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Genetic characterization of Gir cattle based on microsatellite markers

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Abstract
The genetic characterization study of Gir cattle was evaluated using 23 microsatellite markers suggested by FAO (ISAG). The number of alleles observed at different loci ranged from 5 (MM8, HUT27 and CSSM8) to 25 (ILSTS28 and HEL9) with a total of 205 alleles across Gir cattle. The average expected heterozygosity value (0.751±0.027) compared to average observed heterozygosity values (0.376±0.039) indicated a high amount of genetic variability in the Gir population. The Fixation index (FIS) value for all 23 microsatellite loci varied from 0.912 (CSSM066) to - 0.032 (ILSTS005) with a mean of 0.474. The polymorphic information content (PIC) values observed for most of the markers with an average of 0.909 are indicating that all these microsatellite markers are highly informative in the Gir population. The high number of observed alleles and high heterozygosity indicated the presence of high genetic variability in the Gir population and selected microsatellites were highly polymorphic and proved very useful for breed characterization.

Keywords: Microsatellite, genetic characterization, gir cattle, heterozygosity

Introduction
India is a mega-biodiversity in the world and maintains more than its proportionate share of livestock breeds. Approximately 6 percent of the total domestic animal biodiversity exists in India. India has a strong genetic base of 53 well-defined breeds of cattle distributed across the geographical locations of 28 states and nine union territories of India [1]. To meet commercial objectives, the dairy sector requires the establishment of highly standardized cattle herds, which is reflected in breeding program selection practices. The widespread selection and multiplication of superior animals result in a large reduction in the genetic basis of the germplasm, which is the primary source of genetic variation required for the genetic improvement of breeds and their economically important traits. As a result, there is an urgent need to prevent the fast depletion of animal genetic resources [2].

Native breeds have the potential of surviving and sustaining production, reproduction and draught power under various harsh climatic conditions and distinct genetic features. Conservation of such genetic groups is crucial. In such conditions, the first step is the evaluation of genetic resources and the identification of appropriate populations for conservation. Estimating genetic diversity is critical for determining which populations should be prioritized for conservation.

The traditional breeding trait evaluation approaches based on morphological features, physical body measurements, production, reproductive and adaptive traits were used in the description of breeds but have limited ability to identify minor changes in population genetic diversity. The molecular markers are the potential tool to analyze the current germplasm and manipulate it to develop character-specific strains and provide the basis for effective genetic conservation [3]. Microsatellites are the most potent of the several types of molecular markers accessible. They are highly polymorphic, distributed over the genome at a frequency of one every 6 kb nucleotide sequence, co-dominantly inherited and easy to PCR amplification, making them potentially useful. Microsatellite markers can help in assessing the genetic variation within and between breeds and provide a rational basis for ranking the breeds in terms of genetic uniqueness for conservation purposes. Microsatellites have the potential to be used as markers to investigate genetic variation, parentage identification, gene flow, hybridization, genetic distance and diversity in indigenous cow breeds [4].

The present study was carried out to examine the genetic variation in Gir cattle, one of the outstanding dairy cattle breeds of India known for its milk-producing capacity, heat tolerance, resistance to disease and longevity.

Materials and Methods

Blood samples were collected from randomly selected non-related 104 Gir animals from its breeding tract. The DNA was isolated from blood as per John's method using the standard phenol-chloroform extraction method. The evaluation of the quality of DNA was done by agarose gel electrophoresis and the quantity of DNA was estimated by UV spectrophotometer. The 23 microsatellite loci, *viz*., (ETH3, ILSTS034, ETH152, ILSTS28, CSSM066, HEL9, ILSTS033, CSRM60, ILSTS006, ILSTS030, BM1824, MM8, MM12, ILSTS005, HUT27, BM1818, INRA035, ETH10, INRA005, HEL1, CSSM8, CSSM61, CSSM43) were selected for estimation of genetic diversity in Gir cattle (Table 1). PCR reaction was carried out in a final reaction volume of 15 µL in a thermal cycler (Biometra Ltd., Eppendorf). The annealing temperature for panel-I was optimized at 56 °C and panels II, III and IV at 58 °C (Table 1).

The amplified DNA was then genotyped using capillary electrophoresis on automated DNA sequencer (ABI PRISM 3500 Genetic Analyzer). Genotyping reaction containing one µL of diluted PCR products, 8.5 µL of Hi-Di Form amide (Applied Bio systems, USA) and 0.5 µL of GeneScan-500 LIZ; size standard marker (Applied Bio systems, USA). Further analysis of the samples was done using Gene mapper v4.1. The statistical analysis was performed using Gene Alex version 6.503 and Cervus version 3.0.7.

