

Genetic Variability of Guzerat and Senepol Bovine Breeds by Microsatellite Markers

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ABSTRACT

Bovine production plays economic importance in Brazil and Guzerat and Senepol breeds are producer of meat. It was aimed to analyze the genetic variability of Guzerat and Senepol breeds by microsatellite markers. The breeds were collected and genotyped for ten microsatellite loci by automatic sequencer and statistically analysed. A total of 53 alleles were observed being the average number was 5.3 in both breeds. The effective numbers of alleles were 3.36 for Guzerat and 3.11 for Senepol cattle. The Shannon indexes were 1.36 for Guzerat and 1.26 for Senepol cattle. The expected heterozygosity were 0.71 and PIC values were 0.64 in both breeds. The F_{IS} were 0.01 and 0.11 for Guzerat and Senepol breeds, respectively and Hardy-Weinberg equilibrium were $P > 0.05$ for Guzerat and $P < 0.05$ for Senepol cattle. The combined discrimination powers were 0.99 in both breeds and combined exclusion powers (PE1) were 0.99 in both breeds and combined exclusion powers (PE2) were 0.96 and 0.95 for Guzerat and Senepol breeds respectively. There is genetic variability in both breed, but there are evidences of inbreeding enabling genetic drift and should be necessary to use major number of microsatellite loci to analyze with high efficiency the exclusion power (PE2).

KEYWORDS: Cattle, STRs, Diversity, Livestock conservation.

Variabilidade Genética de Bovinos das Raças Guzerá e Senepol por Marcadores Microsatélites

RESUMO

A bovinocultura desempenha função importante na economia Brasileira e as raças Guzerá e Senepol são produtoras de carne. Objetivou-se analisar as variabilidades genéticas das raças Guzerá e Senepol através dos marcadores microsatélites. Os animais foram coletados e genotipados para dez *loci* microsatélites em um sequenciador automático de DNA e analisado estatisticamente. Um total de 53 alelos foi observado com número médio de 3,36 para Guzerá e 3,11 para a raça Senepol. Os índices de Shannon foram 1,36 para Guzerá e 1,26 para raça Senepol. As heteroziosidades esperadas foram 0,71 e os valores de PIC foram 0,64 em ambas as raças. Os valores de F_{IS} foram 0,01 e 0,11 para Guzerá e Senepol, respectivamente e as proporções de Hardy-Weinberg foram não significativas em Guzerá ($P > 0,05$) e significativas em Senepol ($P < 0,05$). Os poderes de discriminação combinados foram 0,99 em ambas as raças e os poderes de exclusões combinados (PE1) foram 0,99 em ambas as raças e os poderes de exclusão combinados (PE2) foram 0,96 e 0,95 em Guzerá e Senepol, respectivamente. Existe variabilidade genética em ambas as raças, mas existem evidências de endocruzamentos por consequência da deriva genética e seria necessário avaliar um maior número de *loci* microsatélites para aumentar a eficiência no poder de exclusão (PE2).

PALAVRAS-CHAVE: Gado, STRs, Diversidade, Conservação animal.

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INTRODUCTION

Bovine livestock in Brazil is a factor of the great economy importance, principally the beef cattle. Thus, Brazil is the country that has a biggest production of bovine livestock in the world. The official number of bovines that has been registered in Brazil by “Pesquisa da Pecuária Municipal” of “Instituto Brasileiro de Geografia e Estatística” (IBGE, 2011) is about 212 million of animals. This number is considering animals used for both meat and dairy production.

Genetic variability of resources it is, actually, a world preoccupation to future for next generations, principally for genetic resources which serve as a food, both animal and vegetable origin. Moreover, the importance to maintain the genetic background as genetic variability source may to contribute with preservations of breeds and in improvement animal programs (Silva Filho et al., 2007).

With advancement of molecular biology has been very importance to study the genetic variability, through many tools. Thus, several studies about genetic variability in Brazilian bovines were made using differences tools, from RAPD (Serrano et al., 2004) to microsatellite markers (Egito et al., 2007). Actually, many papers are being published in differences sciences journals disseminating the current genetic structure of some Brazilian bovine breeds using Microsatellites markers (Carneiro et al., 2007).

