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Molecular Genetic Characterization of Local Buffalo Population of Jammu and Kashmir Region using Microsatellite Markers

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ABSTRACT

In the present study, genetic characterization of local buffalo population, a native to north temperate region of India in Jammu and Kashmir, was carried out for the purpose of breed characterization and assessing existing intra-population genetic diversity. A total 50 blood samples procured at random from genetically unrelated animals of two sexes and different age groups were collected from different locations in the breeding tract of these buffaloes. The multi-locus genotype data were generated using 15 FAO recommended buffalo specific microsatellite markers, which gave amplification and various parameters were estimated through PopGene software (1.3.1). A total of 103 distinct alleles were observed with mean observed and effective number of alleles as 6.8667±0.29 and 5.5683±0.2 respectively across all 15 studied loci. The maximum (9) alleles were contributed by loci (CSM013 and CSM061) and the least (5) by (ILSTS030). The mean Observed and Expected Heterozygosities across all loci were 0.6840±0.01 and 0.8250±0.007 respectively. The polymorphism information content (PIC) value ranged from 0.7227 (CSM038) to 0.8357 (CSM013) with mean PIC of 0.7913±0.008. The Nei's genetic distance measures varied from 0.7606 (CSM038) to 0.8528 (CSM013 and BRN) with mean genetic distance of 0.8167±0.007. The mean Shannon's index value ranged from 1.5220 (ILSTS030) to 2.0320 (CSM013) with mean of (1.7894±0.03). Microsatellite analysis thus revealed high level of polymorphism across studied microsatellite markers and informativeness of the markers for genetic diversity analysis studies which can be utilized to plan future association studies to exploit the uniqueness and adaptability of indigenous buffalo population.

Keywords: Polymorphism, Shannon's index, heterozygosity, genetic distance, PopGene.

The animal genetic resources are the intricate part of agro-ecosystems, providing food, fiber, manure and fuel thus making a large contribution to food and agriculture production. Livestock sector of India, whose overall contribution in total GDP is nearly 4.11% in the world and is considered as an important component of the national economy. Globally, there are nearly 3000 breeds and breed varieties of seven major mammalian species viz., cattle, buffalo, sheep, goat, horse, pig and donkey (F.A.O 1997). Buffalo have been bred, predominantly in Asia, for thousands of years for use by humans. India is the home tract of some of the best buffalo breeds of world and has approximately 55.7 percent (108.7 million, 19th livestock census) of the total world buffalo population with 13 well-recognized breeds based on their phenotypic characteristics, production performances and eco-geographical distribution. But, these breeds constitute only 30% of total buffalo population of India and the remaining does not belong to any well-defined breeds and categorized as nondescript. The Local Buffalo population is also known as Gujjari Buffalo of Jammu and Kashmir region as they are reared by traditional "Gujjar" community, who are a distinct tribe of nomadic pastoralists. This is a domestic water buffalo kept for dairy production and for draught purposes. Most of its home tract is plain including Jammu, Kathua, RS Pura, Samba, Udhampur and rest is hilly or mountainous, including the Pir Panjal Range which separates it from the southwest part of

the Kashmir Valley and part of the Great Himalayas in the eastern districts of Doda and Kishtwar. Estimated Livestock population of the state as per the latest available integrated survey (2011-12) is 160.407 lakhs out of which local buffalo population comprises of 7.889 lakh buffalos. Local buffalo population is light golden brownish to light black in color, sometimes with white markings on the face or legs. Eyes are presenting little or brownish white in color somewhat like walled eye appearance, active and prominent in both males and females. (Fig. 1) Forehead contains light brownish to white tuft of hairs. Horns are slightly less curled as compared to Murrah and Nili Ravi and are circular in cross section.

Livestock genetic diversity in India has been constantly neglected as well as threatened. However, it is likely that many measures with the potential to reduce the risk of genetic erosion will also promote efficient utilization of existing animal genetic resources and so be complementary to wider livestock development objectives. Moreover, the use of Murrah bulls for improvement in the native breeding tract of most other recognized breeds might have further narrowed down the effective size of the respective male populations (Sadana *et al.,* 2006). Thus, genetic monitoring of population is essential, and it has become more feasible with the use of hyper variable markers such as microsatellites and minisatellites (Spencer *et al*., 2000).

