ASSESMENT OF GENETIC DIVERSITY IN CHIEMHOA MALE BUFFALOES FROM TUYENQUANG PROVINCE BY MICROSATELLITES

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ABSTRACT

In recent years, the number of ChiemHoa buffaloes has decreased gradually and tends to be inbreeding and seriously degraded. The evaluation and effective using of ChiemHoa buffaloes genetic resourses help improve the quality of ChiemHoa buffalo population. The study evaluated the genetic characteristics of 10 ChiemHoa male buffaloes for breeding in Tuyen Quang and Mountainous Animal Husbandry Research and Development Center (Song Cong, Thai Nguyen province) from 2019-2021 by 8 microsatellite markers, CSSM057, CSSM029, ILST033, RM099, CSSM022, BMC1013, ETH003, CSSM038. The purpose of the study is to evaluate the genetic distance of the Chiem Hoa buffalo population, thereby orienting the use and exploitation of effective buffalo genetic resources in the future. The results of the buffalo alleles showed that there were 34 polymorphic bands and 4 single-bands. Among these results, 6 microsatellite markers for these buffaloes showed high genetic diversity; ILST033 and BMC1013 markers had average genetic diversity. The genetic distance of these buffaloes range from 0.364 to 0,909 and the average was 0.6. The 205 and 206 buffalo had highest genetic distance; the 03 and 05 buffaloes had lowest genetic distance. These results suggested these buffaloes were high diversity and could use for breeding in Tuyen Quang province.

Keywords: *ChiemHoa buffaloes, Microsatellites, Genetic diversity*

INTRODUCTION

Currently, the Domestic Animal Diversity Information System (DAD-IS) and Domestic Animal Genetic Resources Information System by Food Agriculture Organization (FAO) have been world widely introduced to conservate the domestic animal's gene for the future sustainable social economic development. The diversity of genetic variation within animal species and between animal breeds can be assessed and analyzed by several methods (Toro et al., 2009). Among those, microsatellite marker was commonly used to assess the genetic diversity of domestic farm animals (Cañón, J., 2006; Hillel, J., 2003; Kumar, S., 2006; Peter, C., 2007; San Cristobal, M., 2006).

In Vietnam, buffaloes keeping has been playing a crucial role in agriculture production, as it provides the main power for plowing, transportation in the rural areas, as well as a remarkable amount of manure for soil fertility, finally, adequation consumer's demand on buffalo meat. Apart from that, the by product from buffalo's slaughter such as skin, horns, hair can be utilized for ornamental purposes… Buffalo meat has been increased in market demand, including EU and North America markets because of its low cholesterol content. Thus, increasing buffalo production in Vietnam is of promising potential. Regarding buffalo breeding, there has not yet a breeding program in Vietnam, the uncontrol breeding practice by farmers has led to the decline of buffalo productivity. In fact, in some places, the selection of buffalo has been practicing in reverse where the most suitable males for breeding were slaughter, the rest were used for draughting power and for breeding. In many places, buffaloes have been declining in size and bodyweight. Apart from poor breeding practice, there was also lack of research on buffalo nutrition, thus, the biological potential of buffalo has not yet been exposed.

This study assessed genetic diversity of 10 Chiem Hoa male buffaloes in breeding from Tuyen

Quang province using microsatellite markers. These buffaloes were chosen from different community in Tuyen Quang based on growth characteristics.

MATERIALS AND METHODS

Location and time

Study was conducted in Chiem Hoa district, Tuyen Quang province and in Mountainous Animal Husbandry Research and Development Center (Song Cong, Thai Nguyen province) from 2019-2021.Gene analysis was done at Insitute of Life Science, Thai Nguyen University.

Materials

Chiem Hoa buffaloes: 10 Chiem Hoa male buffaloes currently nurtured and exploited for reproduction, has a large stature in 03 communes in the selected area of Chiem Hoa district, Tuyen Quang province. The buffaloes coded as 01, 02, 03, 04, 05 were breed in Tuyen Quang and the other buffaloes, 205, 206, 207, 208 and 209 were breed in Mountainous Animal Husbandry Research and Development Center.

Microsatellite markers: Eight microsatellite markers recommended by FAO (http://www.fao.org/docrep/014/i2413e/i2413e00.pdf) were CSSM057, CSSM029, ILSTS033, RM099, CSSM022, BMC1013, ETH003, CSSM038 (Table 1).

