IDENTIFICATION OF Y CHROMOSOME HAPLOGROUP AND ESTIMATION OF GENETIC DIVERSITY IN INDIAN BUFFALO BREEDS USING Y SPECIFIC STR MARKERS

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ABSTRACT

The present investigation was to identify Y chromosome haplogroup using STR markers and to study genetic diversity in Indian breeds of buffalo. A total of 139 semen samples of five different Indian buffalo breeds were collected from different semen station of India for the study. DNA was extracted using standard phenol chloroform method. Based of Y specific microsatellite UMN1113 and UMN0304, the population was divided in 47 haplo groups. Subsequently, analysis of microsatellites revealed that overall haplogroup diversity in whole population of 139 buffalo bulls was (0.798±0.048), Murrah breed had maximum (0.899±0.024) and Surti had minimum (0.600±0.215) diversity. AMOVA showed that between breeds variation was 16.69% and differed significantly among the breeds ($P<0.0001$). Further, the pair wise genetic distance (F_{ST} value) between different breeds showed that Mehsana lies in between Surti and Murrah, which also signifies the fact that Mehsana breed originated as cross of Murrah and Surti, while Nili Ravi and Jaffarabadi were clustered together sharing Y specific signatures.

Keywords: *Bubalus bubalis*, buffaloes, genetic distance, haplogroup diversity, Y specific STR

INTRODUCTION

River buffaloes are the back bone of the dairy industry in India, providing a source of milk, meat, skin, hides, fertilizers, fuel, and draft power. As per 20th Livestock Census Buffalo contributes about 20.50% of the total livestock population in India. Its population has increased by 1.06% during 2012 to 2019 with a total of 109.85 million heads (Annual Report, 2019 to 2020) India owns the richest source of germplasm of buffaloes in terms of 17 recognised breeds of riverine buffaloes comprising of breeds like Murrah, Nili Ravi, Jaffarabadi, Marathwadi, Mehsana, Nagpuri, Pandharpuri, Bhadawari, Surti, Banni, Kalahandi, Toda, Luit, Bargur and Chhattisgarh (NBAGR, 2019).

Y-STR is located on the male-specific portion of Y-chromosome, and the coding genes present on it had vital impact in determining male sex, spermatogenesis, and other male-related functions. Liu *et al.* (2002) reported that among

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unrelated males, Y-STRs are polymorphic and are inherited through same paternal line with slight changes through generations. Y chromosome-specific microsatellites are haploid and paternally inherited thus, are of vested interest. Non-recombination in the Y-specific region, which is comprised of about 95% of Y chromosome, indicates that Y chromosome is inherited "en bloc" as a haplotype (Liu *et al*., 2002). Haplotype is a combination of alleles belonging to the linked loci on the same chromosome that are transmitted together in the next generation or a set of alleles for genetic markers (a set of gene or genetic marker DNA sequences) inherited as a unit. A group of related haplotypes which share a common ancestor due to a unique event polymorphism at a specific locus in their DNA sequence is called a haplogroup (Goddard *et al*., 2007). Hooft and co-workers (2007) observed seven, six, and ten haplotypes at the UMN0304, UMN1113 and INRA189 loci, respectively in African buffaloes. As these haplotypes are present in non-recombining region of Y- chromosome, so can be combined into 18 haplogroups. Thus, Y chromosome acts as a haploid which preserve the original combinations of mutational events along male lineages as a single unambiguous haplotypes. These haplotypes can be analyzed within or between populations to study the genetic diversity as there is an increase in variability in male reproductive success in a herd (Nowak *et al.,* 1991). The knowledge of within and between breed genetic variation is mandatory for better implementation of breeding programmes and optimum utilization of animal genetic resources for conservation.

The objective of present study was to evaluate the polymorphism of Y-STR haplogroups and to understand their genetic relationship based on polymorphic analysis in different breeds of Indian buffaloes. Previous studies of haplogroup identification and genetic diversity have been primarily done on African buffalo (*Syncerus caffer*) (Hooft *et al*., 2010). No attempt has been done to explore the Y specific microsatellite haplogroup diversity in Indian buffalo breeds.

