ALLELE FREQUENCIES AND PIC VALUES OF MICROSATellites In THREE South INDIAN CATTLE BREEDS

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Microsatellites are tandem repeats of DNA where basic repeat unit is only a few base pairs long. They are now-a-days replacing all other types of genetic markers because they overcome many of the limitations associated with other types of markers. They generally have higher heterozygosities than RFLPs and, because they arise from defined loci, results are more easily interpreted than patterns generated by minisatellites. They have been reported to be highly polymorphic and in Indian cattle they are now being tried for use as a marker, even though the experiments are in its initial stages. Present work is an initiative to study the polymorphic nature of microsatellites in Ongole, Kangayam and Umblachery.

Materials and Methods

Blood was collected from 28 Ongole, 30 Kangayam and 19 Umblachery animals. DNA was isolated as per the protocol of Montgomery and Sise (1990) with few modifications. Three microsatellites namely ETH 225 (Steffen et al., 1993), HBB (Bishop et al., 1993) and ILSTS 030 (Kemp et al., 1995) were used for the present study. The forward primer of each microsatellite was 5′ end labelled using Polynucleotide Kinase and [γ-32P]ATP as per the protocol on Naom et al., (1995). PCR was carried out in a final volume of 20 ml containing 100 ng of DNA, 2.0 μl of 10 x PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3), 100 mM of each dNTPs, 1.5 mM MgCl₂, 10 μM of labelled primer, 20 μM of unlabelled primer and 0.75 units of Taq DNA Polymerase. Cycling conditions comprised of initial denaturation at 94°C for 3 min, followed by 30 cycles each - denaturation at 94°C for 1 min, annealing (65°C for ETH 225, 60°C for HBB and 55°C for ILSTS 030) for 1 min and extension at 72°C for 1 min. Amplified products were loaded on to six percent sequencing gel, run and autoradiographed following standard procedures. The alleles were sized on the basis of co-migration of M13 sequencing ladder on the same autoradiograph. The alleles were scored manually and allele frequencies calculated.

Polymorphism Information Content (PIC) were calculated using the following formula (Botstein et al., 1980)–

\[ PIC = \sum_{i=1}^{n} P_i^2 \sum_{j=1}^{n} P_i P_j (2P_i P_j)^2 \]

where \( P_i \) is the frequency of the \( i \)th allele.

Results and Discussion

The total number of alleles at ETH 225 locus was 12 in all of three breeds studied with a mean allele size of 145.33 ± 2.32 bp

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and a mean frequency of 0.083 ± 0.014. Ongole showed 8 alleles with a mean allele size of 148.75 ± 2.34 bp and a mean frequency of 0.125 ± 0.032. In Kangayam 7 alleles were present with a mean allele size of 147.86 ± 3.20 bp and a mean frequency of 0.148 ± 0.048 and in Umblachery, 10 alleles were present with a mean allele size of 146.20 ± 2.44 bp and a mean frequency of 0.100 ± 0.018. This study was in concurrence with Steffen et al., (1993) who reported 8 alleles at this locus. Peelman et al., (1998) also got 6, 7 and 8 alleles in four Belgian Cattle breeds. In the present study, the more number of alleles in Umblachery can be attributed towards highly polymorphic nature of microsatellites.

The total number of alleles for HBB locus was 11 with a mean allele size of 174.00 ± 2.00 bp and a mean frequency of 0.091 ± 0.026. In Ongole 8 alleles were present with a mean size of 177.00 ± 1.73 bp and a mean frequenting of 0.125 ± 0.032. Kangayam also showed 8 alleles with a mean allele size of 173.25 ± 2.32 bp and mean frequency of 0.125 ± 0.050; while Umblachery showed 6 alleles with a mean size of 177.14 ± 1.99 bp and mean frequency of 0.143 ± 0.054. This result was in contract to the study of Arranz et al., (1996) who found only two alleles. Breeds studied in the present study are more polymorphic for this locus also.

The number of alleles in total population for ILSTS030 locus was 6 with a mean allele size of 154.33 ± 1.76 bp and mean frequency of 0.167 ± 0.102. Ongole samples showed 3 alleles with a mean allele size of 156.33 ± 2.40 bp and a mean frequency of 0.333 ± 0.193. Kangayam also showed 3 alleles but with a mean allele size of 155.00 ± 1.16 bp and mean frequency of 0.334 ± 0.164; while in Umblachery 4 alleles were present with a mean allele size of 152.00 ± 1.29 bp and a mean frequency of 0.250 ± 0.146. This result was in concurrence with Gortari et al., (1997) who also found 4 alleles in sheep.

PIC value for ETH 225 locus was 0.891 for over all population, while, it was 0.820, 0.751 and 0.871 for Ongole, Kangayam and Umblachery, respectively. PIC values for HBB marker was 0.831 for over all population and it was 0.819, 0.737 and 0.727 for Ongole, Kangayam and Umblachery respectively. PIC values at ILSTS 030 locus was found to be 0.519 for over all population while, it was 0.442, 0.04 and 0.494 for Ongole, Kangayam and Umblachery respectively.

Steffen et al., (loc. cit) report a PIC value of 0.77 for ETH 225 marker, while Peelman et al., (loc. cit) report a PIC value ranging from 0.73 to 0.78 for different breeds. Kemp et al. (1995) have reported a PIC value of 0.65 at ILSTS 030 locus; while in the present study it has lower value. Hence, it can be concluded that microsatellites ETH 225 and HBB are more polymorphic in Indian cattle breeds, and can be used as a marker to characterize these cattle.

**Summary**

The number of alleles for microsatellites loci ETH 225, HBB and ILSTS 030 were found to be 12, 11 and 6 with a mean allele size of 145.83 ± 2.32 bp, 174.00 ± 2.00 bp and 154.33 ± 1.76 bp respectively. The allele frequencies for ETH 225, HBB and ILSTS 030 were 0.083 ± 0.014, 0.091 ± 0.026 and 0.167 ± 0.102 respectively. PIC values for ETH 225 and HBB showed them to be highly polymorphic.
with values of 0.891 and 0.831 respectively, while microsatellite ILSTS 030 was less polymorphic for these breeds with a PIC value of only 0.519.

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