ng of DNA, 0.2 µM of each primer and PCR master mix consisting of 0.2 mM of each dNTP and 2 mM of $MgCl_2$. A negative control, consisting of all the reaction components, except for the template DNA, was also included to detect any possible contamination. Touchdown protocol was run. Initial denaturation of 95°C for 1 min; amplification cycle with steps of denaturation at 95°C for 45 sec, 60–51°C with

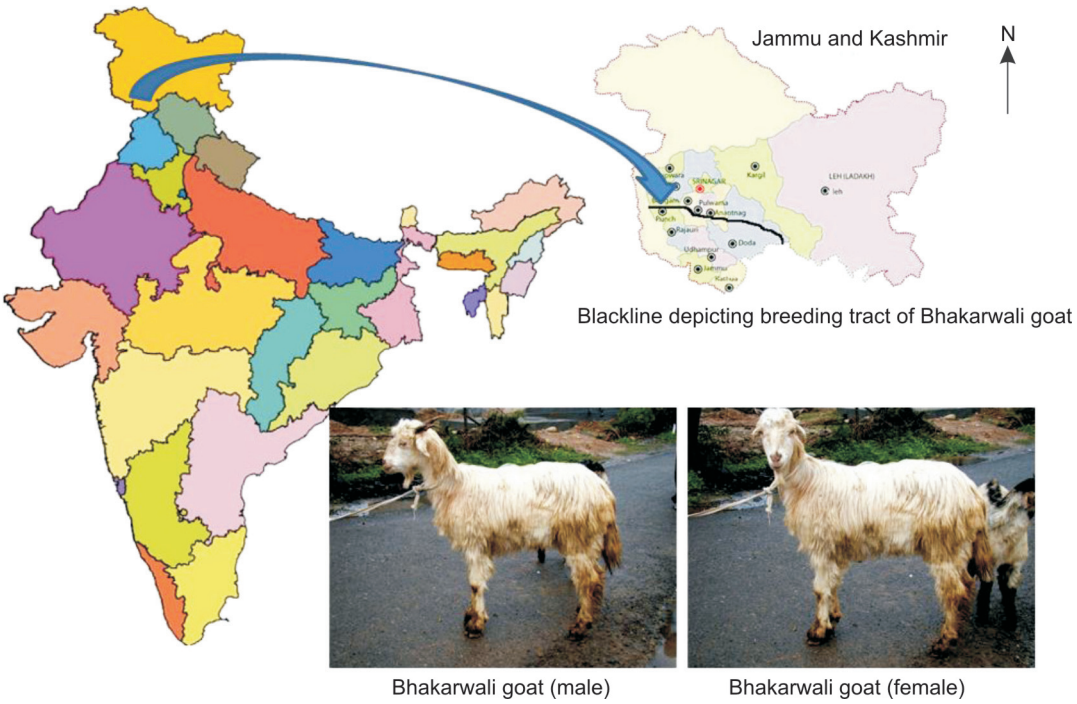

Fig. 1. Bhakarwali goat distribution area (marked on map) and representative animals.

decrease of 3°C every third cycle for 1 min, 72°C for 45 sec and 20 cycles of denaturation 95°C for 45 sec, amplification at 48°C for 1 min, extension at 72°C for 45 sec followed by final extension step at 72°C for 5 min was done. Remaining 2 loci (ILSTS049 and OarAE129) were amplified with specific temperature protocol which consisted of initial denaturation of 95°C for 1 min; 32 cycles of 95°C for 30 sec, specific annealing temperature (58 and 60°C, respectively) for 45 sec, 72°C for 45 sec and final extension step at 72°C for 10 min. The amplification products were electrophoresed on a 1.8% agarose gel treated with ethidium bromide (0.5 mg/ml) for visualization of DNA bands under ultraviolet light. PCR products were multiplexed and genotyping was carried out on an automated DNA sequencer using LIZ 500 as the internal size standard. Allele sizing was done using GeneMapper software v3.7.