Rapid developments in molecular genetics have made it possible to detect genetic polymorphism at the DNA sequence level, i.e. using molecular markers and thus the microsatellite markers have proved to be important and efficient molecular tools for diversity analysis in farm animals due to their several advantages like random distribution across the genome, high degree of polymorphism, co dominance, neutrality with respect to selection and possibility of automated scoring of genotypes and have been used to analyze genetic variations in Buffaloes, cattle, sheep, goat, pigs, horses and chickens (Boycee *et al*., 1996). The present study was therefore undertaken to characterize Buffalo germplasm of Jammu and Kashmir region using suitable FAO specific microsatellite markers.

MATERIALS AND METHODS

Ethical approval

During collection of blood samples from local buffalo population, attention had been paid to minimize pain to the animals and all the samples collection was carried out in accordance with the guidelines laid down by the International Animal Ethics Committee and prevailing local laws and regulations. The approval for carrying out this study was taken from the Institutional Animal Ethics Committee.

Collection of blood samples

Totally, 50 venous blood samples were collected at random from genetically unrelated animals of local

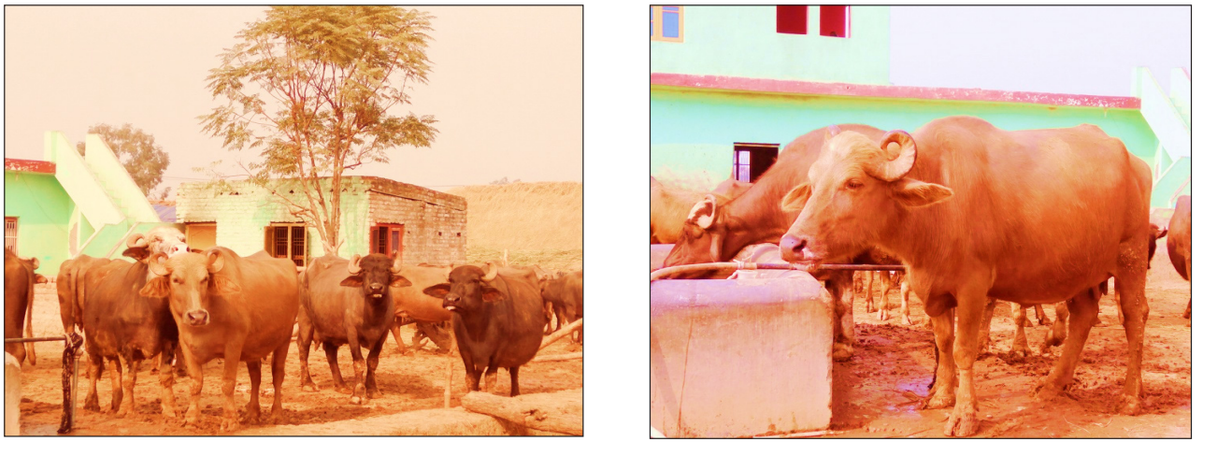

Fig. 1: Local Buffalo Population/Gujjari Buffalo of Jammu and Kashmir

buffalo population belonging to either sex or different age groups from its from different locations of the natural breeding tract (RS Pura, Miran sahib, Kathua, Akhnoor, Samba, Rajouri, Reasi, Udhampur) and adjoining areas of Jammu region and other districts of Jammu and Kashmir as shown in Fig. 2.

Fig. 2: Migratory route along with distribution of Local Buffalo Population/Gujjari Buffalo in Jammu and Kashmir

Isolation of DNA samples

DNA was isolated by phenol-chloroform extraction method (Sambrook and Russel, 2001) from 5-10 ml blood of 50 samples. The quality of DNA was assessed through 0.7% horizontal mini-submarine agarose gel electrophoresis. The purity of DNA was assessed by calculating ratio of optical densities at 260 nm and 280 nm. The samples with OD ratio (OD260/OD280) ranging from 1.7 to 1.9 was used in subsequent experiments and without any smear in 0.7% Agarose gel electrophoresis.

