Indian J Dairy Sci 76(6): 583-587
https://doi.org/10.33785/IJDS.2023.v76i06.010

RESEARCH ARTICLE

Y-chromosome diversity in dairy bulls of Tamil Nadu

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Received: 11 December 2022 / Accepted: 06 June 2023 / Published online: 23 December 2023

Abstract: The Y-chromosome specific microsatellite studies are of particular interest as they could reveal the paternal lineage and domestication pattern among the population distributed over geographical area. As few bulls are used against large number of cows using artificial insemination approach, it is necessary to study the Y-chromosome microsatellite markers to understand the Y-chromosome diversity, paternal lineage and molecular variation among population. The present study included 431 bulls comprising *Bos taurus, Bos indicus* and crossbreds from various frozen semen stations of Tamil Nadu. The microsatellites loci *viz. UMN0103*, UMN0307, BM861, UMN0504, INRA189, INRA124, DDX3Y and UMN2008 were screened. The overall mean number of different alleles, number of effective alleles, Shannon's information index, diversity and unbiased diversity were 2.18 ± 0.16, 1.37 ± 0.06, 0.35 ± 0.04, 0.20 ± 0.02 and 0.21 ± 0.02 respectively. The analysis of molecular variance revealed that the variation within populations accounted for 66 per cent of total variation.

Keywords: Genetic diversity, Microsatellite, Paternal lineage, Y-chromosome

Introduction

Y-chromosome-specific microsatellites are of particular interest as they are haploid and paternally inherited. As there is no recombination in the Y-specific region, which makes up around 95% of the Y chromosome, the Y chromosome is inherited "en bloc" as a haplotype. Y chromosome polymorphisms have been utilized in the analysis of domesticated bovine breeds, showing new perspectives in the paternal origin and also the development of a breed (Edwards et al. 2000; Gotherstrom et al. 2005; Li et al. 2007; Kantanen et al. 2009; Ganguly et al. 2020). Y chromosome polymorphisms have been utilized in the analysis of domesticated bovine breeds, showing new perspectives in the paternal origin and also the development of a breed (Edwards et al. 2000; Gotherstrom et al. 2005; Li et al. 2007; Kantanen et al. 2009).Y chromosome is an efficient indicator for the demographic events namely domestication, migration, population expansions and population bottlenecks (Edwards et al. 2000; Ginja et al. 2010)The microsatellites residing in Y-chromosome aids in understanding and distinguishing taurine, indicine and crossbred patrilines and their introgression (Edwards et al. 2000; Giovambattista et al. 2000; Hanotte et al. 2000; Li et al. 2007).

With effective implementation of artificial insemination programme across the country, the boss taurus breeds *viz.* Jersey and Holstein Friesian have been used for extensively for crossbreeding, and upgrading Bos indicus cattle breeds. This necessitates to understand the paternal lineage and Y-chromosome diversity. As the studies pertaining to paternal lineage of cattle in India are scanty this study was undertaken to evaluate Ibos taurus, Bos indicus and crossbred population of Tamil Nadu.

Materials and methods

Diluted frozen semen samples were collected from 417 bulls of Jersey (78), Holstein Friesian (16), Crossbred Jersey (253), Crossbred Holstein Friesian (36), Kangayam (12), Red Sindhi (18) and Umblachery (4) from various frozen semen stations (Exotic Cattle Breeding Farm (ECBF), Eachenkottai; Nucleus Jersey and Stud Farm (NJF), Udhagamandalam; District Livestock Farm (DLF), Udhagamandalam and District Livestock Farm (DLF), Hosur) of Tamil Nadu. The genomic DNA was extracted from 0.5 mL of semen (0.25 mL per semen straw) by Phenol Chloroform extraction method. The isolated genomic DNA was subjected to horizontal gel electrophoresis for quality check, optical density (260/280 nm) and concentrations were calculated by using spectrophotometer (NanoDrop OneC of Thermo Scientific, USA).

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The samples with $OD_{260/280}$ ratio of 1.7 to 1.9 were considered and used further in the study.

