Journal of Animal and Veterinary Advances 7 (11): 1499-1502, 2008
ISSN: 1680-5593

Genetic Diversity in Buffalo Population of Guilan Using Microsatellite Markers

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Abstract: Genetic Structure of Guilan buffalo population, in the South and South western area of Caspian Sea in Iran, was characterized, using 14 microsatellite markers (CSSM019, CSSM029, CSSM033, CSSM038, CSSM041, CSSM043, CSSM047, CSRM060, CSSM061, CSSM062, CSSM070, BMC1013, BRN and ETH003). The average of observed and effective number of alleles across all loci was found to be 4.14 and 3.17, respectively. CSSM062 was the most polymorphic markers according to its effective number of alleles (4.69), expected heterozygosity (0.79) and Polymorphism information content (0.75). However, CSSM029 showed the lowest effective number of alleles (1.20), expected heterozygosity (0.50) and Polymorphism information content (0.37). Results showed that these markers were suitable in population genetics researches. The mean of observed and expected heterozygosity, Polymorphism information content and Shannon information index were equal to 0.90, 0.67, 0.61 and 1.22, respectively across all loci in the population. It was concluded that a high degree of genetic diversity exist in the Guilan buffalo populations.

Key words: Genetic diversity, microsatellite, polymorphism, buffalo

INTRODUCTION

Guilan is one of the provinces of Iran and locate in the South and South western area of Caspian Sea. The buffalo population of Guilan kept under extensive system of production. They were domesticating during the long period of time. Domestic animals are product of selection, improvement and domestication processes and they have also, undergone the effects of genetic drift, mutation and artificial selection (Giacomoni *et al*., 2008). The domesticated breeds are part of biodiversity. Therefore, the preservation and conservation of these breeds are important. Appropriate management, conservation in development programs and biological and local research are some ways to preserve these local breeds, in order to maintain their genetic characteristics as part of a breeding system (Giacomoni *et al*., 2008). The use of microsatellites in population genetics has so far been mainly reported in buffalo populations (Zhang *et al*., 2007; Kumar *et al*., 2006; Vanhooft *et al*., 2002). There is a considerable potential for using such polymorphic tools in this kind of analysis. In this study, genetic structure of Guilan buffalo population was evaluated by considering the individual multilocus genotype.

MATERIALS AND METHODS

Sampling: A total of 60 blood samples were collected from different area of Guilan province. Blood samples were taken from the Jugular vein and store at -20°C before examination. Buffalo were sampled according to, the size of the herds, geographical distance and distribution.

DNA isolation: Genomic DNA was extracted from 5 mL of the whole blood samples, using the modified salting out method (Miller *et al*., 1988). Agarose gel and spectrophotometer were used to determine the qualification and quantification of DNA. Extracted DNA was diluted in TE (Tris Hcl 10 mM, Na_2EDTA 0.2 mM, pH = 7.5) and the concentration was adjusted to 50 ng μL^{-1}.

Microsatellite selection and amplification: Fourteen microsatellite markers were chosen from joint international society of animal genetics and FAO working group for biodiversity study (Hoffmann *et al*., 2004). Information on the 14 microsatellites investigated is presented in Table 1 (Hoffmann *et al*., 2004). The PCR reactions were conducted in a 15 μL reaction mixture, which included

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Table 1: Characterizations of microsatellite used in the analysis

Marker	Chromosome	Primer sequence (5'-3') forward and reverse	Annealing temperature (°C)	Gene bank (Accession number) or citation
CSSM019	1	TTGTCAGCAACTTCTTGTATCTTT		
		TGTTTTAAGCCACCCAATTATTTG	55	U03794
CSSM029	9	CGTGAGAACCGAAAGTCACACATTC		
		GCTCCATTATGCACATGCCATGCT	55	U03807
CSSM033	17	CACTGTGAATGCATGTGTGTGAGC		
		CCCATGATAAGAGTGCAGATGACT	65	U03805
CSSM038	11	TTCATATAAGCAGTTTATAAACGC		
		ATAGGATCTGGTAACTTACAGATG	55	U03817
CSSM041	21	AATTTCAAAGAACCGTTACACAGC		
		AAGGGACTTGCAGGGACTAAAACA	55	U03816
CSSM043	1	AAAACTCTGGGAACTTGAAAACTA		
		GTTACAAATTTAAGAGACAGAGTT	55	U03824
CSSM047	3	TCTCTGTCTCTATCACTATATGGC		
		CTGGGCACCTGAAACTATCATCAT	55	U03821
CSRM060	11	AAGATGTGATCCAAGAGAGAGGCA		
		AGGACCAGATCGTGAAAGGCATAG	60	AF232758
CSSM061	8	AGGCCATATAGGAGGCAAGCTTAC		
		TTCAGAAGAGGGCAGAGAATACAC	60	(Barker *et al.*, 1997)
CSSM062	9	GTTTAAACCCCAGATTCTCCCTTG		
		AGATGTAACAGCATCATGACTGAA	55	(Barker *et al.*, 1997)
CSSME070	3	TTCTAACAGCTGTCACTCAGGC		
		ATACAGATTAAATACCCACCTG	55	AF303223
BMC1013	3	AAAAATGATGCCAACCAAATT		
		TAGGTAGTGTTCCTTATTTCTCTGG	54	G18560
BRN	11	CCTCCACACAGGCTTCTCTGACTT		
		CCTAACTTGCTTGAGTTATTGCCC	60	X59767
ETH003	3	GAACCTGCCTCTCCTGCATTGG		
		ACTCTGCCTGTGGCCAAGTAGG	65	Z22744

0.25 μM of each primer, 200 μM deoxynucleoside triphosphate, 1.5-3 mM MgCl2, 1 unit of Taq DNA polymerase, 1X PCR buffer and approximately 150 ng of genomic DNA as a template. Optimum PCR amplification conditions were determined for each marker separately. To visualize the Amplified products, they were electrophoresed on denaturing Acrylamid gels. Gels were scanned by GELDOC XR BIORAD. The Gelpro Analyser software (Media Cybernetics), version 3.1, was used to determine the amplified fragment length and assign genotypes to each animal.

