

Full Length Research Paper

Genetic diversity between Gir, and Kankrej cattle breeds using microsatellite markers

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The genetic diversity study of native Gir and Kankrej (*Bos indicus*) cattle populations were evaluated using nine microsatellite markers (ETH-225, CSRM-60, HEL-9, INRA-005, ETH-10, HAUT-24, BM1818, ILSTS-002 and ILSTS-006) suggested by FAO (ISAG). A total of 60 cattle were sampled from different places of local Rajasthan region. For each, 30 individuals were sampled. The mean number of observed and effective alleles in Kankrej were high (5.222 and 3.714) comparatively and the average expected heterozygosity values (0.5403) indicated high diversity in the Kankrej population than Gir (0.4520). High polymorphism information content (PIC) values observed for most of the markers with an average of 0.5116 are indicative of high polymorphism of these markers in Kankrej breed than in Gir (0.4202), which showed high informativeness of all the microsatellite markers in Kankrej breed. Three microsatellites markers (HAUT24, BM1818 AND ILSTS006) did not show amplification in both breeds. INRA005 was the only markers amplified in Kankrej. The allele diversity (mean observed number of alleles was 6.11; mean effective number of alleles was 5.187) and gene diversity (0.2771) values implied a substantial amount of genetic variability in both populations. Reasonably high PIC values observed for most of the markers, with an average PIC value of 0.5116 across all the loci implied that this set of microsatellite are very informative for evaluation of genetic diversity in both the breeds. This informativeness of microsatellite markers showed it can be used for various applications like, conservation, disease diagnosis and polymorphism in different populations.

Key words: Genetic diversity, polymorphism information content (PIC), heterozygosity, polymorphism, marker.

INTRODUCTION

India is rich in farm animal diversity. It has diverse and unique cattle genetic resources which led to their domestication some 10, 000 years ago. These breeds have evolved over the years and gradually adopted themselves against the natural selection of various governing factors like nutrient supply, changing climatic conditions, predators and competition arising within the species. These subpopulations also underwent selection procedures imposed by men for food, fuel, clothing and draught purposes. Various forms of selective pressures related to adaptation in the local environment led to the shaping of entirely new species. According to National

Bureau of Animal Genetic Resources (NBAGR) there are 30 well documented cattle breeds in India based on phenotypic characterization. The western part of India is endowed with excellent cattle breeds viz., Gir as a milch, while Kankrej as dual-purpose cattle breeds. So, these breeds need to be investigated at the genome level to

study their genetic diversity.

Animal breeding, carried out under unidirectional selection pressure, may involve both an increase in the frequency of favorable additive genes as well as break in regulatory homeostatic mechanisms which were

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established during the process of natural selection of these populations (Notter, 1999). According to Hanotte and Jianlin (2005), countries doing intensification and industrialization of agricultural practices (developing countries) are leading to the extinction of native livestock species by cross breeding them. This resulted in the dramatic loss of many documented breeds especially in developing and underdeveloped countries and many more breeds are at the risk and will be lost without even having been adequately characterized or studied. Around 16% of them have become extinct and 15% are considered as rare (FAO, 2000). Hence, there is an urgent need to prevent the rapid erosion of animal genetic resources.

Serious efforts for conservation and evaluation strategies for effective management of domestic animal breeds should be made based on potential use of molecular information in breed conservation, so that full potential of livestock biodiversity is realized leading to improved human health and nutrition and expanded opportunities for livelihood diversification.

Genetic characterization can be done by various methods such as cytogenetic, biochemical and molecular techniques. Microsatellites are the choice of markers (FAO, 2007) and are easily transferred across ungulate taxa, being widely and successfully applied in conservation studies (Slate et al., 1998; Maudet et al., 2002). Mukesh et al. (2009), Pandey et al. (2006), and Chaudhari et al. (2009) used microsatellite markers for assaying native cattle breeds from different regions of India. Similarly, Kankrej cattle breed of Rajasthan (Sodhi et al., 2007), Ongole and Deoni (Metta et al., 2004), Krishna Valley, Ongole breed of cattle (Karthickeyan et al., 2006, 2008) respectively, Siri cattle (Sharma et al., 2008) were also genetically characterized. Kul et al. (2011) determined genetic distance among ruminant species (cattle, goat and sheep) using microsatellite loci whereas population studies for cattles were performed by 16 polymorphic STR loci by Van de Goor et al. (2011).