Primer preparation

15 FAO (DADIS MoDAD) recommended buffalo specific microsatellite markers namely CSSM033, CSSM038, BRN, CSSM032, CSSM013, ETH003, CSSM061, BMC1013, CSSM062, ILSTS030, ILSTS008, HMH1R, ETH121, ILSTS033, RM099 (Table 1), which gave amplification were included in the analysis.

Processing of DNA samples

The microsatellite loci were amplified in programmable thermal cycler (Bio-Rad, S 1000) after optimization. The polymerase chain reaction (PCR) program used involved initial denaturation at 94°C for 5 min and 30 cycles of denaturation at 94°C for 30 s, annealing for 45 s, extension at 72°C for 45 s and final extension at 72°C for 10 min. Documentation of PCR product was done in 1.5% agarose gel electrophoresis at 2-5 v/cm as shown in Fig. 3 and Fig. 4 for CSM032 and ETH121 respectively. The PCR products for different microsatellite loci were resolved on 6% denaturing (urea) polyacrylamide gels along with 50 and 100 bp DNA ladders at 40-45w. Microsatellite alleles were visualized by silver staining.

Statistical analysis

The microsatellite genotype data were analyzed using PopGene version (1.3.1) software Yeh *et al.* (1999) to calculate allele frequencies, observed and effective number of alleles, observed and effective heterozygosities and polymorphism information content (PIC) in the population. It was used to compute summary statistics (e.g., allele frequency, gene diversity, genetic distance, F-statistics, multilocus structure etc.) for single-population and multi-population. The PIC was assessed using allelic frequencies evaluated according to Botstein *et al*., (1980).

Hardy Weinberg equilibrium

The test for deviation from Hardy-Weinberg equilibrium was based on genotypic frequencies and Wright's Fixation index (*F* statistics using F-Stat IS) software (Goudet and Hered 2002) and results were discussed and interpreted accordingly. The exact tests for deviations from Hardy-Weinberg equilibrium (HWE) were performed using the GENEPOP package (Raymond and Rousset 1995).

RESULTS AND DISCUSSION

Various measures of genetic diversity obtained in the present study with local buffalo population of Jammu region are presented in Table 2. All 15 microsatellite loci that have been identified to be polymorphic in a variety of buffalo breeds amplified successfully in local Buffalo as well and produced definite banding patterns from which individual genotypes could be ascertained.

A total of 103 distinct alleles were detected (Table 2) in over 15 studied microsatellite loci with a mean observed number of alleles (Na) of 6.8667±0.29 alleles

Table 1: Primer sequence of different microsatellites used in the study

Sl. No.	Name of primer	Primer sequence (5' -> 3') Forward Reverse	Number of base pairs F' R'	Optimised annealing temperature (°C)
1	CSSM033	CACTGTGAATGCATGTGTGTGAGC CCCATGATAAGAGTGCAGATGACT	24 24	65
2	CSSM038	TTCATATAAGCAGTTTATAAACGC ATAGGATCTGGTAACTTACAGATG	24 24	55
3	BRN	CCTCCACACAGGCTTCTCTGACTT CCTAACTTGCTTGAGTTATTGCCC	24 24	60
4	CSSM032	TTATTTTCAGTGTTTCTAGAAAAC TATAATATTGCTATCTGGAAATCC	24 24	55
5	CSSM013	ATAAGAGATTACCCTTCCTGACTG AGGTAAATGTTCCTATTTGCTAAC	24 24	55
6	ETH003	GAACCTGCCTCTCCTGCATTGG ACTCTGCCTGTGGCCAAGTAGG	22 22	65
7	CSSM061	AGGCCATATAGGAGGCAAGCTTAC TTCAGAAGAGGGCAGAGAATACAC	24 24	60
8	BMC1013	AAAAATGATGCCAACCAAATT TAGGTAGTGTTCCTTATTTCTCTGG	21 25	54
9	CSSM062	GTTTAAACCCCAGATTCTCCCTTG AGATGTAACAGCATCATGACTGAA	24 24	55
10	ILSTS030	CTGCAGTTCTGCATATGTGG CTTAGACAACAGGGGTTTGG	20 20	55
11	ILSTS008	GAATCATGGATTTTCTGGGG TAGCAGTGAGTGAGGTTGGC	20 20	58
12	HMH1R	GGCTTCAACTCACTGTAACACATT TTCTTCAAGTATCACCTCTGTGGCC	24 25	60
13	ETH121	CCAACTCCTTACAGGAAATGTC ATTTAGAGCTGGCTGGTAAGTG	22 22	59
14	ILSTS033	TATTAGAGTGGCTCAGTGCC ATGCAGACAGTTTTAGAGGG	20 20	55
15	RM099	CCAAAGAGTCTAACACAACTGAG ATCCGAACCAAAATCCCATCAAG	23 23	60

