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Research Article

ALLELES AND THEIR FREQUENCIES FOR EIGHT MICROSATELLITES IN RED SINDHI BREED OF CATTLE

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ABSTRACT
The population size of the Red Sindhi breed is reducing because of crossbreeding programmes and mechanization of agriculture in its breeding tract. Hence, the present study was carried out to evaluate the heterozygosity in the population and to characterize this breed by identifying DNA markers using microstatellites. Microsatellites often have multiple alleles and may have heterozygosity frequencies of 70 per cent or more. This makes them highly informative for genetic analysis. A total of eight microsatellite primers were used for microsatellite analysis in genomic DNA of Red Sindhi cattle. The amplified products were analysed for polymorphic alleles and their frequencies. The microsatellite primers such as ILSTS 005, ILSTS 006, ILSTS 008, ILSTS 010, ILSTS 011, ILSTS 012, ILSTS 013 and ILSTS 014 gave 12, 11, 10, 10, 9, 6, 7 and 7 alleles respectively. The level of polymorphism displayed by eight microsatellites in the Red Sindhi breed could be useful in fixing breed specific alleles for genetic characterization of Indian Zebu breeds. The accuracy in genetic analysis and measurement of variability could be further improved by employing more number of microsatellite loci for genetic analysis in this breed.

Key Words: *Red Sindhi, Microsatellites, Alleles, Allele Frequency and Characterization*

INTRODUCTION
Indian sub-continent is blessed with many varieties of indigenous cattle breeds and are categorized into dairy, draft and dual purpose breeds depending upon their utility. Indian zebu cattle breeds are having the special qualities like disease resistance, heat tolerance, surviving and reproducing in adverse climatic conditions and low feed input.

The Red Sindhi cattle are considered one of the few distinct dairy breeds of cattle in the whole Indo-Pak sub-continent. This breed is well adapted to climatic conditions of the country with the potential for high milk production and has been exported to various countries all over the world (Mustafa et al., 2002).

The population size of the Red Sindhi breed is reducing because of crossbreeding programmes and mechanization of agriculture in its breeding tract. Hence, the present study was carried out to evaluate the heterozygosity in the population and to characterize this breed by identifying DNA markers using microstatellites.

Microsatellites often have multiple alleles and may have heterozygosity frequencies of 70 per cent or more. This makes them highly informative for genetic analysis. In addition, the loci are small enough to be analysed using Polymerase Chain Reaction. The efficiency of a marker depends on informativeness of a polymorphic marker. It depends upon the number of alleles and their population frequencies. Marker informativeness is more easily estimated by simply counting the number of heterozygotes in a suitably large sample set. Keeping the back ground information in mind, this study was undertaken to identify the DNA markers in Red Sindhi cattle and to characterize the Red Sindhi cattle by developed DNA markers.

MATERIALS AND METHODS
Primers
A total of eight microsatellite primers were used for microsatellite analysis (Table-1). These microsatellites were originally developed by Brezinsky et al., (1993a,b,c) and Kemp et al.,(1993) from the

genomic library of N' Dama (*Bos taurus*) bull. The primers were reconstituted in TE with a concentration of 40 n moles/ml as stock solution and 40 p moles/ml as working solution. All primers were tested with similar conditions on tested DNA samples.

Genomic DNA

High molecular weight template was prepared from peripheral blood mononuclear cells collected from 30 Red Sindhi cattle maintained at District Livestock Farm, Hosur. The DNA samples were dissolved in TE buffer (pH8.0) to make uniform concentration of 50 ng/ml.

PCR Amplification

The amplification reactions were carried out in 0.2 ml microfuge tubes using a programmable thermal cycler (MJ Research). Each 20µl reaction mix comprised of primers each at 20mMoles,DNTPs each at 50mMoles, 0.5 units of Taq DNA polymerase, 200ng template genomic DNA, 10mM Tris(pH8.3), 50mM KCl, 0.001 Nonidet P40 and 1.5mM $MgCl_2$.

