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GENETIC CHARACTERISTICS OF EGYPTIAN BUFFALO USING DNA MICROSATELLITE MARKERS

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SUMMARY

To evaluate the genetic polymorphism for DNA microsatellite markers of Egyptian buffalo, 471 unrelated Egyptian buffalo were genotyped with 11 microsatellite markers. The data were analyzed with GenALEx6 software. Nine (82%) of the microsatellite markers were polymorphic and two (18%) were monomorphic. A total 198 alleles were detected, with the number of alleles per marker ranging from 17 (RM28 and BM415) to 29 (BMC4203), giving a mean number of 22±1.302 alleles per marker. The effective number of alleles was lower than the observed values with a mean value of 16.502±1.137 per marker. The most frequent alleles were ranged from 0.086(BMC4203) to 0.127 (BM415). The mean observed and expected homozygosity was 0.113 and 0.063, respectively, while the observed and the expected heterozygosity was 0.887 and 0.937, respectively, over all loci. Polymorphism information content values were ranged from 0.909 (BM415) to 0.949 (ILSTS093 andILSTS097). At the nine microsatellite loci, the mean of fixation index was 0.052. Successful genotyping of Egyptian buffalo using these DNA microsatellite markers suggests that the latter can be a valuable resource for genome analysis in Egyptian buffalo.

Keywords: Egyptian buffalo, microsatellite DNA, polymorphism

INTRODUCTION

The Egyptian buffalo (Bubalus bubalis) contributes significantly to the agricultural economy and food security in Egypt. Also, buffalo is the main dairy animal in Egypt, in addition to being an important source of red meat. Annual milk and meat production from buffaloes are 2,640,638 and 169,013.57 tons, respectively, contributing to 49 and 40% from the total national milk and meat production in Egypt, respectively (MALR,2008). Genetic maps provide new insights into genome structure and chromosomal architecture of the genome, and also serve as framework for identification and location of genes linked with economically important traits. Except for water buffalo, the genetic maps have been reported for most of the important livestock species. To develop genetic maps of water buffalo, identification and characterization of polymorphic microsatellite markers is a prerequisite (Nagarajan *et al*., 2009).

DNA markers-based technologies enable the detection of different polymorphic types. Among those, microsatellites or short tandem repeats (STR) or simple sequences repeats (SSR) have been identified in all the eukaryotic species that have been investigated thus far (Ron *et al*., 1996). The use of microsatellites in population genetics has so far been mainly reported in buffalo population (Zhang *et al*., 2007; Kumar *et al*., 2006 and Van Hooft *et al*., 2002).

Several studies had shown that repeated flanking sequences of microsatellite markers are often conserved between related species, allowing cross-species amplification (Schlotterer *et al*., 1991; Moore *et al*., 1994; Kemp *et al*., 1995; Levin *et al*., 1995; Moore *et al*., 1995; Liu *et al*., 1996 and Primmer *et al*., 1996).These markers can be used in the characterization of species populations, genetic diversity (Esmaeilkhanian and Banabazi, 2006) and population studies (Arora *et al*., 2004 and Amirinia *et al*., 2007), as they are hyper variable and widely dispersed through genome. Moreover, they have application in the identification of individuals and parentage testing (Marklund *et al*., 1994; Luikart *et al*., 1999, Seyedabadi *et al*., 2006 and Bhuyan *et al*., 2010).

Researchers applied cattle microsatellite markers for defining the genome make up in buffalo because no systematic studies have been undertaken to develop polymorphic DNA markers specific to these species (Shokrollahi *et al*., 2009). Hassanane *et al*. (2007) indicated the successful genotyping of bovine microsatellites in the Egyptian buffalo genome.

Genetic characterization of each breed is necessary for its effective and meaningful improvement and conservation (Sajid *et al*., 2007). So, it is essential to characterize

buffalo at the molecular level for their effective use in the genetic improvement programs (Saifi *et al*., 2004).

The purpose of the present study was to genetically characterize Egyptian buffalo using 11 DNA microsatellite markers.