per locus for Local Buffalo population of Jammu region which significantly corroborated with the findings in Marathawadi Buffalo (Kathivaran *et al*., 2009) and Pandarpuri, Jaffarbadi, surti and Nagpuri buffaloes (Kumar *et al*., 2006) and three water buffaloes in Turkey (Ozkan *et al*., 2014). These microsatellites exhibited a high level of polymorphism as revealed by a wide range of alleles varying from 5 (ILSTS030) to 9 (CSM013 and CSM061) in Local buffalo population of Jammu region which significantly corroborated with the findings of Navani *et al*. (2002), Kumar *et al*. (2006) and Vijh *et al*. (2008) for Indian riverine buffaloes.

The allele size ranged between 88 bp (RM099) to 240 bp (BMC1013) for local buffalo population of Jammu region. The mean effective number of alleles (Ne) was 5.5683±0.2 for Local buffalo population of Jammu region. The mean effective number of alleles was less than the observed values across all loci and ranged from 4.1771 (CSM038) to 6.7935 (CSM013) in local buffalo population of Jammu region. The overall allelic diversity considered to be a reasonable indicator of genetic variation within the population displayed high genetic variation in local buffalo population. The FAO had specified at least 4 distinct alleles per locus for proficient judgment

Table 2: Measures of genetic diversity at each microsatellite locus in Local Buffalo population

Locus	Sample Size	na	ne	I	Obs het	Exp het	Ave Het	Allele size (bp)	Nei	PIC
CSM013	100	9.0000	6.7935	2.0320	0.6600	0.8614	0.8308	162-172	0.8528	0.8357
CSM032	100	7.0000	5.9172	1.8479	0.6800	0.8394	0.8119	208-224	0.8310	0.8086
CSM061	100	9.0000	6.0024	1.9619	0.7200	0.8418	0.8227	100-126	0.8334	0.8135
CSM038	100	6.0000	4.1771	1.5411	0.7400	0.7683	0.7859	163-187	0.7606	0.7227
BMC1013	100	6.0000	5.5928	1.7565	0.7400	0.8295	0.7802	217-239	0.8212	0.7962
ETH121	100	7.0000	6.1425	1.8567	0.6200	0.8457	0.8118	182-198	0.8372	0.8156
ETH003	100	8.0000	6.5445	1.9756	0.6400	0.8558	0.7760	96-192	0.8472	0.8293
CSM062	100	6.0000	5.2854	1.7209	0.7000	0.8190	0.8294	124-136	0.8108	0.7834
ILSTS033	100	7.0000	6.1652	1.8747	0.8200	0.8463	0.8209	126-138	0.8378	0.8168
ILSTS008	100	7.0000	5.0050	1.6959	0.6800	0.8083	0.7973	168-176	0.8002	0.7702
BRN	100	6.0000	5.7405	1.7674	0.6000	0.8341	0.8097	121-147	0.8258	0.8012
ILSTS030	100	5.0000	4.3066	1.5220	0.8200	0.7756	0.7971	146-158	0.7678	0.7292
CSM033	100	6.0000	4.5620	1.6411	0.5800	0.7887	0.7856	154-175	0.7808	0.7496
HMH1R	100	7.0000	5.2798	1.7793	0.6600	0.8188	0.7905	169-187	0.8106	0.7853
RM099	100	7.0000	6.0096	1.8673	0.6000	0.8420	0.8196	87-119	0.8336	0.8124
Mean	100	6.8667	5.5683	1.7894	0.6840	0.8250	0.8046		0.8167	0.7913
St. Error		0.2	0.2	0.03	0.01	0.007	0.004		0.007	0.008

