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Development of Molecular Markers for Detecting Genetic Relationships Within and Among Six Egyptian Buffalo Locations

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Abstract: Genetic variations was detected in Egyptian buffaloes at six locations; El-Behira (Be), El-Sharkia (Sh), El-Menofia (Me), Kafr El-Sheikh (Kf), El-Menia (Mn) and Sohag (So). Ten individuals from each location were blood sampled. SDS-protein and esterase markers were used to detect the genetic variations within the six studied location. SDS-protein profiles showed lower percentage of polymorphism (21.5%) than esterase profiles (45.7%) within each of the studied locations. Moreover, the mean of the genetic similarity averages within the all studied locations based on protein, esterase and overall markers were 0.951, 0.890 and 0.921, respectively, which indicated the high homogeneity within each of the six studied locations. RAPD marker was used to detect the genetic variations among the six studied locations using 10 random primers against bulked DANs of the 10 individuals of each location. The polymorphism percentage from all used primers was 43.5% among the six studied locations. The similarity average among the six locations based on overall primers was 0.884, which indicated the homogeneity among the Egyptian buffalo locations. The dendrogram of genetic relationship based on overall RAPD primers indicated the possibility of Egyptian buffalo transportation between El-Delta and Upper Egypt among the Nile River. Finally, all studied locations, except Kafr El-Sheikh, were characterized by six unique positive and 17 unique negative markers.It was concluded that these results could have a great impact in Egptian buffalo improving programs.

Key words: Buffalo · Genetic relationship · SDS-PAGE · Esterase · RAPD

INTRODUCTION

The water buffalo (*Bubalus bubalis*) contributes immensely to the agricultural economy through milk, meat, hides and draught power. A larger part of the human population depends on domestic water buffalo than on any other livestock species in the world [1]. There are a total of three indigenous buffalo varieties in Egypt, Menofi, Beheri and Saidi based on minor reported phenotypic differences concerning size, colour and production. However, these phenotypic differences are not well defined to be relied on as taxonomic classification, thus the characterization and identification of breeds at the genomic level is of utmost importance [2]. A world wild interest in indigenous breed characterization has risen for the purpose of biodiversity studies, taxonomic classification, derivative origin and setting policies for management, maintainance and improvement of these breeds. Therefore, to design rational breeding strategies for optimum utilization and conservation of available genetic variability in buffaloes, it is essential to understand their genetic architecture and relationships among various breeds [3].

The polymorphism of blood protein markers gives some useful information in studies of animal breeding such as the relationships among breeds and their evolution. Protein markers generally indicated significant differences of allele frequencies between cattle breeds and an effect of geographical origin on genetic distance [4]. In this case, many researchers employed classical biochemical polymorphic markers such as polymorphic proteins [5, 6] and isozymes [7, 8] to characterize and estimate genetic distances between breeds of goats, to study the genetic diversity within goat breeds and to reconstruct the phylogenetic relations among goat populations.

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DNA markers have come to play a major role in the characterization of livestock breeds. Edwards *et al.* [9] stated that DNA markers provide highly efficient and informative ways of characterizing diversity and also can help to resolve the question of how many different genetic classes are present. Moreover, they calculated the genetic similarities among different European cattle breeds and showed how much diversity is present and its evolutionary relationships with wild relatives. In the same time, a number of studies have been conducted to investigate the genetic diversity in cattle at the DNA level showed that DNA markers can provide information on individual identity, on degrees of relatedness and on how genetic variation is distributed within populations [10, 11].

The aims of the present study were to estimate the level of polymorphisms and similarity values within (using SDS-protein and esterase markers) and among (using ten RAPD primers) six Egyptian buffalo locations (Fig. 1) to provide baseline data for future characterization and conservation efforts and to identify unique RAPD markers and generate fingerprints for each studied location.

MATERIAL AND METHODS

Animals: Blood samples of ten individual animals representing six Egyptian buffalo locations were collected from El-Behira (Be), El-Sharkia (Sh), El-Menofia (Me), Kafr El-Sheikh (Kf), El-Menia (Mn) and Sohag (So) jugular veins into sterile plain glass tubes containing disodium salt of EDTA as anticoagulant .