Table 1: Nucleotide sequence of microsatellite primers

S. No	Code	Nucleotide sequence
1.	ILSTS 005	5' GGAAGCAATGAAATCTATACC 3'
		5' TGTTCTGTGAGTTTGTAAGC3'
2.	ILSTS 006	5' TGTCTGTATTTCTGCTGTGG 3'
		5' ACACGGAAGCGATCTAAACG 3'
3.	ILSTS 008	5' AGCACCTGCTGCATACTACC 3'
		5' GAATCAGTGTCAGTGTTTCCC 3'
4.	ILSTS 010	5' ATGGAGAGCAAATGGTCAGC 3'
		5' ACTACAATGGACATGAGTCCG 3'
5.	ILSTS 011	5' GCTTGCTACATGGAAAGTGC 3'
		5' CTAAAATGCAGAGCCCTACC 3'
6.	ILSTS 012	5' TCTACCACCGATACAGATGG 3'
		5' GAAGTAGGTAGTGCTGGAGG 3'
7.	ILSTS 013	5' CTTGATCCTTATAGAACCTGG 3'
		5' ACACAAAATCAGATCAGT 3'
8.	ILSTS 014	5' CTGACTATGGTGATAATCCC 3'
		5' TCTTTTCCCTTTCCTTCCCC 3'

Thermal Cycling

ILSTS 005 and ILSTS 006 Amplification included an initial denaturing step of 45 seconds at 94ºC. This was followed by 19 cycles of 45 seconds at 89ºC, 1 min. at the annealing temperature of 55ºC and 20 seconds at 70ºC. Final extension was for 3 min. at 70ºC.

ILSTS 008

Amplification reactions were carried out as same as that of ILSTS 005 and ILSTS 006 but the annealing temperature was 58ºC

ILSTS 010, ILSTS 011, ILSTS012, ILSTS 013 and ILSTS 014

Amplification reactions were carried out as same as that of ILSTS 008 but the temperature cycling after initial denaturation was 24 times instead of 19 times.

Analysis of PCR products

The PCR products were made to run on a 2 per cent Agar gel electrophoresis together with100 bp DNA ladder at 100 volts for 90 min. The gel was stained by ethidium bromide for 10 min. and viewed by UV

illumination. The alleles were photographed and were analysed by software aided gel documentation system (comprised of Ultra-lum scanner, Scion image capturing system and Sigma gel package)

RESULTS

DNA samples of Red Sindhi cattle were amplified with eight microsatellite primers and the products were analysed for alleles. The alleles and their frequencies scored in red Sindhi cattle for 8 different primers are depicted in Table-2.

Table 2: Alleles and their frequencies for different microsatellite primers

ILSTS005		ILSTS006		ILSTS008		ILSTS010		ILSTS011		ILSTS012		ILSTS013		ILSTS014	
*	**	*	**	*	**	*	**	*	**	*	**	*	**	*	**
199	0.08	299	0.08	128	0.10	269	0.05	320	0.15	94	0.03	132	0.10	149	0.08
206	0.08	311	0.05	132	0.08	275	0.13	322	0.08	96	0.18	137	0.15	150	0.10
211	0.13	322	0.08	134	0.10	290	0.10	325	0.10	98	0.60	143	0.25	152	0.20
213	0.08	332	0.08	139	0.08	294	0.05	326	0.20	102	0.15	152	0.08	153	0.13
215	0.08	335	0.13	143	0.08	300	0.08	328	0.10	104	0.18	156	0.08	162	0.15
218	0.13	343	0.13	145	0.18	308	0.18	348	0.08	106	0.18	158	0.23	164	0.15
220	0.10	350	0.05	147	0.10	318	0.15	352	0.50			160	0.13	167	0.20
222	0.08	355	0.13	148	0.13	345	0.10	356	0.08						
224	0.05	363	0.13	156	0.05	354	0.10	358	0.18						
229	0.08	371	0.13	158	0.13	363	0.08								
231	0.05	395	0.05												
234	0.10														

