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Assessing genetic variability in Udaipuri goat using microsatellite markers

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Udaipuri is one of the lesser known goats which are found from Dugadda to Yamkeshwar (Ajmer and Udaipur patties) in Pauri Garhwal district of Uttarakhand. It is a meat type goat and small sized with a compact body, tan in colour and covered with short hair. The head is convex, ears are medium and pendulous while their horns are upward and turned backward in majority of goats. They have a tapering muzzle and a roman nose. Their legs are small, lean and straight with a short tail (Fig. 1).

Fig. 1. Flock of Udaipuri goat

Genetic characterization of breed allows evaluation of genetic variability, which is a fundamental element in working out breeding strategies for improvement at genetic level as well as for their sustainable utilization and conservation. The exploitation of DNA polymorphism as molecular markers has unlocked many vistas in genetic characterization, conservation, improvement and molecular evolution studies in livestock species. The objective of this study was to examine the genetic variability of Udaipuri goat using microsatellite markers.

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Approximately 10 ml blood was collected from 30 genetically unrelated animals of Udaipuri goat from its breeding tract. The genomic DNA was isolated using the protocol described by Sambrook and Russel (2001). A set of 20 microsatellite markers were chosen from the list suggested by ISAG & FAO.

The PCR was performed on 100 ng genomic DNA in a final reaction volume of 20 µl containing 10× PCR buffer (with 20mM $MgCl_2$), dNTPs (200 mM each), forward and reverse primer (10 pmol/µl), *Taq* DNA polymerase (1U/ µl) and DNase free water. The PCR protocol involved initial denaturation at 95°C for 5 min, denaturation at 95°C for 1 min, primer annealing at 52–60°C for 30 sec, extension at 72°C for 1 min, 30 cycles of denaturation to extension step and a final extension at 72°C for 10 min. The conformation of PCR amplification was done by 1.5% agarose gel electrophoresis. Eight percent Urea Polyacrylamide gel electrophoresis (PAGE) was employed to resolve the microsatellite alleles. The scoring of the alleles was done manually and size of the alleles was determined by using the INCHWORM (version 1.02) software (http:// www.molecularworkshop.com/programs/inchworm.html). The population variability parameters taken out for molecular study were derived using POPGENE software (Yeh *et al.* 1999). Allelic frequency, the observed and effective numbers of alleles, heterozygosity values were calculated using POPGENE software. The allelic frequencies so obtained were utilized to calculate the Polymorphic Information Content (PIC) values

$$PIC = 1 - \sum_{i=1}^{j} P_i^2 - 2 \sum_{i=j+1}^{j} \sum_{j=1}^{i-1} P_i^2 P_j^2$$

where, P_i and P_j are the frequencies of the i^{th} and j^{th} alleles at a locus, respectively (Botstein *et al.* 1980). Shannon's index values and the tests for the deviation from the Hardy-Weinberg equilibrium were also calculated for all the microsatellite markers under study using the POPGENE software.

All the twenty microsatellite markers were amplified successfully. A total of 96 alleles were observed across all the loci under study in Udaipuri goat population. The observed number of alleles varied from 2 (OarJMP29) to 7 (ILSTS-058, ILSTS-34 and OarFCB-304) with an overall mean of 4.80. The effective number of alleles varied from

Table 1. Microsatellite allelic size, numbers of alleles and genetic variation at the microsatellite loci in Udaipuri goat.