Microsatellite DNA is a class within of the repetitive sequences of DNA in eukaryote genome and it is better known among markers. They are sequences of genome that consist of repeats from one to nine nucleotides in many copies in *tanden* found in vertebrates, insects, fungi and vegetables (Charlesworth et al., 1994). Historically, the microsatellite term is applied in repetitions with two nucleotides (CA) and (GT) and various others names are used to describe these repetitive sequences in *tanden* as Simple Sequence Repeat (SSRs) or Short Tandem Repeat (STRs) (Edwards et al., 1991).

Recently, microsatellites markers are being

applied a lot in conservations studies of various organisms, from vegetable to animals. There are several advantage to use the microsatellite markers in detriment others molecular markers. Initially, microsatellites were applied in mapping genomic project (Weissenbach et al., 1992). It has been extensively used in association analyses of genes to disease susceptible (Rubinsztein 1999) and demonstrated extreme efficiency in paternity analyses of individuals (Edwards et al., 1992) and populations studies (Paetkau et al., 1995). Variation in microsatellite is used in hybridizations studies between correlated species (Roy et al., 1994). Therefore, it was aimed to analyze the genetic variability of two bovine breeds produced in Brazil, Senepol and Guzerat breeds, being a taurine and a zebuine respectively, utilizing microsatellite markers used in paternity exams routine recommend by International Society of Animal Genetics (ISAG).

MATERIALS AND METHODS

A total of 58 animals were casually selected from two differences Brazilian herd, being 29 animals of Senepol breed (Taurine) from a farm in Uberlândia town of Minas Gerais State and 29 animals of Guzerat breed (Zebuine) from a farm in Imperatriz town of Maranhão State. There were collected 5 mL of blood tissue by vacutainer blood collection and after they were conserved on freezer at -20°C to extraction of DNA. Extraction of DNA was realized by Phenol:Chloroform:Isoamyl Alcohol (25:24:1) method Sambrook et al., (1989). A set of ten *loci* of microsatellite markers were selected from *loci* used to paternity exam routine in bovine recommended by International Society of Animal Genetics (ISAG): BM1818, BM1824, BM2113, ETH10, ETH225, ETH3, INRA023, SPS115, TGLA122 and TGLA126 *loci*.

PCR reactions were realized in Multiplex System for a final volume of 15 µL under the following conditions: Promega's STR 10X

Buffer (1X); MgCl₂ (1.5 mmol); every forward primer were marked using fluorescent dyes (FAM, HEX or TAMRA) (1-4 pmol) and reverse primer unlabeled (1-4 pmol); Platinum Taq DNA polymerase® (Invitrogen, USA) (0.5 unit) and genomic DNA (100 ng/μL). PCR reactions were in a Mastercycler PCR Thermocycler (Eppendorf, Germany) under the following conditions: hot start at 94°C for 4 min; 30 cycles of 94°C for 1 min, 58°C for 1 min and 72°C for 1 min; final extension temperature at 72°C for 4 min. Products of PCR were separated and detected in the ABI Prism® 3100 Genetic Analyzer (Applied Biosystems) following the manufacturer's standard protocol. Data was analyzed with GeneScan® v3.7 and Genotyper® v3.7 software (Applied Biosystems).

POPGENE 1.32 software (Yeh et al., 1999) was used to determine the allelic frequencies per *locus* as well as some diversity indexes as: Number of observed alleles (*Na*) per *locus*; number of effective alleles (*Ne*) per *locus* and Shannon index (*SI*). Expected heterozygosity (*He*) per *locus* was estimated according to Ney and Roychoudhury (1974). Polymorphism Informative Content (*PIC*) was estimated according to Botstein et al. (1980).

A exact test was used to determine possible deviations of Hardy-Weinberg equilibrium (HWE) and existence of random associations of genotypes applying Markovian Chain method as well as it was determined the inbreeding coefficient (*F_{IS}*) (Weir and Cocker,

1984) within of each population using GENEPOP 3.1d software (Raymond and Rousset, 1995).

