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Molecular characterization of Mahabubnagar goats based on microsatellite markers

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Abstract
Genetic variation at 15 microsatellite loci was examined in Mahabubnagar goats which are distributed widely in the southern part of Telangana. The observed number of alleles varied from 5 (SRCRSP5) to 13 (SRCRSP15) with an average value of 8.800 whereas the effective number of alleles varied from 4.429 (SRCRSP5) to 11.250 (SRCRSP15) with the overall mean of 7.711. The average observed and expected heterozygosity values were 0.694 and 0.864, respectively. The overall mean PIC observed was 0.845 indicating higher polymorphism in these goats. The inbreeding estimate showed mild to moderate level of inbreeding with F_{IS} value of 0.196. Out of the 15 amplified loci, nine loci deviated significantly from Hardy-Weinberg equilibrium. The high level of genetic variability, however, suggested the scope for further genetic improvement of Mahabubnagar goats.

Keywords: Mahabubnagar goat, genetic diversity, polymorphism, microsatellite markers

1. Introduction
India is a rich repository of goat genetic resources in the form of 20 well defined goat breeds, which constitute about 20-25% of the total goat population with 135.1 million (19th Livestock Census, 2012). The remaining 75 – 80% population is non-descript having mixed features. Goat rearing is an inseparable part of mixed farming system prevailing in arid and semi-arid areas of Telangana. Goats provide dependable source of income to 40% of rural population.
Mahabubnagar goats though yet to be recognised as a breed are well known for their production potential, faster growth rate, prolificacy and typical coat colour patterns spread all over the Mahabubnagar, Nalgonda and Rangareddy districts of Southern Telangana. The phenotypic performance of these goats indicated that the bodyweight ranged from 2.16 ± 0.03 kg at birth to 18.81 ± 0.17 kg at 12 months in males, while in females the values ranged from 2.11 ± 0.03 kg to 16.97 ± 0.15 kg at birth and 12 months of age, respectively (Ekambaram *et al.*, 2010) [5].
Phenotypic and genetic characterization would provide comprehensive information on this group of goats and would pave way for its characterization and recognition as a separate breed. Since there are no molecular genetic studies conducted, an attempt is made to decipher the genetic architecture of these goats by using microsatellite markers which are co-dominant in nature and abundant in the genome.

2. Materials and Methods
The investigation was carried out on 31 unrelated animals of Mahabubnagar goats which are maintained at Livestock Research Station, Mahabubnagar and Institutional Livestock Farm Complex, College of Veterinary Science, Rajendranagar, Hyderabad. Approximately 10 ml blood was collected aseptically from external jugular vein using the vacutainer tube containing EDTA as a anticoagulant. The samples were brought to the laboratory in an ice box and stored at 4 ºC till further processing. The samples were collected at random irrespective of age and sex of Mahabubnagar goats.
In this study, 15 microsatellite primer pairs were used: CSRD247, ILSTS005, ILSTS011, ILSTS029, ILSTS087, INRA063, MAF065, MAF70, OarFCB48, SRCRSP3, SRCRSP5, SRCRSP9, SRCRSP15, SRCRSP23, and TGLA53. Most of primers used were independent and located on different chromosomes.

2.1 Genomic DNA extraction and PCR amplification of microsatellite loci
Genomic DNA was isolated from collected blood using standard Phenol-Chloroform method (Sambrook and Russel, 2001) [17]. Nano drop was used for estimation of the quantity of

genomic DNA and the quality was estimated by electrophoresis of the isolated genomic DNA on 0.8% agarose gels.

Selected 15 microsatellite loci were amplified by Polymerase Chain Reaction (PCR) in 12.5 µl reaction mixture containing 1 µl (100 ng/ µl) of Genomic DNA, 1.25µl of 10X Taq Buffer, 0.25µl dNTPs (10mM/µl), 0.75µl of Forward- Primer (100 pM/µl), 0.75µl of Reverse-Primer (100 pM/µl), 0.75 µl of $MgCl_2$ (25 mM/ µl), 0.5U of Taq Polymerase (1 unit/µl) and 7.25µl of autoclaved MilliQ water was added to make up the final volume. The PCR reaction cycle was accomplished by Initial denaturation for 5 min at 95 ºC, followed by 34 cycles of one min at 94 ºC, 30 sec at optimal annealing temperature, 30 sec at 72 ºC and final extension step at 72 ºC for 5 min. Amplification products along with 50 bp DNA ladder (for scaling) were resolved on 8% polyacrylamide gel and silver staining was used for visualising. The product sizes were estimated with the help of 50 bp ladder as a standard marker.

