GENETIC RELATIONSHIPS AND POLYMORPHISM IN CAMEL POPULATIONS

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The dromedary camel has a single hump, it is known also as Arabian camel. The Arabian camel is the most important livestock animal in Northern and Eastern African countries. It has the best chance to survive in a prolonged drought period. The average life expectancy of a camel is forty to fifty years. Furthermore, due to their instinct for deferent grazing, habits, camel breeds help to keep the ecological balance impact.

The globally total number of camels is said to be 20 million. The Arab countries have about 12.5 million camels or about 70% of the world population (FAO, 2003).

In Egypt, there is different camel breeds used for different purposes, with a different range in body weight. In general, the body weight of the camel is controlled by breed's type, gender and nutrition. These breeds, according to Wardeh *et al.* (1990) are Sudani (common for riding and racing), Falahi or Baladi (used for transportation and agricultural operations), Maghrabi (dual- purpose, for meat and milk) and Mowaled, (hybrid between Maghrabi and Falahi). Ismail *et al*. (2006) studied protein polymorphism for the characterization of four camel breeds in Egypt. They found that homogeneity ratios calculated from protein fingerprinting patterns were 10, 16.7, 50 and 37.3% for Maghrabi, Sudany, Falahy and Mowaled breeds, respectively. El-Seoudy *et al.* (2008) characterized three Egyptian camel breeds (Sudani, Falahi and Maghrabi), using different molecular genetic criteria including native protein, isozyme and ISSR-PCR analysis. The presence of different amplified DNA segments in the studied breeds could be used as genetic markers for each breed. Abo-Elazm (2009) identified some camel breeds depending on biochemical genetic fingerprinting of each breed using protein polymorphism, as well as randomly amplified polymorphic DNA and simple sequence repeats. Cui *et al.* (2007) studied a complete mitochondrial genome sequence of the wild two-humped camel. They found that the mitochondrial genome sequence was 16,680 bp for *Camelus bactrianus* Ferus containing 13 protein-coding, two rRNA, and 22 tRNA genes as well as a typical control region; this basic structure

is shared by all metazoan mitochondrial genomes. For genetic improvement, molecular and biochemical characterizations of camel breeds are the first part for the prevention of germplasm erosion by cross breeding. They are also considered as important tools for the development of indigenous breeds. The selection depending on molecular markers for population studies is an important tool for biodiversity El-Seoudy *et al*. (2008).

The goal of this work is to characterize three camel breeds which found in Egypt using different molecular and biochemical genetic analysis. In order to assess the genetic relationships for the studied breeds, blood samples from these three camel breeds (Maghrabi, Falahi and Sudani) were used. Native plasma proteins and mtDNA using randomly amplified polymorphic DNA (RAPD) primers were applied.

MATRIALS AND METHODS

The present study was carried out in Genetic Engineering and Biotechnology Center (ACGEB), Faculty of Agriculture, Ain Shams University.

Matrials

Animals

Three main breeds of camels which are known in Egypt (Falahi, Maghrabi, and Sudani) were subjected to genetic studies and 17 random samples of females and males for these three breeds were taken for the present study.

Blood samples

A total of 17 blood samples, (three females and three males from each Sudani and Falahi breeds, and three females and two males from Maghrabi breed) were collected during February of 2005 to January of 2007 from Mariout, Delta and Cairo stations.

Blood Collections

Blood samples were collected in vacutainer glass tubes or in plastic vacuette tubes (Greiner, Labortechnik, Tübingen, Germany), which contained EDTA-Na_2 (EDTA disodium) as anticoagulant. Plasma was obtained by centrifugation at 5000 rpm for 15 minutes at 4°C, and the plasma protein (supernatant) was transferred to clean plastic vials and stored at-20°C until electrophoretic analyses were done. The pellets were immediately stored at -20°C for mtDNA extractions.

Methods

Biochemical genetic analysis

Native gel electrophoresis separates proteins based on their size and charge properties. Separation and gel buffer were prepared according to Hames and Rickwood (1981).

Molecular genetic analysis

Camel mitochondrial DNA was isolated using a kit (Mitochondrial/Cytoplasmic Viral DNA Purification Kit) from V-Gene Biotechnology Limited.

The mtDNA was isolated according to the manufacturer's directions.

