African Journal of Biotechnology Vol. 11(65), pp. 12808-12813, 14 August, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2037
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Genetic variation among Northern and Southern Egyptian buffaloes using polymerase chain reaction-random amplified polymorphic DNA (PCR-RAPD)

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Accepted 23 July, 2012

The domestic water buffalo is a species of great economic potential, especially in developing countries like Egypt. Egyptian buffalo have been classified according to minor phenotypic differences and their geographical locations. Few studies have taken place to investigate the genetic variations in Egyptian buffalo using microsatellites analysis. In the present study, 11 random primers were analyzed for the genetic diversity determination between Northern and Southern Egyptian buffaloes using polymerase chain reaction-random amplified polymorphic DNA (PCR-RAPD) analysis. 169 bands were amplified for the analyzed 11 random primers, from which 160 bands (94.67%) for North populations and 168 bands for South population (99.41%). Out of the 160 amplified bands in North populations, 152 bands were polymorphic with a percentage of 89.94% and only one specific band (0.59%). In South population, all 168 amplified bands were polymorphic, nine bands (5.33%) were specific for this population. The identity index and the genetic distance between North and South populations were measured. The results showed that the two tested populations have the same origin and belong to one breed without significant genetic difference between their animals.

Key words: Buffalo, genetic diversity, polymerase chain reaction (PCR), random amplified polymorphic DNA (RAPD).

INTRODUCTION

Genetic variation is critical to the long term survival and evolutionary potential for any species or population. It can become decreased through isolation, inbreeding, and strong selective pressures such as environmental changes, diseases, or extensive mortality. Loss of genetic variability removes genes from the population that could enable certain individuals to survive a major event, reproduce and pass on their genetic material to the next generation. Low genetic variability within an individual or population greatly reduces the ability to respond to a major disease event or adapt to changing environmental conditions.

Various types of markers such as morphological, biochemical and molecular markers are used for the study of genetic diversity of native breeds (Barwar et al., 2008). Morphological markers have low level of variations and hence are not very useful for breed and population characterization (Walsh, 2000) whereas biochemical markers reflect variability in the coding sequences which constitute less than 3% of the total genome (Teneva, 2009). On the other hand, developments in DNA technologies have made it possible to uncover a large number of genetic polymorphisms at the DNA sequence level, and to use them as markers for the evaluation of the genetic basis for the observed phenotypic variability.

Water buffalo includes both river buffalo (2n =50) and swamp buffalo (2n = 48); the Egyptian water buffalo are of the river type. It is a species of great economic potential in Egypt where it is a major source of milk and meat. Egyptian buffalo have been classified according to minor phenotypic differences and their geographical locations such as Beheiri, Menoufi and Balady which are found mainly in North Egypt and Saiedy found in South

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Egypt (DAD-IS, 2004). Few studies have been taken place to investigate the genetic variations in Egyptian buffalo using microsatellites analysis (El-Kholy et al., 2007; El-beltagy et al., 2008). Random amplified polymorphic DNA (RAPD) markers have been used successfully in estimating genetic relatedness among various breeds and populations of chicken (Okumus and Kaya, 2005), sheep (Mahfouz et al., 2008), goat (Rahman et al., 2006), cattle (Hassen et al., 2007) and buffalo (Sajid et al., 2007; Barwar et al., 2008).

The objective of this study was to evaluate the genetic variation between Northern and Southern Egyptian buffaloes since there are still some conflicting opinions on its genetic differences.

MATERIALS AND METHODS

Genomic DNA extraction

Genomic DNA was extracted from the whole blood of 40 unrelated healthy buffaloes, belonging to Northern and Southern Egyptian buffaloes (20 samples each), according to the method described by Miller et al. (1988) with minor modifications. Briefly, 10 ml of blood taken on EDTA were mixed with 25 ml of cold 2X Sucrose-Triton and 15 ml double distilled water. The tubes were placed on ice for 10 min and mixed by inversion several times. After centrifugation, at 5000 rpm for 15 min at 4°C, the pellet was re-suspended by 3 ml of nucleic lysis buffer. The content was mixed with 108 µl of 20% SDS and 150 µl of Proteinase K. The tubes were placed in a water bath at 37°C overnight.

