

Genetic characterization of Malvi cattle using microsatellite markers

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ABSTRACT

Microsatellite markers offer great potential for genetic comparisons within and between populations. They are the best available molecular tools for characterization of breeds. Microsatellite markers (25) were used to screen 70 Malvi cattle. The mean number of alleles was 9.08 per microsatellite locus with the range of 4 (ILSTS011, ILSTS054) to 18 (CSSM663). The range in the allele size was from 88 bp (CSRM60) to 297 bp (ILSTS006). The microsatellite loci deviated significantly from Hardy-Weinberg equilibrium. Sign test and Wilcoxon revealed no Bottleneck in recent past in Malvi population. The mean polymorphic information content was 0.71 ranging from 0.41 to 0.91. The overall means for observed and expected heterozygosity were 0.54 and 0.75 respectively. The overall observed heterozygosity, PIC and Shannon index values were 0.54, 0.71 and 1.67 indicating high gene diversity. The markers used in the present study were highly informative.

Key words: Allele frequency, Malvi, Microsatellite

The name of the Malvi breed has been derived from the place of its origin – the Malwa tract. It has also taken its name after the family of King of Malawa. The breeding tract of Malvi includes the districts of Indore, Dewas, Ujjain, Shajapur, Rajgarh in Madhya Pradesh. Due to popularity of cross of crossbreeding during last 3 decades, the number of Malvi animals have declined in the all districts mentioned above. The major area of the breeding tract is covered with fertile black cotton soil of heavy loam type. The animals are famous for their economic feeding practices and good adaptability to various conditions of work climate and soils. Malvi bullocks are known for their draught capacity. Bullocks are active, strong and excellent in ploughing, lifting water, quick transportation, endurance and ability to carry heavy loads on rough roads but cows are poor yielders. Typically Malvi animals were of medium size, strong and robust. The body has been observed as compact, squarely built and not massive, with well proportionate limbs (Figs 1, 2). The body colour varied from white (69%) to light gray or gray (31%). Iron gray colour was also found.



Fig. 1. Malvi female



Fig. 2. Malvi male

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MATERIALS AND METHODS

The blood samples of 70 unrelated Malvi cattle were collected at random from different villages of the tract. The DNA was isolated as per John *et al.* (1991). The quantity and quality of the isolated DNA samples were tested by spectrophotometer for subsequent analysis. Twenty-five microsatellite markers (FAO 2004) were selected to screen the population of Malvi cattle. Out of 25 only 24 could be amplified in 4 multiplex sets (set I carrying 10 microsatellites pairs, set II carrying 6 pairs, set III carrying 5 microsatellite pairs and set IV carrying 4 microsatellite pairs).

The amplification was carried out using PCR reaction mixture of 15 µl. The mixture was prepared by using 60ng of template DNA, 1.5 mM MgCl₂, 2 picomoles each of forward and reverse primers, 1 units of Hot Start Taq DNA polymerase and 100 mM dNTPs. Amplification was carried out with annealing temperatures of 54 °C (Set I and Set II) and 55 °C (Set III and Set IV). 1 µl of PCR products was mixed with 0.20 µl of size standard fluorescent dye GS Liz 500 and finally volume up to 10 µl with Hi- Di formamide. Sizing was done with ABI 310 genetic analyzer with Liz 500 as international law standard.

Microsatellite fragment analysis: Microsatellite fragment

Table 1. Genetic variability parameters in Malvi breed of cattle

Locus name	No. of alleles	Het Obs	Het exp	PIC
BM1818	9	0.78	0.82	0.80
BM1824	8	0.84	0.80	0.77
CSRM60	11	0.69	0.83	0.81
CSSM663	18	0.05	0.86	0.84
ETH10	6	0.71	0.72	0.66
ETH152	10	0.62	0.79	0.75
ETH185	11	0.38	0.82	0.80
ETH3	6	0.74	0.63	0.58
HAUT27	6	0.31	0.67	0.61
HEL51	12	0.51	0.77	0.74
HEL9	16	0.20	0.92	0.91
HEL1	8	0.43	0.76	0.72
ILSTS005	6	0.66	0.65	0.58
ILSTS006	5	0.18	0.59	0.55
ILSTS011	4	0.37	0.45	0.41
ILSTS030	5	0.72	0.76	0.71
ILSTS034	17	0.48	0.82	0.79
ILSTS054	4	0.82	0.66	0.60
ILSTS033	7	0.70	0.68	0.62
INRA005	6	0.76	0.76	0.71
INRA035	11	0.45	0.88	0.86
INRA063	6	0.57	0.63	0.58
MM12	10	0.68	0.80	0.77
MM8	16	0.39	0.88	0.86
	9.08	0.54	0.75	0.71

Het obs: Heterozygosity observed, Het exp: Heterozygosity expected

sizing was performed by the Gene Mapper version 3.7. All variability parameters were calculated using software Microsatellite analyzer version 4.0 downloaded from http://i122server.vuwien.ac.at/MSA/info.html/MSA_info.html. The PIC was also calculated using marker allelic frequencies with the help of equation (Botstein *et al.* 1980). Exact tests for deviation from Hardy Weinberg equilibrium (HWE) were performed using the GENEPOP package (Raymond and Rousset 1995).

RESULTS AND DISCUSSION

The percentage of polymorphic loci obtained was 100 since all microsatellite loci screened in Malvi cattle exhibited polymorphism. The microsatellite allele number, allele size, polymorphism information content and heterozygosity values are presented in Table 1.

