DEVELOPMENT OF MOLECULAR MARKERS FOR MEASURING GENETIC DIVERSITY AMONG AND WITHIN FOUR SUDANESE AUTOCHTHONOUS CATTLE

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There are many types of cattle in Sudan, and different classifications of Sudanese cattle have been proposed. Various authors have sought to classify the breeds on the basis of their origin and phenotypic characteristics. Of those authors Bennett *et al.* (1954) classified the Sudanese local cattle into three main groups, Northern or Arab, Southern or Nilotic, and the small cattle of Nuba mountains. According to Joshi *et al.* (1957) and Payne (1970), the Northern Sudan cattle include Kenana, Butana, Western Baggara, White Nile and Northern Province cattle. However, these classifications are on the basis of phenotypic characteristics or geographic origin and are not related to genotype, except for the part of genotype reflected by phenotype. With the advent of molecular technology a powerful new tool is available for characterization, classification and estimation of distances between breeds and strains.

More recently molecular markers have come to play a major role in the characterization of livestock breeds. Edwards *et al.* (2000) stated that extensive studies have shown that DNA markers provide highly efficient and informative ways of characterizing diversity and DNA markers and can help to resolve the question of how many different genetic classes are present. The genetic similarities among different classes and how much diversity is present and their evolutionary relationships with wild relatives. A number of studies have been conducted to investigate the genetic diversity in animals and plants at the DNA level, (Edwards *et al*., 2000; Adawy, 2006; Adawy *et al.*, 2006; Hussein *et al*., 2005 & 2006) and showed that DNA markers can provide information on individual identity, on degrees of relatedness and on how genetic variation is distributed within populations.

The aim of the present study is to estimate the level of polymorphism among and within four populations of Sudanese autochthonous cattle to provide baseline data for future characterization and conservation efforts. Also to estimate the genetic similarity and relationship within and between these four Sudanese populations. These populations are the Kenana and Butana cattle which are well known as some of the most promising

tropical breeds of dairy cattle while the Baggara and Nilotic are mainly used for meat production.

MATERIALS AND METHODS

Animal material

Blood samples from a total of 54 animals representing four Autochthonous populations of Sudanese cattle were collected from Nyala, Kosti, Atbara and Umbenain, the homelands of Western-Baggara, Nilotic, Butana and Kenana cattle, respectively.

Genomic DNA isolation

The total genomic DNA was isolated and purified according to Promega Corporation (1999) by Wizard Genomic DNA Purification kit. The method used to measure the amount of DNA was the quantitation of DNA using comparison by gel electrophoresis, *i.e.*, quantitation of DNA by Ethidium bromide fluorescence (Sambrook *et al.,* 1989).

RAPD analysis

Random Amplified Polymorphic DNA (RAPD) was used to detect polymorphism at the DNA level, based on the polymerase chain reaction (PCR). The DNA amplification protocol was performed as described by Williams *et al.* (1990) with some modifications.

A total of 42 random primers were used to screen the DNA of the four populations of Sudanese cattle, the primers generating weak or complex patterns were discarded. Out of the 42 tested primers, only 22 were selected for further analysis. The decamer random primers used in this study were selected from a set of Operon Kits (Table 1). The primers were synthesized at the Agricultural Genetic Engineering Research Institute (AGERI), ARC, Giza, Egypt, on an AB/392 DNA/RNA synthesizer by Applied Biosystems.

Data analysis

Scoring of the data was performed on the basis of the presence or absence of each band for all genotypes by assigning (1) to visible bands and zero (0) to absent bands. Only clear and unambiguous bands were visually scored, the scoring did not consider differences in intensity of the bands among profiles from different samples. The genetic similarities and similarity matrices were calculated among populations using Jaccard's coefficient (Jaccard, 1908). Cluster analyses based on similarity matrix obtained with unweight pair group method of arithmetic average (UPGMA) as described by Jaccard (1908), and relationships within and between populations were represented as dendrograms displaying the hierarchical associations among all genotypes. The similarity matrix contains all of the similarity values relating any two samples in the population. Pairwise comparisons were made between genotypes based on bands that showed polymorphism among all genotypes, and the values used to generate Jaccard's similarity coefficient (Jaccard, 1908).

