Genetic characterization of local goats of Karnataka by microsatellite marker analysis


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ABSTRACT

The diversity status of local goats of Karnataka was studied by using microsatellite marker analysis. The genomic DNA from unrelated local goats were PCR- amplified with a panel of 23 microsatellite markers. Microsatelite PCR products were multiplexed and run on capillary based genetic analyser and the raw data obtained was analysed. Totally 158 alleles were observed and the number of alleles ranged from three (ILSTS005 and OarJMP29) to 13 (RM088). The number of effective alleles ranged from 2.25 (ILSTS005) to 8.40 (RM088) in all the 23 loci studied. The mean observed heterozygosity (Ho) was 0.4698±0.2214 [range 0 (ETH225) to 0.8462 (ILSTS034)] and the mean expected heterozygosity (He) was 0.7471±0.1098 [range 0.5656 (ILSTS005) to 0.9138 (SRCRSP 8)] indicating the heterogenous nature of the local goat population of Karnataka.

Key words: Karnataka, Local goats, Microsatellite.

INTRODUCTION

In India goats are reared mainly by the small and marginal farmers, including landless agricultural labourers. Goat contributes 16% of the total meat production in the country and is the second highest contributor of meat in India (Anonymous 2014). India’s livestock sector is one of the largest in the world with a holding of 11.6% of the world population (Islam et al., 2016). Goat population in the country is 135.17 million numbers as per the 19th Livestock census and Karnataka has 47.96 lakhs of goats that contributes to 3.55 per cent share of the total goats of the country. The availability of meat in India is only about 15g/person/day against the ICMR recommendation of 30g/person/day. Characterization of native breeds is the first step in conservation programme. Various methods like typing of blood groups, biochemical polymorphisms and DNA marker studies are conducted for genetic characterization. Molecular characterization using microsatellite markers may help in estimating the diversity, distinctiveness and population structure as it is not affected by environmental changes. Microsatellites are simple sequence motifs of two to six bases which are tandemly repeated. They are arranged head to tail without interruption by any other base or motif and are located in the non- coding regions (Litt and Luty, 1989). These microsatellites are selectively neutral and are randomly distributed and show co-dominant pattern of inheritance hence used in population diversity studies (Luty et al., 1990).

This study on genetic characterization of local goats using microsatellite markers was undertaken for the first time in Karnataka. As there are no distinct goat breeds native of Karnataka attempt was made to study the local goats expecting uniqueness in different regions. Villages were randomly selected from the four agro-climatic zones viz., Eastern dry zone (EDZ), Central dry zone (CDZ), Southern dry zone (SDZ) and Southern transition zone (STZ) of Southern Karnataka.

MATERIALS AND METHODS

Twenty three microsatellites markers (ETH 225, ETH10, ILSTS002, ILSTS005, ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS049, ILSTS058, ILSTS059, ILSTS065, ILSTS082, ILSTS087, OarHH64, OarFCB48 OarJMP29, OMHC1, RM088, SRCRSP5, SRCRSP8 and SRCRSP23) were chosen for the present study from the list proposed by the Food and Agriculture organization of the United nations (Project MoDAD, http://www.fao.org/dad-is). Whole blood samples (8- 10ml in EDTA) were collected from 50 unrelated indigenous goats distributed in different villages of southern Karnataka. DNA isolation was done High Salt Method as described by Millers et al. (1998). Horizontal agarose gel electrophoresis technique was adapted to check the quality of genomic DNA. The amplification reaction was carried out in thermal cycler in reaction volume of 15.0µl. Each 15.0µl contained 1. 0 µl template DNA(50ng/µl), 0.5 µl each of forward and reverse template primer with a concentration of 2.0 mM, composed of Amplicon Taq DNA Polymerase, NH4+ buffer system, dNTPs and magnesium chloride) and 5.5 µl of

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More than four alleles which indicated the ample genetic variability within the population studied. Lesser than four number of alleles at the loci ILSTS005 and OarJMP29 was also reported in other Indian breeds like Marwari (Kumar et al., 2005a), Kutchi (Dixit et al., 2008b), Zalawadi, Gohilwadi and Surti (Fatima et al., 2008) and in exotic breeds, Angora and Saanen (Luikart et al., 1999). Seventeen loci showed significant deviation from Hardy –Weinberg equilibrium (P<0.0.01). F_e ranged from -0.1782 (ILSTS034) to 1 (ETH225). The genetic distance between the flocks of EDZ and CDZ was the least with 0.5617 and the genetic distance between STZ and EDZ was the widest with 0.7316.