Microsatellite alleles: The number of alleles was 9.08/microsatellite locus. The number of alleles was in the range of 4 to 18. These polymorphic loci generated 218 alleles in the breed. In general the number and sizes of microsatellite allele observed in this study fall within the range mentioned in the Secondary Guidelines for Development of National Farm Animal Genetic Resources Management Plans, published by FAO (2004). The mean number of alleles observed in the study used is greater than that reported in other Indian cattle breeds such as Sahiwal (5.2) and Deoni (5.9) (Mukesh *et al.* 2004) using the same microsatellites. The allele size were ranging from 88 bp (CSRM60) to 297 bp (ILSTS006). The electrophorogram of some representative microsatellites markers showing alleles at different microsatellite in Malvi breed of cattle are shown in Fig 3. The frequencies of microsatellite alleles in some representative loci are shown in Figs 4, 5 and 6.

Polymorphism information content (PIC): The statistical assessment of informativeness of a marker, as denoted by PIC value, was ranging from 0.41 for ILSTS011 to 0.91 for HEL9 with a mean PIC value of 0.71. The high polymorphism information content noticed in this study are in agreement with Belgian cattle breeds for loci ETH10, ETH225, and BM1818 (Peelman *et al.* 1998). The PIC values observed in most of the loci are comparable with those observed in Indian cattle breeds such as Deoni, Haryana, and Sahiwal. The PIC values for loci ILSTS005 (0.58), ILSTS033 (0.62) and ILSTS034 (0.79) were slightly different from PIC (0.69, 0.70 and 0.77 respectively) reported by Karthickeyan *et al.* (2006) in Krishna Valley breed of cattle while ILSTS011 locus had similar PIC value (0.41). Except ILSTS011 all other loci possessed high PIC values indicating that markers are highly informative for characterization of Malvi breed of cattle.

Hardy Weinberg equilibrium: Hardy Weinberg equilibrium test was used to predict whether the population is stable or not. Exact test for deviations from Hardy-Weinberg equilibrium (HWE) was performed using the GENEPOP package (Raymond and Rousset 1995).



Fig. 3. Electrophoregram showing alleles at different microsatellite in Malvi breed of cattle.

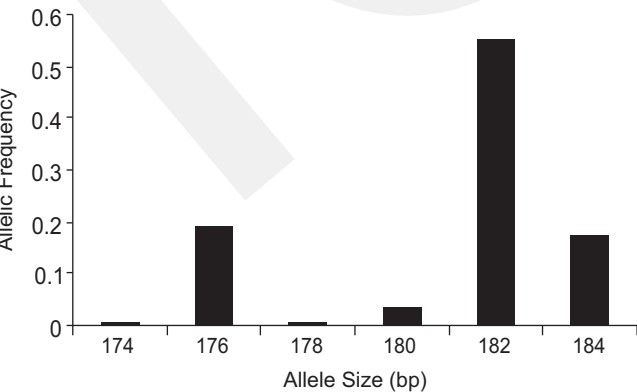


Fig. 4. Allelic frequency distribution at locus INRA063.

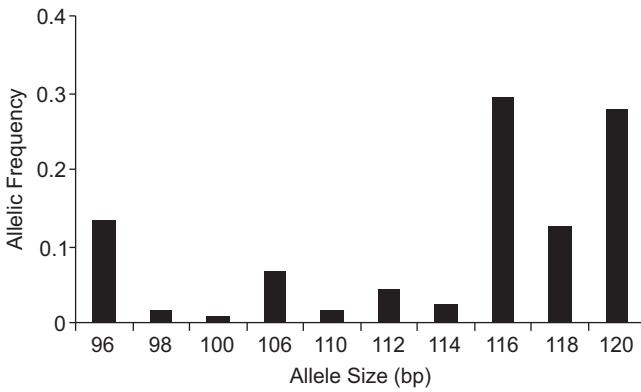


Fig. 5. Allelic frequency distribution at locus MM12.

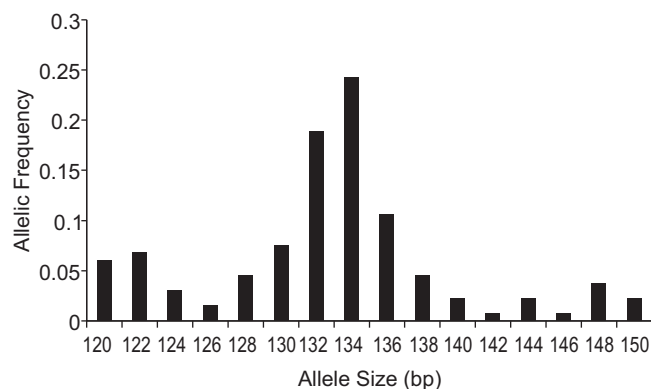


Fig. 6. Allelic frequency distribution at locus ILSTS034.

Significant levels were calculated per locus. Microsatellite loci BM1818, HEL1, ILSTS005, ILSTS030, ILSTS034, ILSTS0554, INRA035, and MM8 were in HWE. Rest of the microsatellite loci deviated significantly ($P < 0.01$) from Hardy-Weinberg equilibrium.

Within population genetic variability: The overall means for observed and expected heterozygosities were 0.54 and 0.75, respectively, with the ranges of 0.05 (CSSM663) to 0.84 (BM1824) and 0.45 (ILSTS011) to 0.92 (HEL9). A diversity index is a mathematical measure of species diversity in a community. The Shannon index is a parameter for determining diversity index. The Shannon index values for all the microsatellites are given in Table 1. The mean value for Shannon index is 1.67, which indicates high gene diversity within Malvi population. The high heterozygosity values

observed indicates more number of polymorphic loci in Malvi cattle.

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