RESULTS AND DISCUSSION

Genetic diversity among the four populations of cattle

The total number of reproducible fragments amplified by the twenty two primers reached 266, out of these 137 was polymorphic fragments. This represented a level of 51.5% polymorphism and an average of 6.23 polymorphic markers per primer among the bulked samples of the four populations (Table 1 and Fig. 1). The size of amplified fragments for the different primers ranged from 100 bp to 2100 bp. Among the four populations, each primer produced multiple bands profiles with a total number of amplified DNA fragments ranging from 7 to 17, while the number of polymorphic fragments ranged from 0 to 15. The high level of polymorphism among populations indicated considerable genetic divergence between the four populations. In this respect, Cao *et al.* (2002) investigated the genetic diversity among four sheep breeds using RAPD markers, in which 16 primers amplified 146 bands and out of these 42 bands were polymorphic among sheep breeds. This represented a level of polymorphism of 29%. In China, Geng *et al.* (2002) showed that the DNA polymorphism frequencies of Chaidamu goat (CG), Chaidamu Cashmere goat (CCG) and Liaoning Cashmere goat (LCG) were 0.84, 0.86, and 0.85 respectively and the size of the DNA fragments was 176-2937 bp.

Genetic diversity within the four populations of cattle

RAPD profiles within each of the four populations, Baggara - Nialawy, Nilotic - Majock, Butana and Kenana cattles are presented in Fig. (2). Fourteen and 12 individual samples and their bulk from Western Baggara, Nilotic, Butana and Kenana cattles, respectively showed polymorphism within the four breeds using 10 RAPD primers. The selected primers produced multiple bands profiles with a number of amplified DNA fragments of (5 to 17), (5 to 14), (5 to 16) and from of (4 to 13) in Baggara, Nilotic, Butana and Kenana, respectively. The total number of deducible fragments reached 96 out of which 22 were polymorphic fragments in Baggara. While, 95, 86 and 87 out of which 35, 21 and 36 were polymorphic fragments in Nilotic, Butana and Kenana cattles, respectively. This represented a level of polymorphism of 23.2%, 36.8%, 24.4% and 41.4% in Baggara, Nilotic, Butana and Kenana cattles, respectively and the size of amplified fragments ranged from 100 bp to 3341 bp. The average number of polymorphic amplicons was 2.2, 3.5, 2.1 and 3.6 markers / primer in Western Baggara, Nilotic, Butana and Kenana cattles, respectively.

In this study, the level of polymorphism among the four populations was higher than that obtained within each of the separate populations. This low level of polymorphism within populations was

probably mainly a consequence of the fact that each population occupies a contiguous geographic area. Each of these cattle populations is owned by a nomadic or semi-nomadic tribe of herdsmen. Consequently, matings within each of the respective populations my not be random and genetic homogeneity is expected. The relatively high level of polymorphism obtained within Kenana cattle (41.4%) may be due to the large geographical spread of the population and the expected migration of cattle through historical times along the Blue and White Nile, Gezira, Khartoum and Southwards to the Ethiopian border. Considerable cross-breeding is known to occur along the White Nile between Kenana cattle and the cattle of some Western Sudan tribes. The lowest level of polymorphism was found within Western Baggara and Butana cattle (23.2% and 24.4%, respectively), indicating that these populations are genetically homogenous. These cattle are located in the Nyala region and are named after the region. It is a closed population with a limited regular migration pattern from South to North during the rainy season and from North to South by the end of the rains in a very regular system in a route that does not allow much exposure to admixture with other populations. This study lends support to the view that there are major genetic differences between these four cattle populations.

Identification of unique RAPD markers

Identification of unique RAPD markers characterizing the various cattle breeds by unique fingerprints could have a number of potential applications including the determination of breeds purity, efficient use and management of genetic resources collection and the establishment of property rights. Table (3) showed that 8 out of 22 RAPD primers (36%) gave unique markers at different molecular sizes. The four cattle breeds were characterized by 42 unique positive and negative markers (20 UPM) and (22 UNM). The Kenana breed was characterized by the highest number of unique markers (15) while, Nilotic was characterized by the lowest number (7). The breeds Baggara and Butana were identified by 10 and 10 unique markers, respectively.