Methods:

Biochemical Analyses:

SDS-PAGE Analysis: The ten collected samples were used to detect protein polymorphism within each of the six locations. Gel preparation, electrophoresis conditions and staining and destaining of gels were done according to Laemmli [12].

Esterase Analysis: The same collected samples were used to detect esterase polymorphism within each of the six locations. Esterase electrophoresis was performed according to Tanksley and Rick [13].

RAPD Analysis:

Genomic DNA Isolation: The n collected samples from each location were used to detect RAPD polymorphism among the six locations. Genomic DNA was extracted according to Sambrook *et al.* [14]. The genomic DNA of each ten samples from each location was mixed in a bulked sample according to Lukyanov *et al*. [15].

Fig. 1: Map showing locations of the sampled individuals of the Egyptian buffaloes

Table 1: Primers name, sequence and GC% content which used in the present study

Primers	Primer sequence 5'→3'	GC content %
C01	TTC GAG CCA G	60
C04	CCG CAT CTA C	60
C06	GAA CGG ACT C	60
C07	GTC CCG ACG A	70
C09	CTC ACC GTC C	70
C11	AAA GCT GCG G	60
C12	TGT CAT CCC C	60
C13	AAG CCT CGT C	60
C17	TTC CCC CCA G	70
C19	CTT GCC AGC C	70

RAPD Analysis: The amplification conditions and PCR mixture were set according to Williams *et al*. [16]. A set of ten decamer random primers was used as listed in Table 1. The amplified products (12.5 ul loaded) were separated on 1.5% agarose gels and the amplified fragments were photographed under UV light using Polaroid Camera.

RESULTS

Genetic variations within the six studied locations:

Protein and Esterase Polymorphisms Within the Six Studied Locations: The banding patterns of the six location protein fractions revealed wide variations of different bands (73 monomorphic and 20 polymorphic bands) as shown in Table 2. El-Sharkia and El-Menia locations showed high polymorphic bands (25.0%), El-Menofia, Kafr El-Sheikh and Sohag locations revealed moderate polymorphic bands (21.4, 22.2 and 20.0%, respectively), while El-Behira location exhibited low polymorphic bands (14.3%).

Table 2: Summary of densitometric analysis for SDS-protein banding patterns within the six tested locations.

Location	Range of molecular weight (KDa)	Range of bands/individual	Range of band frequency	Total bands	Polymorphic bands	Polymorphism%
Be	196.75-16.70	12-14	0.4-1.0	14	2	14.3
Sh	208.88-13.25	12-16	0.1-1.0	16	4	25.0
Me	200.14-19.01	11-14	0.6-1.0	14	3	21.4
Kf	208.22-16.63	14-17	0.1-1.0	18	4	22.2
Mn	219.42-23.01	13-15	0.3-1.0	16	4	25.0
So	229.2-24.29	13-14	0.1-1.0	15	3	20.0
Total				93	20	21.5

Table 3: Summary of densitometric analysis for esterase profiles within the six tested locations.

Location	Range of relative front	Range of bands/individual	Range of band frequency	Total bands	Polymorphic bands	Polymorphism %
Be	0.31-0.91	4-4	0.3-1.0	5	2	40.0
Sh	0.41-0.97	5-5	0.1-1.0	7	4	57.1
Me	0.50-0.98	4-4	1.0-1.0	4	0	0.0
Kf	0.41-0.95	3-5	0.5-1.0	5	2	40.0
Mn	0.29-0.95	4-5	0.1-1.0	7	4	57.1
So	0.23-0.87	4-5	0.1-1.0	7	4	57.1
Total				35	16	45.7

Table 4: Similarity ranges and averages within each of the six studied locations based on protein and esterase markers.