Statistical analysis: The data on body weight and biometry were subjected to statistical analyses as per Snedecor and Cochran (1989) and the results obtained are presented as mean and standard error. Basic genetic parameters including allele frequencies, observed (Na) and effective number of alleles (Ne), observed (Ho) and expected heterozygosity (He) and heterozygote deficit (F_{IS}) in the whole population were calculated by analyzing genetic data with GenAlEx v6.5 software (Peakall and Smouse 2012). Bottleneck v1.2.02 (http://www.ensam.inra.fr/URLB) software was used to test bottleneck events in the population by 2 appraches. The first approach consisted of 3 heterozygosity tests developed by Cornuet and Luikart (1996): (*i*) Sign test, (*ii*) Standardized differences test, and (*iii*) Wilcoxon sign-rank test. The probability distribution was established using 1,000 simulations under 3 models—Infinite allele model (IAM), step-wise mutation model (SMM) and two-phase model of mutation (TPM). The second method was the graphical representation of the mode-shift indicator originally proposed by Luikart *et al.* (1998).

RESULTS AND DISCUSSION

Breeding tract: The state of Jammu and Kashmir represents an intricate mosaic of mountain ranges and hills characterized with river terraces valleys and gorges. It has plain region in the south, Shivalik hills and mid Himalayan mountains northwards up to Pir Panjal range. Bhakarwali goat's habitat was identified to be the hilly tract comprising Poonch (33p 51′N; 74p 08′E), Rajouri (33.38p N; 74.54p E), Reasi (33.08p N; 74.83p E), Udhampur (32p 55′N; 75p 09′E), Jammu (32p 43′N; 74p 54′E), Kathua (32p 17′N; 75p 36′E), Doda (33p 13′N; 75p 54′E), Kishtwar (33p 19′N; 75p 48′E) and Ramban (33.24p N; 75.25p E) districts. These are distributed in approximately 26,293 km^2 area. The rich forests of the state played an important role in maintaining the ecological imbalances. Bhakarwali goats are semi-migratory in nature. During winter season, they are found in foot hills and in summers, they tend to migrate to highland pasture of sub-himalayan range.

Physical characteristics and performance: White and black are most prevalent body colours observed in Bhakarwali goats. A plethora of body colours such as brown, black-white, brown-white, grey-white and mixed colours were also observed in the flocks. Dominant skin colours are white, black and brown. Muzzle colours are black, brown, white, red and others (blue, grey and yellow). Horns are present in both the sexes and are mainly black and brown in colour (Fig.1). Ears are either pendulous (52%) or erect (48%). Bhakarwali breed has long and strong horns with straight screwed and curved shape with variable length as per age. Hoof colours of goats are mainly black, brown and white. Average weight (kg) of adult male at one year of age is 30.15±0.32. Corresponding value for female is 28.60±0.68. Chest girth, body length and height at withers (cm) are 74.82±0.82, 79.26±0.23 and 73.56±0.37 and 71.20±0.59, 71.74±0.71 and 71.82±0.61 for female, respectively (Table 1).

Feeding and management practice: Fodder is mainly home grown or collected from natural resources or even sometimes purchased from market. Group feeding is practiced sometimes although grazing during day time is a routine. Some examples of green fodders are Daman (*Grewia optiva*), Kikkar (*Acacia nilotica*), Sirin (*Albizia lebbeck*), Kachnar (*Bahuinia variegate*), Mango leaves (*Mangifera indica*), Ber (*Zizyphus mummularia*), Subabul (*Leucaena leucocephala*), Neem (*Azadirachta indica*) and Peepal (*Ficus religiosa*) etc. Wheat and maize are provided as dry fodders. Water availability is from natural resources such as waterfalls, canal and rivers. Housing is both open and closed depending upon economic status and is

