Original Research

Molecular Characterization of Mahabubnagar Goats using Microsatellite Markers

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Abstract

Mahabubnagar goats distributed in Mahabubnagar and in adjoining areas of Nalgonda and Rangareddy districts of Telangana, are well known for their production potential. The aim of the study is to estimate the genetic diversity and to asses genetic status of 40 unrelated Mahabubnagar goats using 15 microsatellite markers. A total of 123 alleles were amplified with an average of 10.00 ± 0.458 allels per locus and product size varied from 98 (OarFCB 20) to 287(INRABERN 185). The loci genotyped were found to be polymorphic with moderate to high informativeness. The high mean number of allels implies great allelic diversity which can be exploited for selection and indicate their suitability for diversity analysis. The overall mean expected and observed heterozygosity was 0.863 ± 0.011 and 0.778± 0.034. High heterozygosity values obtained may be due to intermixing of different populations probably during grazing period. The within breed diversity values revealed that there is wide genetic variability and the population was outbred in nature as indicated by negative inbreeding coefficient value in INRA023 and ETH10.All the 15 loci significantly deviated from Hardy Weinberg equilibrium.

Key words: Genetic Diversity, Mahabubnagar Goats, Microsatellite Markers

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Introduction

Goats are the most widely spread domestic species in the world and play an important economic role in developing countries (Adrina *et al.*, 2010). Goat rearing is an inseparable part of mixed farming system prevailing in arid and semi-arid areas of Telangana. Mahabubnagar district in Telangana stands first with 6.86 lakhs of goat population (Livestock Census, 2012). Mahabubnagar goats grow faster, breed more efficiently and has liking for a wider variety of feeds including many weeds and shrubs. The bodyweights

of Mahabubnagar goats ranged from 2.16 ± 0.03 kg at birth to 18.81± 0.17 kg at 12 months of age 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).

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 on this genetic group of goats, 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. Hence, the present study is taken up to estimate genetic diversity and to asses genetic status of Mahabubnagar goat population.

Materials and Methods

Experimental Animals and Sample Collection

A total of about 40 unrelated Mahabubnagar goats (15 males and 25 females), randomly selected from the farmers herds were utilized for the present study. The blood samples were obtained from the breeding tract of Mahabubnagar district. About 8-10 ml of blood was drawn from each animal intravenously from the external jugular vein into vacutainer tubes containing EDTA (2.7%).

Microsatellite Marker Primers

A total of 15 microsatellite marker primers were utilized for genotyping the 40 blood samples obtained. The markers were chosen from the list recommended by FAO (www.fao.org) (Table 1) for studying the genetic diversity and they comprised mostly dinucleotides, located on different chromosomes.

Isolation of Genomic DNA

Genomic DNA was isolated by standard Phenol-Chloroform method (Sambrook and Russel, 2001) with minor modifications.

Polymerase Chain Reaction (PCR)

The PCR amplification of all the microsatellite loci was carried out in 0.2 ml capacity Eppendorf tubes, using thermal cycler (Eppendorf). A master mix for PCR amplification was prepared for required number of samples in 0.2 ml tube as presented in the Table 2 and vortexed. An aliquot of 11.5 µl of master mix per sample was drawn into thin-walled PCR tubes and 1µl (100 ng) of template DNA was added. The PCR tubes were marked for the identification, then spinned briefly for proper mixing. The PCR protocol followed for the amplification of microsatellite marker primers is given in Table 3.