Methods

Sample collection and DNA genomic extraction: A total of 10 Chiem Hoa male buffaloes from different location of Tuyen Quang province were selected base on local standards, interviews with the farmers and the Tuyen Quang buffalo's survey to ensure each buffaloes were representative.The blood sampling for buffalo DNA based on guideline of FAO [1]. The buffalo's ear vein was sterilized with 70 percent alcohol and wipe the area dry with tissue. The blood samples from 10 buffaloes were collected and stored in tubes with EDTA and then transferred to laboratory for DNA extraction. The genomic DNA of buffaloes were isolated using Dneasy Blood & Tissue Kit (Quiagen, Germany) following the protocol and starting with 100 µl anticoagulated blood at Institue of Life Science, Thai Nguyen University. The concentration of genomic DNA samples was measured by UV-Vis at 260/280 nm absorbance and checked on 1% agarose electrophoresis.

PCR Amplification and microsatellite genotyping: Eight microsatellite markers from 15 recommended microsatellite markers for buffaloes by FAO [1]were selected (CSSM057, CSSM029, ILST033, RM099, CSSM022, BMC1013, ETH003, CSSM038). Information of microsatellite markers, 5' fluorescent label for forward primer and alleles amplified per markers is given in Table 1.

The multiplex PCR reactions were carried out in total volume 100 µl consisting of 1 µl DNA (50 ng/µl), 10 µl dNTP, 10 µl PCR buffer, 5 µl working primers, 0.5 µl Taq polymersase and water to 100 µl. The primers which had the same annealing temperature were used together in the multiplex PCR (CSSM038, CSSM029 and ILSTS033; CSSM057, CSSM022 and RM099). The PCR temperature profile followed by denaturation at 94 ^{o}C for 5 min, 35 cycles of denaturation at 94 ^{o}C for 30 sec, 55 – 60 ^{o}C for 1 min (temperature base on different marker given in Table 1), annealing at 72 ^{o}C for 1 min; and extension at 72 ^{o}C for 10 min. The HiDi formamide solution was mixed with 500 bp size marker by ratio 40:1. Mix 10 µl mixed HiDi formamide solution with 1 ul PCR and incubated at 4^{o}C then loaded into ABI 3500 DNA analyzer (Applied Biosystems, USA) for separation and detection and done in

Gyeongsang National University, Korea. Results were analyzed by Gene mapper at Institue of Life Science, Thai Nguyen University to determine allele size and comparison.

Table 1. Buffalo chromosomal location and microsatellite marker for buffaloes

No	Microsatellite markers	Chromosome	Anneaaling temperature (^oC)	Allele range (bp)	Gene bank code
1	CSSM033	17(17)	65	154-175	U03805
2	CSSM038	11(10)	55	163-187	U03817
3	CSSM029	9(7)	55	174-196	U03807
4	CSSM057	9(7)	60	102-130	U03840
5	CSSM022	4q(5)	55-60	203-213	U03806
6	BMC1013	3p(19)	54	217-239	G18560
7	ILSTS033	13(12)	55	126-138	L37213
8	RM099	3p(19)	60	87-119	G29087

Statistical analysis: Allele frequencies, observed number of alleles, expected number of alleles [9-kimura], and observed and expected heterozygosity values [5-Nei M.] were computed using MStoolkit. Allele frequencies were used to calculate PIC for each locus as described by Botstein et al. [2] The genetic similarity between gene samples source was estimated followed by Nei and Li [5].Genetic diversity coefficient (H) for each molecular marker was as follow: $H = 1 - \sum Pi^2$

Where: Pi is the frequency of allele of each molecularmarker.Obtained data was input into NTSYS software PC version 2.0 in order to identify the differences between populations via Jaccard similarity coefficient. Analyzing and evaluating phenotypic correlation coefficients according to the UPGMA grouping method.

RESULTS AND DISCUSSION

The extraction genomic DNA of Chiem Hoa buffaloes

The blood samples of Chiem Hoa male buffaloes were isolated and DNA extracted. Genomic DNA samples were determined concentration at 260/280 nm and checked on 1% agarose electrophoresis (Figure 1). The DNA samples were high purity and could use for other experiment.

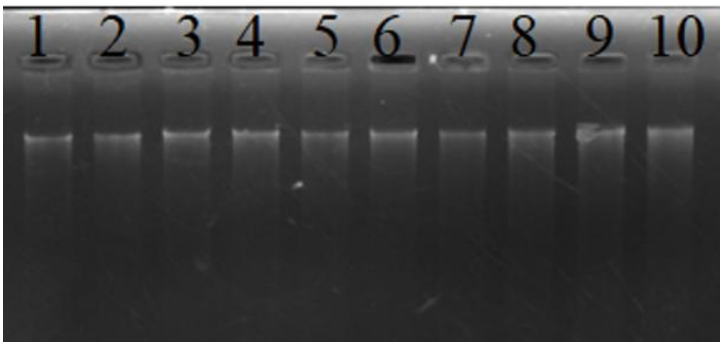

Figure 1. Genomic DNA extration of Chiem Hoa male buffaloes in Tuyen Quang province

Lane 1-10: Genomic DNA extraction from 01, 02, 03, 05, 205, 206,207, 208 and 209 buffaloes.

