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Genetic diversity analysis of Chaugarkha goat using microsatellite markers

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The Chaugarkha is an important small sized meat purpose breed of goats mainly found in Almora district and adjoining areas of Bageshwar, Pithoragarh and Nainital district of Uttarakhand. This breed is well sustained in its home tract due to its hardiness on the denuded cliffs where other agricultural activities are barely profitable. There is a need for characterization, conservation and recognition of this goat breed like other indigenous livestock (Deshmukh *et al*. 2015, Kumar and Gaur 2015 and 2016, Pandey *et al*. 2011). Blood samples (25) of Chaugarkha goats were collected randomly from unrelated animals from its home tract. The standard phenol chloroform extraction method (Sambrook and Russell 2001) was employed to isolate genomic DNA from frozen blood samples using a set of 15 microsatellite markers. The PCR was carried out in a final reaction volume of 24 µl which include 10× PCR buffer (with $MgCl_2$ 20 mM), dNTPs (2 mM each), forward and reverse primer (10 pmole/µl) and Taq DNA polymerase (1.0 U/µl) using PTC-200 PCR machine. All amplification conditions included an initial denaturing step of 5 min at 94°C followed by 35 cycles of denaturation (94°C for 30 sec), annealing (52°C to 60°C for 30 sec) and extension (1 min for 72°C). Duration of final extension was 10 min at 72°C. Microsatellite alleles were resolved on 8% denaturing polyacrylamide gel. To visualize the PCR products, the gel was stained using silver staining. The amplified product was visualized and the scoring of allele was carried out following gel documentation where the image capture and analysis was done by Quantity one[Tm] software. Using the lane-matching algorithm, a 5% level of tolerance was allowed for matching of alleles across the lanes. A judicious blend of manual as well as machine logic was applied by the independent observers, so as to score the alleles in each lane, with respect to within and between population comparisons of samples. After matching of the samples, the genotyping table was prepared in M.S. Excel format which was processed further for feeding into the various population analysis software. The population parameters i.e. heterozygosity values, PIC values and Shannon index were derived using the *POGENE* software and MS-Tools (Peakall and Smouse 2012).

The number of alleles observed in randomly selected DNA samples for a given marker is supposed to represent the number of alleles in the whole population for that particular locus (MacHugh *et al.* 1997). The observed and effective number of alleles for each locus is summarized in Table 1. The observed number of alleles varied from 5 (ILSTS-005, ILSTS-34 and ILSTS-049) to 9 (ILSTS-029, ILSTS-030 and ILSTS-058) with a mean of 7.20±0.34. The effective number of alleles varied from 2.62 (ILST-005) to 6.76 (ILSTS-008) with a mean of 4.82±0.07. In Kanniadu goats of Tamil Nadu, Dixit *et al.* (2011) reported that the observed number of alleles ranged from 5 (RM004) to 13 (RM088, OarE129) with an average value of 8.64±0.48 where as the effective number of alleles ranged from 1.45 (ILSTS034) to 7.89 (ILSTS033 and OMHC1) with the overall mean value of 4.22±0.34. The studied loci in this breed of goat showed low effective number of alleles indicating that only few of the alleles having high frequency might have contributed more to the overall allelic frequency. Nevertheless, the loci revealed high level of allelic diversity among them. The total number of alleles observed and the minimum number of alleles at a locus demonstrated that all microsatellite loci were sufficiently polymorphic.

The average observed heterozygosity ranged from 0.80 (ILSTS-005, ILSTS-019, ILSTS-030, ILSTS-033, ILSTS-034, ILSTS-049 and ILSTS-059) to 0.92 (ILSTS-065), with an average value of 0.83±0.01. The average expected heterozygosity ranged from 0.63 (ILSTS-005) to 0.87 (ILSTS-029), with an average value of 0.79±0.02. The within population average Nei's heterozygosity/gene diversity ranged from 0.62 (ILSTS-005, ILSTS-34) to 0.85 (ILSTS-029) with an average value of 0.77±0.02. Dixit *et al*. (2011) reported that the average observed and expected heterozygosity values were 0.53±0.03 and 0.73±0.02, respectively in Kanniadu goats. The studied loci in this breed of goat showed high level of observed heterozygosity and within population average expected heterozygosity/gene diversity indicating the diverse nature of the goat population studied.