However, these indigenous breeds are already characterized at physical and production level. The present status and declining trend in the populations of these breeds in Rajasthan need be given the assessment at the molecular level. Hence, the present research work was carried out to study the genetic diversity between Gir, and Kankrej cattle breeds using microsatellite markers.

MATERIALS AND METHODS

Sampling and DNA extraction

A total of 30 blood samples each from Gir and Kankrej cattle breeds were collected. All the animals were randomly selected, genetically unrelated and the information was collected after consulting pedigree records maintained and interviewing the owners in detail. Gir blood samples were collected from Vallabh Nagar (Udaipur) and Rawatbhata from various owners in village. Kankrej blood samples

were collected from a local Kankrej farm in Barmer in Rajasthan.

Genomic DNA was isolated using the rapid salt extraction method (Aljanabi and Martinez, 1997) with some modifications. The evaluation of quality and purity of DNA was done through agarose gel electrophoresis and ultraviolet (UV) spectrophotometer. Nine microsatellites (Table 1) were chosen from the available list of 30 microsatellite for genetic diversity study.

PCR reaction was carried out in a final reaction volume of 50 µl in a thermal cycler. The annealing temperature for various markers was optimized as in Table 2. Optimization of microsatellite was carried out according to the microsatellite which is approximately 3 µl for all the microsatellites.

The polymorphic typing of microsatellite marker was done using polyacrylamide gel electrophoresis (PAGE). For typing microsatellites, 8% native PAGE (Hoefer SE 600 series electrophoresis unit) was used. Manual genotyping of animals silver staining of gel (Benbouza et al., 2006) was done. The allele and genotype frequencies were scored by counting the bands using UVP Doc-It[®]LS Image Acquisition Software version 6.3.3. The average heterozygosity and PIC were calculated with appropriate equations for all markers loci.

Data analysis

Effective number of alleles

The measure explain about the number of alleles that would be expected in a locus in each population (Nassiry et al., 2009).

$$A_e = \left(1 / \sum_{a=1}^k p_a^2\right)$$

Where, p_a^2 is the frequency of the a^{th} of k alleles.

Heterozygosity

The probability that any randomly chosen individual is heterozygous for any two alleles at a marker locus having allele frequencies p_i , is

$$\text{defined as heterozygosity. Thus, heterozygosity} = 1 - \sum_{i=1}^n p_i^2,$$

where, $\sum_{i=1}^n p_i^2$ is the homozygosity (Hildebrand et al., 1992). Direct count (DC) heterozygosity (Machado et al., 2003) was obtained as:

$$H(\text{direct}) = \frac{\sum_{i,j} N_{ij}}{N}$$

Where, N_{ij} is the number of heterozygous individuals in the i locus and N is the number of individuals analyzed. It is also known as observed heterozygosity, and the average direct count of heterozygosity over all loci in each tested breed is less than the expected heterozygosity (El Nahas et al., 2008).

The Hardy-Weinberg expected heterozygosity, also defined as Gene Diversity (Nei, 1973) was obtained from observed allele frequencies (Nei, 1978):

$$1 - \sum_{i=1}^n p_i^2$$

Where, p_i is the frequency of the i allele at the i locus and n is the number of alleles at the i locus.

Table 1. Microsatellite markers.