Polymerase chain reaction (PCR) conditions

PCR was performed according to Williams *et al*. (1990) using eight primers, which five of them were A18, B01, B06, B20 and C14 primers from Operon Technologies, Inc with sequences of 5′AGGTGACCGT3′, 5′GTTTCGCTCC3′, 5′TGCTCTGCCC3′, 5′GGACCCTTAC3′ and 5′TGCGTGCTTG3′, respectively. In addition UBC30, UBC31 and UBC76 primers from Invetrogen life technologies were used with sequences of 5′CCGGCCTTAG3′, 5′CCGGCCTTAG3′ and 5′GAGCACCAGT3′, respectively.

PCR conditions were applied as specified by Williams *et al*. (1990). The PCR cycles were as follows: one cycle at 95°C for 5 min; 30 cycles at 94°C for 1 min; at 36°C for 1 min; and at 72°C for 2 min; one cycle at 72°C for 10 min; and a final step was kept at 4°C. PCR was carried out in a 25 µl of volume containing 50 pmoles of each primer, a 1.25 mM of dNTPs, a 50 ng of DNA template, and 1 U of *Taq* DNA polymerase (Gibco). Supplied buffers with the enzyme were used according to the manufacturer's directions. The DNA amplification was performed in a thermal cycler (Perkin ElmerGeneAmp PCR System 2400). After the reaction, an 8 µl of amplified DNA was separated on 1.5% agarose gels (Sigma), which were stained with ethidium bromide and analyzed using Gel Doc system (Bio-Rad.). All PCR products were independently performed at least three independent times to ensure the reproducibility.

Genetic identity and distance

The gels electrophoretic of protein were scanned using Gel Doc (Bio-Rad). The software used for integrating peak areas was the Quantity one Gel Doc 2000 data system (BioRad). The genetic identity and distances were estimated according to Ayala *et al.* (1972). The formula that used to determine the similarity coefficient (F) based on mtDNA fingerprint was according to Nei and Li (1979).

RESULTS AND DISCUSSION

Biochemical genetic fingerprinting

Identification based on native plasma protein

Blood samples were collected from the chosen females and males of Sudani, Falahi and Maghrabi camel breeds. Plasma samples were separated and subjected to native- polyacrylamide gel electrophoresis (Native-PAGE). Figs (1-A and 1-B) showed the electrophoretic patterns of the native protein from female and male animals, respectively.

The major plasma proteins of mammalian blood can be classified as follows: Albumin and Globulin proteins. Globulin proteins are divided into: Immunoglobulins (γ-globulin), Transferrins (ß-globulin), and α-globulins, according to Mordacq and Roberta (1994).

The electrophoretic profiles of the three camel breeds would be described starting from the most cathodal zone as follows:

1- Immunoglobulin (γ-globulins) zone

In female samples, four sets of bands were detected with relative mobilities of 0.05, 0.08, 0.12 and 0.25 which were common for the three studied breeds. However, in the Falahi samples, one more band was seen in only one sample with relative mobility of 0.06. In male samples the relative mobility (Rf) of in all investigated of different camel breeds samples of males. Male samples showed a maximum number of seven bands with relative mobilities of 0.09, 0.13, 0.16, 0.24, 0.28, 0.29 and 0.31. Three sets of bands (0.09, 0.13 and 0.28) were seen in all samples (common bands). However, the other bands were polymorphic which two of them were found in only one sample (0.16 in Maghrabi male and 0.31 in Sudani male). One of these polymorphic bands 0.24 was seen in two samples of Sudani and one sample of Falahi breeds. A band with a relative mobility of 0.29 was found in only two Maghrabi samples and absent in all other samples, which could be considered as a positive marker for Maghrabi breed.

2- Transferrin (ß-globulins) zone

In female samples of Sudani, Falahi and Maghrabi breeds, two sets of bands were detected with relative mobilities of 0.39 and 0.44. However, two bands with Rf of 0.42 and 0.45 were detected in male samples of the three camel breeds. The first band of them 0.42 was a common band, while the second one 0.45 was a polymorphic which was seen in all Maghrabi and Falahi samples but in Sudani breed it was seen in only one sample. Therefore, this band might be considered as a negative marker for Sudani breed.

