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. Its 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 ILS065 had the lowest (2) with 8.26±0.663 mean number of alleles per locus. Expected number of alleles ranged from 1.065 (ILS044) to 6.755 (OMHC1) with a mean value of 3.613±0.367 alleles per locus. The observed heterozygosity ranged from 0.063 (ILS044) to 0.915 (OMHC1) with a mean of 0.629±0.045. Corresponding values of expected heterozygosity varied between 0.061 (ILS044) to 0.852 (OMHC1) with a mean of 0.639±0.043. Heterozygote deficiency was negligible as average FIS 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
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 Poornch, 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 MgCl₂. 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.
Body length 79.26±0.32 66–105 369 71.74±0.71 60–91 1211
Chest-girth 74.82±0.82 65–94 369 71.20±0.59 58–84 1211
Body weight 0–3 day 2.98±0.05 1.5–4.0 188 2.89±0.05 1.3–4.0 336
Height at withers 73.56±0.37 64–90 369 71.82±0.61 58–85 1211

Parameter Male Female
expected heterozygosity (He) and heterozygote deficit (FIS)
effective number of alleles (Ne), observed (Ho) and
presented as mean and standard error. Basic genetic
Snedecor and Cochran (1989) and the results obtained are
biometry were subjected to statistical analyses as per
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 (FIS)
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
test bottleneck events in the population by 2 approaches. 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 km² 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 male and 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 (Bahutania 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.](http://www.example.com/table1.png)

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              |
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; 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 (Kharakar 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.

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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 Bakharwali goat were observed in Black Bengal (0.69; Vrij 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.

FIS 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 FIS in Bakharwali population indicated occurrence of heterozygotes in higher proportion. Negative inbreeding coefficient was observed in 14 out of 23 investigated loci, indicating even occurrence of outbreeding. 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 FIS (0.002) indicated negligible (only 0.2%) shortfall of heterozygotes in Bakharwali 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; Vrij et al. 2010) as well as goats of NEH region Assam hill (8.5%; Zaman et al. 2013), Sikkim Singharey (22.5%; Shivhare 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 Bakharwali 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 Bakharwali 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).

In conclusion, the results of microsatellite analysis suggest that Bakharwali 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. Bakharwali 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 Bakharwali 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 Bakharwali goat.

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