Data analysis: In preliminary, genetic analysis, allele number (N_a) and frequencies were determined by direct counting. Effective number of allele (N_e) was calculated using the following formula (Hedrick, 2000).

$$N_e = \frac{1}{\sum_{i=1}^{n} P_i^2}$$

Observed heterozygosity (H_o) was calculated as the proportion of total heterozygous individuals. Expected (H_e) heterozygosity and Shannon information index (I) were estimated as the following formula, respectively (Hedrick, 2000; Roman *et al.*, 2007):

$$H_e = 1 - \sum_{i=1}^{n} P_i^2$$

$$I = -\sum_{i} P_i \ln P_i$$

Deviation from Hardy-Weinberg equilibrium (HWE) was estimated with χ^2 test. The total number of alleles and their frequencies, effective number of alleles, observed and expected heterozygosity and the Shannon information index were estimated by the statistics program GENALEX 6.0 (Peakall and Smouse, 2006). Polymorphism information content (PIC) was calculated, using HET software (Ott, 2001), version 1.8, as the following formula (Mateescu *et al.*, 2005):

$$PIC = 1 - (\sum_{i=1}^{n} p_i^2) - \sum_{i=1}^{n-1} \sum_{j=i-1}^{n} 2 p_i^2 p_j^2$$

Where, P_i and P_j are the frequency of ith and jth allele and and n is the number of alleles in all above equations.

RESULTS AND DISCUSSION

The number of alleles per locus, effective number of alleles, observed, expected heterozygosity and

Table 2: Genetic diversity parameters in guilan buffalo population

Marker	Na	Ne	Ho	He	PIC	I
CSSM019	3.00	2.55	1.00	0.61	0.52	1.00
CSSM029	2.00	1.20	0.98	0.50	0.37	0.69
CSSM033	6.00	3.81	1.00	0.74	0.70	1.49
CSSM038	4.00	3.38	0.76	0.70	0.65	1.29
CSSM041	3.00	2.43	0.72	0.59	0.52	0.98
CSSM043	3.00	2.91	0.68	0.66	0.58	1.08
CSSM047	6.00	4.01	0.98	0.75	0.72	1.55
CSRM060	5.00	2.24	0.49	0.55	0.52	1.09
CSSM061	5.00	3.33	1.00	0.70	0.65	1.36
CSSM062	5.00	4.69	1.00	0.79	0.75	1.57
CSSM070	4.00	3.62	1.00	0.72	0.67	1.33
BMC1013	4.00	2.69	0.98	0.63	0.55	1.12
BRN	4.00	3.73	1.00	0.73	0.68	1.35
ETH003	4.00	3.01	0.98	0.67	0.61	1.21
Mean	4.14	3.17	0.90	0.67	0.61	1.22
SE	1.17	0.76	0.17	0.08	0.03	0.25

the Shannon information index are shown in Table 2. Significant departures from HWE were detected for all loci ($p \leq 0.001$). Hardy-Weinberg equilibrium is a useful indicator of genotype frequencies within a population and whether they are based on a valid definition of alleles and a randomly mating sample (Short *et al*., 2007). HWE assumes a stable population of adequate size without selective pressures and is used to comparing observed genotype frequencies to those expected within a population (Short *et al*., 2007). Therefore, excess of heterozygote individuals than homozygote individuals, association of loci with some genes of economics importance, migration and high mutation rate of microsatellite may be the cause.

All loci were polymorphic and generating 58 alleles with means of 4.14. The effective number of allele ranged from 1.20 (CSSM029) to 4.69 (CSSM062) across all loci. CSSM062 and CSSM029 were the most polymorphic and monomorphic markers according to their Polymorphism information content and Shannon information index values. CSSM033 and CSSM047 showed the highest and equal number of alleles but CSSM047 was more polymorphic than CSSM033 according to their Ne, PIC and I values.

The Polymorphism Information Content (PIC) is a parameter indicative of the degree of informativeness of a marker. Loci with many alleles and a PIC near 1 are most desirable (Botstein *et al*., 1980). The Polymorphism Information Content (PIC) showed an average of 0.61. The high average of polymorphism information content, displayed by panel of 14 microsatellites, suggested high utility of these markers for population genetic researches (MacHugh *et al*., 1998; Kayang *et al*., 2002).

The expected heterozygosity (H_e) revealed an average of 0.67 with a range of 0.50 (CSSM029) to 0.79 (CSSM062).

The estimated genetic diversity of Guilan buffalo population (0.67) was, however, higher than buffalo populations of northern India (0.60), Anatolian (0.66) and 11 populations of Asian buffalo (0.38-0.61) (Barker *et al*., 1997; Arora *et al*., 2004; Soysal *et al*., 2005). Hence, it can be concluded that Guilan buffalo population possessed a considerable amount of genetic diversity due to the extensive production system, low pressure of artificial selection and possibility of random mating.

ACKNOWLEDGMENT

The current study, was supported in part by grants in aid from Animal Science Research Institute of Iran. We are grateful to many people who help us in this research.

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