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Genetic characterization of indigenous anatolian water buffalo breed using microsatellite dna markers

M.İ. Soysal[1], E. Ozkan[1], S. Kok[1], M. Occidente[2], Y.T. Tuna[1], E.K. Gurcan[1], D. Matassino[2,3]

[1] Trakya University, Agriculture Faculty, Department of Animal Science, 59030 Tekirdağ, Turkey

[2] ConSDABI - NFP.I – FAO - Centro di Scienza Omica per la Qualità e per l'Eccellenza nutrizionali, Benevento, Italy

[3] Dipartimento di Scienze biologiche e ambientali- Università degli Studi del Sannio, Benevento, Italy

Corresponding author: M.İ. Soysal. Trakya University, Agriculture Faculty, Department of Animal Science, 59030 Tekirdağ, Turkey Tel. +90 2822931292 - Fax: +90 2822931479 - Email: misoysal@ttnet.net.tr

ABSTRACT: One indigenous water buffalo population to Anatolia was characterised with 11 cattle autosomal microsatellite *loci*. A set of 4 cattle microsatellite *loci* was found to be polymorphic in the Anatolian buffalo genome. Genotyping of these polymorphic microsatellite *loci* revealed alleles ranging from 3 to 9. The observed heterozygosity ranged from 0.550 to 0.775 and the expected heterozygosity ranged from 0.494 to 0.815. The F_{IS} value within each *locus*, changed from –0.101 to 0.205. Total F_{IS} was 0.043 indicating that Anatolian water buffalo population samples seemed to be in Hardy- Weinberg expectation.

Key words: Microsatellite, Anatolian water buffalo, Genetic diversity, DNA polymorphism.

INTRODUCTION - The number of water buffaloes in the world has decreased rapidly over the past three decades (Georgoudis *et al*., 1998). Most of world buffaloes live in Asia, Egypt, Southern and South-Eastern Europe. Also buffaloes have played an important role in the rural economy of developing Asian country from ancient times.

According to FAO (2000) data, there were about 166 million domesticated buffaloes raised in the five world continents. However, there are about 158 million buffaloes left in the world (FAO statistics, 2003). Roughly 97 percent of them or 153 million of heads are water buffaloes essentially found in the Asian region.

Also, in Turkey the buffalo population declined dramatically over the last decades. The total population according to FAO statistics (2003) is 164.000 heads. The largest number of buffalo population existed in Black sea region. Eastern Anatolian buffalo population has second biggest number of population. Third biggest number of population in the Marmara region existed in Istanbul and surroundings this city. Feeding is based on grazing, straw and concentrates. Their purpose of raising is firstly milk and secondly meat production.

The aim of this study was to estimate the intra-population genetic diversity using microsatellite markers.

MATERIAL AND METHODS - *Sampling and analysis methods.* The number of animals sampled from the Anatolian water buffalo was 40. Blood samples of unrelated animals were collected in slaughterhouse in Silivri of Marmara region. Blood was collected in 10 ml tubes containing K_2EDTA and stored at –20˚C until the DNA was extracted by the standard Phenol – Chloroform technique (Sambrook *et al.* 1989). The microsatellite *loci* used in the study and their characteristics are given in Table 1.

The PCR analyses were carried out using an Applied Biosystems GeneAmp® PCR System 2700 thermal cycler. The reaction mixture was composed of genomic DNA (100 ng), 200µm dNTPs, 2.0 mM $MgCl_2$, 1X PCR buffer, 5 pmol forward and reversed primers and Taq DNA polymerase (0.5 u/sample) in a total volume of 20 µl.

The PCR reactions were carried at following conditions: 1 cycle of initial denaturation for 5 minutes at 94 ^{0}C, 30 cycles of 45 seconds at 94 ^{0}C, 45 seconds at annealing temperature, 1 minute at 72 ^{0}C and 1 cycle of final extension for 10 minutes at 72 ^{0}C. In order to minimize the artifacts caused during the amplification leading to false size estimations, one or more positive controls were used in each PCR reaction together with a negative control. The PCR products were checked on 2% agarose gel together with DNA size markers standards. For all microsatellites, allele size was determined on all samples with a Perkin Elmer ABI Prism 310 Genetic Analyzer using the GeneScan Software (Perkin Elmer).

For the population and for each *locus* number of alleles (n_A), observed heterozygosity (H_0) and unbiased expected heterozygosity (H_e) were calculated using Genetix 4.0 Programs. The averages of n_A, H_0, H_e based on four *loci* were also computed. The population F_{IS} value of Wright's F statistics based on four *loci* were estimated and used to test the deviation from the Hardy Weinberg equilibrium. All of the above computations were performed by using Genetix 4.0 statistical programs.