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Table 1: Details of microsatellite primers used in the present study

S. No.	Locus	Multiplex Group	Annealing Temperature (ºC)	Primer Sequence	Allele Size (bp)	Chromosome Location
1	INRA023	2	58	F: GAGTAGAGCTACAAGATAAACTTC R: TAACTACAGGGTGTTAGATGAACT	196-215	3
2	OarAE54	3	58	F : TACTAAAGAAACATGAAGCTCCCA R : GGAAACATTTATTCTTATTCCTCAGTG	115-139	25
3	SRCRSP8	1	55	F: TGCGGTCTGGTTCTGATTTCAC R:GTTTCTTCCTGCATGAGAAAGTCGATGCTTAG	215-255	-
4	SPS113	3	58	F: CCTCCACACAGGCTTCTCTGACTT R: CCTAACTTGCTTGAGTTATTGCCC	134-161	10
5	INRABERN172	3	58	F: CCACTTCCCTGTATCCTCCT R: GGTGCTCCCATTGTGTAGAC	234-267	26
6	OarFCB20	2	58	F: GGAAAACCCCCATATATACCTATAC R: AAATGTGTTTAAGATTCCATACATGTG	93-112	2
7	MCM527	2	58	F: GTCCATTGCCTCAAATCAATTC R: AAACCACTTGACTACTCCCCAA	165-187	5
8	ETH10	_	55	F: GTTCAGGACTGGCCCTGCTAACA R: CCTCCAGCCCACTTTCTCTTCTC	200-210	15
9	MAF209	_	55	F: GATCACAAAAAGTTGGATACAACCGTG R: TCATGCACTTAAGTATGTAGGATGCTG	100-105	17
10	INRABERN185	_	55	F: CAATCTTGCTCCCACTATGC R: CTCCTAAAACACTCCCACACTA	261-289	18
11	P19(DYA)	_	55	F: AACACCATCAAACAGTAAGAG R: CATAGTAACAGATCTTCCTACA	160-196	-
12	TCRVB6	_	55	F: GAGTCCTCAGCAAGCAGGTC R: CCAGGAATTGGATCACACCT	217-255	10
13	SRCRSP7	-	55	F: TCTCAGCACCTTAATTGCTCT R : GGTCAACACTCCAATGGTGAG	117-131	16
14	BM6444	-	65	F: CTCTGGGTACAACACTGAGTCC R: TAGAGAGTTTCCCTGTCCATCC	118-200	2
15	DRBP1	-	58	F: ATGGTGCAGCAGCAAGGTGAGCA R: GGGACTCAGTCTCTCTATCTCTTTG	195-229	23

Table 2: Composition of master mix for PCR amplification

Components	Volume (µl)	Final Concentration
10× Taq Buffer	1.25	1x
dNTPs (10mM)	0.25	2.5 mM
Primer-Forward (100 pM)	0.6	60 pM
Primer-Reverse (100 pM)	0.6	60 pM
$MgCl_2$ (25 mM)	0.75	1.5 mM
Taq Polymerase (1 unit/µl)	0.5	0.5 unit
Autoclaved Mille Q water	7.55	

After PCR amplification, the amplified products were electrophoresed on 2% agarose (Genei) to resolve the bands. Electrophoresis was carried out @ 5-6 volts/cm, bands were visualized and documented in gel documentation system (Syngene, UK).

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Table 3: PCR reaction conditions

Step	Process	Temperature (°C)	Time
1	Initial denaturation	95	5 min
2	Cyclic denaturation	94	1 min
3	Primer annealing	Depends on primer	30 s
4	Cyclic extension	72	30 s
5	Repeated steps 2 to 4 for 34 cycles		
6	Final extension	72	5 min
7	Hold	4	Forever

Poly Acrylamide Gel Electrophoresis (PAGE)

The PAGE gel mix was prepared by mixing 8 ml of 5x TBE, 8 ml of Acrylamide: Bisacrylamide (29:1) solution and 24 ml of distilled water. Finally, 150 µl of APS and 40µl of TEMED were added and the solution was poured directly in between the two glass plates, because polymerization starts with addition of ammonium persulphate. Flat side of the comb was inserted at the top and the assembly was left undisturbed for complete polymerization. After polymerization, the comb was removed and the gel assembly was placed in the PAGE apparatus partly filled with 1× TBE with the larger glass plate facing the outside. The air bubbles were removed, if any trapped beneath the bottom of the gel. The wells were flushed with TBE.