Seven STRs namely UMN0103, UMN0307, BM861, UMN0504, INRA189, INRA124, DDX3Y and UMN2008 located Y-chromosome were screened. The primers for amplifying microsatellite loci were fluorescent-labelled with dyes *viz.* TET, FAM, ATO550 and ATO565. The details of primers, fluorescent labels and annealing temperature are furnished in the Table 1. The standardized PCR thermocycling protocol is as follow: comprised initial denaturation at 95°C for 5 min; followed by 35 cycles of denaturation at 94°C for 30 sec; annealing at 58°C and 60°C temperature for different microsatellites for 30 sec; extension at 72°C for 30 sec and final extension at 72°C for 10 min.

The PCR products were subjected to assay by multiplexing of the PCR product based on allelic range and fluorescent label, followed by capillary electrophoresis on ABI 3730 instruments (Applied Biosystems). Sizing of microsatellite fragments in the chromatogram was performed by the Peak Scanner v2.0. and GeneMapper® v6.1 software. The STR allelic data were used to ascertain frequency, Shannon's information index, unbiased diversity, Nei's genetic distance, AMOVA were analyzed using GenAIEx 6.5. The pairwise Nei's genetic distances were represented with Multidimensional scaling in two-dimensional space using SPSS v21 software package.

Results and discussion

The markers considered in this study were highly polymorphic except UMN0504. Single genotype 144 bp of UMN0504 was observed in previous study by Ginja et al. (2009) which included 13 Portuguese, three Portugal and five Brahman cattle breeds. However, Ozsensoy et al. (2014) reported three genotypes *viz.* 106, 144 and 146 bp of UMN0504 in Turkish cattle breeds. The haploid allelic frequencies of microsatellite loci are furnished in Table 2.

BM861 locus displayed two major genotypes (156 and 158 bp) of which 156 bp was observed to be fixed in *Bos indicus*. This result is in concordance with the reports by Li et al. (2009), Ginja et al. (2009), Perez-pardal et al. (2011) and Edwards et al. (2011). However, this locus was previously reported to be polymorphic in cattle breeds of Ethiopia (Li et al. 2007), Portuguese (Ginja et al. 2009), Creoles (Ginja et al. 2010), Europe (Perez-pardal et al. 2011), Turkey (Ozsensoy et al. 2014), Polish (Prusak et al. 2015) and *Bos indicus* (Ganguly et al. 2020) with genotypes 156, 158, 160 and 164 bp. The presence of 156 bp genotype in crossbred of our study might be result of using *Bos indicus* bulls for crossbreeding.

The 247 bp genotype of DDX3Y was observed to be fixed in *Bos indicus*; while it was a major genotype in Holstein Friesian, Jersey, crossbred Holstein Friesian and crossbred Jersey. But previous reports indicated that, the genotypes 245 and 249 bp were exclusively found in *Bos indicus* and *Bos taurus* breeds respectively (Ginja et al. 2009; Ginja et al. 2010; Ganguly et al. 2020).

As expected, genotype 88 bp was observed in *Bos indicus* breeds while presence of the same genotype in crossbred was unlooked. Upon karyotyping samples of crossbred bulls possessing 88 bp genotypes revealed acrocentric Y-chromosome in one sample. The underlying reason might be due to fortuitous mating or

Table 1 Details of primers used for amplification of Y-chromosome specific microsatellites