	Protein marker		Esterase marker		
Location	Range	average	Range	average	All markers average
Be	0.92-1.00	0.960	0.75-1.00	0.883	0.922
Sh	0.86-1.00	0.939	0.60-1.00	0.890	0.915
Me	0.88-1.00	0.952	1.00-1.00	1.00	0.976
Kf	0.88-1.00	0.950	0.75-1.00	0.865	0.908
Mn	0.88-1.00	0.938	0.67-1.00	0.814	0.876
So	0.89-1.00	0.968	0.60-1.00	0.889	0.929
All locations average		0.951		0.890	0.921

Table 5: Summary of densitometric analysis for bulked DNAs among the six tested locations using 10 random primers.

Primer	Range of amplicon size (bp)	Range of amplicons / individual	Range of amplicon frequency	Total amplicon	polymorphic amplicons	polymorphism %
C01	1795-265	7-9	0.17-1.00	10	5	50.0
C04	2924-362	4-7	0.17-1.00	7	3	42.9
C06	1501-232	9-11	0.17-1.00	12	5	41.7
C07	2689-372	14-17	0.50-1.00	17	3	17.6
C09	2380-406	9-12	0.17-1.00	13	8	61.5
C11	1500-317	7-8	0.33-1.00	8	1	12.5
C12	1631-392	4-5	0.17-1.00	5	1	20.0
C13	1107-274	7-10	0.50-1.00	10	6	60.0
C17	1276-241	10-13	0.33-1.00	15	9	60.0
C19	1293-272	7-10	0.17-1.00	11	6	54.5
Average				10.8	4.7	43.5

Table 6: Number of amplified fragments and similarity ranges and averages among the six studied locations using 10 random primers.

	Number of amplified fragments / location						Similarity value	
Primer	Be	Sh	Me	Kf	Mn	So	Range	Average
C01	7	9	9	8	8	7	0.71-1.00	0.881
C04	5	5	7	4	4	6	0.73-1.00	0.868
C06	11	9	11	9	9	10	0.80-1.00	0.898
C07	14	15	14	17	16	17	0.90-1.00	0.941
C09	10	12	9	11	11	12	0.63-1.00	0.864
C11	8	8	7	7	7	7	0.93-1.00	0.963
C12	5	4	4	4	4	4	0.89-1.00	0.963
C13	9	7	10	10	7	7	0.71-1.00	0.832
C17	13	12	11	11	10	11	0.70-0.95	0.794
C19	7	10	10	7	8	8	0.67-1.00	0.836
Average	8.9	9.1	9.2	8.8	8.4	8.9	0.794-0.963	0.884

Concerning esterase isozyme, the six location profiles showed wide variations of different bands (19 monomorphic and 16 polymorphic bands) as shown in Table 3. El-Sharkia, El-Menia and Sohag locations showed high polymorphic bands (57.1%), El-Behira and Kafr El-Sheikh locations revealed moderate polymorphic bands (40.0%), while El-Menofia location exhibited no polymorphic bands (0.0%).

Genetic Similarity Within the Six Studied Locations: The ranges and averages of similarity values within each of the six studied locations based on protein and esterase markers are listed in Table 4. Concerning protein marker, all studied locations revealed high similarity averages, which indicated high homogeneity within each location. However, the lower genetic similarity averages were found within El-Menia (0.938) and El-Sharkia (0.939) locations, Kafr El-Sheikh (0.950) and El-Menofia (0.952) locations showed moderate genetic similarity averages, while the higher genetic similarity averages were found within El-Behira (0.960) and Sohag (0.968) locations. Based on esterase marker, the highest genetic similarity average was found within El-Menofia (1.00) location, while the lowest one was observed within El-Menia (0.814) location. However, El-Behira (0.883), El-Sharkia (0.890), Kafr El-Sheikh (0.865) and Sohag (0.889) locations showed moderate genetic similarity averages.

Genetic Variations among the Six Studied Locations:

RAPD Polymorphism among the Six Studied Locations: The total number of reproducible fragments amplified by the used 10 primers among the tested locations reached 108, which 47 of them were polymorphic fragments as presented in Table 5. All used primers recorded high polymorphism which ranged from 41.7 to 61.5%, except C07 (17.6%), C11 (12.5%) and C12 (20.0%) primers which showed low polymorphism.