Table 1. Body weight (kg) and biometry (cm) measurements of Bhakarwali goat

Parameter		Male			Female		
		Average	Range	N	Average	Range	N
Body weight	0–3 day	2.98±0.05	1.5–4.0	188	2.89±0.05	1.3–4.0	336
	3 days–6 month	12.05±0.36	9–14	339	10.65±0.40	8.5–14	587
	6 months	19.68±0.30	12–22	273	18.86±0.42	10–22	588
	12 months weight	30.15±0.32	20–35	369	28.60±0.68	18–35	1196
Chest-girth		74.82±0.82	65–94	369	71.20±0.59	58–84	1211
Body length		79.26±0.32	66–105	369	71.74±0.71	60–91	1211
Height at withers		73.56±037	64–90	369	71.82±0.61	58–85	1211

Taggar *et al.* (2016).

predominantly provided at night. Most of the housing is half-walled; however kids are kept in places fenced with thorny bushes to protect them from predators.

Production traits: Average milk yield of 1,600 individuals was calculated to be 907.58±10.74 g/day (Taggar *et al.* 2016). Observed mean of total lactation days is 205.40 (n=178). Different parameters for milk constituents were recorded (n=102) such as fat (3.04±0.27%), SNF (11.03±0.33%), specific gravity (1.04%) and total solids (14.61±0.57%). Parameters related with the slaughter characteristics were also recorded (n=550) such as: average age (35±0.84 months), weight (39.14±0.66 Kg), carcass weight (22±0.34 Kg), skin weight (3.33±0.07 Kg), skin length (75.63±0.83 cm) and skin width (62.06±0.93 cm). Hairs are utilized for making ropes which are generally cut for the first time after two years of age and then subsequently after every 2–3 years.

Diseases and treatment: Foot and mouth, *Peste des petits ruminants* (PPR), parasitic diseases (Haemonchosis, Liver flukes, Dicroceliosis, Strongylosis, Lumber paralysis and Coccidiosis etc.), mange tics and nutritional deficiency are mainly reported in the field. Treatment facilities are provided by local para-veterinary staff of sheep and animal husbandry department, Jammu and Kashmir government. During the course of current survey farmers were regularly provided with antihelminthics drugs for control of various parasitic diseases and mineral mixture for better health and immunity.

Genetic characterization: Twenty three microsatellite (SSR) markers amplified with isolated DNA samples of Bhakarwali goat. Their observed allele size range in Bhakarwali goat along with microsatellite marker details are provided in Table 2. All the microsatellite loci analyzed for diversity analysis were polymorphic and in total, 190 alleles were observed. Diversity estimates of Bhakarwali goat population viz. number of alleles observed at a locus, number of alleles expected at a locus, polymorphism information content of a locus and heterozygosity both observed and expected are furnished in Table 3. The values of Shannon Information Index ranged from 0.173 to 2.181 with a mean of 1.426±0.110. As most of the markers had high I values; therefore, they can potentially be used for individual identification, linkage mapping and parentage testing.

Allelic diversity: Sufficient allelic diversity was observed in Bhakarwali goat population as OMHC1 showed as high as fifteen alleles. ILSTS065 showed the lowest (2) alleles. However, high value was observed for mean number of alleles per locus being 8.26±0.663. Expected number of alleles ranged from 1.065 (ILSTS044) to 6.755 (OMHC1) with a mean value of 3.613±0.367 alleles per locus. Lower values of expected number of alleles as compared to observed number of alleles in all the population suggested that low frequency alleles were prevalent in this population (Sharma *et al*. 2015).

Genetic variation is a prerequisite for organisms to adapt to ever changing environments. Indian goats in general show higher genetic variation that must have contributed to their adaptability (Sharma *et al.* 2015). Goats of Himalayan region are no exception (Table 4). The mean observed number of alleles in the Himalayan breeds ranged from 4.9±2.220 (Assam Hill, Zaman *et al.* 2013) to 10.4±3.91 (Changthangi, Mishra *et al.* 2010) and mean effective number of alleles from 2.576±0.285 (Sume-Ni,

Table 2. Sequence and characteristics of microsatellite markers selected for diversity estimation of Bhakarwali goat