**Allele number, size and frequency:** The allele sizes were in the range of 200 to 208bp for ETH 10, 146 to 158bp for ETH225, 117 to 127 bp for ILSTS002, 174 to 178bp for ILSTS 005, 173 to 185bp for ILSTS008, 142-162bp for ILSTS019, 159 to 171 bp for ILSTS030, 155 to 177bp for ILSTS034, 145 to 169 bp for ILSTS044, 162 to 176 bp for ILSTS049, 138 to 184 for ILSTS058, 149 to 161 for ILSTS078.

### Table 1: Panel of microsatellite markers

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<th>Multiples sets</th>
<th>Dye</th>
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</table>

n - Number of alleles observed; n_1 - Expected number of alleles; H_1 - Observed heterozygosities; H_2 - Shanon Index; PIC - Polymorphism information content; F_s - F statistics; HWE - Hardy Weinberg equilibrium.

ILSTS059, 131 to 169 for ILSTS065, 108 to 122bp for ILSTS082, 142 to 152bp for ILSTS087, 122 to 128bp for OarHH64, 149 to 161bp for OarFCB48, 120 to 124bp for OarJMP 29, 185 to 197bp for OMHC1, 109 to 147bp for RM088, 102 to 118 for SRCRSP23, 156 to 178bp for SRCRSP5 and 222 to 254bp for SRCRSP8. The highest number of allele was noticed with the loci RM088 with 13 alleles, followed by 11 alleles in three loci ILSTS044, ILSTS058 and ILSTS065. There were 10 alleles for the loci ILSTS019 and SRCRSP8. The least number of three alleles was seen in two loci ILSTS005 and OARJMP 29. The rest of the loci were in the allele range of four to nine. The observed number of alleles were higher in Berari goats that ranged from 9 (ILSTS059, RM004) to 25 (ILSTS005, ILSTS029) with an average value of 15.560±0.947 (Kharkar et al., 2015).

The observed heterozygosity (H) ranged from 0 (ETH225) to 0.8462 (ILSTS034 and ILSTS065) and the expected heterozygosity (He) ranged from 0.5656 (ILSTS005) to 0.9138 (SRCRSP 8). The observed heterozygosity was lower than the expected heterozygosity in all except for two loci, OarHH64 and SRCRSP5. The mean observed heterozygosity in this study was 0.4698 ± 0.2214 which was in agreement with the findings in many other Indian breeds viz., Beetal (Sharma et al., 2008), Ganjam, Attappady, Malabari, Kann Adu, Sangamneri and Osmanabadi goats (Dixit et al., 2010) and it was lower than that observed in Berari goats by Kharkar et al., (2015) which was in the range of 0.834 (RM004) to 0.940 (ILSTS029) with an overall mean of 0.895±0.006.

Polymorphism information content: The Polymorphism information content ranged from 0.0114 (ILSTS059) to 0.9168 (RM088) with an overall mean of 0.7644 ± 0.1895. Based on the PIC values, the local goats of Karnataka were similar to Konkan Kanyal breed of Maharashtra with respect to 12 loci (ILSTS002, ILSTS008, ILSTS019, ILSTS030, ILSTS044, ILSTS049, ILSTS058, ILSTS065, ILSTS082, OarHH64, OarFCB48 and OMHC1). The results were similar to nine loci with respect to Malbari breed (ILSTS002, ILSTS019, ILSTS030, ILSTS049, ILSTS058, ILSTS059, ILSTS082, OarHH64 and OMHC1). The results of PIC were similar with Bengal goats with respect to seven markers (ETH10, ETH225, ILSTS019, ILSTS044, ILSTS049, ILSTS065 and OarFCB48), while it was similar with Kanni Adu with 5 markers (ETH225, ILSTS002, ILSTS008, OarFCB48 and OARJMP29). The average PIC were
higher in other northern Indian breeds like Berari that ranged from 0.814 in RM004 to 0.937 in ILSTS029 with an average of 0.886±0.007 (Kharkar et al., 2015) and in Gaddi breed it was 0.7148 (SRCPS5) to 0.909 (P19 [DYA]) with mean PIC of 0.8105±0.01 (Singh et al., 2015).

