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Assessment of Genetic Variability in Murrah and Surti Buffaloes using Microsatellite Markers

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ABSTRACT

Background: The present study illustrates the genetic diversity of Surti buffaloes using a total of 10 microsatellite markers selected from the list suggested by FAO (ISAG).

Methods: All the 10 microsatellites were successfully amplified by polymerase chain reaction and observed number of alleles ranged from 6 to 12 with a total of 101 alleles across the 10 loci.

Result: The overall mean observed heterozygosity and expected heterozygosity values were 0.617 and 0.772 and ranged from 0.367 to 0.767 and 0.734 to 0.840 respectively. All the 10 primers used in the present study were found to be polymorphic and highly informative with the mean PIC value 0.69 in Murrah, 0.722 found in Surti buffalo. The estimate of heterozygote deficiency varied from 0.039 to 0.509 with a mean positive value of 0.203±0.057, suggesting a deficit of heterozygotes in studied breeds. The sufficiently high mean values of observed number of alleles, observed heterozygosity and PIC for various microsatellites are important to examine the genetic variability of Murrah and Surti buffaloes population in present scenario for creating useful conservation strategies as well as disease diagnose.

Key words: Genetic variability, Microsatellite marker, Murrah, PIC index, Surti.

INTRODUCTION

The dairy sector in India has shown remarkable growth in the past decade in term of milk production. Buffaloes are to be considered as backbone of Indian dairy industries, by contributing more than 56% of total milk production Mishra Bhuyan *et al*. (2010). Apart from high milk yield, buffalo breeds also possess certain favorable traits than indigenous cattle including endurance potential, relatively disease resistance and their adaptability to different agro climatic conditions prevailing in the country. Mechanized and unplanned breeding among the available native breeds of buffalos causes dilution of livestock's breeds. Out of well-defined breeds in India, Murrah and shrutiare considered as traditionally evolved for high milk production. The Murrah buffalo is endowed with genotype which makes it a prominent milch buffaloaround the globe. While shruti buffalo consume less feed and maintain well under extensive system of management. The Murrahand shrutibuffalo deciphers average milk ranging from 1500 to 2500 kg and 900 to 1300 kg per lactation (Sangwan, 2012). Due to these deserving milch traits, theabove breeds have been used in India extensively for grading up of non-descript buffaloes. This necessitates more attention in the genetic improvement of the dairy animal. Molecular and genetic characterization of these breeds is important for the prevention of germplasm erosion. Recentlyvarious molecular techniques have been used for the molecular characterization at DNA level such as RFLP, RAPD, AFLP, microsatellites and SNPs. Microsatellites areshort repetitive DNA sequence stretches present across the genome at the frequency of one at every 6 kb sequence (Schlotter and Tautz, 2000, Bruford and Wayne, 1993). Thus highly polymorphic and dispersed nature of microsatellites make it as a useful tool to study genetic variation, genetic diversity and, gene flow. (Bruford and Wayne, 1993 and Roy *et al*., 1994). In this study, we have attempted to characterize the molecular genetic variability of Murrah and shruti buffaloes of Rajasthan region using microsatellites markers.

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MATERIALS AND METHODS

Sampling and blood collection

A total of 30 blood samples from each buffalo breed were collected randomly as described by Swathi *et al*. (2018) and the information was collected after consulting pedigree records maintained and interviewing the owners in detail. Murrah buffalo blood samples were collected from the unorganized setups in the rural areas of Haryana state (Hisar

and Bhiwani districts). Surti buffalo blood samples were collected from Livestock Research Station (Navania, Udaipur, Rajasthan). Blood was collected in commercial EDTA vials. Samples were transported to the laboratory on ice and stored at 4°C until used.

DNA isolation and quantification

Genomic DNA was isolated using the QIAamp ® DNA Mini Kit with slight modification. The purity and concentration of the isolated genomic DNA were estimated using agarose gel electrophoresis and UV-absorption spectrophotometer respectively. The absorbance at 260 and 280 nm wave length is used to measure the optical density (OD) of the DNA samples. A ratio between 1.4 and 1.9 is considered as a relatively pure DNA sample as it did not show any effect on PCR reaction (Sambrook and Russel, 2001).

