GENETIC DIVERSITY AND CONSERVATION OF ANIMAL GENETIC RESOURCES IN IRAQI BUFFALO USING MICROSATELLITE MARKERS

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ABSTRACT

In our study, conducted in Iraq and Huazhong University, China, divided Iraq into three main regions: a southern area including Basrah, Missan, and Dhi-Qar, a middle area including Al-Qadisiyah, Babil, Karbala and Baghdad, and a northern area including Diyala, Kirkuk and Mosul. The aim of the study was to measure the genetic diversity, polymorphism and heterozygosity in Iraqi buffaloes using microsatellite techniques. Sixty-nine blood samples were collected from unrelated animals. Six microsatellite markers were used (ETH125, CSSM060, BM1706, ETH02, ETH225 and INRA005). The polymerase chain reaction (PCR) was done using specific bovine primers and a genetic analyzer (ABI-3730). Our results revealed that all the six markers amplified the DNA. The marker INRA005 did not show high polymorphism; it only revealed three alleles (137-141 bp). The marker ETH152 showed the highest level of polymorphism; it has sixteen alleles ranged between 192-217 bp. This study showed that there are three main clusters: the first one included Basrah, Baghdad and Al-Qadisiyah, the second consisted of Kirkuk and Missan, while the third consisted of Babil and Mosul.

Keywords: Iraqi buffalo, microsatellites, PCR, genetic diversity

INTRODUCTION

The buffalo contributes effectively in the agricultural economy and food security in the countries of the Indian Subcontinent and South East Asia, through meat, milk, leather and labor. It is well known that the buffalo was domesticated very early in history, but when and where is unknown (Cockrill, 1974). The water buffalo emerged in East Asia (Potts 1996) and mainland South East Asia. It spread north and west to China and to the Indian subcontinent (Lau, 1998). The buffalo has been present in the valley of the Indus River in the Indian subcontinent since about 4000-5000 years ago, but there are areas of independent domestication of water buffalo in Mesopotamia and China earlier than this-about 2500-7000 BC (Haynes *et al*., 1991; Payne, 1991 and Bradley, 2006). Macgregor (1939) classified buffalo into two types according to formal criteria and behavior: the river buffalo in the Indian subcontinent and westerly to the Balkan region and the swamp buffalo in Southeast Asia, India, Nepal and northeast to the valley of the Yangtze River in China. Barker *et al*. (1997) was the first to analyze the genetic diversity of buffalo breeds in South Asia using protein coding spatial microsatellites. They noted a clear distinction between these two types: river and marsh buffaloes, the latter being raised mainly in the swamps of Southeast Asia, and this distinctiojn was confirmed later by Zhang

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et al. (2007). Today, almost all breeds have been described according to geographical location and genetic phenomena. Using the new biotechnology in national research centers, local breeds have been identified in China, India and Pakistan, the Philippines, and Vietnam.In China, four types of buffaloes were identified in accordance with the geographical distribution (Wenping *et al*., 1998). Using molecular data, Zhang *et al*., 2007 classified Chinese buffalo breeds into four groupsthough these are not commensurate with the classification proposed by Wenping (1998). Therefore, there is a need to determine the breeds genetically in order to obtain correspondence between geographic and genetic classifications of the Chinese breeds and the Indian (Kumar *et al*., 2006). The discovery of the existence of genetic differences at the level of DNA can bring about a revolutionary strengthening of programs of genetic improvement of this animal. Current DNA technology can cover most of the requirements for this purpose. Several recent studies have been publishedon the domestic buffalo using microsatellite markers. These include studies of the swamp buffalo in south-east Asia (Barker *et al*., 1997), in India (Kumar, 2006), and in China (Zhang, 2007) and of the river buffalo in Greece and Italy (Moioli, 2001). While many researchers have investigated genetic polymorphism in the buffalo, in Iraq, this issue has not been studied enough. The present study is to fill the gap in this important aspect and to throw light on the genetic situation in Iraq. Further scientific objectives are development of biodiversity restoration approaches in agricultural biodiversity; managing agricultural biodiversity to improve productivity and conserve diversity and making the first stage of animal genetic resources conservation.