Locus	Repeat motif	Primer sequence (5'-3')	Chromosome location	Number of allele	Size (bp)	Reference
BM1818	(GT) ¹³	F:AGCTGGGAATATAACCAAAGG R:AGTGCTTTCAAGGTCCATGC	23	8	258-272	Bishop et al. (1994)
CSRM60	(CA) ¹⁷	F:AAGATGTGATCCAAGAGAGAGGCA R:AGGACCAGATCGTGAAAGGCATAG	10	8	96-116	Moore et al. (1994)
ETH10	(CA) ¹²	F:GTTTCAGGACTGGCCCTGCTAACA R:CCTCCAGCCCACTTTCTCTTCTC	5	7	212-224	Solinas et al. (1993)
ETH225	(CA) ¹⁸	F:GATCACCTTGCCACTATTTTCCT R:ACATGACAGCCAGCTGCTACT	9	10	141-159	Steffen et al. (1993)
HAUT24	(CA) ¹⁹	F:CTCTCTGCCTTTGTCCCTGT R:AATACACTTTAGGAGAAAAATA	22	8	109-129	Thieven et al. (1997)
HEL9		F:CCCATTTCAGTCTTCAGAGGT R:CACATCCATGTTCTCACCCAC	8	13	143-167	Kaukinen et al. (1993)
ILSTS002	(AC) ¹⁷	F:TCTATACACATGTGCTGTGC R:CTTAGGGGTGAAGTGACACG	18	6	123-137	Kemp et al. (1992)
INRA005	(GT) ¹³	F: CAA TCT GCA TGA AGT ATA AAT AT R: CTT CAG GCA TAC CCT ACA CC	12		240-246	Vaiman et al., 1992
ILSTS006	(GT) ²³	F:TGTCTGTATTTCTGCTGTGG R:ACACGGAAGCGATCTAAACG	7	10	281-299	Brezinsky et al. (1993)

Table 2. Annealing temperatures for various marker loci.

Locus	Annealing temperature (°C)	
	Marked	Optimized
INRA 005	58	58
CSRM 60	54	54
ETH 10	58	58
ETH 225	60	58
BM1818	58	55
HEL 9	52	52
ILSTS 002	52	54
ILSTS 006	54	55
HAUT 24	55	55

Polymorphism information content

PIC was calculated following formula (Botstein et al., 1980):

$$PIC = 1 - \sum_{i=1}^n p_i^2 - \left(\sum_{i=1}^n p_i^2 \right)^2 + \sum_{i=1}^n p_i^4$$

Where, p_i = frequency of the marker allele, a_i and n = number of different alleles.

RESULTS

The number of alleles, size range of alleles, direct count heterozygosity, and Hardy Weinberg heterozygosity is given individually for the two breeds in Table 3 and combined in Table 4. This shows the genetic diversity data for both Gir and Kankrej. Number of alleles varies from 7 (ILSTS 002) to 12 (CSRM 60, ETH 225) with little difference between the cattle breeds. Microsatellite ETH 10 allele profiles of Gir and Kankrej on PAGE is given in Figure 1.

The mean observed and effective numbers of alleles were found to be 6.11 and 5.187, respectively across all loci studied. Direct count heterozygosity was found to be maximum for INRA005 (0.7142) and minimum for ILSTS002 (0.3571). The average direct count heterozygosity across all the loci was 0.4364. The Hardy-Weinberg heterozygosity was maximum for INRA 005 (0.9006) and minimum for ILSTS002 (0.8046) across all the loci studied within both the breeds. The average Hardy-Weinberg heterozygosity was found to be 0.2771. INRA005 was found to be highly informative with the highest PIC value (0.8945). The least informative marker was ILSTS002 (0.7784). The mean of all PIC values was 0.5323.

In Gir, maximum direct count heterozygosity was observed for CSRM60 (0.8571), and minimum was observed for ILSTS002 (0.5714). The average H (DC)

Table 3. Direct count heterozygosity H (DC) and Hardy-Weinberg heterozygosity H (HW) and PIC values for and Gir and Kankrej breeds of cattle.

Locus	GIR					KANKREJ				
	A _o	A _e	H (DC)	H (HW)	PIC	A _o	A _e	H (DC)	H (HW)	PIC
CSRM60	10	5.233	0.8571	0.8080	0.7789	8	0.4239	0.7143	0.7642	0.7335
ETH 10	6	4.6130	0.7857	0.7832	0.7374	9	9.480	0.8571	0.8945	0.8886
ETH225	8	5.336	0.8126	0.7857	0.7786	8	4.739	1	0.7889	0.7692
HEL9	8	6.222	0.7857	0.8393	0.8204	6	5.485	0.7857	0.8177	0.6797
ILSTS002	6	6.7575	0.5714	0.8520	0.6699	4	3.299	0.8557	0.6969	0.6389
INRA 005	0	0	0	0	0	10	10.05	0.7142	0.9005	0.8945
BM1818	0	0	0	0	0	0	0	0	0	0
HAUT24	0	0	0	0	0	0	0	0	0	0
ILSTS006	0	0	0	0	0	0	0	0	0	0
Average	4.222	3.1290	0.4236	0.4520	0.4202	5.222	3.719	0.5474	0.5403	0.5116
Average w/o 3 markers	6.333	4.694	0.6354	0.6780	0.6303	7.833	5.579	0.8212	0.8104	0.7674