The tube contents were transferred to a 15 ml polypropylene tube and 2 ml of saturated NaCl were added and shaken vigorously for 15 s. After centrifugation at 3500 rpm for 15 min at 4°C, the supernatant was transferred to a clean 15 ml polypropylene tube and mixed with absolute ethanol. The precipitated DNA was washed twice in 70% ethanol and exposed to air to dry completely. The DNA was dissolved in 200 µl TE buffer in 1.5 ml microfuge tube and kept overnight in an incubator at 37°C. DNA concentration was determined and diluted to the working concentration of 50 ng/µl, which is suitable for polymerase chain reaction using Nano Drop1000 Thermo Scientific spectrophotometer.

Polymerase chain reaction (PCR)

PCR was used for DNA amplification using 11 random primers prepared by the University of British Colombia (UBC), canada (Table 1). The PCR cocktail consisted of 1.0 µM of a random primer and 0.2 mM dNTPs (Fermentas), 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin (w/v), 0.1% Triton X-100 and 1.25 units of *Taq* polymerase (Fermentas). The cocktail was aliquoted into tubes with 100 ng of buffalo DNA. The reaction mixture was preheated at 94°C for 5 min followed by 45 cycles; 1 min, denaturation at 94°C, 2 min annealing at 35°C and 2 min extension at 72°C; followed by final extension at 72°C for 5 min.

Gel electrophoresis and RAPD-PCR analysis

The PCR amplified products (25 µl) from buffalo DNA samples were separated electrophoretically on 1.5% (w/v) agarose gel in 1x TBE buffer containing ethidium bromide and visualized under UV light using Gel Documentation System (Syngene Bio Imaging). Two types of molecular size markers were used, 100 bp (Axygen) and 1-Kb (Fermentas) ladders.

The sizes of amplified bands were determined using Gel-Pro analyzer and RAPD banding patterns were scored for the presence (1) and the absence (0) of bands for each sample. The scores were then pooled for constructing a single data matrix. The statistical analysis of the data was performed using the free software "Popgene version 1.31" (Yeh et al., 1999) including the calculation of allele frequencies according to Nei (1987). The identity index and genetic distance between the two studied populations were measured and UPGMA dendrograms were formed according to Nei (1978).

RESULTS

11 random primers were tested to investigate the genetic differences between Northern and Southern Egyptian buffaloes using PCR-RAPD analysis: namely UBC 204, 205, 208, 701, 705, 713, 725, 731, 743, 745 and 747.

The results show that the number of bands amplified by the 11 random primers in the two investigated buffalo populations were 169 bands with a mean of 15.36/primer. 159 bands (94.08%) were shared between Northern and Southern populations. 160 bands (94.67%) were amplified in Northern population and 168 (99.41%) bands were amplified in Southern population (Table 3). In Northern population, 152 out of 160 amplified bands were polymorphic with a percentage of 95% and only one specific band (0.63%). In Southern population, all the 168 amplified bands were polymorphic, from which nine bands (5.36%) were found to be specific for this population.

The number of bands amplified by the 11 random primers in the two buffalo populations ranged from 12 bands (UBC 204 and UBC 745) to 17 bands (UBC 208, UBC 725, UBC 731, UBC 743 and UBC 747). The band frequencies ranged from 0.0000 to 1.0000 in the two Egyptian buffalo populations. Table 2 shows the band frequency for the 11 investigated primers in both Northern and Southern population.

The results showed that eight common bands were present in all the 20 Northern samples. Three common bands were produced by primer number 205 (band 7, 8 and 11) and one common band produced by each of primer numbers 208 (band 8), 701 (band 3), 705 (band 9), 725 (band 5) and 745 (band 11). However no common bands were detected in Southern buffalo samples. The results also show that primer numbers 205, 701, 713, 725, 731, 743 and 745 had created specific bands in Southern population with frequencies of 0.6307, 0.6985, 0.1210 and 0.0955, 0.1210 and 0.1472, 0.1472, 0.2313, 0.0230, respectively. Only one band was found to be specific in Northern population by primer number 701 with a frequency of 0.0253 (band 13).