Microsatellite typing

A total of10Microsatellite were selected on the basis of higher variability from recommended list of FAO (FAO, 2011 and Jakhesara *et al.,* 2010). Markers with good heterozygosity, ability to co-amplify in multiplex panel with no multiple peaks were preferred. Microsatellite primer pairs were custom synthesized by GCC Biotech Pvt. Lt. India based on their allele size, the level of polymorphism and the level of genetic diversity in Surti and Murrah buffalo breeds (FAO 2011). All the primers as supplied by the manufacturer were initially added with 50 µl of TBE buffer (pH 8.0). After that, each primer was reconstituted in sterilized DNase free Mili Q water to arrive at a final concentration of 10 p moles/µl.

PCR was carried out in a final reaction volume of 25 µl. All PCR components were thawed and spun for a few seconds prior to use. Initially, the PCR conditions were standardized for annealing temperatures, they were attempted in accordance with literature of each marker given (Crawford *et al*., 1995) but some markers required temperature optimization. Gradient PCR was attempted for several markers to determine their exact annealing temperature. PCR cycling conditions were 5 min at 95°C, after that 30 cycles of 1 min at 94°C, 1 min at annealing temperature (52-58°C) depending on the primer and PCR conditions standardized, 1 min at 72°C and the final extension of 7 min at 72°C. The polymorphic typing of microsatellite marker was done on 8% native polyacrylamide gel electrophoresis (PAGE) at 80 V (Hoefer SE 600 series electrophoresis unit) and visualized by ethidium bromide staining. Allele size was estimated utilizing a 100 bp ladder (Promega, USA). The genotype of each individual buffalo at 10 different loci was recorded by manual counting and data was further analyzed using different statistical tools.

Statistical analysis and Bioinformatics approach

Allele frequencyobserved number of alleles (N_a), Effective number of alleles (N_e), Observed heterozygosity (H_{obs}) and Expected heterozygosity (H_{exp}) were calculated by GenAlEx 6.5 version (Peakall and Smouse 2006, 2012). Hardy-Weinberg equilibrium (HWE) was estimated using GENEPOP 1.2 version (Raymond andRousset, 1995). Fixation Index (F_{is}) was estimated using FSTAT version 2.9.3 (Goudet, 2001). Polymorphic Information content (PIC) for each locus was estimated according to (Botstein *et al*., 1980).

RESULTS AND DISCUSSION

To assess the genetic variability within the two buffalo breeds *i.e*. Murrah and Surti were conducted using 10 microsatellite markers and they were successfully amplified, produced clear banding pattern. Genetic variations that can be effectively measured within and between populations by using various statistical parameters *viz*. Allele frequency, Observed and effective number of alleles, Observed and expected heterozygosity, F-statistics, Hardy-Weinberg equilibrium and Polymorphic information content. Total 162 alleles were found across all 10 microsatellite loci and allele frequency of selected markers is shown in a Table 1and 2.

The most polymorphic marker in Murrah buffalo were CSSM033, CSSM019 and CSRM060 with a total of ten alleles individually beside that in Surti buffalo highly polymorphism were found in CSSM047 with total of twelve alleles and least polymorphic loci in Murrah buffalo was ETH003 with five alleles and in Surti buffalo, CSSM022 and ETH003 with six alleles individually. The overall allele diversity, considered to be a reasonable indicator of genetic variation with in the population. The range of alleles comparable to earlier reported buffalo breeds (Navani *et al*., 2002; Pundir *et al*., 2000; Arora *et al*., 2004; Barker *et al*.,1997; Sukla *et al*., 2006; Aminafshar *et al*., 2008).

Table 1: Allele frequency across 10 microsatellite loci in Murrah buffalo.

Allele/n	CSSM033	CSSM043	CSSM047	CSSM019	CSRM060	CSSM022	ETH003	CSSM061	ILSTS005	ILSTS030
1	0.033	0.417	0.033	0.2	0.267	0.133	0.333	0.05	0.117	0.083
2	0.167	0.05	0.217	0.1	0.183	0.417	0.217	0.183	0.067	0.217
3	0.033	0.433	0.267	0.033	0.05	0.283	0.217	0.117	0.45	0.05
4	0.367	0.017	0.017	0.067	0.083	0.083	0.217	0.3	0.05	0.283
5	0.05	0.067	0.033	0.333	0.117	0.05	0.017	0.067	0.117	0.083
6	0.017	0.017	0.05	0.033	0.017	0.033		0.133	0.133	0.283
7	0.017		0.35	0.133	0.017			0.05	0.067	
8	0.033		0.033	0.033	0.017			0.05		
9	0.25			0.017	0.183			0.05		
10	0.033			0.05	0.067					

