GENETIC DISTANCES AMONG NINE SUDANESE SUB-BREEDS OF AUTOCHTHONOUS CATTLE AS MEASURED BY RAPD AND AFLP MARKERS

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Sudan is mainly an agricultural country with a large livestock population estimated to be 39.4 million heads of cattle. These types and sub-types are adapted to the wide variety of environments. Many reputed authors have proposed various classifications of Sudanese cattle depending on morphological phenotypic markers in the assessment of genetic diversity of native cattle. These markers are affected by environmental factors and the developmental stage of the animal. Studies based on protein markers (Blott *et al.*, 1998; Kantanen *et al.*, 1999) generally indicated significant differences of allele frequencies between cattle breeds and an effect of geographical origin on genetic distance. Several studies have been performed to investigate genetic variability on the molecular level (Karp *et al.*, 1997; O'Brien, 1994). Molecular markers have played a major role in the genetic characterization and detection of variation in genomic DNA. A number of techniques of classical fingerprinting have been particularly useful for genetic analysis. Of these techniques, RAPD plays a useful role in genetic analysis of livestock, especially in relatedness among either breeds or species (Cushwa and Medrano, 1996). Likewise, Gwakisa and Kemp (1994) created DNA pools of two cattle subspecies (*Bos indicus* and *Bos taurus*) to identify specific RAPD markers and suggested the usefulness of RAPD as genetic markers for cattle breed differentiation. Amplified fragment length polymorphism (AFLP) is another powerful DNA marker technique based on the detection of DNA restriction fragments by PCR amplification (Vos *et al.*, 1995). In cattle (Ajmone-Marsan *et al.*, 1997) and rat (Otsen *et al.*, 1996), the AFLP markers are distributed over the genome as the restriction sites and allow estimation of relative distances between individual animals genome. Differences in AFLP patterns have proven informative for the divergence of the wild and domestic cattle species (Lenstra and Bradley, 1999; Buntjer *et al.*, 2002), for introgression of zebu in African cattle (Nijman *et al.*, 1999), for zebu-banteng hybridization and for investigating genetic relationships of pigs (Ovilo *et al.*, 2000) and goats (Ajmone-Marsan *et al.*, 2001). The identification of polymorphism between different local Sudanese breeds is useful for

conservation and planned usage of local breeds in cross breeding. This identification of polymorphism will permit the study of the potential association between polymorphism in the breed and economically important traits and diseases resistance.

The aims of this research are: (1) To estimate the level of polymorphism among 9 sub-breeds representing 4 Sudanese cattle breeds using RAPDs and AFLPs markers. (2) To identify unique DNA markers and generate fingerprints for each sub-breed. (3) To study the genetic relationships among these sub-breeds using Jaccard coefficient. (4) To compare the ability of molecular markers in detecting and measuring the genetic diversity of these sub-breeds.

MATERIALS AND METHODS

Animal material

Blood samples of 54 individual animals representing 4 cattle breeds were collected from their home-land, Nyala, Kosti, Atbara and Umbenain for the Baggara, Nilotic, Butana and Kenana cattle, respectively. According to the cluster analysis of RAPD data within breeds, the dendrogram distinguished two sub-breeds within each of the Baggara, Nilotic and Butana cattle while distinguished 3 sub-breeds within Kenana cattle (Adawy *et al.*, 2007). The DNA of these nine sub-breeds was screened with RAPD and AFLP to identify markers characterizing each cattle sub-breed.

DNA isolation

Genomic DNA was isolated and purified according to Promega Corporation (1999) by Wizard Genomic DNA Purification Kit.

RAPD analysis

A total of 23 RAPD primers were used to screen the DNA of the 9 sub-breeds representing four populations of Sudanese cattle. The decamer primers were selected from a set of Operon Kits (Table 1). RAPD products (10µl loaded) were separated on 2% agarose gels and band patterns were photographed under UV light.

