GENETIC DIVERSITY STUDY OF RATHI AND SAHIWAL BREEDS OF CATTLE THROUGH MICROSATELLITE MARKERS

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ABSTRACT

The aim of present study was to understand the existing genetic diversity and structure of native cattle breeds (Rathi and Sahiwal) adapted to the north-western arid and semi-arid region of India based on microsatellite loci. Since cattle domestication from Neolithic (8,000-10,000 years ago) today the population has reached 1.5 billion and further it's likely to be 2.6 billion by 2050. High magnitude of numbers, breed management, market need of traceability of breed product, conservation prioritization and IPR issues due to germplasm flow/exchange, has created a critical need for accurate and rapid breed identification. Till date breed identification methods based on molecular data analysis has great importance. The study describes the genetic variability within and among two indigenous bovine breeds' viz. Rathi and Sahiwal based on five microsatellite markers (BM1818, CSRM60, ETH10, ETH225 and INRA005). High PIC values observed for most of the markers with an average of 0.5313 are indicative of high polymorphism of these markers in rathi breed whereas in Sahiwal 0.4202, which showed high in formativeness of all five microsatellite markers in Rathi breed. Reasonably high PIC values observed for most of the markers, with an average PIC value of 0.5116 across all the loci imply that this set of microsatellite are very informative for evaluation of genetic diversity and characterization in both the breeds. This in formativeness of microsatellite markers showed it can be used for various applications like, conservation, disease diagnosis and polymorphism in different populations.

Key words: Diversity analysis, genetic structure, indigenous breed, microsatellite markers

Introduction

The cattle genetic resources in India are represented by 30 morphologically characterized breeds ranging from dairy to pure draught types, very tall to short stature breeds, and highland cattle to those adapted to arid climate. Most of these native cattle breeds are adapted to particular agro-climatic conditions and are an integral component of the Indian agricultural system. Some of the features like adaptability to extreme climatic conditions, subsistence on poor feed and better capabilities to withstand environmental stress/diseases make Indian native cattle a rich source of highly evolved gene pool of immense economic importance. Under the present production system, which places emphasis on increased milk production, the population size of some Indian native cattle breeds would either be reduced or their typical characteristics diluted. Such a scenario might result in the loss of important gene combinations and the variability important for breed improvement. In the existing context, it has become essential to preserve the existing diversity in Indian cattle breeds as per globally accepted criterion. However, the genetic relationship between native cattle breeds of India is largely unknown. Over the years, categorization of these cattle breeds has often been based on morphological data and information gained from the local breed keepers. Hence, an effort to deduce the genetic structure among the Indian native cattle breeds becomes pertinent for the development of future management strategies. For this purpose, microsatellites markers previously exploited for diversity studies in cattle populations from Africa, Europe, and Asia (Moazami-Goudarzi *et al*., 1997).

The discovery of molecular markers such as microsatellite DNA sequences and the development of methods for their analysis (identification) opened up new possibilities for such studies. Microsatellite markers are highly polymorphic and occur within the whole genome, which makes them a very useful tool for the identification of native breeds. The relationship or linkage between a marker and the level of a quantitative trait can indicate the probable localization of the gene affecting this trait on the same chromosome and in the area of the marker's locus Kury[3] *et al*. (1997). In the last decade, microsatellite markers were extensively used to determine the genetic diversity and relationships among cattle breeds that has been documented in many studies (Rogiæ *et al*., 2011; Medugorac *et al*., 2009; Jordana *et al*., 2003; Metta *et al*., 2004 and Mukesh *et al*., 2004).

In this study, we describe the genetic diversity and relationships among the two native cattle breeds adapted to arid and semi-arid regions of north-western India. Each of the breeds included in this study are well known for their regional contribution in sustaining their livestock keepers and represent different utility types.

Materials and Methods

Sampling and genomic DNA extraction

Blood samples were collected randomly from 60 animals of two indigenous cattle breeds from their respective breeding tracts within the hot arid and semi-arid regions of the Rajasthan state of India. Genomic DNA was extracted using the protocol of Qiamp mini kit with some modifications.