MATERIAL AND METHOD

Collection of samples

The Y-STR gene distribution in Indian buffalo populations was studied using healthy and distinct males randomly selected from five diverse breeds (Murrah, Mehsana, Jaffarabadi, Nili Ravi and Surti) located in different semen stations of India *viz*., Frozen Semen Bank (Hissar), Semen Bank Nabha (Nabha), Deep Frozen Semen Production Centre (Rishikesh), State Frozen Semen Production and Training Institute (Patan), Frozen Semen Station (Jagadhari) and Semen Station Bhopal.

DNA extraction

The standard phenol-chloroform method was used for the extraction of genomic DNA from 0.25 ml of semen sample (Sambrook *et al*., 2001). To break di sulphide bonds in sperm membrane strong antioxidant Dithiothreitol (DTT) was used. Further, the precipitation step was done using ethanol. The DNA concentration was estimated using Nanophotometer (Implen Gmbh, Munich, Germany). Working solutions of 50 ng/µL was prepared using nuclease free MQ water and stored at -20^{o}C until further analysis.

Y-STRs and Primer sequence

Three microsatellites *viz*., UMN0304, UMN1113 and INRA189, were selected on the

basis of specificity, localization on the Y-specific region in cattle, polymorphism, ease of haplotype scoring, and possibility of multiplexing the PCR reactions and the primer pairs were employed as previously described in cattle (Liu *et al*., 2003) as well as African buffalo (Hooft *et al*., 2010). The forward primer was labelled with fluorescent dye (ABI Prism primer) as shown in Table 1.

PCR reaction mixture and PCR programme

PCR reaction was performed for STRs (UMN1113, UMN0304, INRA189) by using specific set of primers (table1) in a 15 µL of final volume. The reaction mixture was prepared by mixing 1.5 µL of 10X buffer, 1.5 mM of $MgCl_2$, 200 µM dNTPs, 10 pmol of each forward and reverse primer, 1Unit of *Taq DNA polymerase*, approximately 50 ng/µL of genomic DNA as template and nuclease free water to make up the final volume up to 15 µL.

The standardized thermocycling protocol for STRs was: Initial denaturation at 95ºC for 5 minutes, followed by 35 cycles of denaturation at 94ºC for 30 seconds, annealing at 58ºC for 30 seconds, extension at 72ºC for 30 seconds and final extension at 72°C for 10 minutes.

Genotyping and Allele calling procedure

Each sample was genotyped separately for three STRs (UMN1113, UMN0304, INRA189). For each reaction, 1 µL of PCR product, 0.2 µL of LIZ 500 and 8.8 µL Hi dye was mixed to make a final volume of 10 µL. The final reaction mixtures of 96 samples were taken in a 96 wells genotyping plate for one group at a time. The plate was centrifuged at 1000 rpm for 1 minute to ensure that product is settled at bottom of the well. The plate was then subjected to denaturation at 95°C for 5 minutes following snap chilling on ice. The prepared plate was then subjected to the capillary electrophoresis on ABI 3730 instruments (Applied Biosystems, USA).

Sizing and allele calling was carried out from data generated by using GeneMapper version 3.7 software (Applied Biosystems). For allele calling the approximate size range for STRs were used as per previously reported (Table 1). The +A peaks and shutter peaks were neglected automatically by GeneMapper software and only the true alleles were sorted for each microsatellite.