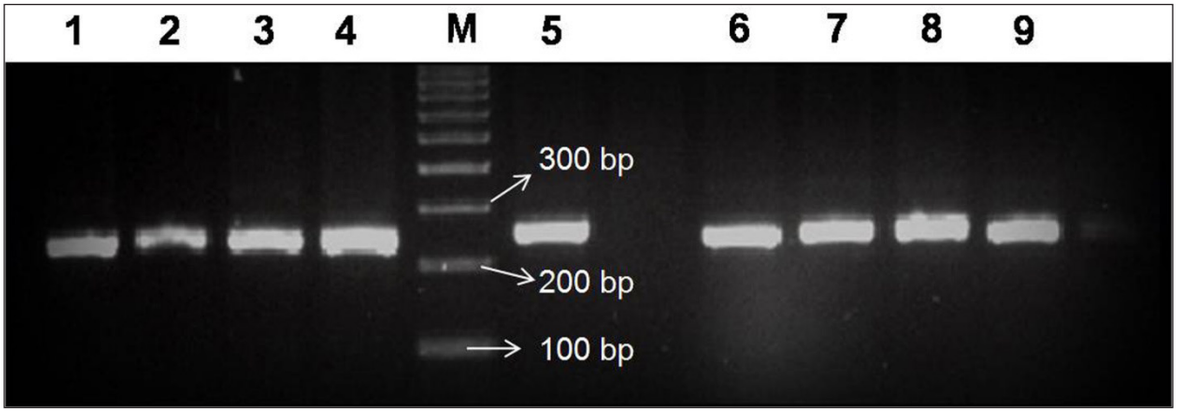

Fig. 3: UV illumination of PCR product (Microsatellite CSM032 (208-224 bp) run on agarose gel electrophoresis. M=100 bp DNA marker; Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9 = PCR products of CSM032 lying between range of 208-224 bp

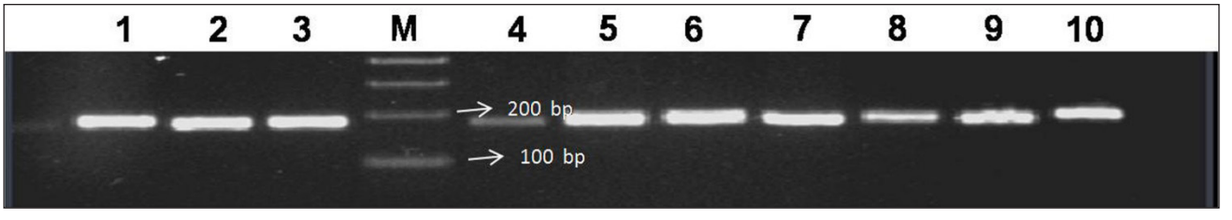

Fig. 4: UV illumination of PCR product ETH121 (182-198bp)) run on agarose gel electrophoresis. M=100 bp DNA marker; Lanes 1, 2, 3, 4, 5, 6, 7, 8, 9 &10 = PCR products of ETH121 lying between range of 182-198 bp

of genetic distance between and within breeds. Hence, all 15 microsatellite markers studied, exhibited ample polymorphism for evaluating intra-population genetic variability in Local buffalo population of Jammu region. The results obtained in Local buffalo almost corroborated with Pandharpuri with the observed number of alleles having mean value of 5.86±0.23 and Bhadawari and Surti Buffaloes having mean value of 7.1±0.19 (Vijh *et al*., 2008; Arora *et al*., 2004).

The mean Shannon's index value was high (1.7894±0.03) in Local buffalo population of Jammu region and ranged from 1.5220 (ILSTS030) to 2.0320 (CSM013) thus showing higher gene diversity in the both the existing population.

Heterozygosity is an appropriate measure of genetic variability within and between populations when populations are expanding. Therefore, heterozygosity values were used as an estimate for variability of studied local buffalo population of Jammu region.