Genetic similarity among and within the four cattle populations

The RAPD data matrix was utilized to estimate the genetic similarity among and within the four populations using the Diversity Data-base Fingerprinting Software (BIO-RAD). The four populations had genetic similarities that ranged from 78.4% to 84.8% with an average of 81.6%. This result is similar to that observed by Rincon *et al.* (2000) who used RAPD markers to analyse Cerole cattle genome polymorphism and showed that the highest similarity was observed in the comparison of Holstein Friesian and Herford (0.81) while the less related fingerprints were observed between Cerole and Herford cattle (0.77). These results were obtained from a total of 215 loci ranging between 300 and 2500 bp. In the present study the lowest genetic

similarity was between Nilotic and Kenana cattle, and the highest was between Baggara and Nilotic.

The genetic similarity within Baggara (Nialawy) cattle ranged from 92.9% to 98.9% with an average of 95.9%. Within Nilotic (Majock) cattle genetic similarity ranged from 88.2% to 96.3% with an average of 92.2%. The maximum and minimum genetic similarity within the Butana and Kenana cattle was 98.7%, 97.2% and 88.0%, 80.3% with an average of 93.3% and 88.7%, respectively. The lowest genetic similarity was found within Kenana cattle and to a lesser extent within the Nilotic cattle. The highest genetic similarity was within Baggara cattle followed by Butana cattle. In this respect, Appannavar *et al.* (2003) used RAPD markers as a demonstration of variability within genomes and to detect the degree of genetic similarities and divergence among different types of Deoni cattle viz. Balankya, Wannera and Wghya cattle. They used six random primers and produced low to high numbers of polymorphic bands between DNA of different Deoni types. Of the 48 RAPD markers they used, 33 were common to all Deoni types, 3 were individual specific and 12 were polymorphic for different Deoni types. The mean average percentage values among Deoni types showed that Balankya and Wannera had less genetic divergence when compared to Waghya.

Genetic relationships

The dendrogram constructed from UPGMA cluster analysis among the four cattle populations based on RAPD data is presented in Fig. (3). The data distinguished two groups. The first group comprised Kenana and Butana, and the second group comprised Baggara and Nilotic. The genetic relationship was 0.80 between these two main groups. The genetic relationship of Kenana and Butana was 0.82, while it was 0.85 between Nilotic and Baggara. This indicates that the genetic relationship between Nilotic and Baggara cattle is relatively closer than that between Kenana and Butana cattle. This is to be expected in view of the migratory routes of the nomadic and semi-nomadic tribes owning these cattle. Geographically, the members of each of these two groups are close to each other and it is expected that some gene flow must have occurred within each group at some time in the past.

The dendrograms constructed from UPGMA cluster analysis within the four cattle populations based on RAPD data for the Baggara (Nialawy), Nilotic (Majock), Butana and Kenana cattle are presented in Fig. (4). The data distinguished two main groups within each of the first three populations, with genetic relationships of 0.94% for Baggara, 0.91% for Nilotic cattle and 0.91% for Butana cattle. Kenana cattle dendrogram distinguished three main groups with genetic relationship of 0.86 % and 0.90%.

Based on the RAPD dendrograms there is a genetic variation within the four populations and that they may be considered distinct entities or 'breeds'. However, it is also clear that the genetic diversity within each of the populations didn't confirm the genetic homogeneity` expected on the basis of their geographical location, which may reflect undocumented migrations and gene flow. It is concluded that there is sufficient diversity among these populations to permit their conservation as separate breeds and there is an evidence of strains within these populations. For the purposes of conservation, each of these populations will have to be considered individually, and appropriate strategies will have to be devised for the long-term maintenance and use of genetic variation among and within breeds.