MATERIALS AND METHODS

Blood samples: Blood samples were collected from 96 individuals from the three main regions in Iraq (approximate 24 from the south and 24 individuals from the north of the country and 48 from the middle region of Iraq) all the blood samples were from unrelated animals. Six microsatellite markers recommended by FAO and ISAG for domestic buffalo diversity studies were used (Hoffmann *et al*., 2004). Forward primers were end-labeled with fluorescent dyes (6-FAM, TET and HEX). Genotypes for each marker were determined using ABI 3730 DNA Sequencer (Applied Biosystems) with the internal size standard Gene-Scan (Applied Biosystems).

Data analysis

Allele frequency, the number of alleles per locus, observed heterozygosity (HO) and expected heterozygosity (HE) were calculated using microsatellite toolkit. To remove the bias of sample size, a corrected mean number of alleles (MNA) value for each locus was obtained by computing the means of 96 random samples of 24 individuals for each population except buffaloes of the middle area, which was 48 individuals. Wilcoxon signed rank tests were conducted to examine the significant differences on MNA and HE between each pair of populations. Exact tests for deviations from Hardy-Weinberg equilibrium (HWE) were performed for each population–locus combination using GENEPOP version 3.4 with the P-values obtained by the Markov Chain randomization test. FST values per pair of populations (Weir & Cockerham, 1984) were computed and tested using the FSTAT program (Goudet, 2002). Two approaches were employed to investigate the genetic relationships among the populations. First,

Nei's DAgenetic distances (Nei *et al*., 1983) were calculated and then used to construct the neighbor-joining tree using MEGA software. Secondly, principal component analysis (PCA) with gene frequency was conducted.

RESULTS

Genetic variability: In all 70 alleles were detected across the six microsatellite loci .The total number of alleles per locus (TNA) varied from three (INRA005) to 16 (ETH152). The MNA across thesix loci in Iraqi indigenous buffaloes was 5.98. The results of the present study showed that all the loci used in this study amplified the DNA of the Iraqi buffalo and showed a high level of polymorphism except INRA005 (Table 1).

Heterozygosity: From the results of the present study, all the six loci showed variable rate of heterozygosity The expected heterozygosity ranged from 0.134 in the INRA005 marker to 0.869 in the ETH152 marker, while the observed heterozygosity varied from 0.145 in the INRA005 marker to 0.916 in the CSSM060 marker. Most loci showed heterozygosity rates above 0.5, which means these markers are highly informative markers (Table 2).

Relationships among populations: A neighbor-joining tree on the basis of genetic distances was constructed (Figure 1), and for the first time in Iraq, three main clusters have been detected in this genetic tree map. The first of these clusters includes Basrah, Baghdad, and Qadisseah provinces, the second consisted of the buffalo of both Kirkuk and Missan provinces, while the third cluster included both Babil and Mosul provinces. The least genetic distance was between the buffalo of Baghdad and Qadisseah provinces, which was 0.083, while the highest distance was between the buffalo of Mosul and Babil and between the buffalo of Mosul and Kirkuk provences, which was about 0.458.