S. No	Microsatellite	Repeat motif	Primer sequence (5'-3')	FL	AT(°C)
Panel 1					
1	BM861	$(GT)_6C(TG)_{10}$	Forward: TTG AGC CAC CTG GAA AGC	TET	60°C
			Reverse: CAA GCG GTT GGT TCA GAT G		
2	DDX3Y	$(TA)_9(TC)_9$	Forward: TGA ACC ACT AGG GAG GTC ATC	FAM	60°C
			Reverse: TTC CAA TTT AGC TGT GGT TAT CTG		
3	INRA124	$(GT)_4A(TG)_9$	Forward: GAT CTT TGC AAC TGG TTT G	FAM	60°C
			Reverse: CAG GAC ACA GGT CTG ACA ATG		
4	INRA189	$(TG)_{22}$	Forward: TAC ACG CAT GTC CTT GTT TCG G	FAM	60°C
			Reverse: CTC TGC ATC TGT CCT GGA CTG G		
Panel 2					
5	UMN0103	$(CA)_{22}$	Forward: ACA CAG AGT ATT CAC CTG AG	TET	58°C
			Reverse: ATT TAC CTG GGT CAA AGC AC		
6	UMN0307	$(CA)_{18}$	Forward: GAT ACA GCT GAG TGA CTA AC	ATO550	58°C
			Reverse: GTG CAG ACA TCT GAG CTG TG		
7	UMN0504	$(CT)_2GT(CT)_3(GT)_2$	Forward: AGG CCA TCT GCA TAG TGA AG	FAM	58°C
			Reverse: TGC TGG ACT GCT CAT CTC TG		
8	UMN2008	$(CA)_2GA(CA)_{11}G(CA)_3(TG)_{17}$	Forward: CAA GCA TAT CAG TGG CCT GG	ATO565	58°C
			Reverse: GCT GCA AGG AAA CTA TTT CA		

AT: Annealing temperature (°C); FL: Fluorescent Label

artificial insemination with bulls having *Bos indicus* lineage. The frequency of 104 bp genotype and 100 bp were previously reported in Turkish (Ozsensoy et al. 2014), Creole (Ginja et al. 2010), Portuguese (Ginja et al. 2009), European (Perez-pardal et al. 2011) cattle breeds. However, in this study, these genotypes were exclusive to Holstein Friesian, Jersey, crossbred Holstein Friesian and crossbred Jersey and thus testifies the taurine paternal lineage.

Supported by previous finding of Perez-pardal et al. (2011), microsatellite UMN0103 displayed two loci in *Bos indicus* breeds as expected while single locus in Holstein Friesian, Jersey, crossbred Holstein Friesian and crossbred Jersey. The two loci were separated and named as UMN0103a and UMN0103b for ease of analysis. The two allelic combinations found in this study *viz.* 116/124 bp and 114/124 bp were supported by findings of Ginja et al. (2009), Ginja et al. (2010), Ganguly et al. (2020).

Table 2 Haploid allele frequencies of Y-chromosome specific microsatellite markers in various genetic groups of cattle

Locus	Allele size (bp)	Jersey (n=75)	Holstein Friesian (n=16)	Crossbred Jersey (n=264)	Crossbred Holstein Friesian (n=29)	Kangayam (n=12)	Red Sindhi (n=18)
BM861	152	-	-	0.004	-	-	-
	154	-	-	0.004	-	-	-
	156	0.080	-	0.452	0.069	1.000	1.000
	158	0.920	1.000	0.540	0.931	-	-
DDX3Y	247	0.773	0.813	0.818	0.862	1.000	1.000
	249	0.227	0.187	0.182	0.138	-	-
INRA	88	-	-	0.049	-	1.000	1.000
189	100	-	0.250	0.012	0.517	-	-
	104	0.840	0.688	0.871	0.483	-	-
	106	0.160	0.062	0.068	-	-	-
UMN010	114	-	-	0.023	-	0.417	0.833
3a	116	-	-	0.133	0.069	0.500	0.167
	124	0.067	1.000	0.394	0.897	-	-
	126	-	-	0.011	-	-	-
	128	0.933	-	0.439	0.034	0.083	
UMN010	124	0.067	1.000	0.545	0.966	0.917	1.000
3b	126	-	-	0.012	-	-	-
	128	0.933	-	0.443	0.034	0.083	-
UMN030	145	-	-	0.004	-	-	-
7	147	0.013	-	0.004	-	-	-
	149	0.013	-	-	-	-	-
	151	0.014	-	0.030	-	-	-
	153	0.960	-	0.792	-	0.167	0.889
	155	-	-	0.008	-	0.833	0.111
	157	-	-	0.010	-	-	-
	159	-	1.000	0.152	1.000	-	-
UMN050	144	1.000	1.000	1.000	1.000	1.000	1.000
4							
INRA124	130	0.040		0.155		0.583	0.556
	132	0.960	1.000	0.845	1.000	0.417	0.444
UMN200	134	0.067	-	0.030	-	-	-
8a	136	-	-	0.008	-	-	-
	140	0.933	1.000	0.932	1.000	0.667	0.889
	144	-	-	0.011	-	-	-
	146	-	-	0.019	-	0.250	0.111
	148	-	-	-	-	0.083	-
UMN200	136	-	-	0.008	-	-	-
8b	140	0.853	0.813	0.515	0.690	0.084	0.223
	144	0.040	-	0.034	-	-	-
	146	0.014	0.187	0.125	0.034	0.583	0.444
	148	0.093	-	0.318	0.276	0.333	0.333