The similarity or identity index and the genetic distance between the two studied populations were measured. The unbiased measure of genetic identity between the two populations was 0.8756 with a genetic distance of 0.1329 (Table 4) and the dendrogram is shown in Figure

Table 1. Nucleotide sequence and PCR conditions of the random primers used in this study.

Random primer	Primer sequence	PCR condition
204	TTC GGG CCG T	Hot starting at 94°C for 5 min 45 cycles: 94°C for 1 min 35°C for 2 min 72°C for 2 min Final extension at 72°C for 5 min
205	CGG TTT GGA A	
208	ACG GCC GAC C	
701	CCC ACA ACC C	
705	GGA GGA AGG G	
713	CCC TCC CTC T	
725	GGG TTG GGT G	
731	CCC ACA CCA C	
743	CCA CCC ACA C	
745	GGG AAG AGG G	
747	CCA CCA ACC C	

Table 2. Band frequencies for random primers in tested buffalo populations.

Band number	North population (%)	South population (%)	Band number	North population (%)	South population (%)	Band number	North population (%)	South population (%)
	Marker UBC 204			**Marker UBC 205**			**Marker UBC 208**	
204-1	0.1938	0.3970	**205-1**	0.4523	0.5736	**208-1**	0.5528	0.0230
204-2	0.0513	0.2615	**205-2**	0.0513	0.3970	**208-2**	0.1938	0.6985
204-3	0.0780	0.1743	**205-3**	0.5000	0.2313	**208-3**	0.1056	0.0465
204-4	0.2584	0.3604	**205-4**	0.1633	0.1472	**208-4**	0.3292	0.0465
204-5	0.0253	0.0465	**205-5**	0.1056	0.6985	**208-5**	0.2254	0.4359
204-6	0.4523	0.1210	**205-6**	0.0000	**0.6307**	**208-6**	0.6838	0.2313
204-7	0.7764	0.0955	**205-7**	*1.0000*	0.1472	**208-7**	0.2929	0.4778
204-8	0.7764	0.2615	**205-8**	*1.0000*	0.1472	**208-8**	*1.0000*	0.3258
204-9	0.5528	0.3258	**205-9**	0.0513	0.1743	**208-9**	0.1938	0.3258
204-10	0.5000	0.6307	**205-10**	0.4084	0.6307	**208-10**	0.1633	0.2313
204-11	0.3675	0.2313	**205-11**	*1.0000*	0.1472	**208-11**	0.6838	0.2929
204-12	0.1633	0.5233	**205-12**	0.0253	0.0230	**208-12**	0.4084	0.5736
			205-13	0.6127	0.4778	**208-13**	0.4523	0.5736
			205-14	0.0253	0.1743	**208-14**	0.0780	0.5233
			205-15	0.5000	0.1210	**208-15**	0.5528	0.0955
						208-16	0.5000	0.0955
						208-17	0.3675	0.0707
	Marker UBC 701			**Marker UBC 705**			**Marker UBC 713**	
701-1	0.1938	0.6985	**705-1**	0.0780	0.0465	**713-1**	0.1056	0.1743
701-2	0.0000	**0.6985**	**705-2**	0.5528	0.3604	**713-2**	0.0513	0.1472
701-3	*1.0000*	0.0465	**705-3**	0.6838	0.5736	**713-3**	0.0000	**0.1210**
701-4	0.4084	0.4359	**705-4**	0.6838	0.3604	**713-4**	0.2584	0.3258
701-5	0.1056	0.6985	**705-5**	0.1633	0.1743	**713-5**	0.0513	0.0465
701-6	0.2584	0.0955	**705-6**	0.7764	0.6307	**713-6**	0.5528	0.2615
701-7	0.4084	0.5736	**705-7**	0.1056	0.0230	**713-7**	0.0000	**0.0955**
701-8	0.5528	0.0230	**705-8**	0.5528	0.3604	**713-8**	0.4084	0.2023
701-9	0.0253	0.6307	**705-9**	*1.0000*	0.6985	**713-9**	0.1056	0.0707
701-10	0.6127	0.4359	**705-10**	0.5000	0.5736	**713-10**	0.6127	0.3258
701-11	0.6127	0.6307	**705-11**	0.5528	0.3604	**713-11**	0.2254	0.1472
701-12	0.2929	0.0230	**705-12**	0.2929	0.1210	**713-12**	0.7764	0.3970