Haplogroup identification and frequency distribution

The allelic sizes for each STRs from raw data of capillary electrophoresis was obtained by using GeneMapper software. Further the allelic data was analysed using GenAIEx 6.5 to find out haplotype at each microsatellite (UMN1113, UMN0304 and INRA189) respectively. Further the microsatellite data was analysed using GenAIEx 6.5 to find out the different haplogroups and their frequencies based on group of microsatellites (UMN304, UMN1113 and INRA189). Thus, considering all three microsatellites, a haplogroup (N) can be written as (N = n1, n2, n3), where n1 = (1….,13), n2 = (1….17), n3 =1; n1 = UMN1113, n2 = UMN0304, n3 = INRA189, respectively. Further, the haplogroup frequency was used to calculate the Na = No. of Different Alleles, Ne = No. of Effective Alleles = 1 / (Sum pi^2), I = Shannon's Information Index = -1* Sum (pi * Ln (pi)), uh = Unbiased Diversity = (N / (N-1)) * h. Where pi is the frequency of the ith allele for the population and Sum pi^2 is the sum of the squared population allele frequencies.

Genetic variation and distance analysis

The pairwise F_{ST} distance between different

population and Analysis of Molecular Variance (AMOVA) was performed to split total variation into within and between variation components using ARLEQUIN ver. 3.5.2.2. Further, the Extent of diversification and clusters were studied by constructing Multi dimentional scaling analysis using SPSS ver. 16.0.

RESULTS AND DISCUSSIONS

The Y-STR UMN1113, UMN0304 and INRA189 were found to have eight, ten and one alleles, respectively. Contrasting to autosomal microsatellites, various bovine Y-chromosomal microsatellites occur as multi-copies which collectively constitute a haplotype (Liu *et al*., 2003). We observed thirteen and seventeen haplotypes at the UMN1113 and UMN0304 loci, respectively.

As INRA189 had only one allele it was automatically neglected. There is no recombination, thus, haplotypes can be combined into 47 haplogroups as presented in Table 2. In African buffalo, microsatellite UMN1113, UMN0304 and INRA189 were used to formulate 18 haplogroups based on six haplotypes of UMN1113 namely 131-133 (1), 133 (2), 131-159 (3), 131-157 (4), 131-155 (5), 131-153 (6), and seven haplotypes of UMN0304 namely 213-223-225 (1), 215-225 (2), 205-213-223 (3), 205-215 (4), 205-213-221 (5), 205-215-221 (6), 213-221 (7) (Hooft *et al*., 2010). In present study haplogroups were tend to be more in number but still depicted the same trend as in African buffalo.

In present study, the breed wise distribution of haplogroup depicted that majority of haplogroups belonged to Murrah breed and least were present in Surti breed as shown in Figure 1. Breed wise distribution of haplogroup frequency showed that haplogroup number twenty-seven, thirty-one, and thirty seven were present with maximum frequency as shown in Figure 2. Further, the unbiased diversity based on individual microsatellite and haplogroup was analysed and found that overall Murrah breed was most diverse breed (0.899) in the study (Table 3) which correlated the sample collection of Murrah buffalo from different states of India.

Total diversity found in the population was 0.798 which depicted a moderately high diversity, corresponding to the diversity found in African buffalo 0.737 in KMP and 0.475 in HiP populations (Hooft *et al*., 2010). The results indicate higher genetic diversity in riverine buffalo as compared to African populations.

Using the haplogroups frequencies, the analysis of molecular variance was carried out, which showed that the variation among breeds (Murrah, Mehsana, Jaffarabadi, Nili Ravi and Surti) was highly significant at P-value (<0.0001) with percentage of variation 16.7% (Table 4). It depicted that there is significant variation.

The pair wise genetic distance analysis revealed that there is no significant difference between (Murrah and Mehsana), (Surti and Mehsana) and (Jaffarabadi and Nili Ravi). Nili Ravi and Surti were found to be genetically most distinct breeds (F_{ST} value 0.383*) as shown in Table 5. To further investigate the breed differentiation, F_{ST} values were used to perform Multidimensional scaling analysis (MDS) using SPSS software and three main clusters namely Murrah, Surti / Mehsana and Jaffarabadi / Nili Ravi were observed. Nili Ravi and Jaffarabadi breeds were found to be clustered in first quadrant, Surti and Mehsana in second quadrant and Murrah in third quadrant. Earlier MDS analysis based on autosomal microsatellite analysis in Indian buffalo breeds showed the close proximity of Mehsana, Surti and Murrah whereas

Table 1. Details of primers and annealing temperature.