Genetic Similarity and Relationships among the Six Studied Locations: The ranges and averages of similarity values among the six studied locations using 10 random primers are listed in Table 6. The tested primers gave high

Table 7: Similarity values among the six studied Egyptian buffalo locations based on overall RAPD primers

Location	Be	Sh	Me	Kf	Mn
Sh	0.873				
Me	0.854	0.907			
Kf	0.845	0.908	0.882		
Mn	0.825	0.893	0.877	0.947	
So	0.840	0.888	0.893	0.913	0.912

Table 8: Unique positive and negative-location-specific markers among the tested Egyptian buffalo locations using RAPD markers

	Unique positive RAPD markers			Unique negative RAPD markers		
Location	Marker fragment size (bp)	Primer	Total number of marker / location	Marker fragment size (bp)	Primer	Total number of marker / location
Be	232	C06	3	397	C01	
	2380	C09	296	452	C01	
	392	C12	265	406	C06	
				1133	C09	7
				978	C09	
					C19	
					C19	
Sh				885	C13	
				555	C13	2
Me	413	C01		2136	C09	
	1249	C04	2	1934	C09	
				626	C09	3
Mn				552	C06	
				723	C09	
				766	C13	3
So	469	C19	1	1795	C01	
				701	C17	2
Total	6				17	

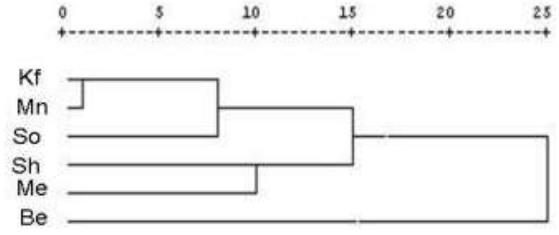

Fig. 2: Dendrogram of genetic relationship among the six studied Egyptian buffalo locations based on overall RAPD primers

similarity averages among the six locations which ranged from 0.794 (using C17 primer) to 0.963 (using C11 and C12 primers). However, similarity values among the six locations based on overall primers are given in Table 7. The highest similarity value was between Kafr El-Sheikh and El-Menia (0.947) locations followed by between Kafr El-Sheikh and Sohag (0.913) locations, while the lowest one was between El-Behira and El-Menia (0.825) locations.

The dendrogram based on RAPD markers among the six locations was classified into two main clusters, the first one was divided into two sub-clusters as shown in Figure 2. Within the first cluster, Kafr El-Sheikh, El-Menia and Sohag locations were grouped together in the first sub-cluster, while the second one included El-Sharkia and El-Menofia locations. The second cluster was comprised only El-Behira location.

Identification of Unique Rapd Markers for the Studied Locations: Identification of unique RAPD markers characterizing the various buffalo breeds by unique fingerprints could have a number of potential applications including the determination of breed purity, the management of genetic resources collection and the establishment of property rights. Table 8 shows that eight primers out of 10 primers (80%) gave unique markers for only five locations (83%) at different fragment sizes. These markers were scored from the presence of a unique band for a given location. However, these locations were characterized by six positive and 17 negative markers. The largest number of location-specific markers were scored for El-Behira location (10 markers) followed by El-Menofia location (5 markers), El-Menia and Sohag locations (3 markers) and El-Sharkia location (2 markers). On the other hand, C09 primer generated the largest number of RAPD-specific markers (7 markers) followed by C01 primer (4 markers), C06, C13 and C19 primers (3 markers) and C04, C12 and C17 primers (one marker).

DISCUSSION

Concerning biochemical polymorphism within the six studied location, the average of protein polymorphism was 21.5% indicating low protein variation, while the average of esterase polymorphism was 45.7% indicating moderate esterase variation . Similar result was observed by El-Seoudy *et al*. [17] who detected lower genetic differences using native protein marker than six isozyme systems within the examined animals of three Egyptian camel breeds.

The average of similarity indices reflects the range of homogeneity and inbreeding within each tested populations. However, the mean of the genetic similarity averages within the all studied locations based on protein, esterase and overall markers were 0.951, 0.890 and 0.921, respectively, which indicated the high degree of homogeneity and inbreeding within each one of the six studied locations .