Table 1: Microsatellite markers used for characterization of Gir cattle breed

Panel	Markers	Primer sequence		Dye Labelled	Amplicon size range
Panel I	CSRM60	F	AAGATGTGATCCAAGAGAGAGGCA	FAM	69-121
		R	AGGACCAGATCGTGAAAGGCATAG		
	ILSTS006	F	TGTCTGTATTTCTGCTGTGG	FAM	276-302
		R	ACACGGAAGCGATCTAAACG		
	MM12	F	CAAGACAGGTGTTTCAATCT	TAMARA	91-139
		R	ATCGACTCTGGGGATGATGT		
	ILSTS030	F	CTGCAGTTCTGCATATGTGG	TAMARA	146-160
		R	CTTAGACAACAGGGGTTTGG		
	BM1824	F	GAGCAAGGTGTTTTTCCAATC	TAMARA	176-196
		R	CATTCTCCAACTGCTTCCTTG		
	ILSTS005	F	GGAAGCAATGAAATCTATAGCC	FAM	177-193
		R	TGTTCTGTGAGTTTGTAAGC		
	MM8	F	AGGACAGAAAAGACT	FAM	132-148
		R	CTCAAGATAAGACCACA		
Panel II	HUT27	F	TTTTATGTTCATTTTTTGACTGG	HEX	130-152
		R	AACTGCTGAAATCTCCATCTTA		
	BM1818	F	AGCTGGGAATATAACCAAAGG	HEX	235-285
		R	AGTGCTTTCAAGGTCCATGC		
	INRA035	F	ATCCTTTGCAGCCTCCACATTG	FAM	97-123
		R	TTGTGCTTTATGACACTATCCG		
	ETH10	F	GTTCAGGACTGGCCCTGCTAACA	FAM	203-223
		R	CCTCCAGCCCACTTTCTCTTCTC		
	INRA005	F	CAATCTGCATGAAGTATAAATAT	ROX	132-150
		R	CTTCAGGCATACCCTACACC		
	HEL1	F	CAACAGCTATTTAACAAGGA	FAM	210-246
		R	AGGCTACAGTCCATGGGATT		
Panel III	CSSM66	F	ACACAAATCCTTTCTGCCAGCTGA	FAM	172-210
		R	AATTTAATGCACTGAGGAGCTTGG		
	ETH3	F	GAACCTGCCTCTCCTGCATTGG	FAM	97-123
		R	ACTCTGCCTGTGGCCAAGTAGG		
	ILSTS034	F	AAGGGTCTAAGTCCACTGGC	HEX	126-196
		R	GACCTGGTTTAGCAGAGAGC		
	HEL9	F	ATTCAGTCTTCAGAGGT	TET	144-170
		R	CACATCCATGTTCTCACC		
	ILSTS033	F	TATTAGAGTGGCTCAGTGCC	ROX	128-162
		R	ATGCAGACAGTTTTAGAGGG		
	ILSTS28	F	TCCAGATTTTGTACCAGACC	HEX	130-160
		R	GTCATGTCATACCTTTGAGC		
	ETH152	F	AGGGAGGGTCACCTCTGC	TAMARA	184-206
		R	CTTGTACTCGTAGGGCAGGC		
Panel IV	CSSM61	F	AGGGCCATATAGGAGGCAAGCTTAC	FAM	125-165
		R	TTCAGAAGAGGGCAGAATACAC		
	CSSM43	F	AAAACTCTGGGAACTTGAAAACTA	HEX	240-275
		R	GTTACAAATTTAAGAGACAGAGTT		
	CSSM8	F	CTTGGTGTTACTAGCCCTGGG	ROX	180-210
		R	GATATATTTGCCAGAGATTCTGCA		
	ILSTS17	F	GTCCCTAAATCGAAATGCC	HEX	95-130
		R	GCATCTCTATAACCTGTTCC		
	CSSM57	F	GTCGCTGGATAAACAATTTAAAGT	TAMRA	124-93
		R	TGTGGTGTTTAACCCTTGTAATCT		
	ILSTS61	F	AAATTATAGGGGCCATACGG	ROX	125-165
		R	TGGCCTACCCTACCATTTCC		

Results and Discussion

The number of alleles, PIC, heterozygosity and the effective number of alleles for the Gir cattle is given in Table 2. Among 25 microsatellite loci tested, all were found to be polymorphic. Overall allele diversity is a reasonable indicator of genetic variation within the population. A total of 205 distinct observed alleles were detected with a mean of 11.957 alleles (Table 2). These microsatellites exhibited a high level of polymorphism as revealed by a wide range of alleles, which varied from 5 (MM8, HUT27 and CSSM8) to 25 (ILSTS28 and HEL9). The overall effective numbers of alleles were less than the observed values across all the loci and ranged from 1.676 (ETH10) to 10.094 (ILSTS034). The mean of observed alleles and effective alleles were found to be 11.957±1.221 and 5.222±0.559, respectively across all loci. The mean number of effective alleles was found to be less as compared to observed alleles supported by kale *et al.*, 2010 study [5] in which the mean observed and effective number of alleles were found to be 5.29±1.25 and 3.55±1.13, respectively.