Locus name/ primer name	Repeat motif in cattle	Primer sequence (5'-3')	Label	Standardized Annealing temp. (°C)	Allele size range in cattle (Lui *et al.*, 2003)
UMN0304-F	$(TAAA)_3TA(CA)_{16}$	TGATATTCACAAGGCCGCTG	VIC	58°C	210-232 bp
UMN0304-R		GGCTGTGGTATACTATGGAG			
UMN1113-F	$(CA)_{10}TA(CA)_4$	ACAGCACTTCTTAACAAAGC	NED	58°C	124-140 bp
UMN1113-R		TAGCCACACATCATGTTC			
INRA189-F	$(TG)_{22}$	TACACGCATGTCCTTGTTTCGG	FAM	58°C	148-156 bp
INRA189-R		CTCTGCATCTGTCCTGGACTGG			

Table 2. The distribution of Haplogroup among different bulls of buffalo.

Code	No. of bulls	UMN1113	UMN0304	Code	No. of bulls	UMN1113	UMN0304
1	1	10	13	24	1	6	13
2	1	1	1	25	1	6	14
3	4	11	2	26	1	6	16
4	1	11	3	27	31	6	2
5	2	12	2	28	6	6	3
6	2	12	3	29	2	6	4
7	1	13	15	30	4	6	5
8	1	2	2	31	18	6	6
9	1	3	11	32	7	6	7
10	1	3	14	33	3	6	8
11	1	3	15	34	3	6	9
12	2	3	2	35	1	7	15
13	1	3	3	36	1	7	17
14	1	3	6	37	11	7	2
15	1	3	9	38	2	7	5
16	1	4	13	39	2	7	6
17	1	4	16	40	1	7	7
18	4	4	2	41	1	8	10
19	1	4	3	42	1	8	14
20	1	4	6	43	3	8	15
21	3	5	2	44	1	8	2
22	1	6	11	45	1	9	10
23	1	6	12	46	2	9	15
				47	1	9	8

Table 3. Haplogroup diversity in Indian breeds of buffalo.

Breeds	Diversity (± standard deviation)		
	UMN0304	UMN1113	Haplogroup
Jaffarabadi	0.805±0.119	0.889±0.091	0.694±0.147
Mehsana	0.855±0.041	0.438±0.121	0.775±0.079
Murrah	0.692±0.055	0.462±0.072	0.899±0.024
Nili Ravi	0.731±0.058	0.858±0.035	0.625±0.076
Surti	0.900±0.161	0.900±0.161	0.600±0.215
Total	0.797±0.038	0.710±0.106	0.798±0.048

Table 4. Analysis of molecular variance (AMOVA) analysis.

Source of variation	Df	Sum of squares	Component of variance	Percentage of variation
Between population	4	8.940	0.07964 Va	16.69*
Within population	134	53.275	0.39758 Vb	83.31
Total	138	62.216	0.47722	
F value (P-value)		0.1669 (<0.0001)		Significant

Table 5. F_{ST} pair wise genetic distance between different Indian breeds and P value significance (+ denotes significant, - denotes non-significant).

Breeds	Murrah	Mehsana	Surti	Jaffarabadi	Nili Ravi
Murrah		-	+	+	+
Mehsana	0.02389		-	+	+
Surti	0.07868	-0.0062		+	+
Jaffarabadi	0.17802	0.2579	0.34715		-
Nili Ravi	0.22089	0.30274	0.38287	-0.0774	