Locus no.	Dye	Type of repeat	Allele size range	Chromosome number	Gene bank accession
ETH225	VIC	(CA)18	146–160	14	Z14043
ILSTS044	NED	(GT)20	145–177	Ann	L37259
ILSTS008	FAM	(CA)12	167–195	14	L23483
OarHH64	PET	–	120–138	4	212a
ILSTS059	FAM	(CA)4(GT)2	105–135	13	L37266
ILSTS065	PET	(CA)22	105–135	24	L37269
OarJMP29	NED	(CA)21	120–140	Ann	U30893
ILSTS033	PET	(CA)12	151–187	12	L37213
OarFCB48	VIC	(CT)10	149–181	17	M82875
OMHC1	NED	–	179–209	Not reported	228a
ILSTS005	VIC	(nn)39	174–190	10	L23481
ILSTS019	FAM	(GT)10	142–162	Ann	L23492
ILSTS058	PET	(GT)15	136–188	17	L37225
ILSTS087	NED	(CA)14	142–164	Ann	L37279
ILSTS029	PET	(CA)19	141–191	3	L37252
ILSTS049	NED	(CA)26	160–184	11	L37261
ILSTS30	FAM	(CA)13	159–179	2	L37212
ILSTS34	VIC	(GT)29	153–185	5	L37254
ILSTS022	PET	(GT)21	186–202	Ann	L37208
RM088	FAM	(CA)14	109–147	4	U10392
RM4	NED	(CA)13	105–127	15	U32910
ILSTS082	PET	(GT)17	100–136	2	L37236

Table 3. Measures of genetic variation in Bhakarwali goat population

Locus	N	Na	Ne	I	Ho	He	F_{IS}
ETH225	48	9	3.022	1.459	0.479	0.669	0.284
ILSTS044	48	4	1.065	0.173	0.063	0.061	–0.021
ILSTS08	47	7	2.839	1.290	0.511	0.648	0.212
OarHH64	47	8	2.336	1.340	0.511	0.572	0.107
ILSTS059	46	8	4.038	1.586	0.652	0.752	0.133
ILSTS065	47	2	1.667	0.590	0.553	0.400	–0.382
OarJMP29	48	3	1.314	0.455	0.271	0.239	–0.134
OarAE129	46	11	6.054	1.998	0.891	0.835	–0.068
OarFCB48	48	12	6.583	2.078	0.875	0.848	–0.032
ILSTS033	48	10	2.808	1.544	0.625	0.644	0.029
OMHC1	47	15	6.755	2.181	0.915	0.852	–0.074
ILSTS019	47	6	4.710	1.647	0.574	0.788	0.271
ILSTS005	48	8	2.298	1.258	0.625	0.565	–0.106
ILSTS058	48	12	5.519	2.049	0.750	0.819	0.084
ILSTS087	45	8	5.287	1.781	0.867	0.811	–0.069
ILSTS30	47	9	5.995	1.922	0.681	0.833	0.183
ILSTS34	48	10	1.990	1.204	0.500	0.498	–0.005
ILSTS29	46	8	1.609	0.911	0.391	0.379	–0.034
ILSTS049	46	7	3.248	1.417	0.717	0.692	–0.037
RM088	46	8	3.819	1.593	0.913	0.738	–0.237
ILSTS082	48	13	4.567	1.859	0.792	0.781	–0.014
ILSTS022	46	8	3.187	1.450	0.761	0.686	–0.109
RM04	48	4	2.396	1.015	0.542	0.583	0.070
Mean	47.087	8.261	3.613	1.426	0.629	0.639	0.002
SE	0.198	0.663	0.367	0.110	0.045	0.043	0.033

Na, No. of different alleles; Ne, No. of effective alleles = 1/(Sum pi^2); I, Shannon's information index = –1× Sum (pi × Ln (pi)); Ho, Observed heterozygosity, No. of Hets/N; He, Expected heterozygosity = 1- Sum pi^2; F, Fixation index = (He-Ho)/He = 1– (Ho/He). Where pi is the frequency of the i^{th} allele for the population and Sum pi^2 is the sum of the squared population allele frequencies.