Table 2: Genetic characteristics of 23 microsatellite loci in Gir cattle

S.N.	Locus	N	Na	Ne	I	Ho	He	uHe	F	Pic
1	ETH3	94	16	8.529	2.367	0.149	0.883	0.887	0.831	0.933
2	ILSTS034	74	18	10.094	2.571	0.230	0.901	0.907	0.745	0.941
3	ETH-152	92	9	4.289	1.659	0.435	0.767	0.771	0.433	0.889
4	ILSTS-28	87	25	8.973	2.576	0.379	0.889	0.894	0.573	0.887
5	CSSM066	79	14	7.312	2.233	0.076	0.863	0.869	0.912	0.928
6	HEL-9	103	25	9.851	2.652	0.214	0.898	0.903	0.762	0.921
7	ILSTS-033	100	16	9.307	2.418	0.410	0.893	0.897	0.541	0.937
8	CSRM 60	103	17	5.935	2.186	0.252	0.832	0.836	0.696	0.971
9	ILSTS 006	101	6	2.941	1.261	0.545	0.660	0.663	0.175	0.809
10	ILSTS 030	103	7	2.566	1.142	0.427	0.610	0.613	0.300	0.857
11	BM 1824	86	16	6.231	2.215	0.233	0.840	0.844	0.723	0.975
12	MM8	75	5	2.813	1.180	0.280	0.645	0.649	0.566	0.992
13	MM12	93	8	3.896	1.607	0.237	0.743	0.747	0.682	0.874
14	ILSTS005	97	7	2.771	1.279	0.660	0.639	0.642	-0.032	0.857
15	HUT 27	98	5	3.189	1.355	0.378	0.686	0.690	0.450	0.800
16	BM1818	97	13	3.068	1.573	0.381	0.674	0.678	0.434	0.967
17	INRA035	102	13	6.328	2.134	0.735	0.842	0.846	0.127	0.922
18	ETH10	93	7	1.676	0.853	0.194	0.403	0.405	0.520	0.846
19	INRA005	97	9	6.018	1.891	0.289	0.834	0.838	0.654	0.889
20	HEL 1	98	12	2.577	1.296	0.571	0.612	0.615	0.066	0.917
21	CSSM8	102	5	2.446	1.053	0.539	0.591	0.594	0.088	0.990
22	CSSM61	102	9	4.918	1.797	0.255	0.797	0.801	0.680	0.877
23	CSSM43	102	13	4.372	1.933	0.784	0.771	0.775	-0.017	0.918
	Mean	94.696	11.957	5.222	1.793	0.376	0.751	0.755	0.474	0.909
	SE	1.853	1.221	0.559	0.115	0.039	0.027	0.027	0.059	0.016

(**Note:** Na: No. of alleles, Ne: No. of effective alleles, I: Shannon's Information Index, Ho: Observed heterozygosity, He: Expected heterozygosity and µHe: Unbiased expected heterozygosity, PIC: Polymorphism Information Content and FIS: Fixation Index)

Heterozygosity is an appropriate measure of genetic variability within a population. In the present study observed heterozygosity values ranged from 0.076 (CSSM066) to 0.784 (CSSM43) whereas the range of expected heterozygosity was 0.403 (ETH10) to 0.901 (ILSTS034). The mean heterozygosity was observed to be 0.376±0.039 and mean heterozygosity was expected to be 0.751±0.027 across all the loci. The mean observed heterozygosity values, though lower than the expected values, exhibited failure of significant differences using the ANOVA test (p>0.05) suggesting random mating in Gir cattle. The high value of expected heterozygosity indicated that the population has retained the presence of several alleles although at a small frequency. All these findings imply a substantial amount of genetic variability in Gir cattle [6].

Fixation index also known as the inbreeding coefficient is the proportion of the variance in the subpopulation contained in an individual, showing a heterozygote deficit within a population [7]. The FIS value for all 23 microsatellite loci ranged from 0.912 (CSSM066) to -0.032 (ILSTS005) with a mean of 0.474 (Table 2) showing a considerable level of inbreeding present in the Gir population due to lack of systematic breeding. This type of result was reported for Sahiwal, Deoni and Hariana cattle, wherein significant heterozygote deficiencies have been observed [4].

The PIC values across the loci were found to be highest (0.992) for locus MM8 and least (0.800) for the HUT27 locus. The high PIC values observed for most of the markers (Table 2) are suggestive of the fact that these microsatellites might prove to be quite useful for the evaluation of genetic diversity in other native Indian cattle breeds [8].

Conclusions

The present study is a valuable attempt to explore Gir cattle genetic variation using microsatellite DNA markers. More research involving more native Indian cattle breeds would be beneficial in determining their recent origins and linkages. Estimating genetic variation is necessary to investigate heterosis in future complimentary crosses within Zebu breeds and crosses between Zebu and Exotic breeds. This would aid in the development of rational breeding plans.

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