Power of discrimination (*PD*) per *locus* was estimated according to Kloosterman et al. (1993). Probability of exclusion (*PE*) was determined in two situations: (*PE1*), estimated by 1a equation, when the genotypes of matrix, offspring and bull are compared, this equation is appropriated when the matrix is confirmed as genitor at time of birth and (*PE2*), estimated by 2a equation, when at least one parental genotype not available (Jamieson and Taylor, 1997). Values per *locus* of *PD*, *PE1* and *PE2* were combined (*PDC* and *PEC*) to all *loci* evaluated (*k loci*) according to Fisher (1951).

RESULTS AND DISCUSSION

A total of 53 alleles were observed for ten *loci* analyzed in both breeds. However, the less polymorphism *loci* were BM1824 and INRA23, in the Guzerat and Senepol breeds, respectively, while the most polymorphism *loci* were BM1818 in Guzerat breed and BM2113 and TGLA126 in Senepol breed. The average proportion of effective allele in relation to observed allele was about 0.60 in the two breeds. Therefore, TGLA126 *locus* was the great proportion of effective allele in relation to observed allele (0.87) in Guzerat breed, while BM1818 (0.77) and BM1824 (0.77) *loci* were in Senepol breed (Tab. 1 and 2).

Table 1. Parameters of diversity in Guzerat breed.

<i>Loci</i>	<i>Na</i> ^a	<i>Ne</i> ^b	<i>SI</i> ^c	<i>He</i> ^d	<i>PIC</i> ^e	<i>Fis</i> ^f	<i>PHWE</i> ^g
BM1818	7	3.68	1.57	0.75	0.70	0.25	0.07
BM1824	3	2.16	0.91	0.56	0.48	-0.05	1.00
BM2113	6	4.75	1.65	0.82	0.76	0.02	0.88
ETH10	5	2.59	1.17	0.64	0.56	-0.05	0.15
ETH225	6	4.00	1.56	0.78	0.72	-0.01	0.48
ETH3	5	3.40	1.36	0.73	0.65	0.09	0.92
INRA23	6	2.07	1.08	0.53	0.49	-0.01	0.11
SPS115	5	3.86	1.41	0.77	0.69	-0.13	0.81
TGLA122	6	3.64	1.54	0.75	0.70	0.06	0.51

TGLA126	4	3.48	1.30	0.74	0.66	-0.10	0.44
Average	5.30	3.36	1.36	0.71	0.64	0.01	0.25 ⁱ
Sd ^h	1.16	0.85	0.24	0.10	0.10	0.11	

^aNumber of observed alleles, ^bnumber of effective alleles, ^cShannon index, ^dexpectedheterozygosity, ^epolymorphism informative contend, ^fdeficit of heteroziosity, ^gProbability for Hardy-Weinberg equilibrium, ^hstandard deviation and ⁱprobability for all *loci*.

Table 2. Parameters of diversity in Senepol breed.

<i>Loci</i>	<i>Na</i> ^a	<i>Ne</i> ^b	<i>SI</i> ^c	<i>He</i> ^d	<i>PIC</i> ^e	<i>Fis</i> ^f	<i>PHWE</i> ^g
BM1818	5	3.84	1.45	0.76	0.69	0.27	0.04
BM1824	4	2.18	0.96	0.56	0.49	0.62	0.39
BM2113	7	5.22	1.73	0.84	0.78	-0.13	0.26
ETH10	5	3.83	1.47	0.77	0.70	-0.01	0.26
ETH225	5	2.16	1.10	0.55	0.50	0.31	0.00
ETH3	5	3.37	1.33	0.73	0.66	-0.26	0.04
INRA23	3	1.42	0.57	0.32	0.29	0.33	0.02
SPS115	6	2.57	1.21	0.64	0.58	0.00	0.55
TGLA122	6	3.05	1.31	0.69	0.61	-0.16	0.70
TGLA126	7	3.48	1.46	0.74	0.67	0.10	0.37
Average	5.30	3.11	1.26	0.71	0.64	0.11	0.00 ⁱ
Sd ^h	1.25	1.08	0.32	0.15	0.14	0.27	

^aNumber of observed alleles, ^bnumber of effective alleles, ^cShannon index, ^dexpectedheterozygosity, ^epolymorphism informative contend, ^fdeficit of heteroziosity, ^gProbability for Hardy-Weinberg equilibrium, ^hstandard deviation and ⁱprobability for all *loci*.