**Pair-wise $F_{IS}$ and Shannon’s information index:** $F_{IS}$ ranged from -0.0328 (SRCRSP5) to one (ETH 225). The positive values of $F_{IS}$ (inbreeding in individual relative to the sub population) ranged from 0.0074 (OarHH64) to 1 (ETH225). The gene diversity as indicated by Shannon Index (I) ranged from 0.9431 (ILSTS005) to 2.3102 (RM 088) with a mean of 1.5608 ± 0.4241. In general the higher polymorphism observed across loci in this study on local goats of Karnataka strengthened the suitability of these markers for genetic diversity studies in goats. The Shannon’s index was more than 2 with respect to the RM088, SRCRSP8, ILSTS065, ILSTS044, ILSTS058 and ILSTS019 with 2.3102, 2.1847, 2.1306, 2.1078, 2.0405 and 2.0258 respectively. The rest of the markers had Shannon’s index value of more than one except one marker ILSTS 005 with the index value of 0.9431. The Shannon’s index for the four agro-climatic zones (EDZ, CDZ, SDZ and STZ) was 0.8943, 1.1095, 0.6178 and 0.8965, respectively) appears to be lesser, probably due to the small sample size studied. However, the overall Shannon’s information index was high indicating high polymorphism across the loci, thus suggesting the suitability of these markers for further genetic diversity studies in goats.

**Test for Hardy-Weinberg equilibrium:** There was significant deviation from Hardy – Weinberg equilibrium ($P < 0.01$) with 14 loci (ETH 225, ILSTS002, ILSTS005, ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS049, ILSTS058, ILSTS059, ILSTS082, OarFCB48, RM088), whereas the remaining nine loci (ETH10, ILSTS065, ILSTS087, OarHH64, OarMP29, OMHC1, SRCRSP5, SRCRSP8, SRCRSP23) did not show any significant deviation ($P > 0.05$).

**Genetic diversity status of local goats of the different agro-climatic zones:** All the loci studied were showing polymorphism in all the four agro-climatic zones (EDZ, CDZ, SDZ and STZ). The ILSTS002 was not seen STZ and the loci SRCRSP 23 was not found in SDZ. The mean number of alleles observed in the four agro-climatic zones viz., EDZ, CDZ, SDZ and STZ were 3.0, 4.0, 2.22 and 2.70, respectively. Distribution of all the 23 markers in the four zones studied is presented in Fig-1.

The number of alleles for different loci for EDZ ranged from one (ETH 225, ILSTS008, ILSTS059, OarFCB 48) to seven (SRCRSP8). More than four alleles were observed in eight loci (ETH10, ILSTS 019, ILSTS034, ILSTS065, ILSTS082, ILSTS087, RM088 and SRCRSP8).
The mean observed heterozygosity and the expected heterozygosity observed in the goat populations of the four agro-climatic zones, EDZ, CDZ, SDZ and STZ, were 0.5123 and 0.5937, 0.4133 and 0.6431, 0.4053 and 0.4352, 0.6242 and 0.6362, respectively. The Shannon index was highest in CDZ (1.1095) and lowest in SDZ (0.6178) followed by the other two zones EDZ (0.8943) and STZ (0.8965). Based on the results a Dendrogram depicting the divergence of goat flocks of different zones was created and depicted in Fig-2.

In conclusion the microsatellite analysed in this study were informative and should be used in further genetic diversity studies of goats of other regions in Karnataka.

REFERENCES