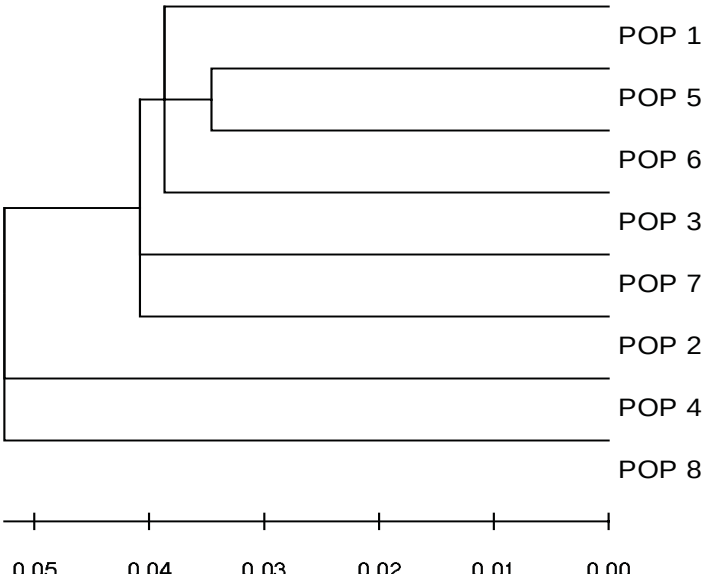

Figure 1. Genetic linkage map of the Iraqi buffalo.

Table 1. Polymorphism of 6 microsatellite loci in 3 Iraqi indigenous buffalo populations.

Locus	Total number of alleles (TNA)			Mean number of alleles (MNA)
	Southern area	Middle area	Northern area	
ETH152	8	9	7.5	8.16
CSSM060	6.5	7.5	6.5	6.83
BM1706	6.5	8	8.5	7.66
ETH02	6.5	6	6.5	6.33
ETH225	4.5	4.75	4.5	4.58
INRA005	2.5	2	2.5	2.33
Mean	5.75	6.20	6	5.98

Table 2. Mean of heterozygosity of six loci in the Iraqi buffaloes.

Locus	Ho and He *	Southern area	Meddle area	Northern area
ETH152	Ho	0.875	0.729	0.75
	He	0.865	0.865	0.869
CSSM060	Ho	0.916	0.833	0.791
	He	0.782	0.827	0.824
ETH02	Ho	0.75	0.770	0.791
	He	0.740	0.753	0.766
BM1706	Ho	0.75	0.791	0.708
	He	0.786	0.833	0.085
ETH225	Ho	0.625	0.770	0.75
	He	0.577	0.628	0.693
INRA005	Ho	0.208	0.145	0.458
	He	0.197	0.134	0.365

*HO: Observed heterozygosity, HE: Expected Heterozygosity.

DISCUSSION

Most of the microsatellite loci used here were highly informative in the Iraqi buffalo. In the present study, a high level of genetic variability was revealed in Iraqi buffalo. The mean expected heterozygosity of eight populations varied between 0.197 in the INRA005 to 0.865 in the ETH152 loci. The mean observed heterozygosity was 0.208 in the INRA005 and 0.916 in the CSSM060 loci. Our results were supported by many studies conducted in other countries, all of which showed the weakness of the INRA005 marker to the degree of the absence of polymorphism like in Iranian buffalo (Mirhoseinei *et al*., 2005) or weak polymorphism in the Egyptian buffalo (Zeinab, 2005), with some exceptions as in the French buffalo which showed more than three allelesand in the Italian buffalo, in which it reached six alleles (Ciampolini *et al*., 1995). These differences may be due to the differences in the breed or cluster included in the study.

Among the six microsatellite loci used in this study, the ETH152 marker showed the presence of 16 alleles, and this can be considered the highest number of alleles of this marker that has ever reported. In a study on microsatellite markers in the Indian buffalo, this marker showed only three alleles (Kale, 2010), while in the Iranian buffalo it has between five and sevenalleles only (Seyed *et al*., 2005). In Egyptian buffalo the number of alleles for this marker was eight (Zeinab, 2005). All of these studies and many other studies did not reach the number in the present study-the marker ETH152 had 16 alleles only in Iraqi buffalo. This fact supports our theory that the Iraqi buffalo originated in Iraq and was not imported from India.

CONCLUSION

We have presented the first study of genetic diversity of Iraqi domestic buffalo using microsatellite markers recommended by FAO and ISAG. Results indicate distinct genetic variation, high levels of genetic differentiation and genetic structure with three major clusters. These findings could provide an objective basis for classification and conservation of indigenous buffalo resources.

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