RESULTS AND CONCLUSIONS - Heterologous cattle microsatellite markers have been tested on Anatolian buffalo genome. A set of 11 (TGLA227, ILSTS005, CSSM66, BM1818, ETH10, ETH225, ETH3, HAUT24, HEL5, TGLA122, TGLA126) cattle microsatellite *loci* was analysed in Anatolian buffalo samples. Four cattle microsatellite *loci* were found to be polymorphic in the Anatolian water buffalo genome. The number of alleles per *locus* varied from 3 (ILSTS005) to 9 (BM1818). The mean number of alleles per *locus* was about 6.75. Allele number distribution at the four analysed *loci* is given in Table 2. The observed heterozygosity ranged from 0.550 to 0.775, and the expected heterozygosity ranged from 0.494 to 0.815.

Arora *et al*. (2003) studied microsatellite characterization of Tarai Buffalo of India. The Tarai buffalo is riverine with 50 chromosomes, which is similar to Anatolian water buffalo population which is called as subgroup of Mediterrenean water buffaloes. Arora *et al*. (2003) examined heterologous cattle microsatellite *loci* and used them for molecular genetic characterization of Tarai genome. A set of 22 cattle microsatellite *loci* was found to be polymorphic in the Tarai genome. Genotyping of these polymorphic microsatellite *loci* revealed alleles ranging from two to seven. Observed heterozygosity changed from 0.1316 to 0.9231. The value of mean observed heterozygosity was 0.60 in the Tarai buffalo population and expected heterozygosity changed from 0.1246 to 0.8149. BM1818, CSSM66 and ILSTS005 microsatellite *loci* were found be polymorphic in the Tarai buffalo population and also Anatolian water buffalo population. Anatolian water buffalo population heretozygosity was found to be similar in Tarai buffalo population.

Moioli *et al.* (2001) compared genetic diversity between Greek, Italian and Egyptian buffalo populations using 13 polymorphic microsatellite *loci*. The number of alleles per *locus* varied from two (ILSTS005) to 19 (ETH03). Only for two *loci* (CSSM33 and ILSTS005), all detected alleles were found in all three populations (Italian, Greek and Egyptian). ILSTS005 *locus* showed 3 alleles in Anatolian water buffalo population. Observed average heterozygosity was 0.135, 0.151 and 0.158 in the Italian, Greek and Egyptian populations, respectively. It was lower, although not significantly different from the expected heterozygosity (0.173, 0.176 and 0.190 respectively for the Italian, Greek and Egyptian). Compared with the above values, in Anatolian water buffalo population observed and expected heterozygosity were very high.

The Anatolian water buffalo population F_{IS} value within *locus* ranged from –0.101 to 0.205 and the total F_{IS} was 0.043. This result shows that, Anatolian water buffalo population samples seemed to be in Hardy Weinberg expectation. Ultimately, the present study revealed the presence of high degree of genetic diversity within the water buffalo populations of Turkey.

Table 1. Nomenclature of the microsatellite loci used in the study, their primer sequences, Polymorphism information contents (PIC), annealing temperature, chromosome localization and references.

Locus Name	Primer Sequence	PIC	Annealing Temp. (0C)	Chromosome Number	Reference
TGLA227	CGAATTCCAAATCTGTTAATTTGCT				
	ACAGACAGAAACTCAATGAAAGCA	0,87	55	18	Steigleder et al. (2004)
ILSTS005	GGAAGCAATGAAATCTATAGCC				
	TGTTCTGTGAGTTTGTAAGC	0.42	55	10	Arora et al. (2003)
CSSM66	ACACAAATCCTTTCTGCCAGCTGA				
	AATTTAATGCACTGAGGAGCTTGG	0.49	58	14	Arora et al. (2003)
BM1818	AGCTGGGAATATAACCAAAGG				
	AGTGCTTTCAAGGTCCATGC	0.40	58	23	Arora et al. (2003)

Table 2. Characteristics of bovine microsatellite markers tested on Anatolian Water Buffalo population.

LOCUS	Number of alleles (n_A)	Observed Heterozygosity (H_O)	Expected Heterozygosity (He)	F_{IS}
TGLA227	7	0.600	0.743	0.205
ILSTS005	3	0.550	0.494	-0.101
CSSM66	8	0.775	0.707	-0.084
BM1818	9	0.750	0.815	0.092
Mean	6.75	0.668	0.689	

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