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Molecular characterization of Nagori cattle using microsatellite markers

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Abstract
The present study estimates genetic variability in nagori cattle (n=30), a draught breed of India, by use of 15 microsatellite markers recommended by the Food and Agriculture Organization. Microsatellite genotypes were derived and allelic and genotypic frequencies, heterozygosities and gene diversity were estimated. A total of 123 alleles were distinguished by the 15 microsatellite markers used. All the microsatellites were highly polymorphic, with mean (± s.e.) allelic number of 8.2±1.143, ranging 2-14 per locus. The observed heterozygosity in the population ranged between 0.52 and 0.758, with mean (± s.e.) of 0.610 ± 0.019, indicating considerable genetic variation in this population.

Keywords: genetic diversity, microsatellite markers, nagori cattle and heterozygosities

1. Introduction
India is one of the mega bio-diversity centers of the world. As per the 19th Livestock Census (2012) [6], the cattle population in India is 48.12 million, of which 160.50 million are indigenous. Mechanization, unplanned and indiscriminate breeding among native stocks as well as human bias in favor of certain breeds have directly or indirectly lead to the dilution of indigenous germplasm (FAO, 2000). Hence, there is an urgent need to prevent the rapid erosion of animal genetic resources. Indian agriculture is an economic symbiosis of crop and livestock production with cattle as the foundation. Sadly, the population of indigenous cattle (*Bos indicus*) is declining (8.94 % in last decade) and needs immediate scientific management. Genetic characterization is the first step in the development of proper management strategies for preserving genetic diversity and preventing undesirable loss of alleles.
The Nagori breed of cattle is primarily a draught breed, small in size and capable of much endurance. The breeding tract of Nagori breed is Bikaner, Jodhpur and Nagaur district of Rajasthan. The breed takes its name from the name of the home tract i.e., Nagaur district. They are basically White in color and are upstanding, very alert animals with long and narrow face. The Nagori breed is one of the most famous draught breed of India and are generally appreciated for fast draught activity. Average milk yield per lactation of Nagori cattle is 603 kg. The lactation yield ranges from 479 to 905 kg.
Microsatellites are short DNA sequence stretches, in which a motif of one to six bases is tandemly repeated and these sequences differ in repeat number among the individuals (Schlotter and Tautz, 2000). Microsatellites are highly polymorphic, dispersed throughout genome at a frequency of one at every 6 kb sequence (Bruford and Wayne, 1993) [1] and amenable to PCR amplification to make them potentially useful DNA markers in gene mapping studies. The present study was carried out with an objective of assessing the molecular genetic variability of Nagori cattle using microsatellite markers.

2. Materials and Methods
2.1 DNA samples
The microsatellite analysis was carried out on a sample of 30 unrelated Nagori cattle collected from its breeding tract. Genomic DNA was isolated from peripheral blood using standard Phenol Chloroform method (Sambrook *et al*., 2001) [11] with minor modifications. The purity and concentration of DNA samples were estimated by UV spectrophotometer. The quality of DNA samples were also checked by agarose gel (1%) electrophoresis.

2.2 Microsatellite markers and PCR amplification
A total of 15 microsatellite primer sets, specific for cattle, were used in the study as recommended by FAO and International Society for Animal Genetics (BM1818, CSRM60, ETH10, ETH225, INRA005, BM2113, ETH3, ETH152, HEL1, HEL5, ILSTS022, INRA035, INRA063, ILSTS002, ILSTS006). PCR was carried out in 25µ1 reaction volume containing

1.5 mM Mgcl2, 200µM dNTPs, 50ng of each primer, 100 ng of template DNA and 0.5U of Taq DNA polymerase. PCR cycling conditions were: 5 min at 95 ºC, followed by 35 cycles of 45 sec at 95 ºC, 45 sec at annealing temperature (52-64 ºC) of each primer, 45 sec at 72 ºC, and final extension of 10 min at 72 ºC. Amplified PCR products were checked on 2% agarose gel containing ethidium bromide and visualized through UV illumination and then microsatellite marker scoring was done using PAGE (Koreth *et al.* 1996) [5] and genotypes were scored manually.

2.3 Statistical analysis for microsatellite and AFLP data

Analyses of the bands were done using a software aided gel-documentation system (UVP) and genotypes of the individual animals were scored manually. Microsatellite allele frequencies, effective number of alleles, observed and expected heterozygosity, F-statistics were calculated and test of Hardy-Weingberg equilibrium was carried out using software GenAlEx version 6.5.The polymorphism information content (PIC) was calculated according to Nei (1978) using the individual frequencies in which the allele occurred at each locus (http:// www.genomics.liv.ac.uk/animal/pic.html.).