3- Slow α-globulin zone

Investigated female samples showed two sets of bands with relative mobilities of 0.47 and 0.51. The first band (0.47) was found in all Maghrabi samples and only one Falahi sample. On the other hand, the second band was detected in only one sudani sample and two samples each of Falahi and Maghrabi breeds. In male samples, a maximum number of four bands were represented. Three of these bands with Rf of 0.49, 0.55 and 0.56 were seen in Sudani males. In Falahi males, four bands with Rf of 0.49, 0.51, 0.55 and 0.56 were doserved. Maghrabi males exhibited three bands with Rf of 0.49, 0.51 and 0.55. All of these four bands were polymorphic as they were detected in some samples and absent in the others.

4- Fast α-globulin zone

Investigated female samples had one band with relative mobility of 0.57 which considered as a common band for all studied breeds. However, in male samples of the three breeds, two sets of bands were detected with Rf of 0.61 and 0.69 which were not necessary existed in all

studied samples and considered as polymorphic bands.

5- Albumin zone

In female samples, albumin was recorded as a single band with Rf of 0.70. However, in male samples albumin was noticed as a single band with Rf of 0.80. In all samples, albumin bands were broad and heavily stained which indicated that albumin concentration was high.

Albumin is the most abundant protein in mammalian plasma (35-45 mg/ml) which was common in all investigated camel samples. This indicates that albumin concentration is due to the activity of certain locus or different loci of higher expression. Since the mobility of albumin fraction in all tested samples was the same, these points out that albumin in the three camel breeds has similar molecular weight and probably rely on the same genetic background. (Hames and Rickwood, 1981).

Based on the combined data of males and females; gained from plasma protein profiles, the polymorphic ratio of camel breeds were 17.4, 40.9 and 41.7% for Maghrabi, Sudani and Falahi breeds, respectively.

Table (1) showed the similarity matrix among the three tested breeds based on plasma protein analysis of female and male samples. The highest similarity value 0.85 was recorded between Falahi and Subani breeds. However, the lowest similarity value 0.76 was observed between Maghrabi and Falahi breeds, followed by while the similarity value of 0.77 which was recorded between Sudani and Maghrabi breeds.

Molecular genetic fingerprinting

RAPD-PCR profiles of mitochondrial DNA

In the present investigation, RAPD-PCR for mtDNA was used to study the similarity between the three camel breeds. Eight RAPD primers were used to detect fragments of male and female samples of the three camel breeds. All of these primers generated polymorphic PCR products. The following is a full description of these results.

The result of A-18 primer is illustrated in Fig. (2a) for females and males of the three camel breeds individual samples. The molecular size of PCR products generated by this primer were ranged from ≈222 to 1564 bp with a total of 15 fragments which showed a 100% of polymorphic ratio which comprises the whole polymorphic bands, one was unique (a band appeared only in one sample). However, Sudani and Falahi breeds with a total of 9 fragments each, showed two polymorphic bands, 6 were unique and one was common band. While Maghrabi breed showed a total of 13 fragments, 10 bands were polymorphic, two were unique and one was common band.

Primer B-01 resulted in a total of 12 fragments with molecular size ranging from ≈457 to 1810 bp (Fig. 2b). It showed

100% polymorphic ratio which form the whole polymorphic bands, three bands were unique. However Sudani breed with a total of 8 fragments, four bands were polymorphic and four were unique. Falahi breed with a total of 11 fragments, five bands were polymorphic and 6 were unique. While Maghrabi breed showed a total of four fragments, one band was polymorphic and one common band and two bands were unique.

Primer B-06 resulted in a total of 12 fragments with molecular size ranging from ≈380 to 1390 bp (Fig. 2c). It showed 100% polymorphic ratio. However, Sudani breed with a total of five fragments, four bands were polymorphic and one was unique. Falahi breed recorded a total of 11 fragments, four out of them were polymorphic bands, five were unique and two bands were common bands. While Maghrabi breed showed a total of 9 fragments, three bands were polymorphic, two were unique and four bands were common.

Primer B-20 resulted in a total of 8 fragments with molecular size ranging from ≈375 to 2155 bp (Fig. 2d). It showed 100% polymorphic ratio, two bands were unique. However, Sudani breed with a total of 6 fragments, one was polymorphic band and five were unique. Falahi breed recorded a total of 6 fragments, three bands were polymorphic and common. While Maghrabi showed a total of 7 fragments, four bands were polymorphic and three were unique.