Table 2. Contd.

701-13	**0.0253**	0.0000	**705-13**	0.6127	0.6307	**713-13**	0.5000	0.3258
			705-14	0.5528	0.1743	**713-14**	0.6127	0.3970
			705-15	0.6127	0.6307	**713-15**	0.0780	0.1210
			705-16	0.3675	0.2615	**713-16**	0.3292	0.4359

Marker UBC 725			**Marker UBC 731**			**Marker UBC 743**		
725-1	0.2584	0.3258	**731-1**	0.0253	0.0230	**743-1**	0.0000	**0.2313**
725-2	0.1340	0.1472	**731-2**	0.0253	0.0707	**743-2**	0.1633	0.0465
725-3	0.3675	0.2615	**731-3**	0.1056	0.0955	**743-3**	0.2254	0.0955
725-4	0.0000	**0.1210**	**731-4**	0.0780	0.0465	**743-4**	0.0253	0.3604
725-5	*1.0000*	0.2615	**731-5**	0.5000	0.0465	**743-5**	0.6127	0.2313
725-6	0.0253	0.0465	**731-6**	0.1056	0.3258	**743-6**	0.5528	0.3970
725-7	0.4084	0.7868	**731-7**	0.6838	0.2313	**743-7**	0.2584	0.1472
725-8	0.2254	0.1210	**731-8**	0.0000	**0.1472**	**743-8**	0.1938	0.0230
725-9	0.7764	0.2615	**731-9**	0.0253	0.2023	**743-9**	0.0513	0.3258
725-10	0.0253	0.2615	**731-10**	0.7764	0.0465	**743-10**	0.0780	0.2615
725-11	0.1633	0.0955	**731-11**	0.1938	0.3604	**743-11**	0.4084	0.2615
725-12	0.6838	0.4778	**731-12**	0.6838	0.2929	**743-12**	0.4084	0.3258
725-13	0.5000	0.6985	**731-13**	0.2254	0.3604	**743-13**	0.5528	0.3604
725-14	0.1938	0.1472	**731-14**	0.4084	0.2023	**743-14**	0.1633	0.1210
725-15	0.5528	0.1472	**731-15**	0.1056	0.1472	**743-15**	0.1056	0.0230
725-16	0.0780	0.4778	**731-16**	0.0780	0.0465	**743-16**	0.0780	0.0230
725-17	0.0000	**0.1472**	**731-17**	0.0513	0.0707	**743-17**	0.3675	0.0465

Marker UBC 745			**Marker UBC 747**		
745-1	0.2929	0.2929	**747-1**	0.0253	0.1472
745-2	0.1056	0.2929	**747-2**	0.0253	0.2023
745-3	0.1938	0.0707	**747-3**	0.4084	0.0955
745-4	0.6838	0.3970	**747-4**	0.5528	0.2929
745-5	0.1056	0.0707	**747-5**	0.0253	0.3604
745-6	0.1938	0.4778	**747-6**	0.5528	0.2615
745-7	0.1633	0.0465	**747-7**	0.1633	0.4359
745-8	0.5000	0.4778	**747-8**	0.5528	0.4778
745-9	0.2929	0.1472	**747-9**	0.0513	0.2313
745-10	0.0253	0.1210	**747-10**	0.0253	0.1743
745-11	*1.0000*	0.3258	**747-11**	0.6127	0.2023
745-12	0.0000	**0.0230**	**747-12**	0.4523	0.3970
			747-13	0.4523	0.1743
			747-14	0.2254	0.1472
			747-15	0.4523	0.2023
			747-16	0.2254	0.3258
			747-17	0.0780	0.2023

Specific bands are in **bold typeface,** while common bands are in *Italics.* (%): the percentage for band appearance within the population.