MATERIALS AND METHODS

Selection of Buffaloes and Blood Sample Collection:

A total of 471 unrelated multiparous lactating buffaloes (Different families having no blood relation) represented seven different farms in six different governorates were utilized in this experiment. Also, there was no pedigree information available on these animals. A volume of 10 ml peripheral blood was collected from the jugular vein in Falcon tubes supplied with proper amount of EDTA. Field blood samples (471) were placed on a cooling gel in an ice box immediately after their collection and brought to the Animal Biotechnology Lab., established by a grant no.218, financed by Science and Technology Development Fund (STDF) and located in Faculty of Agriculture, Cairo University, Giza, Egypt and stored temporarily at -20°C before DNA extraction.

DNA Extraction:

DNA was isolated from the peripheral leukocytes using Fermentas® kits, Cat. No. k0512, Fermentas Life Science, EU, according to Sambrook and Russel, 2000.

The Yield, concentration and purity of DNA of the samples were quantified using ScanDrop® 200, Anyltikajena, UK. The whole genome of each sample was run in 0.8% agarose gel through a horizontal gel electrophoresis system (mini gel, Biometra® EU). Standard DNA/DNA ladder was used and all samples were brought at the same concentration level (50 ng/ µl).

Microsatellite DNA Markers Selection:

Microsatellite DNA markers are highly polymorphic and abundant often found in noncoding region of genes (Rohrer *et al*., 1998). A total of 11 Microsatellite DNA markers located in chromosome 7 in buffalo were utilized. The information about these DNA markers is given in Table 1.

Amplification of the microsatellite Markers and Genotyping:

The PCR was carried out on 50 ng of the genomic DNA in a 20 µl reaction volume of 50 mM KCL, 10 mM Tris-Hcl (pH 8.8), 200 µM dNTP, 1.5 mM MgCl2, 5 Pmol of each primer and 1.0 U Taq DNA polymerase. The amplification was realized using thermal cycler (G-Storm®, Gene Technologies, UK) machine. The primary denaturation was done at 95 °C for 3 minutes followed by 10 cycles of denaturation at 94°C for 30 sec., the annealing temperature at 58.5- 59.5°C (decrease in temperature set 1°C after each cycle) for 30 sec. and the extension at 72°C for 45 sec. Following these cycles with variant annealing temperatures (Table 1), 30 cycles with constant annealing temperature at 54°C were performed and the reaction ended with final extension at 72°C for 5 minutes and final storage temperature of 4°C.

Data Analysis:

The GenALEX version 6 package software (Peakall and Smouse, 2006) was employed to calculate allele frequencies and sizes, effective number, observed and expected heterozygosity, and fixation index. Polymorphic information content was estimated using R program (Gregory *et al*., 2011).

RESULTS AND DISCUSSION

Nine (82%) of the studied markers were polymorphic and two (18%) were monomorphic of 471 unrelated Egyptian buffaloes. Nagarajan *et al*. (2009), stated that a total of 571 microsatellite markers had been characterized for water buffalo. They found that among the amplified cattle markers, 85% of the markers were polymorphic, this percentage was in agreement with our study, and slightly high when compared with the other studies on water buffalo (Moore *et al*., 1995 and Navani *et al*., 2002). Navani *et al*. (2002) reported that 56% of cattle microsatellite markers provided polymorphic band patterns when tested in 25 buffaloes. Results of the amplification of the bovine microsatellite in buffalo and sheep genomes may be referring to the sharing of a common ancestry for cattle, buffalo and sheep after the divergence of subfamily bovine (Bos Taurus) from the family bovidae (Mattapallil and Ali,1999).

The number of alleles per locus (Na), effective number (Ne) of alleles, observed (Ho), expected (He) heterozygosity, and allele size are shown in Table 2. A total of 198 alleles were detected with an average number of alleles per polymorphic locus was 22±1.302, ranging from 17 (RM28 and BM415) to 29 (BMC4203). Vijh *et al*. (2008) found that the number of alleles per locus ranged from 11 to 26 allele on Indian water buffalo. Also, Weibin *et al*. (2007) reported that a total of 247 alleles were detected with the number of alleles ranging from 13 to 33, giving a mean number of 21 alleles per locus in Qinchuan cows. These differences in the number of alleles are

due to the type of breed studied and the genetic polymorphism within the breed itself (Vallejo *et al*., 2003).