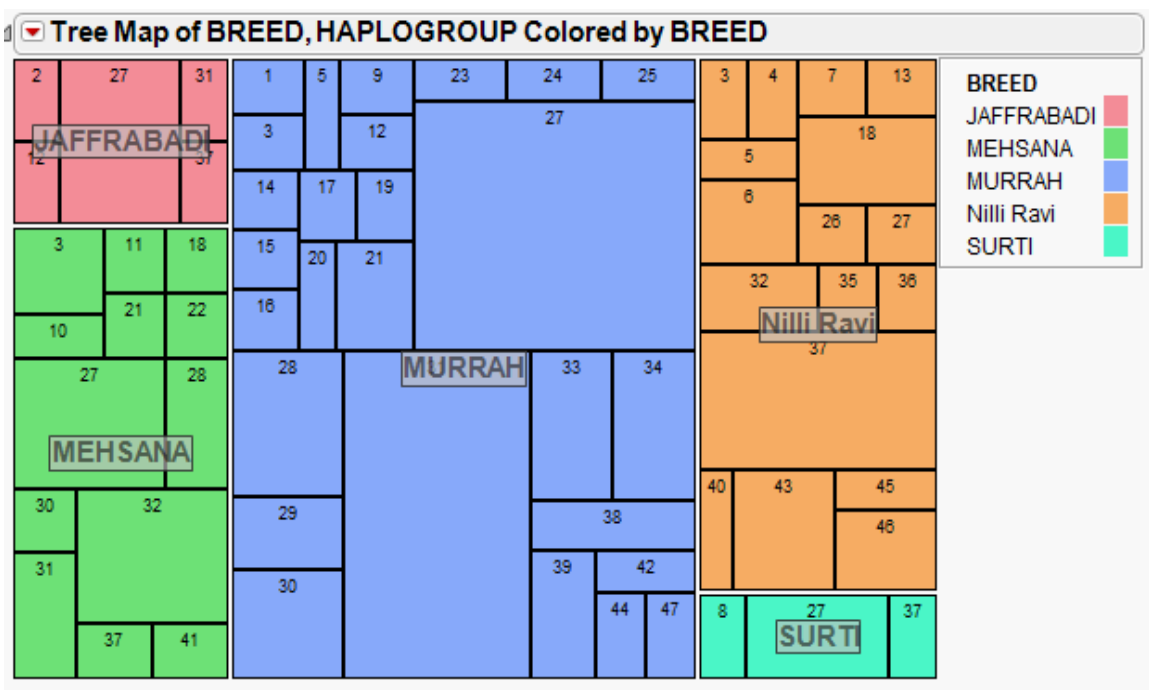

Figure 1. Breed wise distribution of Haplogroup.

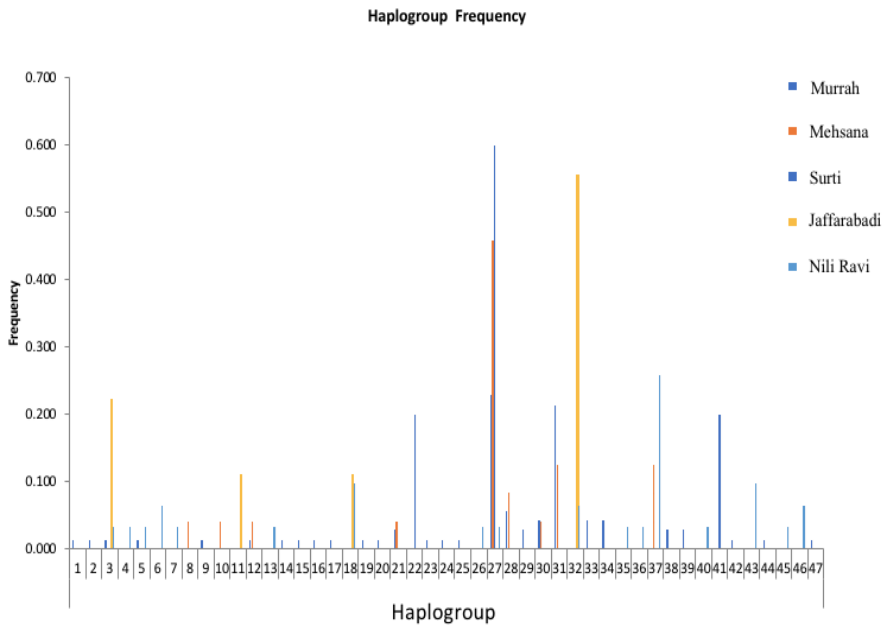

Figure 2. Breed wise distribution of Haplogroups and their frequencies.