Primer C-14 resulted in a total of 10 fragments with molecular size ranging from ≈325 to 1641 bp (Fig. 2e). It showed 100% polymorphic ratio, one band was unique. However, Sudani breed with a total of four fragments, one band was polymorphic and three were unique. Falahi breed with a total of 7 fragments, three bands were polymorphic and four were unique. While Maghrabi breed showed a total of 9 fragments, 6 bands were polymorphic and three were unique.

Primer UBC-30 produced a total of 14 fragments with molecular size ranging from ≈223 to 2360 bp (Fig. 2f). It showed 100% polymorphic ratio, one band was unique. However, Sudani breed with a total of 10 fragments, showed three bands polymorphic and 7 were unique. Falahi breed exhibited a total of 10 fragments, four bands were polymorphic, five were unique and one was common. While Maghrabi breed showed a total of 12 fragments, 9 bands were polymorphic, two were unique and one was common.

Primer UBC-31 noticed a total of 11 fragments with molecular size ranging from ≈301 to 790 bp (Fig. 2g). One common band was detected by this primer with molecular size of 341, eight polymorphic bands and two unique bands, with a recorded higher polymorphism (90.9%). However, Sudani breed with a total of 8 fragments, two bands were unique and 6 were common. Falahi breed with a total of 9 fragments, three bands were polymorphic, unique and common. While Maghrabi breed showed a total of 9

fragments, four bands were polymorphic and unique and one was common.

Primer UBC-76 resulted in a total of 9 fragments with molecular size ranging from ≈246 to 1370 bp (Fig. 2h). It showed 100% polymorphic, one band was unique. However, Sudani breed with a total of 7 fragments, two bands were polymorphic and common and three were unique. Falahi breed with a total of 7 fragments, four bands were polymorphic, two were unique and one was common. While Maghrabi breed showed a total of 8 fragments, four bands were polymorphic, three were unique and one was common.

Based on the combined data obtained through the polymorphism of RAPD profiles, the similarity coefficient values among the studied three camel breeds (Sudani, Falahi and Maghrabi) were calculated according to Dice (1945) equation's. The similarity coefficients between each two of the three camel breeds such as Falahi and Maghrabi, Sudani and Maghrabi and Falahi and Sudani breeds showed 0.33, 0.35 and 0.42, respectively, (Table 2). However, the highest similarity value (0.42) was recorded between Sudani and Falahi breeds as well as biochemical analysis.

Genetic relationships based on plasma protein and RAPD-PCR of mitochondrial DNA analyses

Cluster analysis based on plasma protein and RAPD-PCR of mtDNA (Table 3) revealed the similarity matrix among the three camel breeds such as Falahi and Maghrabi, Sudani and Maghrabi and Sudani and Falahi breeds which were 0.42, 0.45 and 0.53, respectively. The highest similarity index (0.53) was recorded between Sudani and Falahi breeds.

The data obtained from plasma protein and RAPD-PCR from mitochondrial DNA analyses were used to draw the precise relationships among the three tested camel breeds (Fig. 3). The resultant dendrogram revealed two clusters. Consisted of Sudani and Falahi which the first one breeds, while the second one comprised Maghrabi breed only.

The dendrograms produced by each of the two used approaches (protein and mtDNA analysis) showed similar clusters of the three breeds. Besides, when the combined data of the two analyses was used to construct this dendrogram, the same cluster was obtained which means more relatedness between these breeds. This is why one figure only was presented for their relationships (Fig. 3). This was in agreement with Wathig *et al.* (2007) who stated that dromedary camels entered Sudan is believed to be of Egyptian origin. This conclusion was based on a specimen of camel hair rope of an old kingdom found at Fayum in Upper Egypt 2980-2475 BC.

On the protein level, the fingerprinting profile showed that homogeneity ratio was very high in Maghrabi breed (82.6%) while Sudani and Falahi breeds were almost similar where the polymorphic ratio were 40.9% and 41.7% for these breeds, respectively. However, at the mi-

tochondrial DNA level, the results recorded high polymorphic ratio in the three camel breeds (84.2%, 84.3% and 87.3%) for Sudani, Falahi and Maghrabi, respectively.