AFLP analysis

This was performed as described by Vos *et al.* (1995) using the large genome system AFLP I (Gibco BRL, USA) (Cat.No. 10544). About 300 nanograms of DNA were digested with *EcoR*1 and *Mse*1 enzymes and digested fragments were ligated with adaptors. The ligated DNA fragments were amplified in 25 µl volume. Pre-amplification using primers with-one 3' selective nucleotide was carried out. Each reaction (25 µl final volume) contained the following: 75 ng each of primers *EcoR*I-core and *Mse*I-core; 0.2 mM of each dATP, dCTP, dGTP, dTTP (Boehringer Manheim); 1.0 u Taq (Fermentas); 1x reaction buffer (Fermentas) and 300 ng of digested ligated DNA. Samples were subjected to 30 cycles of the following PCR programme: 94°C for 30 s, 60°C for 30 s, 72°C for 60 s. PCR

products were diluted by the addition of 50 µl of buffer (1 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). Diluted pre-amplification products were used as templates for selective amplifications. Nineteen *EcoR*I and *Mse*I selective primer combinations were employed in this study (Table 2). Selective amplification reactions (final volume of 20 µl) contained the following: 16 ng *EcoR*I primer, 15 ng *Mse*I primer, 0.2 mM of each dATP, dCTP, dGTP, dTTP, 0.5 u Taq and 1x reaction buffer. The following amplification profile was used: 1 cycle of 94°C for 30 s, 65°C for 30 s, 72°C for 60 s; 11 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 60 s and 23 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 60 s. Half volume of formamide dye (98% formamide, 10 mM EDTA pH 8.0, 0.5 mg ml¯1 Xylene Cyanol FF) was added. Samples were denatured for 5 min in a boiling water bath and thereafter electrophoresed through preheated (80 W for 30 min) denaturing polyacrylamide gels at a constant power of 80 W for 1 hr 45 min. The gel was silver stained according to the protocol described by the manufacture (Promega Corp., USA, Silver Sequence DNA Staining Reagents Cat. No. Q4132).

Data analysis

The amplified fragments were scored visually as present (1) and absent (0). The Dice coefficients of similarity were estimated according to Dice coefficient: GS(ij)= 2a/(2a+b+c), where GS(ij) is the measure of genetic similarity between individuals i and j, (a) is the number of bands shared by i and j, b is the number of bands present in I and absent in j, and (c) is the number of bands absent in i and in j. The genetic similarities (GSs) and similarity matrices were calculated using the Jaccard's coefficient (Jaccard, 1908). Cluster analysis based on similarity matrix was obtained with unweighted pair group method of arithmetic average (UPGMA), and relationship among and within breeds were visualized as dendrogram displaying the hierarchical associations among all genotypes.

RESULTS AND DISCUSSION

In a previous work (Adawy *et al.*, 2007) the genetic diversity within 4 Sudanese cattle populations (Western Baggara, Nilotic, Butana and Kenana) was studied using RAPD markers and the dendrograms revealed two sub-breeds within each of the Baggara, Nilotic and Butana cattle, while distinguished 3 sub-breeds within Kenana cattle. The dendrogram of Western Baggara cattle (Nialawy) distinguished two main groups with a genetic distance of 0.94. Two main groups were also distinguished with a genetic distance of 0.91 among Nilotic cattle (Majock) and Butana cattle. While for Kenana cattle the dendrogram distinguished three main groups with a genetic distance of 0.86, 0.90 and 0.92. These results suggested that the genetic diversity within local Sudanese populations didn't confirm what was expected on the basis of their geographical locations, which may reflect undocumented migrations, gene flow and identify an original genetic resource. This led to the conclusion that there is genetic

diversity within these breeds to permit their conservation, and there is strong evidence of sub-breeds. Therefore, in the present investigation the genetic diversity of the nine sub-breeds of cattle has been studied using RAPD and AFLP molecular markers to identify markers that distinguish the sub-breeds.

Genetic diversity using RAPD analysis

As shown in (Table 1), a total of 239 bands were amplified using 23 RAPD primers. The bands ranged in size from 200bp to 1100bp (Fig. 1). The number of amplified DNA fragments per primer ranged from 6 to 17 bands and the percentage of polymorphism ranged from zero% to 100%. Across the cattle genotypes the primers OPA-02, OPC-02 and OPC-05 produced the highest number of polymorphic bands (14, 14 and 12), while the lowest number of polymorphic bands (zero) were exhibited by primers OPA-04 and OPO-12.