value (0.4236) across all the loci indicates substantial and very good number of heterozygotes in the population. Similarly, maximum Hardy-Weinberg heterozygosity was observed for ILSTS002 (0.8520) and minimum was observed for ETH10 (0.7832), with an average of 0.4520. The highest PIC value (0.8204) was observed at HEL9 locus and least (0.6699) at ILSTS002 locus for Gir cattle. Reasonably high PIC values were observed for most of the markers with an average of 0.4202.

In Kankrej, maximum direct count Heterozygosity was observed for ETH 225 (1) and minimum for INRA005 (0.7142). The average of H (DC) was found to be 0.5474, maximum Hardy-Weinberg heterozygosity was observed for INRA005 (0.9005) and minimum for ILSTS002 (0.6969), with an average of 0.5403. The highest

PIC value (0.8945) was observed at INRA005 locus and least (0.6389) at ILSTS002 locus for the Kankrej, with an average of 0.5116.

Three markers HAUT 24, BMI818 and ILSTS 006 were not amplified in both Gir and Kankrej.

DISCUSSION

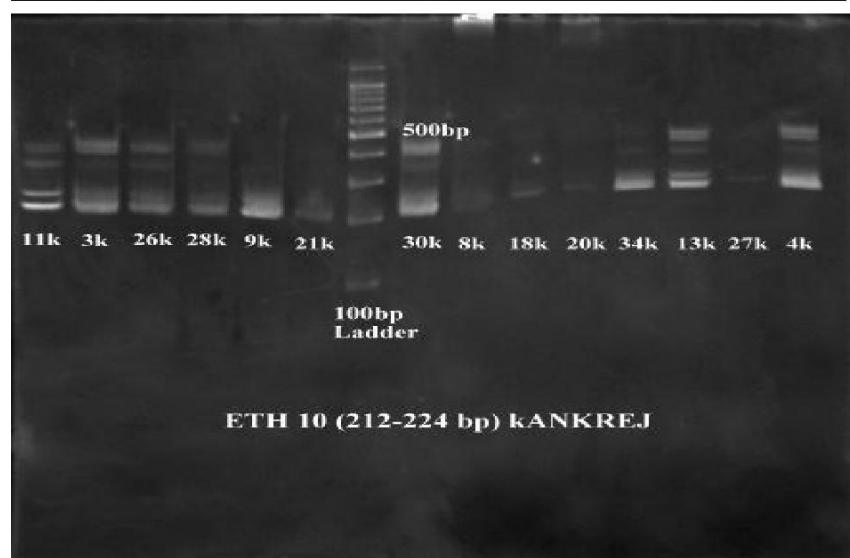
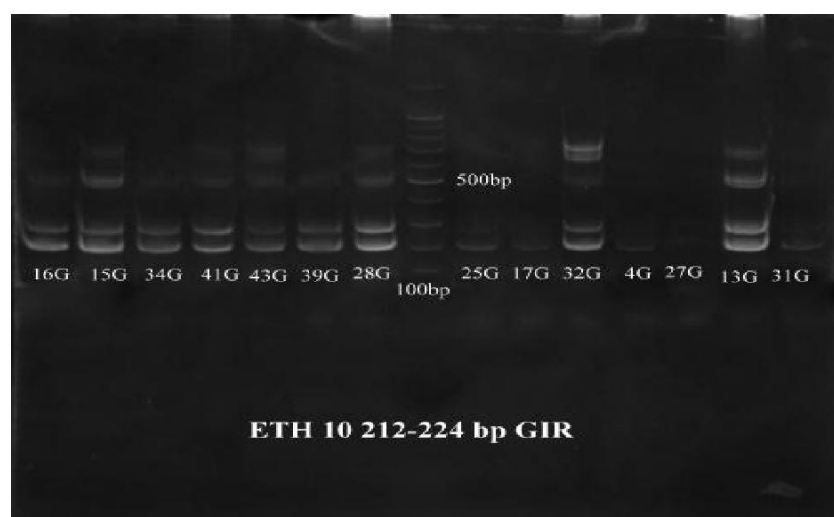
In the present study, genetic polymorphism of Gir and Kankrej was analyzed using nine microsatellite markers. Six microsatellites were amplified in both the breeds. The amplification by CSRM 60, ETH 10, ETH 225, ILSTS 002 and INRA 005 are in accordance with the results reported by Kale et al. (2010). Marker INRA005 was amplified only in Kankrej. Hence, this marker can be used as a breed specific marker for Kankrej cattle breed. The rest three microsatellite