In conclusion, the results revealed almost equality of polymorphism within each of Falahi and Sudani breeds either when protein or mitochondrial DNA was analyzed. This was an expected c(onclusion since Sudani breed was said to be descended from Falahi breed (Wathig *et al.,* 2007). Besides, this might be due to the high level of hybridization between these two breeds. Moreover, similarity value between Falahi and Sudani breeds recorded the highest value when protein or mtDNA were analyzed. However, this similarity value was higher in protein analysis than that of mtDNA analysis. When these results were used to draw the genetic tree of the three breeds, the dendrogram revealed that Sudani and Falahi breeds belong to the same cluster and Maghrabi breed was in another one. The same relation was obtained when protein or mtDNA analyses were used. This was in agreement with El-Seoudy *et al*. (2008) who used SDS-PAGE for milk or plasma protein and isozymes analyses to draw the same breeds relationships. Abo-Elazm (2009) also got the same result when used SSR-PCR analysis on the same three breeds.

SUMMARY

Three Egyptian camel breeds were used in the present study (Sudani, Falahi and Maghrabi). These breeds were identified depending on biochemical and molecular genetic fingerprinting of each breed by using protein polymorphism and random amplified polymorphic DNA (RAPD) for mitochondrial DNA. Eight primers were amplified for the detection of genetic polymorphism in these breeds using mitochondrial DNA as template. The results of protein analysis indicated that the least polymorphic breed was Maghrabi (17.4%) followed by Sudani breed (40.9%), while the highest was Falahi breed (41.7%). However, when mitochondrial DNA was analyzed, the highest polymorphic ratio was seen in Maghrabi breed (87.3%), while Falahi (84.3%) and Sudani (84.2%) breeds. The similarity index depending upon combination of protein and mtDNA analyses indicated that similarity values between Sudani and Falahi breeds was 0.53, Sudani and Maghrabi breeds was 0.45 and Falahi and Maghrabi breeds was 0.42. The dendrogram was constructed to show the phylogenetic relationships among the three breeds.

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Table (1): Similarity matrix for the three camel breeds based on plasma protein analysis.

Breed	Sudani	Falahi	Maghrabi
Sudani	1.000		
Falahi	0.85	1.000	
Maghrabi	0.77	0.76	1.000

Table (2): Similarity matrix for the three camel breeds based on RAPD-PCR of mtDNA analysis.

Breed	Sudani	Falahi	Maghrabi
Sudani	1.000		
Falahi	0.42	1.000	
Maghrabi	0.35	0.33	1.000

Table (3): Similarity matrix for the three camel breeds based on plasma protein and RAPD-PCR of mitochondrial DNA analyses.

Breed	Sudani	Falahi	Maghrabi
Sudani	1.000		
Falahi	0.53	1.000	
Maghrabi	0.45	0.42	1.000

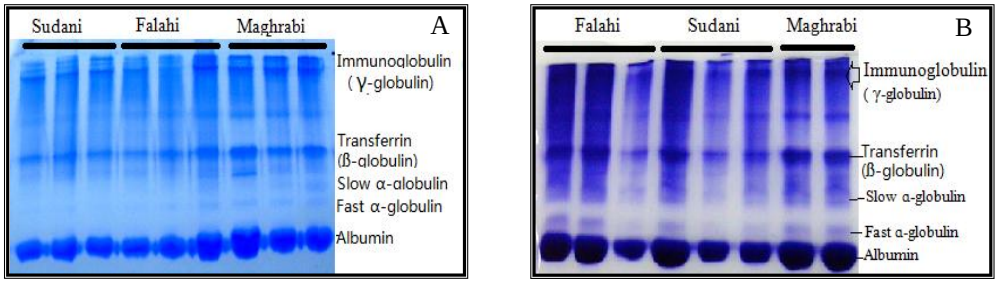

Fig. (1): Electrophoretic patterns for female (A) and male (B) plasma proteins of Sudani, Falahi and Maghrabi camel breeds using Native-PAGE analysis.