Genetic diversity using AFLP analysis

AFLP technique permitted the production of 1250 selectively amplified DNA fragments ranging from 50 bp to 2600 bp in size and the identification of 1123 (89.8%) polymorphic markers as shown in Table (2) and Fig. (2). The number of AFLP bands/primer combination varied from primer to another with an average of 65.9 bands per primer combination. In this respect, DNA fingerprinting of Korean cattle using AFLP markers was studied by Chung *et al.* (2000) using 13 primer combinations. They reported that in the Hanwoo breed, the number of AFLP bands per primer combination varied from 37 to 102 bands with an average of 66.1 bands and a total of 859 markers were generated; among them 568 bands were polymorphic (66%). In Italian Holstein dairy cows, AFLP fingerprinting was used by Ajmone *et al.* (1997) they employed 16 primer combinations which generated 1098 bands out of which 248 were polymorphic averaging 15.5 bands per primer combination and ranging from 6 to 27 bands. Ajmone *et al.* (1999) investigated variation among Italian Friesian, Italian Brown and Maremmana cattle and reported that 316 fragments were produced when they used AFLP to study the polymorphism and 111 of these fragments were polymorphic. This high level of polymorphism among the currently studied breeds may be due to gene flow between populations resulting from migration. Apart from the regular movements of nomadic tribes with their animals, drought often forces the movement of some tribes in search of pasture and water. This often leads to admixture of animal populations and crossbreeding. In addition, all Northern Sudan cattle have the same origin as stated by Mason and Maule (1960) who indicated that Kenana, Baggara and Butana are sub-types of the Northern Sudan-shorthorned Zebu cattle which resulted from the inter-breeding of Sanga cattle with shorthorned-Zebu cattle

during tribal migrations before recorded history.

Genetic similarity

The breeder can use genetic similarity information to make informed decisions regarding the choice of genotypes to cross for the development of populations in order to maximize the expression of heterosis. In this study, the genetic similarities between pairs of each sub-breed were calculated using Jaccard's coefficient. The genetic similarity based on AFLP primer combinations among the 9 sub- breeds ranged from 51.5 to 74.4. The highest genetic similarity (74.4) was scored between sub-breed 7 and sub-breed 9 which both belong to Kenana breed. While the lowest similarity (51.5) was scored between sub-breeds 4 (Nilotic) and sub-breed 6 (Butana), which are different breeds from different locations (data not shown). These results are in good agreement with those previously obtained by Ajmone *et al.* (2000 and 2002) who found that the mean genetic distance within Italian Friesian, Italian Brown and Maremmana cattle was 85%. Jiang *et al.* (2003) showed that the genetic similarity within six autochthonus goat populations using AFLP ranged from 0.951 to 0.970 and from 0.745 to 0.758 between populations. In Italy, Ajmone *et al.* (1999) reported that within 4 goat breeds, the genetic diversity was 87% for the majority of breeds and genetic similarities between pairs of individuals of the same breeds averaged 0.70.

Phylogenetic relationships among the nine cattle sub- breeds

In the present work, the RAPD data revealed genetic similarities ranging from 97% to 85% and the dendrogram obtained from RAPD analysis had four groups. Based on the genetic similarity among cattle breeds, the RAPD dendrogram assigned the four cattle breeds into two clusters, the first one included Nilotic while the second cluster included the other three breeds in separated group (Kenana, Western and Butana). The genetic relationship was 84% between Nilotic and the rest breeds, while it was 87% between Butana and Western Baggara and was 0.85 between Kenana and each of Butana and Baggara. On the other hand the genetic relationships within the four breeds were 86%, 97%, 89% and 92% in Nilotic, Kenana, Butana and Western Baggara, respectively.

The AFLP dendrogram among the nine cattle sub- breeds classified the sub-breeds into two main clusters. The two sub-breeds of Nilotic in a separate cluster and the rest of sub-breeds in the second cluster as shown in Fig. (3) with a genetic relationship of (0.56) between the two clusters, while, it was 0.57 between Western Baggara and (Butana and Kenana). Finally, the genetic relationship was 0.65 between Butana and Kenana. The AFLP genetic relationships within Western Baggara, Nilotic, Butana and Kenana cattles were 0.71, 0.66, 0.73 and 0.74, respectively.

These RAPD results indicate high similarity between the related sub-breeds comparing with AFLP dendrogram and this could be attributed to the high multiplex ratio of the AFLP assay and the potentiality of the AFLP technology to detect larger number of polymorphic bands.