2.2 Statistical Analysis

Data were analyzed using Excel Microsatellite tool kit for calculation of allele frequency, observed number of alleles (N_a), effective number of alleles (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), polymorphic information content (PIC), within breed inbreeding estimate (F_{IS}) and Hardy Weinberg equilibrium (HWE).

3. Results and Discussion

The various measures of genetic variation in Mahabubnagar goats are presented in Table 1. A total of 132 alleles were observed for 15 loci. The observed number of alleles varied from 5 (SRCRSP5) to 13 (SRCRSP15) with an average value of 8.800. The effective number of alleles varied from 4.429 (SRCRSP5) to 11.250 (SRCRSP15) with the overall mean of 7.711. All the loci had lower values for effective number of alleles in comparison to observed number of alleles. The allele size varied from 98bp (SRCRSP23) to 296bp (ILSTS011). The mean number of alleles in the present study was similar to the reports of Dixit *et al*. (2011) [4] in Kanniadu goats and Mahmoudi *et al.* (2009) [9] in Markhoz goats. These values are higher than those obtained by Verma *et al.* (2015) [20] in Odisha local breeds and Maletsanake *et al*. (2013) [11] in Tswana goats. The population with low mean number of alleles have low genetic variation which could be due to either genetic isolation or population bottlenecks or founder effects. The high mean number of alleles implies great allelic diversity which can be exploited for selection and indicate their suitability for the diversity analysis.

The Polymorphic information index (PIC) values varied from 0.738 (SRCRSP5) to 0.904 (SRCRSP15). All the 15 loci had PIC values greater than 0.50. These high values of PIC indicated higher polymorphism and greater heterogeneity in the breed. PIC estimated in the present study are comparable with those values obtained in Gaddi goats, which varied from 0.714 to 0.909 (Singh *et al.*, 2015) [18] and with Mehsana goats having average PIC 0.79 (Mishra *et al.*, 2012) [12]. In contrast, lower PIC values were obtained for Ghumusar (Mishra *et al.*, 2013b) [13], Taleshi (Mahmoudi and Babayev, 2009) [9], Mehsana (Aggarwal *et al.*, 2007) [1] and Bengal and Chegu goats (Behl *et al.*, 2007) [2]. Based on the PIC values, the microsatellite markers can be well utilized for molecular characterization of goat breeds.

The heterozygosity measurement (Table 1) clearly depicted the level of variability in these goats. The values of observed heterozygosity varied from 0.379 (MAF065) to 0.900 (OarFCB48 and SRCRSP3) with overall mean of 0.694. Present finding are in agreement with the heterozygosity observed by Hykaj and Hoda (2014) [7] in Albanian goats (0.673), Mishra *et al.* (2013a) [14] in Berari goats (0.67). However, lower heterozygosity was observed by Zaman and Shekar (2015) [21] in indigenous goats (0.484); Dixit *et al.* (2011) [4] in Kanniadu goats (0.53).

The expected heterozygosity varied from 0.774 (SRCRSP5) to 0.911 (SRCRSP15) with an average of 0.862. The values obtained in the present study are in accordance with the values obtained by Singh *et al.* (2015) [18] in Gaddi goats (0.843), Sadeghi *et al.* (2010) [16] in Raeini goats (0.805) and higher than the values obtained by Rout *et al.* (2012) [15] in Jamunapari goats (0.769), Verma *et al.* (2010) [19] in Sangamneri goats (0.697).

The Shannon information index (Table 1) showed that all the loci were highly informative indicating the high polymorphism across the loci and index value varied from 1.546 (SRCRSP5) to 2.491 (SRCRSP15) with the average value of 2.071. The mean values obtained in the present study were little higher than the values obtained by singh *et al*. (2015) [18] in Gaddi goats (1.950); Mahmoudi *et al.* (2012) [10] in Korki Jonub Khorasan goats (1.759); Dinesh *et al.* (2005) [3] in Jakhrana goats (1.105).