Table 4. Diversity estimates in Himalayan goat breeds

Diversity estimate	Bhakarwali goat (this study)	Changthangi goat (Mishra *et al.* 2010)	Chegu goat (Vijh *et al.* 2010)	Assam Hill goat (Zaman *et al.* 2013)	Gaddi goat (Singh *et al.* 2015)	Sumi-Ne goat (Verma *et al.* 2019)
Na	8.261±0.663	10.4±3.91	7.41±0.201	4.9±2.220	9.00±0.82	5.043±.380
Ne	3.613±0.367	4.59±2.07	4.99±0.145	2.68±1.590	6.587±0.56	2.576±0.285
Ho	0.629±0.045	0.602±0.236	0.71±0.008	0.43±0.285	0.748±0.02	0.347±0.040
He	0.639±0.043	0.741±0.145	0.80±0.006	0.48±0.281	0.843±0.01	0.499±0.050
F_{IS}	0.002±0.033	0.177	0.112	0.085	-	0.258±0.064
I	1.426±0.110	1.717±0.459	-	1±0.606	1.950±0.08	1.008±0.105

"-" not provided.

Verma *et al.* 2019) to 6.5874±0.56 (Gaddi; Singh *et al.* 2015). Accordingly, Bharkarwali population also presented a considerable amount of allelic diversity (Table 4). Much higher allelic diversity has been observed in case of some indigenous goat populations thriving in plains (Kharkar *et al.* 2015, Nath *et al.* 2014 and Bhat *et al.* 2013). Higher allelic diversity has been described for Black Bengal (8.53±0.26; Vijh *et al.* 2010), Mahboobnagar (8.8±0.55; Raghavendra *et al*. 2017), Bidri (8.48±0.88) and Nandidurga 8.22±0.66) (Tantia *et al.* 2018) goats also.

Gene diversity: Heterozygosity refers to genetic variability in a population. Observed heterozygosity in Bhakarwali goat (Table 3) was nearly equal to that of expected heterozygosity, suggesting occurrence of random mating among the individuals in this population. The observed and expected heterozygosity ranged from 0.063 (ILSTS044) to 0.915 (OMHC1) and 0.061 (ILSTS044) to 0.852 (OMHC1) with a mean of 0.629±0.045 and 0.639±0.043, respectively. Among hilly-area breeds, similar value for mean observed heterozygosity (0.602) was spotted in the first and only registered goat breed of Jammu and Kashmir, Changthangi (Mishra *et al.* 2010). Even higher values were observed in Chegu (0.80) and Gaddi (0.748) goat of Himachal Pradesh (Singh *et al.* 2015). Mean observed heterozygosity was less in case of goat populations of North Eastern Hill (NEH) region Assam hill goat (0.48;

Table 5. Population bottleneck analysis in Bakharwali goat

Model used		IAM	TPM	SMM
Sign test (No. of loci with heterozygosity excess)	Exp	13.5200	13.4900	13.3600
	Obs	14	9*	4*
	P-value	0.5213	0.0007	0.0000
Standardized differences test	T2 value	0.1720	–4.137*	–13.377*
	P value	0.4316	0.0000	0.0000
Wilcoxon test (one tail for H excess)	P value	0.2410	0.9697	1.0000

*Null hypothesis that population is under mutation-drift equilibrium is rejected.

Zaman *et al.* 2013), as well as Sumi-Ne goat breed of Nagaland (0.49; Verma *et al.* 2019). In case of non-mountainous breeds, mean observed heterozygosity values similar to that of Bhakarwali goat were observed in Black Bengal (0.69; Vijh *et al.* 2010), Nandidurga (0.60; Tantia *et al.* 2018) and Mahboobnagar (0.69; Raghavendra *et al.* 2017) goats, on the other hand, higher values were noticed in Osmanabadi (0.71; Bhat *et al.* 2013), Sanagamneri (0.73; Nath *et al.* 2014) and Berari (0.79; Kharkar *et al.* 2015) goats.