The dendrogram based on the combined data among the nine sub-breeds and within the four breeds classified the sub-breeds into two main clusters as shown in (Fig. 4). The first cluster comprised Western Baggara and the second included the other breeds with genetic relationship of 0.63. The second cluster grouped Nilotic cattle in a separate group and both Butana and Kenana in the second group with a genetic relationships 63% while, the relationships between Kenana and Butana exhibited 69. This data indicate that the highest genetic relationship was exhibited between Kenana and Butana (0.69) comparing with (0.63) between the other breeds. On the other hand, the genetic relationships within the four breeds according to the present combined data were 0.77, 0.72, 0.78 and 0.81 within Baggara, Nilotic, Butana and Kenana cattles, respectively. In this respect, Ajmone *et al.* (1999) presented a dendrogram and concluded that the Italian Friesian and Italian Brown cattle were slightly more closely related to each other than they were to Maremmana cattle. Jiang *et al.* (2003) studied the cluster analysis in six autochthonous goat populations using AFLP and found that Chengdu Grey goat (CGG) is the most distant population, while Chuandong White goat (CWG) and Yangtze River Delta White goat (RDWG) were the closest populations, followed by Bonjio goat (BG), Hui goat (HG) and Matou goat (MG).

Adawy *et al*. (2005) and Meszaros *et al*. (2007) explained that the difference between the dendrograms constructed using various types of markers may be due to the information covering different parts of the genome. RAPD markers bind randomly to different parts of the genome. The results of the present work are consistent with the geographic location and distribution of the cattle breeds and coincides to some degree with their historical classification mentioned by Mason and Maule (1960), Rouse (1972), Payne (1970) and Joshi *et al*. (1957). Joshi *et al*. (1998) estimated phylogenetic relationships among Hanwoo, Chinese Yanbian and foreign breeds. They reported that the Hanwoo breed was the most closely related to the Chinese Yanbian cattle, followed by the Hereford and Holstein breeds. Moreover, Adawy *et al*. (2006), Hussein *et al*. (2006) concluded that different molecular markers can be used as a complementary tool and a convenient way is to combine the information from a large number of markers.

Characterization of the cattle breeds by RAPD and AFLP unique markers

The AFLP analysis permitted the distinction among the four cattle breeds and the characterization of each breed by specific unique markers. A total of 61 unique markers (24 UPM and 37 NUM)

were identified by 14 AFLP primer combinations (Table 3), Western Baggara was characterized by the highest number of unique markers (24) and the Butana was characterized by the lowest unique markers (8). In addition, current RAPD analysis revealed a total of 12 unique markers (6 UPM and 6 UNM) which were identified by 8 RAPD primers. The Western Baggara was characterized by the highest number of unique markers (5) while the Kenana was characterized by the lowest number of unique markers (1) as shown in Table (4).

Characterization of the cattle sub breeds by RAPD and AFLP unique markers

In the present study, RAPD and AFLP markers were successful in characterizing the 9 sub-breeds of 4 Sudanese cattle. Nineteen AFLP primer combinations detected unique specific markers which identified the nine cattle sub-breeds. Each of these primer combinations revealed unique markers characterizing one or more sub breed. The total number of unique markers across the 9 sub-breeds was 184 including 119 positive markers (UPM) and 65 unique negative markers (UNM). A number of 34, 34, 10, 22, 22, 13, 10, 17, and 22 unique markers characterized the sub-breeds from 1 to 9, respectively (Table 3). Twelve RAPD primers characterized 8 from 9 cattle sub-breeds. The primers OPA-02, OPA-04, OPC-01, OPC-02, OPC-03, OPC14, OPC15, OPO-04, OPO-09, OPO11, OPZ-06 and OPZ-08 revealed unique markers characterized one or more sub-breeds. The total number of unique markers was (24), the sub-breed (3) (Nilotic) was characterized by the highest number of unique markers (6). Then sub-breed (7) characterized with 5 (UNM), while most of the sub-breeds were characterized with only one unique marker (Table 6). Gwakisa *et al*. (1994) identified two RAPD primers that produced fingerprints which distinguished between DNA pools of three Zebu (*Bos indicus*) cattle breeds. Data from the fingerprints were used to estimate genetic homogeneity and divergence between the breeds. They suggested that these data will be useful in breed conservation efforts. Similar interpretation has been previously reported by Kemp and Teale (1994) who collect DNA pools of two cattle subspecies (*Bos indicus* and *Bos taurus*) to identify distinguishing RAPD markers.