The samples were prepared by mixing 1 µl loading dye and 4 µl amplicons and then loaded into wells. Along with the amplicons, a 50 bp ladder (Fermentas) was also loaded into a lane to compare the size of the amplicons generated. The mixture was carefully loaded into the wells with a micropipette (long tips). The electrodes were connected to a power pack. The gel was normally run at 1-8 V/cm. The gel was run until the marker dyes migrated the desired distance. Then the electric power was turned off and disconnected the leads. The glass plates were taken out of the tank and laid the glass plates on the bench. By using a thin spatula, the corner of the upper glass plate was lifted gently. After confirming that the gel remained attached to the lower plate, the upper plate was pulled out gently, without causing any damage to the gel. The gel was stained by silver nitrate as described by Cominicini *et al.* (1995) with minor modifications. The bands of the gel were visualized under UV light of gel documentation system. The product sizes were estimated with the help of 50 bp ladder as a standard marker. The types of bands and their genotypes were documented and used for further analysis.

Statistical Analysis

The microsatellite genotype data were analyzed using GenAlEx software to calculate number of alleles (mean and effective), the allele size, allele frequency, Shannon's information index, observed

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heterozygosity, expected heterozygosity, unbiased heterozygosity, polymorphic information content, coefficient of inbreeding, out crossing rate in the population.

Results and Discussion

The quality and quantity of 40 DNA samples isolated from the blood of 40 Mahbubnagar goats were analysed by using the Nano drop technique by recording the optical absorbance ratio values of 260/280 nm and concentrations (ng/ µl) directly. The overall mean absorbance ratio of the isolated genomic DNA was 1.86 which was similar to the ratios recommended for pure DNA preparations free from protein (Sambrook and Russell, 2001). Genomic DNA was subjected to agarose gel electrophoresis using 0.8% agarose followed by PAGE revealing polymorphism of microsatellite markers. A total of 123 alleles were amplified in the present study across the 15 microsatellite loci studied (F. The loci SRCRSP8 (10), SPS113 (10), INRABERN172 (12), OarFCB20 (10), ETH10 (10), INRABERN185 (11), P19 (DYA) (10), TCRVB6 (10), BM6444 (13), DRBP1 (10) have amplified a total of 10 and more than 10 alleles, whereas the rest of the loci had less than 10 alleles. The differences in allele number and size may be attributable to the study of unrelated local populations spread over distant geographical area, which harboured high degree of genetic variation. The product size (bp) varied from 98 for OarFCB20 to 287 for INRABERN185.

The number of alleles at a locus varied from 5(MAF209) to 13 (BM6444). The number of alleles identified in each locus was in line with the recommendation by FAO suggesting at least 5 different alleles per locus for estimation of genetic diversity between the breeds. The mean number of alleles per locus in the present study was similar to the reports of Hassen *et al.* (2012) in six Ethiopian indigenous goats; Mishra *et al.* (2012b) in Konkan Kanyal goat populations. The overall mean number of alleles per locus was 10.000 ± 0.458. The effective number of alleles varied from 3.609 (MAF209) to 9.440 (INRABERN172) and the overall mean effective number of alleles was 7.685 ± 0.360. The allele size varied from 98 (OarFCB20) to 287(INRABERN185). The allele frequency ranged from 0.013 (234bp atTCRVB6) to 0.436 (101 bp at MAF209).The overall mean Shannon's information index was 2.138 ± 0.056, ranged from 1.440 (MAF209) to 2.397 (BM6444) which are little higher than the reports of Singh *et al.* (2015) in Gaddi goat breed (1.950); Zaman and Shekar (2015) in indigenous goats populations (1.010); Mishra *et al.* (2013a) in Berari goats (1.76).