The value of the effective number of allele (N_e) ranged from 2.711 (CSSM043) to 6.00 (CSRM060) with mean value of 4.409 in Murrah buffalo (Table 3) besides in Surti buffalo ranged from 3.261(CSSM019) to 7.143 (CSSM043) with mean value of 4.99 (Table 4). The effective number of allele (N_e) at each locus were less than the observed number of allele and comparable to earlier reported buffalo breeds. Allelic diversity in terms of numbers of alleles across all the loci along with the effective number of alleles is greater in comparison to earlier reported studies in buffalo breeds *viz.* 5.24 in Nagpuri buffalo (Kataria *et al*., 2009), 5.03 in Riverine buffalo (Zhang *et al*., 2008), 4.14 in Guilan buffalo (Aminafshar *et al*., 2008), 5.86 in Pandharpuri buffalo (Vijh *et al*., 2008), 2.0 in Murrah buffalo (Sukla *et al*., 2006) and 6.63 in Surti buffalo (Kumar *et al*., 2006).

Table 2: Allele frequency across 10 microsatellite loci in surti buffalo.

Allele/n	CSSM033	CSSM043	CSSM047	CSSM019	CSRM060	CSSM022	ETH003	CSSM061	ILSTS005	ILSTS030
1	0.017	0.183	0.033	0.4	0.067	0.033	0.1	0.033	0.05	0.067
2	0.433	0.067	0.017	0.017	0.15	0.35	0.067	0.133	0.1	0.233
3	0.167	0.033	0.033	0.367	0.117	0.25	0.067	0.2	0.1	0.383
4	0.288	0.067	0.267	0.067	0.2	0.083	0.2	0.05	0.383	0.05
5	0.033	0.25	0.217	0.05	0.217	0.167	0.15	0.183	0.15	0.217
6	0.05	0.033	0.05	0.067	0.15	0.117	0.417	0.067	0.033	0.033
7	0.017	0.133	0.133	0.017	0.05			0.217	0.1	0.017
8		0.033	0.05	0.017	0.017			0.1	0.067	
9		0.05	0.067		0.017			0.017	0.017	
10		0.083	0.083		0.017					
11		0.067	0.017							
12			0.033							

Table 3: Observed number of allele (N_a), effective number of allele (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F) for murrah buffalo breed.

Locus	N_a	N_e	H_o	H_e	F	PIC
CSSM033	10	4.306	0.633	0.768	0.175	0.736
CSSM043	6	2.711	0.800	0.631	-0.268	0.562
CSSM047	8	4.054	0.867	0.753	-0.150	0.714
CSSM019	10	5.279	0.533	0.811	0.342	0.788
CSRM060	10	6.000	0.633	0.833	0.240	0.815
CSSM022	6	3.543	0.333	0.718	0.536	0.673
ETH003	5	3.965	0.700	0.748	0.064	0.671
CSSM061	9	5.902	0.667	0.831	0.197	0.717
ILSTS005	7	3.863	0.633	0.741	0.145	0.461
ILSTS030	6	4.467	0.633	0.776	0.184	0.739
Mean±SE	7.700±0.616	4.409±0.330	0.643±0.046	0.761±0.019	0.147±0.072	0.67

Table 4: Observed number of allele (N_a), effective number of allele (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F) for surti buffalo breed.

Locus	N_a	N_e	H_o	H_e	F	PIC
CSSM033	7	3.333	0.900	0.700	-0.286	0.6529
CSSM043	11	7.143	0.633	0.860	0.264	0.8302
CSSM047	12	6.406	0.300	0.844	0.645	0.8061
CSSM019	8	3.261	0.800	0.693	-0.154	0.6465
CSRM060	10	6.522	0.833	0.847	0.016	0.8318
CSSM022	6	4.265	0.433	0.766	0.434	0.7304
ETH003	6	3.922	0.033	0.745	0.955	0.552
CSSM061	9	6.383	0.867	0.843	-0.028	0.8242
ILSTS005	9	4.813	0.567	0.792	0.285	0.7384
ILSTS030	7	3.896	0.533	0.743	0.283	0.6091
Mean±SE	8.50±0.65	4.99±0.466	0.590±0.088	0.783±0.020	0.241±0.119	0.700

All the loci were highly polymorphic in studied population. Polymorphic information content value in murrah ranged from 0.461(ILSTS005) to 0.815 (CSRM060) with mean value of 0.67 while in Surti buffalo ranged from 0.552 (ETH003) to 0.831 (CSRM060) with a mean value of 0.70 (Table 3 and 4). The overall mean PIC value were comparable with the Martinez *et al*.(2006) in Murrah buffalo and the mean PIC value of present study was higher than the reports in South Kanara buffaloes (Kathiravan *et al*., 2009); Murrah (Bhuyan *et al*., 2010) and in Banni buffaloes (Mishra *et al*., 2009c). All loci had high PIC value, indicating utility of these markers for Population assignment (MacHugh *et al*., 1997) as well as genome mapping (Kayang *et al*., 2002).