The results obtained in this study point out the usefulness of the AFLP marker system for fingerprinting cattle breeds and sub-breeds with high accuracy. This could be attributed to the high multiplex ratio of the AFLP assay. Therefore, the use of AFLP fingerprint in cattle breeding programs is highly recommended to assess cattle purity, selection of parental lines that could provide the highest heterotic effect, prediction of heterotic response of hybrids and protection of breeder rights.

From the above mentioned results and discussion it is concluded that there is genetic diversity in the breeds under study to permit their conservation as separate entities. There is evidence of breeds sub-

division (sub-breeds), two sub-breeds were identified for Western Baggara, Nilotic and Butana, while three sub-breeds were identified for Kenana cattle. More detailed studies can be used for further identification of these sub-breeds in order to allow the construction and formulation of proper conservation policies. The genetic diversity of the breeds under study didn't confirm the high degree of relationship expected on the basis of previous historical classification studies and their geographical distribution. They may reflect undocumented migrations and gene flow. However, these may identify original genetic resources. To permit the conservation of these breeds every breed type must be taken individually, and the key issues after identification, are the importance of synchronizing germplasm to production management, environments and appropriate strategies for long-term maintenance and use of genetic variation among and within breeds. This study will be useful for characterizing and discriminating between each type, for selection of parents for future breeding programs and developing strategies for future collections. The identification of polymorphism between the different breeds of cattle is useful in conservation and planned usage of breeds in cross breeding, will permit the study of the potential associations between polymorphism in the breed economical traits and diseases resistance.

SUMMARY

Nineteen AFLP primer combinations and 23 RAPD primers were used to estimate genetic diversity among nine sub-breeds representing four Sudanese autochthonous cattle namely Baggara (Nialawy), Nilotic (Majock), Butana and Kenana cattle. The level of polymorphism as revealed by RAPD and AFLP among the 9 cattle sub- breeds was 48.5% and 89.8%, respectively. The RAPD and AFLP data matrices were utilized to estimate the genetic similarity using Jaccard's coefficient. Based on UPGMA cluster analysis the RAPD and AFLP dendrograms separated Nilotic breed in one cluster and the rest breeds in the second cluster with genetic distances of 84% and 56%, respectively. Each of RAPD and AFLP dendrogram assigned the groups of each cattle breed in a separate sub cluster. The dendrogram based on the combined data from RAPD and AFLP analysis clearly assigned the cattle breeds into two clusters, the sub-breed Western Baggara in one cluster and sub-breeds of the other three breeds in the second cluster. The results assessed the potentiality of the RAPD and AFLP technology for characterizing each breed at the molecular level and generating unique fingerprint. This could have a great impact in cattle improving programs.

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Table (1): Number of amplicons generated and percentage of polymorphism among the nine sub breeds cattle as revealed by RAPDs.

RAPD Primers	Total No. of amplicons	Polymorphic amplicons	% of polymorphism
OPA-02	14	14	100.0
OPA-04	14	0	0.0
OPA-15	6	3	50.0
OPC-01	11	11	100.0
OPC-02	14	14	100.0
OPC-03	12	3	25.0
OPC-05	13	12	92.3
OPC-09	9	3	33.3
OPC-13	10	8	80.0
OPC-14	17	9	52.9
OPC-15	11	5	45.5
OPC-17	6	3	50.0
OPD-10	8	2	25.0
OPE-16	12	3	25.0
OPE-18	13	2	15.4
OPO-01	6	2	33.3
OPO-04	6	2	33.3
OPO-09	8	5	62.5
OPO-11	9	4	44.4
OPO-12	8	0	0.0
OPZ-06	10	4	40.0
OPZ-07	11	3	27.3
OPZ-08	11	4	36.4
Total	239	116	48.5

Table (2): Number of amplicons generated and percentage of polymorphism among the nine sub breeds cattle as revealed by AFLP.

Primer combination	Code	Total no. of amplicons	Polymorphic amplicons	Percentage of polymorphism
E-AAG/M-CAC	2/2	78	62	79.5
E-AAG/M-CAG	2/3	82	80	97.6
E-AAG/M-CAT	2/4	60	59	98.3
E-AAG/M-CTA	2/5	75	69	92.0
E-ACA/M-CAA	3/1	81	54	66.7
E-ACC/M-CAA	4/1	59	55	93.2
E-ACC/M-CAC	4/2	14	13	92.9
E-ACC/M-CAG	4/3	91	76	83.5
E-ACC/M-CAT	4/4	68	68	100.0
E-ACG/M-CTA	5/5	44	40	90.9
E-ACG/M-CTC	5/6	60	51	85.0
E-ACG/M-CTT	5/8	31	25	80.6
E-ACT/M-CAT	6/4	66	64	97.0
E-AGC/M-CAA	7/1	74	72	97.3
E-AGC/M-CAC	7/2	95	82	86.3
E-AGC/M-CAG	7/3	145	132	91.0
E-AGG/M-CAA	8/1	49	46	93.9
E-AGG/M-CAC	8/2	36	34	94.4
E-AGG/M-CAG	8/3	42	41	97.6
TOTAL		1250	1123	89.8