1, according to Nei (1978).

DISCUSSION

The results of this study showed that the two populations shared more than 94% of the detected bands and that the Northern population showed one specific band whereas the southern population showed nine specific bands. The specific band detected in the Northern population was only present in one individual out of the 20 individuals analyzed. In Southern population, seven of the specific bands were present in 4.5 - 41% of the individuals. However, the other two specific bands were present in 91 and 86% of the analyzed individuals.

Table 3. Numbers of amplified, polymorphic, shared and specific bands in the two tested buffalo populations.

Random primer	T.A.B.	North population				South population			
		A.B.	P.B.	Sh.B.	Sp.B.	A.B.	P.B.	Sh.B.	Sp.B.
UBC 204	12	12	12	12	0	12	12	12	0
UBC 205	15	14	11	14	0	15	15	14	1
UBC 208	17	17	16	17	0	17	17	17	0
UBC 701	13	12	11	11	1	12	12	11	1
UBC 705	16	16	15	16	0	16	16	16	0
UBC 713	16	14	14	14	0	16	16	14	2
UBC 725	17	15	14	15	0	17	17	15	2
UBC 731	17	16	16	16	0	17	17	16	1
UBC 743	17	16	16	16	0	17	17	16	1
UBC 745	12	11	10	11	0	12	12	11	1
UBC 747	17	17	17	17	0	17	17	17	0
Total	169	160	152	159	1	168	168	159	9

T.A.B., Total amplified bands; A.B., amplified bands; P.B., polymorphic bands; Sh.B., shared bands; Sp.B., specific bands.

Table 4. Unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) between tested populations according to Nei (1978).

Population	North population	South population
North population	-------	0.8756
South population	0.1329	-------

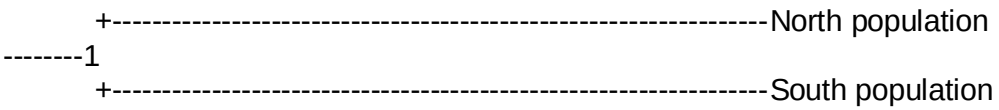

Figure 1. UPGMA dendrogram based on Nei's (1978) genetic distance.

The results obtained by El Beltagy et al. (2008), investigating the genetic differences between Delta (Northern) and Southern Egyptian buffalo populations using two microsatellite multiplexes, agree with our results. Hassan et al. (2009), sequencing the complete mitochondrial D-Loops, described the Northern and Southern populations as two subpopulations. However, El Kholy et al. (2007) using microsatellites analysis, reported that the two populations were not genetically distinct from each other.

Our results show that the two populations belong to one breed since the genetic identity and the genetic distance between the Northern and Southern populations were found to be 0.8756 and 0.1329, respectively. This confirms the previous findings of El Kholy et al. (2007), Abdel-Aziem et al. (2010), El Beltagy et al. (2008) and Hassan et al. (2009).

The Egyptian buffalo breed was reported to be of the Mediterranean type where Moioli et al. (2001) in their study on buffalo from three Mediterranean countries: Italy, Greece and Egypt, reported that differentiation rates between Egyptian buffalo and those of Italy and Greece was 0.070, which indicates that they are of the same type since differentiation rates of up to 0.15 indicates moderate differentiation between populations (Hartl, 1980).

Genetic diversity of buffalo populations has been investigated worldwide using microsatellites and RAPD analysis such as in the Mediterranean region (Moioli et al., 2001), Turkey (Soysal et al., 2007; Gargani et al., 2009), India (Vijh et al., 2008; Barwar et al., 2008) and Pakistan (Sajid et al., 2007).

It is concluded that the two tested Northern and Southern populations belong to one breed since the genetic identity between them is nearly reaching the maximum value, while the genetic distance was very low.

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