All the markers considered for this study are highly informative to characterize Murrah and Surti buffalo population and showed potential to detect genetic diversity in population. The Heterozygosity estimates genetic variability within a population in a considerable manner. Observed and expected heterozygosity at individual loci are present in Table 3. The expected gene Heterozygosity (H_{exp}) ranged between 0.631 (CSSM043) to 0.833 (CSRM060) in Murrahbuffalo with an overall mean value of 0.761 besides in Surtibuffaloranged between 0.693 (CSSM019) to 0.860 (CSSM043) with an overall mean of 0.783. The overall observed Heterozygosity (H_o) across 10 loci ranged between 0.333 (CSSM022) to 0.867 (CSSM047) in Murrahbuffalowith an overall mean of 0.643 and between 0.033 (ETH003) to 0.900 (CSSM033) in Surti buffalo with an overall mean of 0.590. The mean observed heterozygosity detects in the present study in comparison to previous studied Indian buffalo breeds 0.624 in Bhadawari (Tantia *et al*., 2006), 0.45 in Nagpuri buffalo (Kataria *et al*., 2009), 0.631 in Murrah (Joshi *et al*., 2012), 0.684 in Brazilian Murrah (Marrero *et al*., 2015), 0.423 in Pandharpuri (Mishra *et al*., 2008) and 0.69 in Murrah (Kumar *et al*., 2006). In contrast to our expectation low heterozygosity and variability were observed, which might be due to fact that the sample were collected fromspecificarea and farms, even though samples selected were from unrelated individuals but still chances of closed related animals can't be ignored and the presence of more homozygosity in the individual samples could be the reason for low heterozygosity.

However, despite their good results, productivity still appears to be low. In present scenario there is an urgent requirement to increase productivity through better selection of genetically distant animals for mating. The production potential of low producing non-descript buffaloes can be increased rapidly by mating with superior sires breeds like murrah and shruti. Thus, it is required to increase the variability on the existing breeds and breeding policy resulting in developing future strategies for buffalo development.

The present study revealed that expected heterozygosity (H_{exp}) was higher than observed heterozygosity (H_o) hence, showing departure from Hardy Weinberg Equilibrium (HWE) and possibility of in breeding. This was also reflected in positive F_{is} value (0.147±0.072) in Murrah which ranged from -0.268 to 0.536 and in Surti (0.241±0.119) which ranged from -0.286 to 0.955. The mean F_{is} value is almost in similar range with earlier reported study of buffalo breeds (Triwitakorn *et al*., 2006; Arora *et al*., 2003; Mishra *et al*., 2009c).

CONCLUSION

Out of 10 microsatellite loci explored, all 10 loci gave amplification with an average all mean number of alleles 10.1 indicating high informativeness. The observed heterozygosity at various loci was lower than the expected heterozygosity. The PIC values for all the 10 loci were more than 0.5 hence they were regarded as highly informative. The most polymorphic markers were CSSM033, CSSM047 and CSRM060 with a total of 12 alleles each and least polymorphic loci was CSSM022 with 6 alleles in both the breeds.The allelic diversity was found higher in Surti with mean 8.50 than the Murrah buffalo (7.70). The observed heterozygosity was found higher in Murrah breed with mean 0.643 than the Surti buffalo (0.590). The overall mean of inbreeding coefficient (0.203) revealed considerable heterozygote deficit with in the population which could be due to selective breeding, presence of null alleles. The fixation index value observed higher in Surti (0.241) than the Murrah (0.147) which shows that Surti buffalo is more heterozygote deficiency than Murrah buffalo. Significant departure of the population studied from HWE might be because of heterozygote deficiency due to selective breeding over several generations. Sufficiently high overall mean values of observed number of alleles (10.1), observed heterozygosity (0.617) and PIC (0.715) for the microsatellite markers used in the present study supported their suitability for the genetic diversity studies.

Conflict of interest

All authors declare that they have no confllict of interest.

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