The average number of effective (Ne) alleles per locus was 16.502±1.137. Observed heterozygosity (Ho) varied from 0.517 (ILSTS097) to 0.995 (BM143), while expected heterozygosity (He) varied from 0.916 (BM415) to 0.952 (ILSTS093). The overall mean of Ho and He values were 0.887±0.048 and 0.937±0.004, respectively. These results are in agreement with the study of Aminafshar (2008), who concluded that there were high mean percentages observed heterozygosity in three populations of Iranian buffalo using 15 cattle microsatellite.

Takezaki and Nei (1996) reported that the average heterozygosity must be between 0.3 and 0.8 in a population, in order to be a useful marker tool for measuring the genetic variation. Our results for mean heterozygosity were higher than that range. Therefore, the identified markers in this study are a suitable tool for measuring the genetic variation.

Mirhoseinie *et al*. (2005) concluded that the obtained results from heterozygosity indicated that the loci with more alleles contain higher rate of heterozygosity in both cattle and buffalo species.

At every microsatellite locus, allele size range was distinctive (Table 2). And at every locus, there was a most frequent allele (Table 3). At BM415 and RM28, the most frequent allele was 139 and 102 bp, respectively, which had an allele frequency of 0.127 and 0.122, respectively.

Polymorphism information content (PIC), fixation index (F) and Shannon's information index (I) in Egyptian buffalo genome are shown in Table 3. Polymorphism information content (PIC) was estimated using allele frequencies in each polymorphic microsatellite locus, ranged from 0.909 (BM415) to 0.949 (ILSTSO93 and ILSTSO97), and mean PIC was 0.933. The PIC is a parameter indicative of the degree of informative of a marker and another important measure of DNA polymorphism. The PIC reflects the probability that a given offspring of a parent carrying a rare allele at a locus will allow deduction of parental genotype at a locus (Babar *et al*., 2009). Genetic markers with PIC values of less than 0.25 are considered to be less informative and those with values more than 0.5 are reckoned as distinctly informative in population genetic studies (Botstein *et al*., 1980). Loci with many alleles and a PIC near one are most desirable (Botstein *et al*., 1980). Following the criteria of Botstein *et al*., (1980), in this study, all the nine microsatellite loci appeared to be highly informative (PIC>0.5) and thus will be useful to evaluate the genetic diversity in Egyptian buffalo.

Fiona and Tracey (1998), reported that the PIC values are generally slightly smaller than heterozygous values, if large numbers of unrelated animals are genotyped. The number of unrelated animals used to calculate these values does vary and thus reverse the trend that PICs are slightly lower than heterozygous values. This is in agreement with our study.

The fixation indices of BM415, BM1329, BMC4203 and BMS483 microsatellite loci were negative and the others were positive (Table 3). The mean of fixation indices was 0.052, reflecting that the degree of heterozygote defect at these loci was high.

In conclusion, this study declared that a large fraction of bovine DNA microsatellite markers can be amplified and is polymorphic in the Egyptian buffalo. Also, these DNA markers are applicable for population genetic studies on the Egyptian buffalo.

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Table 1. Characterization of the relevant DNA microsatellite markers in chromosome six in cattle

Marker Name	Physical map name	Primer sequence	Annealing Temperature °C	Minimum Allele size (bp)	Maximum Allele size (bp)	No. of alleles in cattle
ILSTS93	D6S22	TGAAATATACCTGAGTAGCAGC TTGTTTTAACTCCCCACCCC	58.7	179	202	19
BM1329	D6S14	TTGTTTAGGCAAGTCCAAAGTC AACACCGCAGCTTCATCC	58.7	137	161	9
BM143	D6S13	ACCTGGGAAGCCTCCATATC CTGCAGGCAGATTCTTTATCG	58	90	122	13
BM415	D6S10	GCTACAGCCCTTCTGGTTTG GAGCTAATCACCAACAGCAAG	54	141	173	15
RM28	D6S4	CTACAGTCATGGGTCTGAAAGAG ATCTTCAGCCTGGCCTGAGAG	62	94	110	5
BMC4203	D6S20	GCAAATGTAAGCTGAAGGCC CCTGGGAAATCCCATGGAC	60	144	170	10
ILSTS97	D6S23	AAGAATTCCCGCTCAAGAGC GTCATTTCACCTCTACCTGG	58	234	244	3
AFR227	D6S18	GACCAACTGAGTGCATGCACG TCATTGAGCAGGAGTAGGATTGAGA	58	96	120	11
BMS483	D6S51	GGTATGAGACCAGGTGTGGG CAGGGCCACATTTCCAAG	56	109	117	5
ILSTS90	D6S21	TAGTACCATACCCAGGTAGG	58	143	147	3
BM4528	D6S12	GCCAAAACACACAAGTGTGC CAGAATCCATACACATGTCAACA AGGAACAGGTATAGGAATATTGGA	58	238	276	7