The polymorphism of the molecular marker

Table 2. The genetic polymorphism of 10 Chiem Hoa male buffaloes using microsatellite markers

Locus	PIC value	Total allele	Expected heterozygous value (H_e)	Observed heterozygosities (H_0)
CSSM057A	0.61211605	5	0.673563218	0.4
CSSM029A	0.69138519	4	0.765517241	0.866666667
ILST033A	0.43236049	4	0.487356322	0.533333333
RM099A	0.74027407	6	0.802298851	0.8
CSSM022A	0.52357615	4	0.626811594	0.5
BMC1013A	0.27096049	3	0.301149425	0.333333333
ETH003A	0.68528738	5	0.751322751	0.785714286
CSSM038A	0.71897778	7	0.772413793	0.733333333

Table 3. Microsatellite loci characteristics: Distance and number of alleles per each locus of studied buffaloes

Locus	Expected alleles distance	Number of alleles	Allele distance	Allele type in the group
CSSM057A	102-130	5	108-124	108, 116, 118, 120, 124
CSSM029A	174-196	4	183-189	183, 185, 187, 189
ILSTS033A	154-175	3	117-139	117, 119, 124, 139
RM099A	87-119	6	85-101	85, 91, 95, 97, 99, 101
CSSM022A	203-213	3	200-218	200, 208, 210, 218
BMC1013A	217-239	3	224-230	224, 226, 230
ETH003A	96-192	5	94-109	94, 96, 102, 108, 109
CSSM038A	163-187	6	162-193	162, 172, 174, 180, 181, 191, 193
Total		***35***		
Mean		4.75		

The genomic DNA from these buffaloes were used for DNA amplified by microsatellite marker and load to ABI 3500 for alleles analyses.

The Polymorphic information content (PIC) isa index to assess the genetic diversity of the population. The PIC index can be used to evaluate the magnitude of genetic morphinism:

when PIC>0.5 which meant higher diversity of the locus; PIC<0.25 meant lower diversity of the locus; and 0.25<PIC<0.5 was the intermediate locus diversity. Our microsatellite markers analysis with 8 primer pairs for 10 tested male buffaloes showed in Table 2. Based on the classification of Botstein et al. [2], our data showed that there were 6 microsatellite markers which had the PIC index higher than 0.5 (accounted for 75%) meaning that there was a high genetic diversity in the samples, whereas there were 2 microsatellite markers (ILST033 and BMC1013) which had the PIC index between the range of 0.25<PIC<0.5 (25%) meaning the medium genetic diversity of the samples. The genetic diversity of the tested buffalo was shown by the expected zygote value and the number of allele/locus (data presented in Table 3). This proved that the used markers were highly diversified for Chiem Hoa buffaloes.

When the polymorphism and monomorphism were analyzed by using microsatellite markers, it was shown that 8 molecular markers with the number of bands from 4 to 7 the average was 4.75 polymorphic band. The results showed 29 polymorpholism alleles and 6 monomorphism alleles, alleles for each molecular markers (Table 4, 5).

Table 4. Number of alleles of studied buffaloes by the microsatellite markers

CSSM057	**SL**	**CSSM029**	**SL**	**ILST033**	**SL**	**RM099**	**SL**
108	1	183	7	119	2	85	1
116	1	185	4	124	2	91	6
118	4	187	5	139	16	95	2
120	11	189	4			97	8
124	3					99	2
						101	4
Total	**20**		**20**		**20**		**23**
CSSM022	**SL**	**BMC1013**	**SL**	**ETH003**	**SL**	**CSSM038**	**SL**
200	7	224	2	94	4	172	4
208	6	226	17	96	6	174	4
210	1	230	1	102	4	180	1
				108	2	181	7
				109	2	191	2
						193	2
Total	**14**		**20**		**18**		**20**

Table 5. Allele frequency

CSSM057A	%	CSSM029A	%	ILST033A	%	RM099A	%
108	5,00	183	35,00	117	0,00	85	4,35
116	5,00	185	20,00	119	10,00	91	26,09
118	20,00	187	25,00	124	10,00	95	8,70
120	55,00	189	20,00	139	80,00	97	34,78
124	15,00		0,00		0,00	99	8,70
						101	17,39