The PIC values ranged from 0.24 in ILSTS-002 to 0.54

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Table 1. Genetic variation at 15 microsatellite loci in Chaugarkha goat

Locus	Size in base pair	N_a	N_e	H_o	H_e	N_h	PIC	I
ILSTS-002	145-192	7	3.09	0.84	0.69	0.68	0.24	1.37
ILSTS-005	365-540	5	2.62	0.80	0.63	0.62	0.25	1.14
ILSTS-008	180-302	6	4.15	0.88	0.77	0.76	0.38	1.55
ILSTS-019	250-390	7	5.43	0.80	0.83	0.82	0.35	1.79
ILSTS-029	135-168	9	6.76	0.88	0.87	0.85	0.30	2.00
ILSTS-30	145-185	9	6.44	0.80	0.86	0.84	0.28	1.98
ILSTS-033	130-165	8	5.02	0.80	0.82	0.80	0.28	1.80
ILSTS-34	158-185	5	2.64	0.80	0.63	0.62	0.24	1.14
ILSTS-044	278-330	7	3.85	0.84	0.76	0.74	0.26	1.57
ILSTS-049	170-192	5	4.28	0.80	0.78	0.77	0.54	1.53
ILSTS-058	125-282	9	6.10	0.84	0.85	0.84	0.31	1.97
ILSTS-059	125-300	8	5.12	0.80	0.82	0.80	0.29	1.84
ILSTS-065	122-285	7	5.90	0.92	0.85	0.83	0.40	1.88
ILSTS-082	100-138	7	5.56	0.88	0.84	0.82	0.39	1.79
ILSTS-087	105-140	7	5.30	0.84	0.83	0.81	0.34	1.73
Mean		7.2	4.18	0.83	0.79	0.77	0.32	1.67
S.E		0.34	0.07	0.01	0.02	0.02	0.02	0.07

N_a, observed number of alleles; N_e, expected number of alleles; H_o, observed heterozygosity; H_e, expected heterozygosity; N_h, Nei's heterozygosity; PIC, polymorphic information content; I, Shannon's information index

in ILSTS-049 with an average of 0.32±0.02. Fatima (2006) reported the PIC values ranged from 0.16 (Oar JMP-029) to 0.82 (ILSTS-087) in the Zalawadi population, 0.35 (Oar JMP-029) to 0.86 (ILSTS-030) in Gohilwadi population and 0.34 (ILSTS-065) to 0.81 (RM-088) in Surti population. The average PIC value was 0.60 across the populations and 0.56, 0.64 and 0.60 for Zalawadi, Gohilwadi and Surti populations, respectively. According to Botstein *et al.* (1980), polymorphic markers are classified as highly informative having a PIC value greater than 0.50, reasonably informative with the PIC value ranging between 0.25 to 0.50 and slightly informative if the value is below 0.25. Thus, the markers like ILSTS-002 and ILSTS-034 were slightly informative, markers like ILSTS- 005, ILSTS-008, ILSTS-019, ILSTS-029, ILSTS-030, ILSTS-033, ILSTS-044, ILSTS- 058, ILSTS-059, ILSTS-082 and ILSTS-087 were found to be reasonably informative and the marker ILSTS-049 was found to be highly informative. The Shannon's information index represents the relative abundance of genetic information of a specific locus to the total information available of the loci (Shannon and Weaver 1949). The Shannon diversity index is an index that is commonly used to characterize species diversity in a community and accounts for both abundance and evenness of the species present. The values were found to be in the range of 1.14 (ILSTS-034) to 2.00 (ILSTS-029) with an average of 1.67±0.07. Fatima (2006) reported that the mean values for Shannon index pooled over the populations was found to be 1.50 and individually for Gohilwadi, Zalawadi and Surti estimates were 1.25, 1.44 and 1.33, respectively. The high value of Shannon's information index indicated the suitability of markers for studying the genetic variability in goat species.

All the parameters (observed number of alleles, effective number of alleles, Shannon's information index, PIC value and gene diversity) estimated to know the genetic variation showed that all the microsatellite markers used were highly informative, indicating the suitability for genetic diversity studies in goats. All the studied loci were polymorphic and reflected substantial genetic diversity in Chaugarkha goat population. It is important to preserve the genetic integrity of breeds in order to prevent their disappearance, as well as to develop similar research for their conservation. In this way, the genetic potential of different breeds can be used more effectively as a resource for unexpected future demands.

SUMMARY

Genetic variability within Chaugarkha breed of goat was studied using microsatellite markers from 25 blood samples of unrelated animals. A set of 15 microsatellite markers were used for PCR and the DNA profiles were scored from the gel that were further analyzed using standard population analysis software. The observed number of alleles varied from 5 to 9 with a mean of 7.20±0.34. The effective number of alleles varied from 2.62 to 6.76 with a mean of 4.82±0.07. The average observed heterozygosity ranged from 0.80 to 0.92 with an average value of 0.83±0.01. The average expected heterozygosity ranged from 0.63 to 0.87 with an average value of 0.79±0.02. The within population Nei's average expected heterozygosity/gene diversity ranged from 0.24 to 0.85 with an average value of 0.32±0.02. The PIC values ranged from 0.24 to 0.54 with an average of 0.32±0.02. The values of Shannon's information index were in the range of 1.14 to 2.00 with an average of 1.67±0.07. The level of genetic variability indicated the possibility for

further genetic improvement of Chaugarkha goats.

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