3. Results and Discussion

The ratio of optical density in this study at 260 and 280 nm were consistently between 1.69 to 1.80 indicating the de-proteinization and good quality of DNA (Sambrook and Russell, 2001) [11]. Similar observations were recorded by many authors in various breeds of catlle in Ongole and Deoni (Muralidhar *et al*. (2003); Srinivas *et al*. (2006) [14]; in Punganur cattle (Chennakesavulu *et al*. (2003)

Various within breed diversity estimates, Viz. Mean number of alleles (Na), Effective number of alleles (Ne), Observed heterozygosity (Ho), Expected heterozygosity (He), Polymorphism information content (PIC), Fixation indices (FIS) at each microsatellite marker analysed in nagori cattle are presented in Table 1. A

A total of 123 alleles were amplified across 15 microsatellite loci. Allele size ranged from 74 to 300 bp. The number of alleles per locus varied from 2 to 14, with an overall mean of 8.2±1.143. The observed number of alleles demonstrated that almost all the microsatellite loci utilized in the present study were sufficiently polymorphic. The mean number of alleles observed is more than the number reported in Deoni (5.9) and Sahiwal (5.2) cattle (Mukesh *et al*., 2004) [8], in Hariana (4.2), Sahiwal (4.1) and Tharparker (3.6) cattle (MacHugh *et al*., 1997) [7] and Deoni (5.9) (Srinivas *et al*., 2006) [14]. Sodhi *et al*. (2006) reported the mean number of alleles as 5.82 in Red Kandhari and 5.86 in Deoni cattle. The effective number of alleles (Ne) is an estimate of the number of alleles with equal frequencies corresponding to a particular PIC value. The mean effective number of alleles was 2.655±0.140, values were in range of 4.167 (INRA005) to 2.00 (ETH10, ETH152 and ILSTS022). The PIC values which is the statistical assessment of informativeness of markers, ranged from 0.375 (ETH10, ETH152 and ILSTS022) to 0.717(INRA005), with a mean PIC of 0.5398. Mostly all the loci showed high PIC values of more than 0.5 indicating that these markers are highly informative for characterization of Nagori cattle. The overall mean observed heterozygosity and expected heterozygosity were 0.610 and 0.973 respectively. The higest observed heterozygosity 0.758 was due to the presence of more heterozygotes individuals for the samples analysed. The high expected heterozygosity reflects the existence of variation in the breed. The mean expected heterozygosity values are slightly higher than that of Umblachery (0.61) cattle (Karthikeyan *et al*., 2007) [4]; Sahiwal (0.61), Hariana (0.66) and Deoni (0.70) cattle (Mukesh *et al*., 2004) [8]. Negative Fis values indicate that individuals in a population are less related under a model of random mating and lower values of FIS indicate no relationship with the individual. The FIS value calculated in present study ranged from -1.00 (ETH10) to -000 (ETH225), with a mean of -0.756 across the loci in this study indicated the presence of excess heterozygosity in the investigated cattle population and out breeding as the samples were colleted randomly from breeding tract. The $\chi 2$ test for Hardy – Weinberg equilibrium revealed that there was highly significant deviation of observed allele frequencies from expected for all the loci studied. This indicated that the population was not in equilibrium status which was due to long term selection being practiced in the population. The disequilibrium exhibited in most of loci revealed that there might be unobserved null alleles.

Table 1: Mean number of alleles (Na), Effective number of alleles (Ne), Shannon's Information Index (I), Expected heterozygosity (He) Polymorphism Information content (PIC) and Fixation Indices (FIS) at various microsatellite loci studied.

Locus	N	Na	Ne	I	He	F	PIC
BM1818	14	4.000	2.947	1.224	0.661	-0.514	0.7007
CSRM60	6	3.000	2.323	0.918	0.569	-0.756	0.5000
ETH10	7	2.000	2.000	0.693	0.500	-1.000	0.3755
ETH225	3	4.000	3.000	1.242	0.668	0.000	0.6218
INRA005	14	5.000	4.126	1.497	0.758	-0.226	0.7177
BM2113	11	3.000	2.659	1.038	0.624	-0.603	0.5532
ETH3	11	4.000	2.373	0.993	0.579	-0.729	0.4986
ETH152	4	2.000	2.000	0.693	0.500	-1.000	0.3755
HEL1	8	3.000	2.667	1.040	0.625	-0.600	0.5547
HEL5	14	4.000	3.161	1.240	0.684	-0.463	0.6257
ILSTS022	3	2.000	2.000	0.693	0.500	-1.000	0.3755
INRA035	13	3.000	2.661	1.038	0.624	-0.602	0.5537
INRA063	2	3.000	2.667	1.040	0.625	-0.600	0.5547
ILSTS002	4	3.000	2.667	1.040	0.625	-0.600	0.5547
ILSTS006	9	3.000	2.571	1.011	0.611	-0.636	0.5356

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