markers (HAUT24, ILSTS006, and BMI818) showed no amplification in both the breeds. ILSTS 006 was not amplified which is consistent with the result reported by Navani et al. (2002) for buffaloes however, the same marker was amplified in previous report by Kale et al. (2010).

Overall, H (DC) was found to be maximum for INRA005 hence it can be used for paternity testing in these breeds and was minimum for ILSTS002 (0.3571). The average direct count heterozygosity across all the loci was 0.4364, indicating substantial number of heterozygotes for these markers in both populations. Maximum Hardy-Weinberg expected heterozygosity (gene diversity) was obtained for INRA 005 (0.9006) and minimum for ILSTS002 (0.8046) indicating the highest and lowest range of heterozygosity for these markers. The average Hardy-Weinberg

Table 4. Genetic diversity data of nine microsatellites in Gir and Kankrej.

Locus	Allele number		Allele size range (bp)		H (DC)	H (HW)	PIC
	A _o	A _e	Minimum	Maximum			
CSRM 60	12	5.398	95	160	0.3928	0.8147	0.7892
ETH 10	11	7.546	200	295	0.3928	0.8674	0.8523
ETH 225	12	8.377	130	195	0.4464	0.8806	0.8697
HEL 9	9	7.875	140	195	0.3928	0.8731	0.8617
ILSTS 002	7	5.117	145	200	0.3571	0.8046	0.7784
INRA 005	10	10.05	135	180	0.7142	0.9006	0.8945
BM1818	0	0	0	0	0	0	0
HAUT 24	0	0	0	0	0	0	0
ILSTS 006	0	0	0	0	0	0	0
Average	6.11	5.187			0.4364	0.2771	0.5323

**Figure 1.** Microsatellite ETH 10 allele profile of Gir and Kankrej on PAGE.

heterozygosity was found to be 0.2771, hence both the populations showed moderate gene diversity. INRA005 was found to be highly informative in both breeds (PIC=0.8945) and ILSTS002 was least informative (PIC=0.7784). Hence, reasonably high PIC values were observed for most of the markers, with an average PIC value of 0.5323 across all the loci which are indicative of the usefulness of these microsatellites for biodiversity evaluation in these breeds.

Amplification using the established primer from ILSTS006, BM1818, and HAUT 24 was not successful in both breeds of cattle but in Rathi and Tharparkar breed where these markers were amplified (Sodhi et al., 2006). This shows the absence of the STS loci in both genomes.

DNA sequences flanking microsatellites are conserved within the Bovidae family. In sheep and goats, 63% primer sequences of cattle are conserved (Kemp et al., 1995) and 75% are conserved in bubaline genome (Navani et al., 2002). There was a significant number of heterozygotes at many loci as indicated by H (DC) values for Gir and Kankrej. This shortage of homozygotes and excess of heterozygotes in the population is helpful in the genetic diversity study and also absence of number of factors, namely sample relatedness, population heterogeneity or null alleles. Thus, this showed more heterogeneity and more variability and thus this population is at a lesser risk of extinction and their germplasm can be used as a reference for future comparative study. The results can be useful in the development of breeding strategy for genetic improvement study.

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