Loci	Size range	Observed number of alleles	Effective numbers of alleles	Obs. Hetr	Exp. Hetr	Nei's Hetr	PIC	I	HWE χ^2
ILSTS-058	139–195	7	4.03	0.46	0.76	0.75	0.72	1.59	83.21**
ILSTS-059	110–150	6	4.90	0.86	0.80	0.79	0.75	1.66	91.61**
ILSTS-34	146–176	7	5.48	0.60	0.83	0.82	0.79	1.77	38.30*
ILSTS-005	167–195	5	2.39	0.33	0.59	0.58	0.55	1.19	45.86**
ILSTS-019	138–164	5	4.09	0.66	0.76	0.75	0.71	1.46	21.92*
ILSTS-049	151–171	5	3.82	0.30	0.75	0.73	0.69	1.46	60.10**
ILSTS-008	142–158	5	4.45	0.40	0.78	0.77	0.74	1.55	55.40**
ILSTS-087	164–184	4	3.71	0.90	0.74	0.73	0.68	1.34	23.71**
ILSTS-033	154–168	5	3.04	0.06	0.68	0.67	0.62	1.29	95.86**
ILSTS-044	146–170	4	2.33	0.23	0.58	0.57	0.50	1.02	25.96**
ILSTS-30	159–183	3	1.83	0.03	0.46	0.45	0.40	0.70	31.31**
ILSTS-002	95–133	5	2.87	0.16	0.66	0.65	0.61	1.27	101.45**
ILSTS-065	121–141	5	4.39	0.30	0.78	0.77	0.75	1.53	60.10**
ILSTS-029	142–192	5	3.78	0.33	0.74	0.73	0.69	1.43	64.30**
ILSTS-082	99–125	5	4.85	0.40	0.81	0.79	0.76	1.59	55.78**
RM-088	107–159	5	3.54	0.40	0.73	0.72	0.67	1.38	29.75**
OarFCB-304	135–189	7	3.43	0.36	0.72	0.71	0.69	1.47	73.23**
OarJMP-29	118–138	2	1.60	0.03	0.38	0.37	0.31	0.56	26.50**
RM-4	131–143	3	1.89	0.36	0.47	0.47	0.41	0.80	22.95**
ETH-225	118–166	3	2.22	0.03	0.55	0.54	0.50	0.89	52.65**
Mean		4.80	3.43	0.36	0.68	0.67	0.63	1.30	
St. Dev		1.36	1.12	0.25	0.12	0.13	0.13	0.33	

*Significant (P≤0.05); **Significant (P≤0.01); NSNot Significant.

1.60 (OarJMP-29) to 5.48 (ILSTS-34) with a mean of 3.43. These values were comparable to those obtained by Zaman *et al.* (2013) in Asom Hill goat. The observed heterozygosity varied between 0.03 (ILSTS-30, OarJMP-29 and ETH-225) to 0.90 (ILSTS-087), with an average of 0.36 which was comparable with Jakhrana goat (0.39) by Kumar *et al.* (2005). The expected heterozygosity ranged from 0.38 (OarJMP-29) to 0.83 (ILSTS-34) with an average of 0.68 within the Udaipuri goat population. The Nei's heterozygosity or genetic diversity varied from 0.37 (OarJMP29) to 0.82 (ILSTS-34) with an average of 0.67. The Nei's heterozygosity observed in Chaugarkha goat (0.77) by Ganie *et al.* (2017) was comparable to those found in Udaipuri goat. The PIC values (Table 1) ranged from 0.31 (OarJMP-29) to 0.79 (ILSTS-34) with an average value was 0.63. The PIC value (0.60) in Surti goat as reported by Dixit *et al.* (2013) was quite comparable to the present investigation. Shannon's Index values (Table 1) were observed to vary from 0.56 (OarJMP-29) to 1.77 (ILSTS-34) with an average value of 1.30. This value was comparable to Surti goat by Fatima (2006). However, lower value of Shannon's Index was reported by Sharma *et al.* (2008) in Barbari goat and in Asom Hill goat by Zaman *et al.* (2013). The estimated value of PIC and Shannon's Information Index seen in Udaipuri goat was sufficiently high indicating the suitability of the markers for studying the genetic variability within the goat population. The genetic information obtained from the selected marker loci is fairly abundant which signifies the appropriateness of these loci for studying the diversity analysis within the population. The chi square (χ^2) test values (Table 1) revealed that out of 20 microsatellite loci studied, all twenty microsatellite markers deviated significantly from HWE. Amie-Marini *et al.* (2014) reported that in Malaysian goat breeds, all the 30 loci under their study deviated significantly from HWE. The significant deviation from HWE in Udaipuri goat might have resulted due to smaller population size as a consequence of non-random mating within the population.

SUMMARY

The study was undertaken for genetic diversity analysis in Udaipuri goat found in Pauri Garhwal district of Uttarakhand using a set of twenty microsatellite markers. Blood samples were collected from 30 unrelated animals from the breeding tract. All the twenty microsatellite loci were amplified successfully and a total of 96 alleles were observed across all the loci. The expected heterozygosity ranged from 0.38 to 0.83 with a mean of 0.68. The Nei's heterozygosity values showed a variation from 0.37 to 0.82 with a mean value of 6.7. The PIC value ranged from 0.31 to 0.79 with a mean value of 0.63. The Shannon's Information Index values were observed to vary from 0.56 to 1.77 with an average value of 1.30. All the twenty microsatellite loci deviated significantly from the Hardy-Weinberg equilibrium. The genetic variability suggested the possibilities of genetic improvement of Udaipuri goat in future.

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