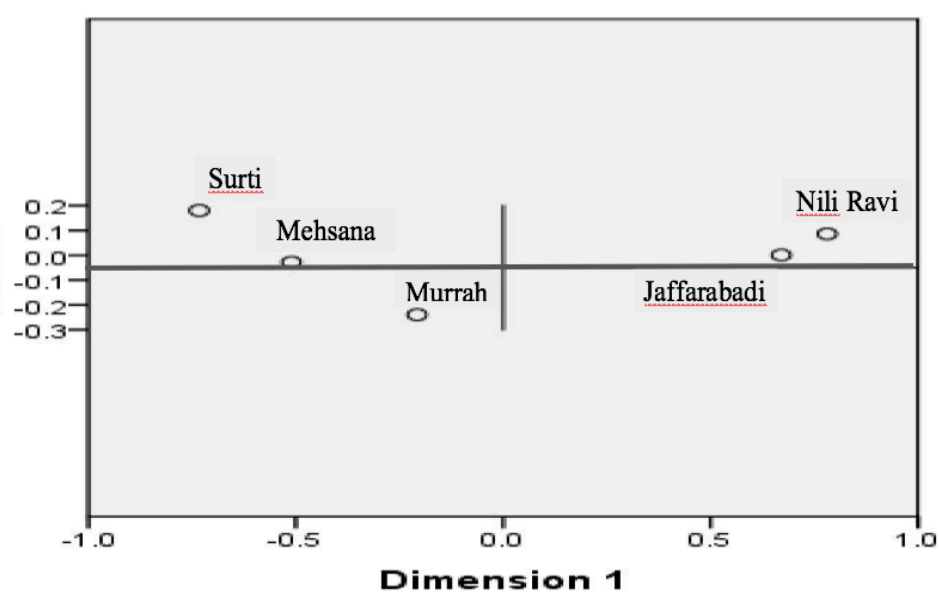

Figure 3. Multidimensional scaling plot depicting clusters of breed.

Jaffarabadi was found to form different cluster (Mishra *et al*., 2010). Genetic relationship and diversity analysis of Indian water buffalo showed that Mehsana was close to Surti (F_{ST} = 0.021) as compared to Murrah (F_{ST} = 0.10) and Jaffarabadi was distinct from Mehsana (F_{ST} = 0.131) (Vijh *et al*., 2008). In accordance with previous genetic diversity analysis, the study depicted that Mehsana was present in between Murrah and Surti. Jaffarabdi tends to form a different cluster in first quadrant as shown in Figure 3.

CONCLUSION

We have presented the first study to identify haplogroups and to assess the genetic diversity in Indian buffalo breeds using Y specific microsatellite markers. Based on the information of two locus (UMN1113 and UMN0304), we estimated 47 haplogroups among 139 unrelated buffalo bulls.

Haplogroup distribution signified that Murrah samples were collected from four different Semen stations thus the most diverse breed in population while Surti was limited to one place had least diversity. The pair wise genetic distance (F_{ST} value) between different breeds showed that Mehsana lies in between Surti and Murrah, which also signify the fact that Mehsana breed originated as cross of Murrah and Surti, while Nili Ravi and Jaffarabadi were clustered together sharing Y specific signatures. This genetic diversity analysis of Indian buffalo based on Y specific microsatellite will help in conservation prioritization and will provide an insight about Y chromosome signature shared among different Indian buffalo breeds. Our results indicated that Y-STRs at two loci had polymorphisms in Indian buffalo breeds, with interesting genetic diversity.

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