SUMMARY

Twenty-two arbitrary RAPD decamer primers were used to investigate the genetic similarity and genetic relationships among and within four populations of Sudanese cattle namely, Baggara (Nialawy), Nilotic (Majock), Butana and Kenana. The DNA samples from 54 animals representing the four populations were collected from their homeland. The level of polymorphism among the four populations was 51.5%. While, 23.2%, 36.8%, 24.4% and 41.4% polymorphism were recorded within Western Baggara (Nialawy), Nilotic (Majock), Butana and Kenana autochthonous cattle, respectively. Cluster analysis based on similarity matrices using UPGMA distinguished two groups. The first group encompassed Kenana and Butana and the second included Baggara and Nilotic with a genetic relationship of 80%. The genetic relationship within each of the two groups was 82.0% and 85.0%, respectively. The dendrogram distinguished two subgroups within each of the three populations, Baggara, Nilotic and Butana. While, it distinguished three groups within Kenana cattle, with a degree of genetic relationship ranging from 86.0% to 93.0%. It is concluded that there is sufficient diversity between these populations to permit their conservation as separate breeds and there is an evidence of sub-breeds within these populations.

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Table (1): Number of bands generated and polymorphism percentage as revealed by RAPD among the four cattle population.

No.	Primers	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism %
1	OP-A02	17	2	15	88.2
2	OP-A04	17	5	12	70.6
3	OP-A05	10	5	5	50.0
4	OP-A15	11	5	6	54.5
5	OP-C01	9	4	5	55.6
6	OP-C02	14	7	7	50.0
7	OP-C03	9	9	0	0.0
8	OP-C05	17	3	14	82.3
9	OP-C09	7	2	5	71.4
10	OP-C13	10	3	7	70.0
11	OP-C14	17	11	6	35.3
12	OP-C15	17	11	6	35.3
13	OP-C17	13	6	7	53.8
14	OP-C18	11	7	4	36.4
15	OP-D10	8	4	4	50.0
16	OP-E16	12	4	8	66.7
17	OP-E18	13	8	5	38.5
18	OP-O01	9	4	5	55.6
19	OP-O04	10	7	3	30.0
20	OP-O09	14	10	4	28.6
21	OP-O11	14	6	8	57.1
22	OP-O12	7	6	1	14.3
Total		266	129	137	51.5

Table (2): Number of bands generated and polymorphism percentage within four Sudan autochthonous cattle as revealed by RAPDs markers.

No.	Primers	Western Baggara cattle			Nilotic cattle			Butana cattle			Kenana cattle		
		Total bands	Polymorphic bands	Polymorphism %	Total bands	Polymorphic bands	Polymorphism %	Total bands	Polymorphic bands	Polymorphism %	Total bands	Polymorphic bands	Polymorphism %
1	OP-C01	11	1	9.1	11	6	54.5	7	0	0.0	11	11	100.0
2	OP-C02	9	2	22.2	14	2	14.3	5	0	0.0	9	6	66.7
3	OP-C04	12	0	0.0	9	3	33.3	10	0	0.0	7	0	0.0
4	OP-C05	13	4	30.8	12	5	41.7	16	8	50.0	12	10	83.3
5	OP-C09	10	5	50.0	8	7	87.5	8	5	62.5	4	1	25.0
6	OP-C12	7	1	14.3	7	2	28.6	9	0	0.0	7	0	0.0
7	OP-C13	7	4	57.1	11	4	36.4	7	1	14.3	10	4	40.0
8	OP-D10	5	1	20.0	6	2	33.3	5	2	40.0	5	2	40.0
9	OP-E16	5	4	80.0	5	3	60.0	7	0	0.0	9	0	0.0
10	OP-E18	17	0	0.0	12	1	8.3	12	5	41.7	13	2	15.4
Total		96	22	23.2	95	35	36.8	86	21	24.4	87	36	41.4

Table (3): Unique markers characterizing the four cattle populations as revealed by RAPDs.