Table (3): Unique markers characterizing the four cattle breeds as revealed by AFLPs.

Primer comb.	Western Baggara		Nilotic		Butana		Kenana	
	UPM	UNM	UPM	UNM	UPM	UNM	UPM	UNM
2/2		80		275, 137		230		185, 180, 175, 173, 100, 98
2/3	163			325				
2/4	138							225
2/5		310, 65, 55, 35		375				
3/1	280, 175	350		149				145, 135, 82
4/1	163, 128, 108			20	170			
4/3	161, 137			310, 278, 220			186	
4/4		350, 340, 220			272			
5/6	128							
6/4	70							
7/1					500, 250		120	177
7/2		1350, 700, 190		1020	320		180	
7/3		160			810		82	240
8/1					270		500, 235	800
Sub-total	11	13	-	10	7	1	6	13
Total	24		10		8		19	

Table (4): Unique markers characterizing the four cattle breeds as revealed by RAPDs.

Primer	Western Baggara		Nilotic		Butana		Kenana	
	UPM	UNM	UPM	UNM	UPM	UNM	UPM	UNM
OPC-01	860							
OPC-02				1000	220			
OPC-03		1500						
OPC-05	430	2000			400			
OPA-02			750					
OPA-15						780		
OPE-16		550						350
OPD-10			460					
Sub-total	2	3	2	1	2	1	-	1
Total	5		3		3		1	

Table (6): Unique markers characterizing the nine sub-breeds representing the four cattle breeds as revealed by RAPDs.

RAPD primer	Western Baggara			Nilotic			Butana			Kenana		
	1		2	3		4	5	6		7	8	9
	UPM	UNM	UNM	UPM	UNM	UNM	UNM	UPM	UNM	UNM	UNM	UNM
OPA-02							850		870			600
OPA-04					1050		1300, 1100					
OPC-01										800, 1100, 1300		
OPC-02					1300				1800	600, 500		
OPC-03			2000									
OPC-14					1400							
OPC-15	500	1000										
OPO-04												1100
OPO-09					700						1300	
OPO-11								1150				
OPZ-06				1500	1600							
OPZ-08							800					
Sub-total	1	1	1	1	5		4	1	2	5	1	2

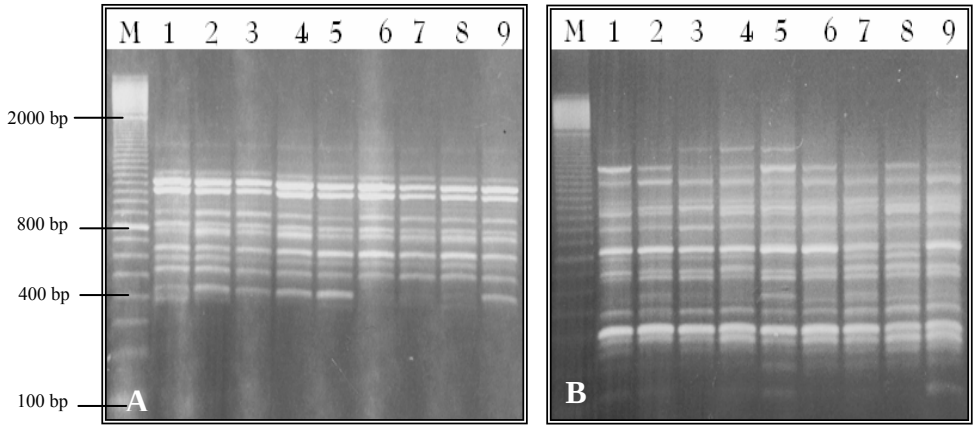

Fig. (1): RAPD profiles of the nine cattle sub- breeds; Lanes (1, 2) Baggara, (3, 4) Nilotic, (5, 6) Butana and (7, 8, 9) Kenana cattle. M: molecular weight standard (100 bp ladder); A: primer OPC-15 and B: primer OPC-14.