Average Shannon index (*SI*) were over 1 in the two breeds. The expected heterozygosity varied from 0.53 to 0.82 in Guzerat breed, while Senepol breed varied from 0.32 to 0.84 and the average heterozygosity were over 0.70 in two breeds. The values of *PIC* varied from 0.48 to 0.76 in Guzerat breed, while Senepol varied from 0.29 to 0.78 and the average heterozygosis were over 0.60 in both breeds (Tab. 1 and 2).

The inbreeding coefficients (*F_{IS}*) varied from -0.10 to 0.25 in Guzerat breed, while Senepol breed varied from -0.26 to 0.62. All breeds had positive average heterozygosis deficit indicating presence of consanguine breeding. Nothing *locus* had significant ($P > 0.05$)

deviation from HW equilibrium in Guzerat breed, while Senepol breed had some *loci* with significant deviations from HWE ($P < 0.05$) (Tab. 1 and 2).

The values of *PD* parameter varied from 0.70 to 0.90 and combined of 0.99 in Guzerat breed, while Senepol breed varied from 0.43 to 0.89 and combined of 0.99. The *PE1* values varied from 0.28 to 0.58 and combined of 0.99 in Guzerat breed, while Senepol breed varied from 0.16 to 0.62 and with combined of 0.99. The *PE2* values varied from 0.14 to 0.35 and with combined of 0.96, while Senepol breed varied from 0.05 to 0.45 and combined of 0.95 (Tab. 3).

Table 3. Analyses of discrimination and exclusion powers.

<i>Loci</i>	Guzerat breed			Senepol breed		
	PD	PE1	PE2	PD	PE1	PE2
BM1818	0.81	0.52	0.33	0.87	0.50	0.33
BM1824	0.72	0.28	0.14	0.72	0.30	0.15
BM2113	0.90	0.58	0.41	0.89	0.62	0.45
ETH10	0.74	0.36	0.20	0.87	0.52	0.34

ETH225	0.80	0.53	0.35	0.66	0.33	0.16
ETH3	0.87	0.45	0.28	0.78	0.46	0.29
INRA23	0.70	0.32	0.15	0.43	0.16	0.05
SPS115	0.86	0.49	0.32	0.79	0.39	0.22
TGLA122	0.87	0.52	0.33	0.80	0.42	0.26
TGLA126	0.83	0.45	0.28	0.85	0.48	0.31
Combined	0.99	0.99	0.96	0.99	0.99	0.95

Comparing the polymorphism with those observed in some European breeds (Kantanem et al., 2000), they were very similar and compared with Brazilian breeds as: Nelore that was more polymorphic than this work (Cevini et al., 2006; Carneiro et al., 2007). *O_{SI}* indicated high genetic variability (over 1.0) as affirmed by Fatima et al. (2008) evaluating goats breeds and by Arif et al. (2010) evaluating antelope breeds using microsatellite markers. Average expected heterozygosities and average *PIC* indicated high genetic variability in both breeds as compared with the taurine and zebuine breeds created in Brazil, including the Guzerat breed (Egito et al., 2007). Same conditions were observed by Carneiro et al. (2007) analyzing Nelore breed on Amazon conditions. The deficits of heterozygosity were literally same those observed in some breeds as Harijana and Hissar (Rehman and Khan, 2009), Brown Swiss breed (Dalvit et al., 2008), Hallikar cattle (Shekar et al., 2011) and taurine and zebuine breeds from Brazil (Egito et al., 2007). Therefore, some breeds were not observed deficits of heterozygosity as Kangayam cattle (Karthickeyan et al., 2009) and Burlina and Hostein Frisian breeds (Davit et al., 2008). Although all *loci* have showed considered variability, they were viable for analyses of discriminations as those observed in the Nelore breed (Carneiro et al., 2007) and Yugoslav Pied cattle (Stevanovic et al., 2010), but should be necessary to extend the number of *loci* to have more efficiency in paternity analyses (Silva Filho et al., 2007).

CONCLUSION

All breeds analyzed indicated considerable genetic variability using 11 microsatellite

loci, but the deficit of heterozygosity may be due to consanguine breeding within each breed being necessary the use of molecular markers assisted selection (MAS) by animal improvement program and to use more microsatellite *loci* in these breeds for better efficiency in paternity analyses.

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