However, the two loci of UMN0103 observed in crossbred cattle (crossbred Holstein Friesian and crossbred Jersey) might be due to deployment of bulls with indicine paternal lineage for crossbreeding programme.

Among two genotypes 130 and 132 bp observed in INRA124, former was major genotype in *Bos indicus* breeds; while the latter was major genotype in *Bos taurus* and crossbred cattle. However, 130 bp genotype was reported to be exclusive to *Bos indicus* and North-Ethiopian cattle breeds (Ginja et al. 2009; Ginja et al. 2010; Prusak et al. 2015; Ganguly et al. 2020). In the same studies mentioned, genotype 132 was observed in bulls with taurine lineage.

UMN2008 displayed two loci as expected, because of its location in pseudo autosomal region of Y-chromosome (Stafuzza et al. 2009). The two loci were separated and renamed as UMN2008a and UMN2008b for ease of analyses. The genotypes were observed in combination *viz.* 140/148, 140/144, 140/146, 134/140, 136/136 bp and 140/140. The genotypes identified are in concordance with previously reported genotypes by Alyethodi et al. (2016).

The overall mean number of different alleles, number of effective alleles, Shannon's information index, diversity and unbiased diversity across the breeding sires of six genetic groups maintained in the organized farms of Tamil Nadu were 2.18 ± 0.16, 1.37 ± 0.06, 0.35 ± 0.04, 0.20 ± 0.02 and 0.21 ± 0.02 respectively.

Y-chromosome based genetic studies on populations which included both *Bos taurus* and *Bos indicus* breeds revealed overall mean diversity index of 0.75 (Li et al. 2007), 0.20 (Ginja et al. 2009), 0.42 (Edwards et al. 2011) and 0.11 (Prusak et al. 2015). Though diversity index varies with respect to the population under study, the diversity of cattle population considered in this study was substantially low (0.21 ± 0.02). Ganguly et al. (2020) reported diversity index of 0.485 and zero in Red Sindhi and Kangayam cattle breeds respectively. However, in this study substantial amount of diversity was observed in Kangayam (0.36 ± 0.08) and Red Sindhi (0.19 ± 0.78) breeds. Studies reported relatively zero (Ginja et al. 2009; Ginja et al. 2010) and 0.021 (Perez-pardal et al. 2011) diversity in Holstein Friesian breed. Similar diversity index was observed in Holstein Friesian population in this study (0.11 ± 0.06). Diversity in Jersey breed was observed to be 0.16 ± 0.34, which is in concordance with the report by Ginja et al. (2009).