Table 2. Genetic estimates for the Egyptian buffalo

Microsatellite markers	N	Na	Ne	Ho	He	Allele size (bp)
RM28	471	17	12.671	0.870	0.921	94-126
BM415	436	17	11.841	0.975	0.916	129-161
BM143	373	19	14.059	0.895	0.929	100-136
BM1329	323	24	17.943	0.994	0.944	123-169
AFR227	449	21	15.809	0.911	0.937	90-130
BMC4203	446	29	20.141	0.951	0.950	136-192
ILSTS093	179	24	20.698	0.899	0.952	183-229
ILSTS097	203	24	20.375	0.517	0.951	220-266
BMS483	393	23	14.976	0.972	0.933	100-144
Mean	364	22	16.502	0.887	0.937	--
SE		1.302	1.137	0.048	0.004	--

N = number of samples per marker; Na =Number of different alleles; Ne= number of effective alleles; Ho= Observed heterozygosity; He= Expected heterozygosity**.**

Table 3. Most frequent alleles and their frequencies, Polymorphism Information Content (PIC) and Fixation Index (F) of Egyptian buffalo

Microsatellite markers	Allele	Frequencies	PIC	F
RM28	102	0.122	0.916	0.055
BM415	139	0.127	0.909	-0.065
BM143	110	0.113	0.924	0.036
BM1329	135	0.088	0.941	-0.052
AFR227	112	0.104	0.933	0.028
BMC4203	156	0.086	0.948	-0.0003
ILSTS093	195	0.064	0.949	0.055
ILSTS097	236	0.076	0.949	0.056
BMS483	116	0.111	0.929	-0.042

التوصيف الوراثى للجاموس المصرى بإستخدام واسمات التوابع الدقيقة

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لتقييم تعدد المظاهر الوراثية لعدد من واسمات التوابع الدقيقة فى الجاموس المصرى، تم الفحص الوراثي لعدد ٤٧١ من الجاموس المصري ليس بينها قرابة بإستخدام عدد ١١ من واسمات التوابع الدقيقة. تم تحليل البيانات بإستخدام برنامج الـ GeneAlex . أشارت النتائج أن تسعة (٨٢%) من واسمات التوابع الدقيقة كانت متعددة المظاهر الوراثية و أن أثنين (١٨%) منها كانت أحادية المظاهر الوراثية.

تم تحديد عدد ١٩٨ أليل، وتراوح عدد الأليلات لكل واسم من الواسمات مابين ١٧ (RM28, BM415) إلى ٢٩ (BMC4203) حيث كان المتوسط العام للأليلات لكل واسم وراثي هو 22±1.302. العدد الفعال للأليلات كان أقل من القيمة الملحوظة بمتوسط 16.502±1.137 لكل واسم وراثي. تراوحت تكرارات الأليلات الأكثر تكراراً فى العشيرة مابين 0.086 (BMC4203) إلى 0.127 (BM415). تراوح متوسط قيمة التجانس الملحوظ و المتوقع مابين 0.113 و 0.063، على الترتيب. بينما تراوح متوسط عدم التجانس الملحوظ و المتوقع بين 0.887 ، 0.937 على الترتيب. لكل المواقع. تراوح قيمة تعدد المظاهر بين 0.909 (BM415) إلى 0.949 (ILSTS093 , ILSTS097)كان متوسط دليل التثبيت لتسعة من واسمات التوابع الدقيقة 0.052 . أشار نجاح التوصيف الوراثي للجاموس المصري بإستخدام واسمات التوابع الدقيقة إلى إمكانية إستخدامها في التحليل الوراثي للجاموس المصري.