Genetic diversity measures the genetic variation among the populations in terms of estimates of heterozygosity. The observed heterozygosity was calculated based on the number of heterozygotes at respective loci over total number of individuals. The overall mean observed heterozygosity was 0.778 ± 0.034, ranged from 0.513 (DRBP 1) to 1.000 (ETH10) which was higher when compared to the studies of Aggarwal *et al.* (2007) in Mehsana goats (0.652); Mishra *et al.* (2013a) in Berari goats (0.67); Mahrous *et al.* (2013) in Barki (0.611), Ardi (0.671) and Zaraibi (0.628) goats; Hykaj and Hoda (2014) in Albanian

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goats (0.673); Zaman and Shekar (2015) in indigenous goats (0.484). The expected heterozygosity was calculated based on the assumption that the population was under Hardy-Weinberg equilibrium (Nei, 1973). In the present study, the highest expected heterozygosity was observed for the locus INRABERN172 (0.894) and the lowest for MAF209 (0.723) with a mean of 0.863 which are in accordance with the values obtained by Singh *et al.* (2015) in Gaddi goats (0.843) and Sadeghi *et al.* (2010) in Raeini goats (0.805). The unbiased expected heterozygosity ranged from 0.732 (MAF209) to 0.905 (INRABERN172) and the mean value is 0.874 ± 0.011.

The level of inbreeding estimated based on the observed heterozygosity, ranged from -0.135 (ETH10) to 0.416 (DRBP1).The overall mean inbreeding coefficient (F_{IS}) was 0.101 ± 0.035 which was lower than the values obtained in studies of Dixit *et al.* (2011b) in Kanniadu goats (0.25); Dixit *et al.* (2010) in southern Indian goats breeds (0.20) and also higher when compared to reports of Mishra *et al.* (2013b) in Ghumusar goats (0.002) and Mahrous *et al.* (2013) in Egyptian and Saudi goat breeds (0.053). Three out of fifteen loci had negative inbreeding coefficients. The overall mean PIC value was 0.848 and across the loci the values ranged from 0.684 (MAF209) to 0.884 (INRABERN172) and all the loci were found to be more than 0.50. Therefore, a wide range of PIC values were reported 0.53 to 0.91 in Ghumusar goats (Mishra *et al.*, 2013b); 0.532 to 0.814 in Taleshi goats (Mahmoudi and Babayev, 2009). The mean PIC values were 0.79 in Konkan Kanyal goats (Mishra *et al.*, 2012b), 0.724 in Mehsana goats (Aggarwal *et al.*, 2007) indicating that these microsatellite markers can effectively be used for molecular characterization, genome mapping, genetic diversity and breeding programme implemented in the studied goat population. The overall mean outcrossing rate was observed to be 0.842 in the present study and the values among the 15 loci varied from 0.412 (DRBP1) to 1.31 (ETH10). In the present study, all the 15 loci studied were found to be polymorphic in nature, thus reaching 100% polymorphism.

In each locus, for all the possible genotypes observed and expected genotypic frequencies were calculated. The observed genotypic frequency varied from 0 (in many loci) to 12 (MAF209) and the expected genotype frequency ranged from 0.006 (TCRVB6) to 7.4 (MAF20 9). The populations were tested for departure from Hardy-Weinberg equilibrium for all the loci. Among the pop ulation studied all the loci (INRA023, OarAE54, SRCRSP8, SPS113, INRABERN172, OarFCB20, MCM527, ETH10, MAF209, INRABERN185, P19 (DYA), TCRVB6, SRCRSP7, BM6444 and DRBP1) significantly deviated from Hardy-Weinberg equilibrium.

Conclusion

Microsatellites are quite reliable markers to be used in the studies to investigate genetic variety and genetic structures of population. The results also reveal the presence of higher genetic variability and such high variation provides an excellent base for designing genetic improvement program. However, further studies

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are required to identify association of markers which could be further employed in MAS (marker assisted selection) and QTL (Quantitative Trait Loci) programs.

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