F_{IS} value indicating heterozygote deficiency in the population ranged from –0.382 (ILSTS065) to 0.284 (ETH225) with an overall mean of 0.002±0.033. A small positive value of F_{IS} in Bhakarwali population indicated occurrence of heterozygotes in higher proportion. Negative inbreeding coefficient was observed in 14 out of 23 investigated loci, indicating even occurrence of out breeding. This may be because of introduction of other goat germplasm (mainly Beetal) from adjoining states. Awareness should be created among farmers for maintaining germplasm in pure form and not to inter mix with other goat populations available in the region. The overall mean of F_{IS} (0.002) indicated negligible (only 0.2%) shortfall of heterozygotes in Bhakarwali population. It was not significant as compared to heterozygote deficiency reported in other hill goat breeds of the same region (Table 4), Changthangi (17.7%; Mishra *et al.* 2010) and Chegu (11.2; Vijh *et al.* 2010) as well as goats of NEH region Assam hill (8.5%; Zaman *et al.* 2013), Sikkim Singharey (22.5%; Shivahare *et al.* 2017) and Sumi-Ne (25.8%; Verma *et al.* 2019) as well as goat breeds of plains such as Bidri (13.6%) and Nandidurga (13.7%) (Tantia *et al.* 2018). The population of Bhakarwali goat at present is sufficiently large (8.8 lakh). As a result, random breeding is going on and is reflected in the observed high allelic as well as genetic diversity and absence of heterozygote deficiency or inbreeding.

Bottleneck inspection: To estimate the excess of heterozygotes, sign, standardized differences and wilcoxon sign rank tests were utilized under all the three mutation models (IAM, TPM and SMM). The results revealed (Table 5) that Bhakarwali population has not undergone any recent reduction in population size. Heterozygosity excess was not significantly (P>0.05) lower as per all the three tests under IAM and for Wilcoxon test under TPM and SMM. However, heterozygosity excess was significantly less (P>0.05) for Sign and Standardized tests under TPM and SMM thus Mode-shift indicator test to detect potential bottleneck was also applied.

Graphical representation utilizing allelic class and proportion of alleles showed a normal 'L'-shaped distribution (Fig. 2). Abundance of low frequency (<0.10) alleles negated chances of bottleneck as the non-bottleneck populations that are near mutation-drift equilibrium are expected to have a large proportion of alleles with low frequency (Tantia *et al.* 2018).

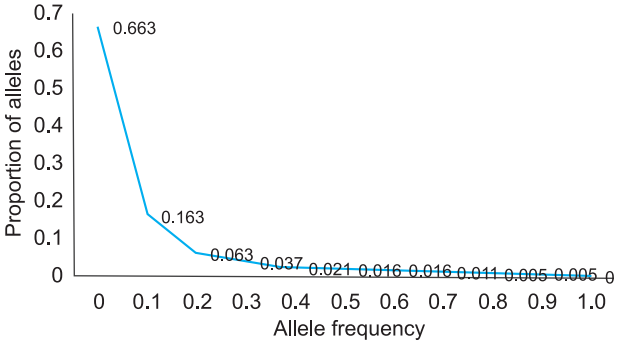

Fig. 2. Graphic representation of proportion of alleles and their distribution in Bhakarwali population.

In conclusion, the results of microsatellite analysis suggest that Bhakarwali breed represents a unique germplasm of goat genetic resources of the country. Tremendous level of heterozygosity and polymorphism indicates abundance of genetic variation in this native goat breed. Bhakarwali breed is unique in terms of adaptability to wide range of temperature across the region. It is facing threat due to the reduction in forest cover and winter grazing pasture on one hand and intermixing with goat germplasm from neighbouring states on the other hand. Absence of specific breeding policy for Bhakarwali goat in the state is another contributing factor. So, there is a need for planning scientific breeding, feeding and management practices to increase the number of productive goats for enhanced profitability and to maintain purity of the breed. The important information generated by microsatellite markers on genetic variation and population structure will pave way towards management and conservation of Bhakarwali goat.

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