F- Stat analysis (Table.1) showed that 13 out of 15 loci had positive F_{IS} values, which indicated significant heterozygote deficiency at these loci. However, two loci showed negative values for F_{IS} indicating significant heterozygote excess. The overall mean within population inbreeding coefficient was 0.196 and it ranged from -0.023 (OarFCB48) to 0.571 (MAF065). The mean F_{IS} value obtained in the present study was lower than the values obtained by Dixit *et al.* (2011) [4] in Kanniadu goats (0.25), Dinesh *et al.* (2005) [3] in Jakhrana goats (0.278) and higher than those of Mishra *et al.* (213b) in Ghumusar goats (0.002), Aggarwal *et al.* (2007) [1] in Mehsana goats (0.156) and Gour *et al.* (2006) [6] in Jamunapari goats (0.189). The possible reasons for heterozygote deficiency cited in the literature include the existence of null alleles (non-amplifying alleles), selective breeding, physical linkage, inbreeding, high mutation rate and size homoplasy of microsatellite loci (Dixit *et al.* 2011) [4], besides the small size of studied population.

The Hardy-Weinberg equilibrium statuses of the populations were tested for all the loci. Among the population studied, nine loci (SRCRSP3, SRCRSP5, SRCRSP9, SRCRSP15, SRCRSP23 and ILSTS015, ILSTS011 and MAF065, MAF70) were significantly deviate from Hardy Weinberg Equilibrium (Table 2). The presence of null alleles might have resulted in the over estimation of particular homozygote or heterozygote and hence the deviation in the frequencies.

Table 1: Sample size (N), Mean number of alleles (Na), Effective number of alleles (Ne), Shannon's Information Index (I), Observed heterozygosity (Ho), Expected heterozygosity (He) and Unbiased Expected heterozygosity (uHe), Fixation Indices (FIS),Polymorphism Information content (PIC) and Out crossing rates at various microsatellite loci studied.

Locus	n	N_a	N_e	I	H_o	H_e	F_{IS}	PIC
CSRD247	31	10	8.580	2.206	0.839	0.883	0.051	0.872
ILSTS005	30	9	7.595	2.099	0.433	0.868	0.501	0.854
ILSTS011	29	8	6.596	1.979	0.483	0.848	0.431	0.831
ILSTS029	31	10	9.707	2.287	0.742	0.897	0.173	0.888
ILSTS087	29	6	5.051	1.697	0.759	0.802	0.054	0.773
INRA063	31	7	6.121	1.870	0.613	0.837	0.267	0.815
MAF065	29	10	8.582	2.204	0.379	0.883	0.571	0.872
MAF70	29	8	7.281	2.029	0.690	0.863	0.201	0.847
OarFCB48	30	9	8.333	2.156	0.900	0.880	-0.023	0.868
SRCRSP3	30	11	9.574	2.318	0.900	0.896	-0.005	0.886
SRCRSP5	28	5	4.429	1.546	0.571	0.774	0.262	0.738
SRCRSP9	29	6	5.479	1.741	0.690	0.817	0.156	0.791
SRCRSP15	30	13	11.250	2.491	0.867	0.911	0.049	0.904
SRCRSP23	31	10	8.467	2.214	0.710	0.882	0.195	0.870
TGLA53	31	10	8.619	2.222	0.839	0.884	0.051	0.873
Mean	29.867	8.8	7.711	2.071	0.694	0.862	0.196	0.845
SE	0.256	0.554	0.488	0.067	0.043	0.010	0.047	-

Table 2: Summary of Chi-square tests for Hardy-Weinberg Equilibrium

Locus	d.f	Chi-square	Probability	Significance
CSRD 247	45	45.775	0.440	ns
ILSTS 005	36	106.909	0.000	***
ILSTS 011	28	58.419	0.001	***
ILSTS 029	45	56.972	0.109	ns
ILSTS 087	15	22.422	0.097	ns
INRA 063	21	30.561	0.081	ns
MAF 065	45	114.886	0.000	***
MAF 70	28	53.102	0.003	**
OARFCB 48	36	44.430	0.158	ns
SRCRSP 3	55	112.634	0.000	***
SRCRSP 5	10	32.929	0.000	***
SRCRSP 9	15	35.810	0.002	**
SRCRSP 15	78	120.183	0.002	**
SRCRSP 23	45	68.461	0.014	*
TGLA 53	45	49.434	0.301	ns

Ns=not significant, * P<0.05, ** P<0.01, *** P<0.001

4. Conclusion

The results of this study provided valuable information about the genetic structure of Mahabubnagar goats. The results revealed high genetic variation within the breeds. However, further studies are required to identify association of markers with desirable traits such as high prolificacy for which this population is known to exploit the potential of the Mahabubnagar goats.

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