The observed and expected heterozygosity values on the basis of allele frequency are also presented in Table 2. The mean observed and expected heterozygosities were 0.6840±0.01 (range of 0.5800 (CSM033) to 0.8200 (ILSTS033 and ILSTS030) and 0.8250±0.007 (range of 0.7683 (CSM038) to 0.8614 (CSM013)) in Local buffalo population of Jammu region which significantly corroborated with the result findings in Murrah, Mehsana, Toda, Surati, Pandharpuri and Nagpuri buffaloes as revealed by (Kumar *et al.,* 2006) and in Nilli Ravi, Murrah, Tarai, Jaffrabadi as revealed by (Vijh *et al*., 2008) and (Arora *et al*., 2004) in Bhadawari and Tarai where the observed heterozygosity was less than that of expected heterozygosity. The low Ho reveals presence of more homozygote individual in the samples analyzed. Though few loci exhibited lower heterozygosity values, most of the loci showed relatively higher expected heterozygosity except at one loci i.e., ILSTS030, that might be due to low selection pressure, large population size and immigration of new genetic materials.

The genetic variability of a population is usually measured as average heterozygosity per locus while the gene differences between two populations may be measured by Nei's standard genetic distance. The average heterozygosity ranged from 0.776 (ETH003) to 0.8308 (CSM013) with mean of 0.8046±0.004 and the values for Nei's measures varied from 0.7606 (CSM038) to 0.8528 (CSM013 and BRN) with mean genetic distance of 0.8167±0.007 in Local buffalo population of Jammu region.

The PIC values, which denotes the statistical assessment of informativeness of a marker were high and ranged from 0.7227 (CSM038) to 0.8357 (CSM013) with mean PIC of 0.7913±0.008 (Table 2) in local buffalo population of Jammu region. This may be due to the fact that there was increased level of heterozygosity and allele richness in the population which are the good indicators of genetic polymorphism in present study on local buffalo population of Jammu region. These values are indicative of the fact that the markers used were highly informative for analysis of genetic diversity in local buffalo population of Jammu region. The genetic marker showing PIC values higher than 0.5 are normally considered as informative in population genetic analysis.

Mean PIC value of 0.53 and 0.669 lower than the values obtained in the present study, was earlier reported in the Nagpuri Buffalo (Kataria *et al.,* 2010) and Karnese Buffalo of South kanara region (Kathivaran *et al.,* 2009) respectively and higher mean pic value of 0.933 as reported in Egyptian Buffalo (Abou-Bakr *et al*., 2014). The present PIC values were comparable with Egyptian Buffalo Breeds (0.736 -0.862.) as reported by *El-Kholy et al.* (2007) using bovine microsatellite markers and mean value of >0.5 in Iraqi water buffaloes. (Jaayid and Maytham 2014) In contrast, Acosta *et al*. (2014) obtained lower PIC values for Cuban water buffalo (0.495), Gullian Buffaloes of Iran (0.61) by Aminaafshar *et al*. (2008).

CONCLUSION

In the present investigation, an attempt has been made to genetically characterize Buffalo germplasm of Jammu and Kashmir region using suitable FAO specific Microsatellite markers in its breeding tract in Jammu region and its adjoining areas using 15 FAO recommended buffalo specific microsatellite markers. Microsatellite analysis revealed high level of polymorphism and informativeness of studied microsatellite markers in genetic diversity analysis in Local Buffalo Population. The high PIC values as observed in the study are indicative of high informativeness of studied markers for genetic diversity analysis in local buffalo Population. Most studied microsatellite markers

had desired neutrality, thus proving to be good candidates for genetic characterization and diversity analysis. The information gathered could be utilized to plan breeding, improvement and conservation programs for this valuable Buffalo germplasm resource and future association studies to exploit its unique adaptability traits. The significant level of variability in this population reflects that the local buffalo population contains a valuable and substantial amount of genetic diversity among the studied breed and thus there is good scope for bringing effective genetic improvement, conservation and designing future breeding policies for these buffaloes but the study needs to be extended to include more microsatellites in a large sample size to further validate the research.

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