***** *Indicates allele size in bp and **indicates allele frequency*

DISCUSSION

Genomic DNA samples of Red Sindhi breed of cattle were amplified with ILSTS 005 and the product gave polymorphism with allele sizes varying from199 to 234. The allele frequencies ranged from 0.05 to 0.13. Out of 12 alleles observed, alleles 211 and 218 were more frequently detected in 30 Red Sindhi animals. Karthickeyan et.al (2008) used ILSTS 005 and observed 4 alleles viz, 182, 186, 190 and 194 in the Ongole breed of cattle. Brezinsky et.al (1993b) used ILSTS 005 and observed 4 alleles viz. 181, 183, 185 and 193 in N'Dama (5 animals) and Friesian(5 animals) cattle. They considered only full sib animals and hence the limited number of alleles. In the present study numbers of alleles were more because the samples collected were from unrelated animals in a large population distributed in a wider area.

The products after amplification with ILSTS 006 revealed polymorphism with allele sizes varying from 299 to 395. The observed allele frequencies ranged from 0.05 to 0.13. In this locus, alleles 335, 343, 355, 363 and 371 were present more frequently than other alleles in the population. Karthickeyan et al., (2009) observed 4 alleles from 286 to 300 in Kangayam with the same primer. Brezinsky et al., (1993c) observed 7 alleles viz. 290, 292,294,296,298, 300 and 304 in N'Dama, Friesian, Boran and Zebu cattle.

DNA samples amplified with ILSTS 008 and the resultant product gave allele sizes ranged from 128 to 158 with allelic frequencies 0.05 to 0.18. Alleles 145, 148 and 158 appeared frequently. These results were comparable with similar work carried out by Kemp et al., (1993) with observed alleles such as 173, 175, 179 and 181 in 5 animals each of N'Dama, Friesian, Boran and Zebu breeds.

ILSTS 010 primer product showed polymorphism with allele size varying from 269 to 363 with allele frequencies ranging from 0.05 to 0.18. These results were comparable with similar work carried out by Brezinsky et al., (1993a) in 5 animals each of N'Dama, Friesian, Boran and Zebu breeds and the observed allele sizes 281, 287 and 291.

Products of ILSTS 011 gave alleles from 320 to 358 with allele frequencies 0.05 to 0.20. Alleles 320, 326 and 358 were frequently detected in this breed. Similar reports were reported by Karthickeyan et al., (2008) for Ongole breed in different population. They observed allele sizes of 262, 268 and 274.

ILSTS 012 primed product of Red Sindhi cattle revealed alleles from 94 to 106 with corresponding allele frequencies ranging from 0.03 to 0.18. Alleles 96, 98, 104 and 106 were more frequently detected in this breed group. Brezinsky et al., (1993a) found that alleles 85, 95, 97 and 99 were detected.

Products of ILSTS 013 showed polymorphism with allele sizes ranging from 132 to 160 with allele frequencies from 0.08 to 0.23. Alleles 143 and 158 were abundant in this breed. Brezinsky et al., (1993a) observed alleles of microsatellite locus ILSTS 013 in N'Dama, Friesian, Boran and Zebu breed of South Africa as 121, 123, 125, 127 and 129.

DNA samples of cattle were amplified with ILSTS 014 and the products showed polymorphism. The allele sizes scored were 149 to 167 with allele frequencies ranging from 0.08 to 0. 20. Alleles 152 and 167 were predominant in this breed. Brezinsky et al., (1993a) observed alleles of microsatellite locus ILSTS 014 in N'Dama, Friesian, Boran and Zebu breed of South Africa as 129, 131 and 133. They also observed Mendelian inheritance of the allele's 131 and 133 in 8 full siblings in N'Dama and Boran calves.

The level of polymorphism displayed by eight microsatellites in the Red Sindhi breed could be useful in fixing breed specific alleles for genetic characterization of Indian Zebu breeds. The accuracy in genetic analysis and measurement of variability could be further improved by employing more number of microsatellite loci for genetic analysis in this breed.

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