CSSM022A	%	BMC1013A	%	ETH003A	%	CSSM038A	%
200	50.00	224	10.00	94	22.22	172	20.00
208	42.86	226	85.00	96	33.33	174	20.00
210	7.14	230	5.00	102	22.22	180	5.00
			0	108	11.11	181	35.00
				109	11.11	191	10.00
						193	10.00

The genetic relationship between tested buffaloes

The inbreeding coefficients of 10 buffaloes were analyzed and data presented in Table 6 ranged from 0.364 to 1.000 and the average was 0.6 (Table 6, Figure 2). These results showed that the studied buffaloes having a high inbreeding coefficient. The highest inbreeding coefficient was between buffalo number 205 and 206 with the value of 0.909, whereas the lowest inbreeding coefficient was between buffalo number 03 and 05. Due to the low sample size of population, the tested animals were obtained from different location and from unknown origin therefore the higher inbreeding coefficient did not prove the relationship of all tested individuals.

Table 6. The inbreeding coefficient of 10 tested buffaloes

Tag No.	01	02	03	04	05	205	206	207	208	209
01	1.000									
02	0.455	1.000								
03	0.667	0.545	1.000							
04	0.727	0.606	0.576	1.000						
05	0.576	0.636	0.364	0.545	1.000					
205	0.576	0.576	0.667	0.545	0.576	1.000				
206	0.606	0.667	0.697	0.576	0.545	0.909	1.000			
207	0.515	0.697	0.606	0.667	0.697	0.697	0.667	1.000		
208	0.606	0.606	0.455	0.636	0.727	0.485	0.455	0.606	1.000	
209	0.545	0.667	0.515	0.636	0.606	0.545	0.576	0.606	0.576	1.000

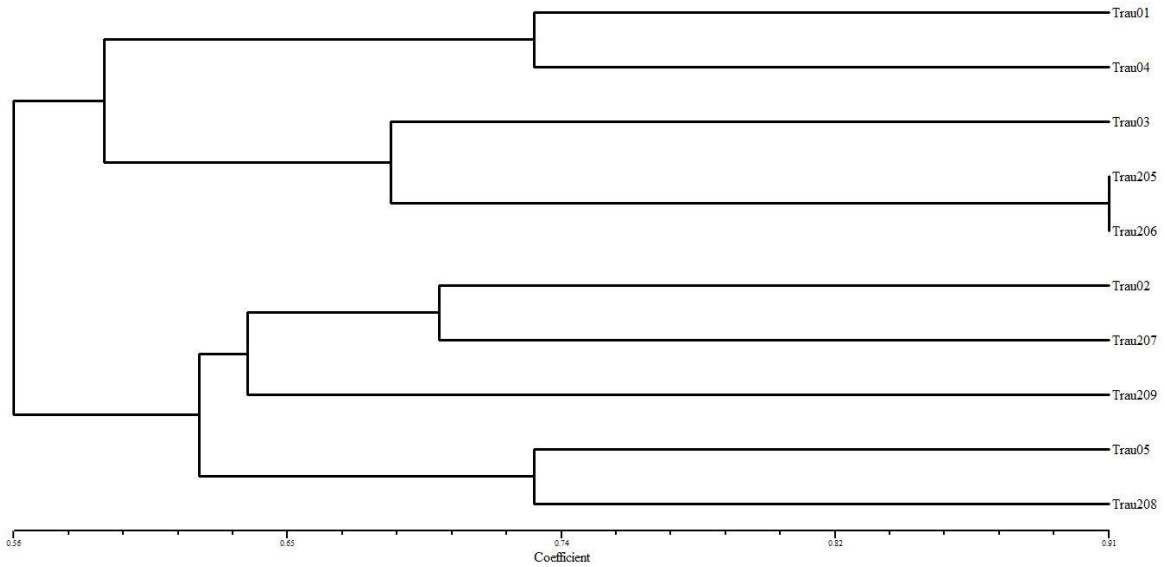

Figure 2. The genetic distance between 10 Chiem Hoa male buffaloes

CONCLUSION

This study assessed the genetic diversity of 10 Chiem Hoa male buffaloes in breeding in Tuyen Quang, Vietnam by using eight microsatellite markers. These buffaloes are high diversity with 6 microsatellite makers with PIC>0.5 (75%) and 2 markers have PIC between 0.25 and 0.5. The analyzed alleles had 29 polymorphic bands and 6 monomorphic bands with an average value of 4.75 bands polymorphic bands per microsatellite marker. About the genetic distance, the buffaloes used in this study were genetically different. Buffalo 205 and buffalo 206 have the highest similarity with a coefficient of 0.909, buffalo 05 and buffalo 03 have the lowest similarity. These results suggested these buffaloes were high diversity and could use for breeding in Tuyen Quang province.

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