Breed Primer	Western Baggara		Nilotic		Butana		Kenana	
	UPM	UNM	UPM	UNM	UPM	UNM	UPM	UNM
OP-A05	1000				600			2000
								1700
OP-C01	860						1200	
OP-C02	2000	200		1000	220	1500	1900	
							200	
OP-C05	430	2000	300		400		1500	750
			1700				1100	700
			1600				500	400
OP-C09	1000			1200				2000
								1400
				700				
OP-C13		350		1500	1900	2000		
					900	800		
					700			
OP-C17		1350				1400		1050
OP-D10		1100						300
Total	5	5	3	4	6	4	6	9

(UPM) Unique positive markers, (UNM) Unique negative markers

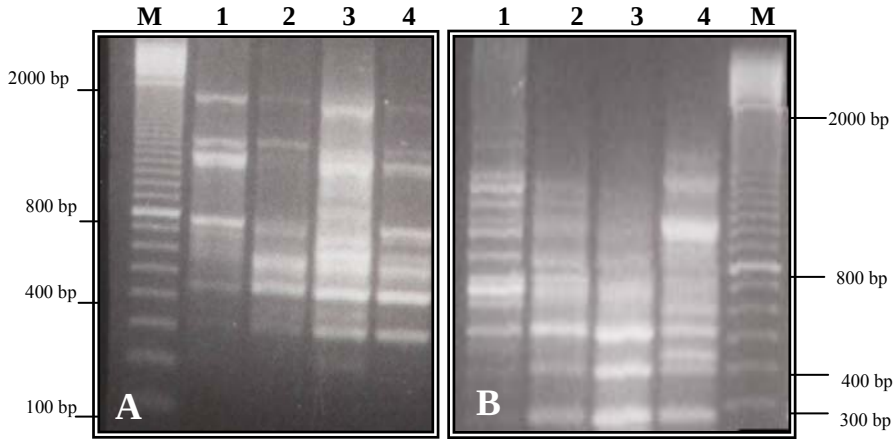

Fig. (1): RAPD profiles of (1) Western Baggara (Nialawy), (2) Nilotic (Majock), (3) Butana and (4) Kenana cattles with primers OP-C13 (A) and OP-C02 (B). M: 100bp ladder.

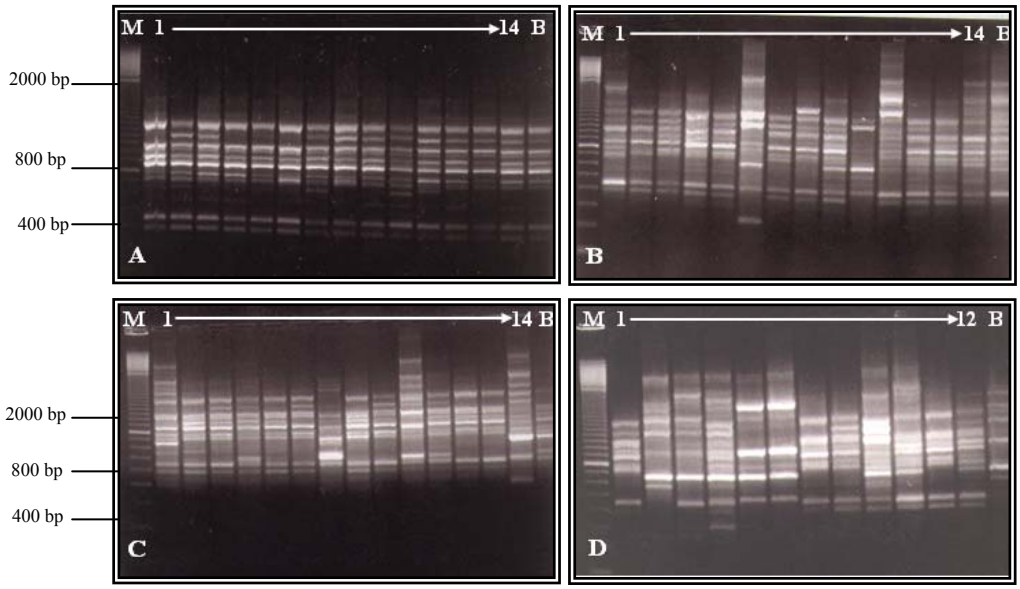

Fig. (2): RAPD profiles within Western Baggara–Nialawy cattle (A), Nilotic–Majock cattle (B), Butana cattle (C) and Kenana cattle (D) as revealed by primer OP-C05, M: marker 100 bp ladder, B: Bulk.