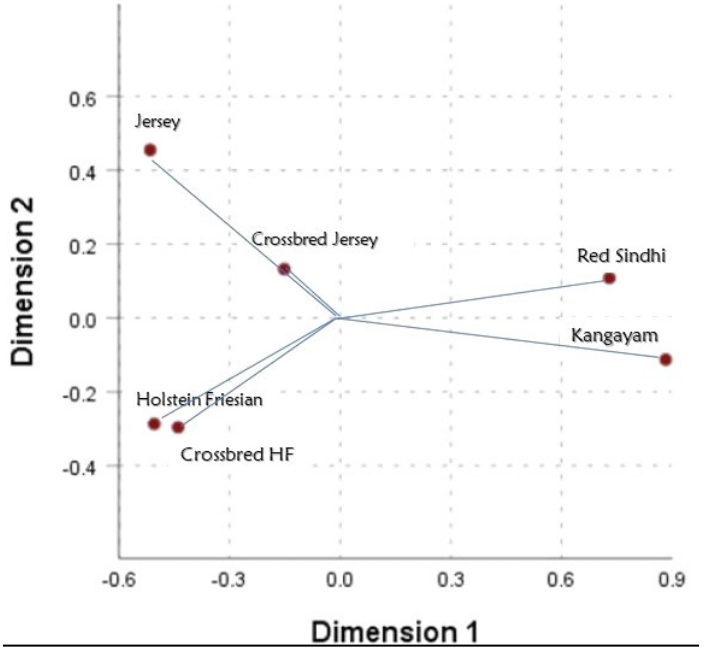

Fig. 1 Multi-dimensional scaling plot for various genetic groups based on Nei's genetic distances estimated from microsatellite data

Table 3 Nei's genetic identity (above diagonal) and genetic distance (below diagonal) based on microsatellite alleles across various genetic groups

Genetic group	Jersey	Holstein Friesian	Crossbred Jersey	Crossbred Holstein Friesian	Kangayam	Red Sindhi
Jersey	-	0.680	0.915	0.671	0.443	0.515
Holstein Friesian	0.386	-	0.810	0.985	0.495	0.516
Crossbred Jersey	0.089	0.211	-	0.811	0.648	0.712
Crossbred Holstein Friesian	0.399	0.015	0.210	-	0.522	0.542
Kangayam	0.815	0.704	0.434	0.649	-	0.898
Red Sindhi	0.664	0.662	0.340	0.613	0.107	-

Analysis of molecular variance revealed that the variation within populations accounted for 66 per cent, whereas the variation among populations accounted for 34 per cent of total genetic variation. The mutli-dimensional scaling plot depicting the relative genetic distances between populations considered in this study is presented in Figure 1. Nei's genetic distances of Holstein Friesian, crossbred Holstein Friesian, Jersey, crossbred Jersey, Kangayam and Red Sindhi cattle were established based on the allelic frequency of the microsatellite loci (Table 3) revealed shortest genetic distance (0.107) between Holstein Friesian and crossbred Holstein Friesian depicting common paternal lineage between them. The longest genetic distance was observed between Jersey and Kangayam (0.815) representing colossal distance between *Bos indicus* and *Bos taurus* paternal lineage. The shorter distances between Holstein Friesian and crossbred Holstein Friesian, Jersey and crossbred Jersey, and Kangayam and Red Sindhi indicated different clusters with common Y-chromosomal microsatellite allelic composition.

Conclusions

To conclude, the UMN0504 locus was monomorphic with 144 bp genotype. The genotypes 156, 247 and 130 bp of BM861, DDX3Y and INRA124 respectively, were observed in higher frequencies in Kangayam and Red Sindhi. The UMN0103 and UMN2008 microsatellites showed two loci each. The presence of two loci for UMN0103 was specific to *Bos indicus* lineage; while UMN0103 was confined to single locus in *Bos taurus* lineage. The 114 and 116 bp genotypes of UMN0103 were observed in combination with 124, 126 and 128 bp genotypes. 88 bp genotype of INRA189, a *Bos indicus* specific genotype was also observed in crossbred Jersey along with Kangyama and Red Sindhi. Based on microsatellite allelic composition the study revealed three distinct clusters; with the *Bos indicus* breeds (Kangayam and Red Sindhi) in one, Holstein Friesian and its crossbred forming the other, and Jersey and its crossbred grouped in another; showing common paternal lineage among them.

Acknowledgements

Authors are thankful to the Commissioner and Director of Animal Husbandary and Veterinary Services and the Managing Director, Aavin illam, TCMPF Ltd. for proving the data pertaining to semen production and frozen semen samples of bulls to carry out the research work.

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