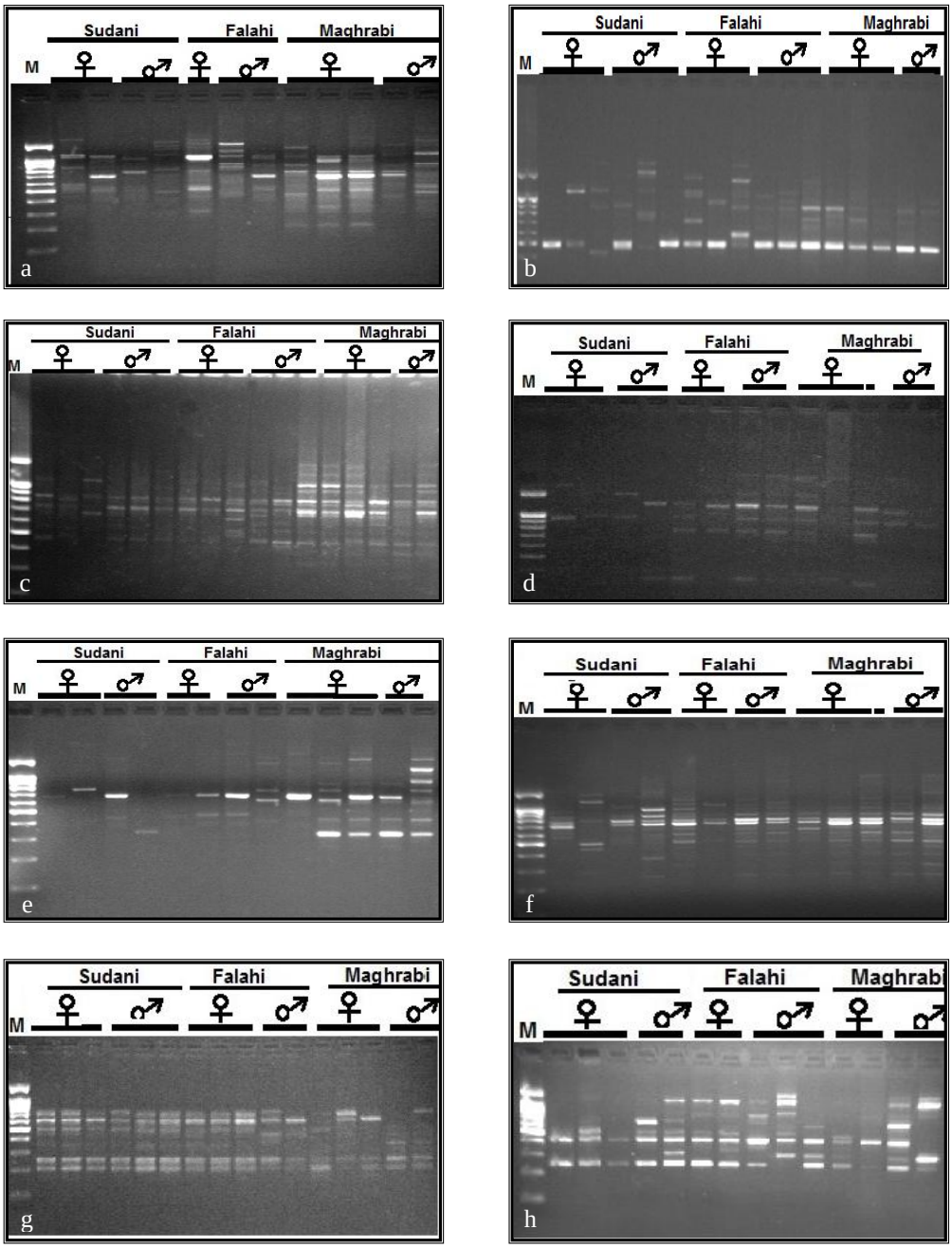

Fig. (2): Mitochondrial DNA polymorphism of females and males for the three camel breeds amplified with A-18 (a), B-01 (b), B-06 (c), B-20 (d), C-14 (e), UBC-30 (f), UBC-31 (g) and UBC-76 (h) primers. DNA marker 1.5 kb (BIORON) (100 base pair ladder) with the following 11 discrete fragments in base pairs (1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100) was used as standard DNA.

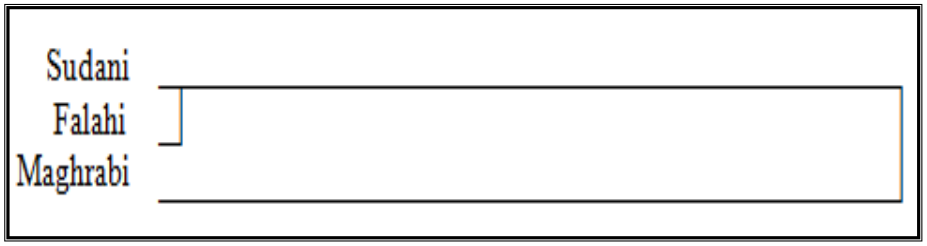

Fig. (3): Dendrogram for the genetic distances among the three camel breeds based on plasma proteins and RAPD-PCR of mtDNA analyses.