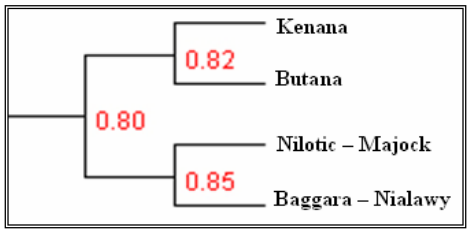

Fig. (3): RAPD dendrogram showing genetic relationships among the four cattle.

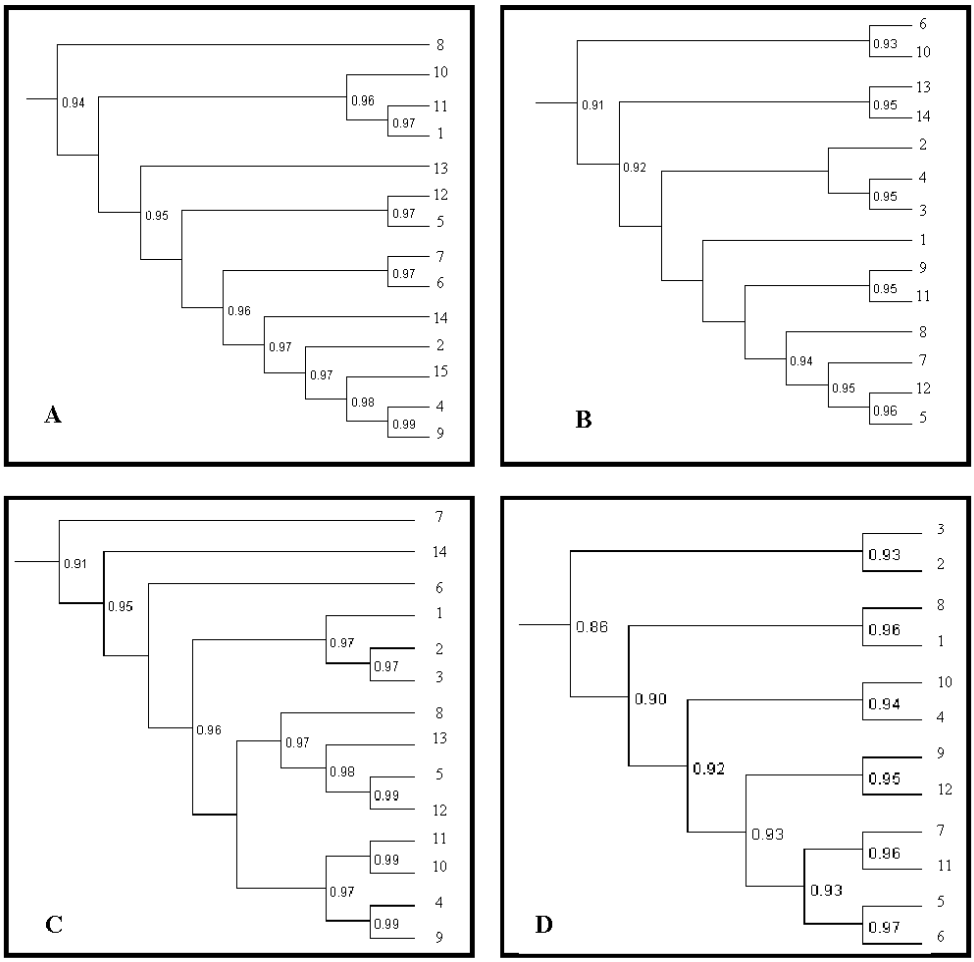

Fig. (4): RAPD dendrograms revealed by UPGMA within: Baggara cattle-Nialawy (A), Nilotic cattle-Majock (B), Butana cattle (C) and Kenana cattle (D).