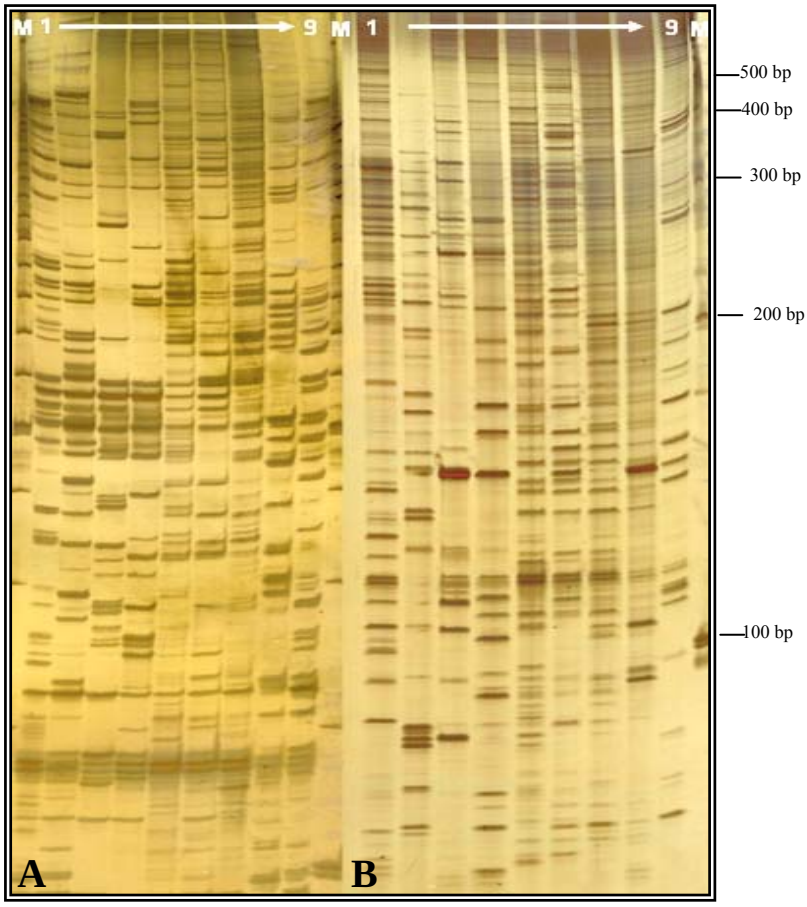

Fig. (2): AFLP profiles of nine sub-breeds; Lanes (1, 2) Baggara, (3, 4) Nilotic, (5, 6) Butana and (7, 8, 9) Kenana cattles. M: molecular weight standard (100 bp ladder); A: primer combination 2/3; B: primer combination 2/4.

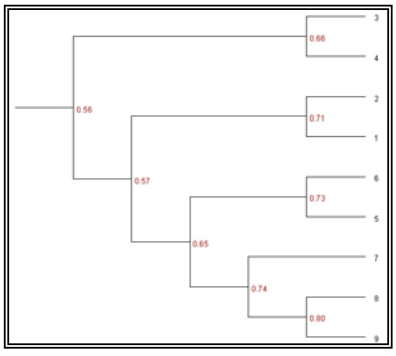

Fig. (3): Dendrogram of genetic relationship generated from AFLP data by UPGMA among 9 sub-breeds of 4 breeds (1, 2 Baggara, 3, 4 Nilotic, 5, 6 Butana and 7, 8, 9 Kenana) cattles.

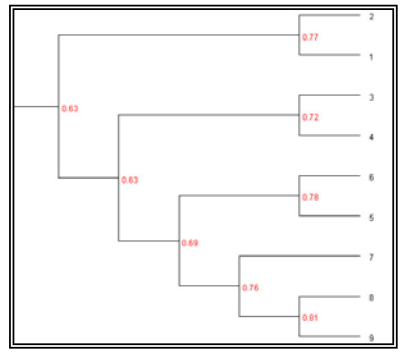

Fig. (4): Dendrogram of genetic relationship generated from combined data by UPGMA among 9 sub-breeds of 4 breeds (1, 2 Baggara, 3, 4 Nilotic, 5, 6 Butana and 7, 8, 9 Kenana) cattles .