Concerning protein marker, recently many authors have been reflected this point such as Anous *et al.* [18] who assessed the genetic structure within each of three Egyptian goat populations using serum protein marker. They found that Barki population had the highest average value (0.69) followed by Baladi population (0.65), while Zaraibi population had the lowest value (0.55) and this may reflect a higher degree of inbreeding in both Barki and Baladi populations compared to Zaraibi population. They concluded that protein analysis is a sensitive method for studying the genetic structure of goat populations.

Concerning isozyme marker, Deza *et al*. [6] studied Mdh and esterase variations among some native goats from different locations and found individual differences among goat samples using these isozyme markers. Moreover, El-Seoudy *et al.* [19] reported that Mdh was not able to distinguish among breeds, sexes or even between different locations, while esterase had a value to discriminate among goat populations. In the other hand, Nyamsamba *et al*. [20] could not obtain any clear genetic differences among eight native goat populations by studying their isozyme variations. Recently, Anous *et al.* [18] used Mdh and esterase isozymes to detect individual variations within each of three Egyptian goat populations. They found that the greatest was in Zaraibi breed, while the lowest was in Barki breed.

Concerning DNA polymorphism among the six studied locations, the polymorphism average recorded form all used RAPD primers was 43.5 % among the bulked individual DNAs of the six studied locations which

indicated moderate DNA variation . This result was similar with those obtained by El-Seoudy *et al*. [17] who detected 43.6% of polymorphism average among three Egyptian camel breeds using 14 ISSR primers.

The similarity average among the six studied locations based on the the profiles of the ten used RAPD primers was 0.884, which indicated the homogeneity among these Egyptian buffalo locations. The same feature was observed by El-Seoudy *et al*. [17] who found that the similarity average among three Egyptian camel breeds was 0.805 using 14 ISSR primers.

The dendrogram based on RAPD markers clustered Kafr El-Sheikh location from El-Delta with El-Menia and Sohag locations from Upper Egypt which indicated the possibility of Egyptian buffalo transportation between El-Delta and Upper Egypt among the Nile River. However, RAPD analysis was in a partial agreement within the geographical distribution and succeeded in distinguishing among the six studied locations.

Various studies have been reported for the same purpose such as El-Seoudy *et al*. [19] who characterized two local breeds of Egyptian goat (Baladi and Zaraibi) using 10 RAPD primers. They concluded that RAPD analysis was a useful tool in charactering goat breeds. Moreover, Kumar [3] used 27 SSR loci to define genetic variation and relationships among eight Indian riverine buffalo breeds. Analysis of molecular variance refuted the earlier classification of these breeds proposed on the basis of morphological and geographical parameters. However, they concluded that their results will be useful for development of rational breeding and conservation strategies for Indian buffaloes. In addition, Adawy *et al*. [10] used 19 AFLP primer combinations and 23 RAPD primers to estimate genetic diversity among four Sudanese cattle. Each of AFLP, RAPD and the combined data dendrogram assigned the individuals of each cattle breed in a separate sub-cluster. They concluded that AFLP and RAPD markers characterized each breed at the molecular level and generated unique fingerprint.

Twenty three unique RAPD markers were detected by eight primers out of the ten used primers (80%) which gave unique markers (six positive and 17 negative markers) for only five locations (83%) at different fragment sizes . These twenty three location-specific markers indicating the possibility of differentiating the closely related locations using molecular markers. Similar result was obtained by Adawy *et al*. [11] who identified 20 unique positive and 22 unique negative markers which characterized four Sudanese cattle populations using RAPD analysis. They concluded that there was a sufficient diversity among these populations to permit their conservations as separate breeds.

It is concluded that there is a low genetic variation within these locations to permit their conservation as separate breeds at the biochemical level. Moreover, the results assessed the potentiality of RAPD technology for characterizing each breed at the molecular level and generating unique fingerprint. This could have a great impact in Egyptian buffalo improving programs.

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