Panel of microsatellite markers and PCR typing

A total of five bovine specific microsatellite markers (BM1818, CSRM60, ETH10, ETH225, INRA005)

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recommended in the MoDAD project for cattle genetic diversity analysis were used.PCR was carried out in a final reaction volume of 25 µl. containing 5 µl of 5 x assay buffer, 2 µl $MgCl_2$, 1 µl dNTPs, 1 µl of each primer, 0.25 µl of Taq DNA polymerase and rest adjusted with nuclease free water. A master mix for minimum of 15 samples was prepared and a liquated 22 µl in each 0.2 ml PCR micro-centrifuge tube. 3 µl of DNA sample was added in respective tubes to make the final volume. PCR cycling conditions employed were: 5 min at 94°C, followed by 30 cycles of 30 sec at 94°C, 1 min at annealing temperature (52-64°C) corresponding to each primer, 30 sec at 72°C and final extension of 10 min at 72°C. The amplification was checked on 2% agarose gel.

Polyacrylamide gel electrophoresis (PAGE)

Microsatellite marker scoring was done using PAGE (Koreth *et al.,* 1996). For typing microsatellites, 8% native PAGE was run. The gel was stained for about half an hour with gentle rocking. The gel was analyzed under UV light and documented by UVP gel-doc system**.**

Statistical analysis

Analyses of the bands were done using a software aided gel-documentation system (UVP) and genotypes of the individual animals were scored manually. The results were evaluated including (i) calculation of the frequency of the alleles identified at particular loci after (Lubieniecka *et al.,* 1999), (ii) the polymorphism information content (PIC) after (Botstein *et al.,* 1980).

Results and Discussion

The present study was initiated with the objective to assess genetic variability within two cattle breeds viz. Rathi, and Sahiwal using some microsatellite markers. Breed characterization requires basic knowledge of genetic variations that can be effectively measured within and between populations. The number of alleles, size range of alleles and polymorphism information content (PIC) is given for the two breeds in Table 2. The effective numbers of alleles were found to be 6.422 and 3.429, respectively across all loci studied. ETH10 was found to be highly informative with the highest PIC value (0.866). The least informative marker was BM1818 (0.6134). The mean of all PIC values was 0.5323.

The effective number of alleles per locus in a population varied from 3.429 (ETH10) to 6.422 (ETH225) (Table 2). Lower values of expected number of alleles as compared to observed number of alleles in all the populations suggested that there were many low frequency alleles in the populations. The mean observed number of alleles across all the loci was 6.86 and was higher than other indigenous cattle breeds reported by Metta *et al.* (2004); Pandey *et al.* (2006).

Previously the allelic diversity in the Indian livestock breeds has been observed to be higher than that reported for the European counterpart (Joshi *et al.*, 2012), that has been attributed to lack of artificial selection pressure. Allelic diversity of similar magnitude has also been reported in Tharparkar, Rathi and Orissa cattle populations of India (Sodhi *et al.,* 2008; Sharma *et al.,* 2012). Measures of genetic diversity based on allelic richness are considered important in conservation genetics as marker-assisted methods for maximizing number of alleles conserved have been shown to be effective (Bataillon *et al.,* 1996). It is also relevant in long-term perspective, as selection limits are determined by the initial allelic composition rather than by heterozygosity (Petit *et al*., 1998).

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Table 1: List of microsatellite markers

Locus	Repeat Motif	Primer Sequences 5'-3'	Chromo-some location	size (bp)
BM1818	$(GT)_{13}$	F:AGCTGGGAATATAACCAAAGG	23	258-272
		R:AGTGCTTTCAAGGTCCATGC		
CSRM60	$(CA)_{17}$	F:AAGATGTGATCCAAGAGAGAGGCA	10	96-116
		R:AGGACCAGATCGTGAAAGGCATAG		
ETH10	$(CA)_{12}$	F:GTTCAGGACTGGCCCTGCTAACA	5	212-224
		R:CCTCCAGCCCACTTTCTCTTCTC		
ETH225	$(CA)_{18}$	F:GATCACCTTGCCACTATTTCCT	9	141-159
		R:ACATGACAGCCAGCTGCTACT		
INRA005	$(GT)_{13}$	F: CAA TCT GCA TGA AGT ATA AAT AT	12	240-246
		R: CTT CAG GCA TAC CCT ACA CC		

Table 2: Genetic diversity data of five microsatellites in Rathi and Sahiwal.

Locus	Allele No.		Allele size range (bp)		PIC
	N_a	N_e	Min.	Max.	
BM1818	6	3.879	280	330	0.6134
CSRM60	8	5.114	95	160	0.7892
ETH 10	4	3.429	200	295	0.8666
ETH225	9	6.422	130	195	0.8626
INRA 005	7	4.345	130	260	0.8253
Average	6.8	4.3678			0.5323

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