MOLECULAR GENETIC IDENTIFICATION OF SOME CAMEL BREEDS IN EGYPT

ALIA A. EL-SEOUDY^{1,3}, A. Z. ABDELSALAM^{1,3} E. E. THARWAT² AND FATMA A. M. ABDELSALAM³

1. Dept. of Genetics. Fac. Agric., Ain Shams Univ., Cairo, Egypt

2. Dept. of Animal Production, Fac. Agric., Ain Shams Univ., Cairo, Egypt

3. Ain Shams Center for Genetic Engineering and Biotechnology, Ain Shams Univ., Cairo, Egypt

The Arabian camel (*Camelus dromedaries*) is the most important livestock animal in the arid and semi-arid areas of Northern and Eastern African countries. It has the best chance to survive a prolonged drought period. Furthermore, due to their instinct for deferred grazing, camel herds help to keep the ecological balance intact.

The total number of camels globally is said to be 20 million. The Arab countries have about 12.5 million camels or about 70% of the world population (FAO, 2003). Egypt is a camel importing rather than a camel breeding country. It has a low density of camels (low camel to human ratios).

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, from these breeds, according to Wardeh *et al.*, (1990): Sudani (common for riding and racing), Falahi or Baladi (used for transportation and agricultural operations), Maghrabi (dual-purpose, for meat and milk), Mowaled, (hybrid between Maghrabi and Falahi).

In the present study, three camel breeds were chosen: Sudani, imported from Sudan. Falahi spreads through the Nile delta and Nile strip. While Maghrabi comes from the north coast. These breeds are considered as the most common in Egypt.

For genetic improvement, molecular characterization of camel breeds is the first part for the prevention of germplasm erosion by cross breeding. It is also considered as an important tool for the development of indigenous breeds. The selection depending on molecular markers for population studies is an important tool for biodiversity conservation.

The objectives of the present study were to: Discriminate the three camel breeds (Sudani, Falahi and Maghrabi) by:

- 1. Native protein and isozymes electrophoresis.
- 2. Molecular genetic markers using ISSR.

MATERIALS AND METHODS

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

Camel breeds and blood collection

Blood samples of Maghrabi camel were kindly supplied by King Maruit Research Station. While blood samples of Sudani and Falahi (Baladi) were collected from camel market in Berkash, Giza, Egypt. Blood samples were collected from healthy males and females of the three camel breeds. These samples were drawn from the jugular vein of camels into sterile interior plain glass tubes after the addition of EDTA disodium salt (EDTA-Na₂) as anticoagulant in each case.

Biochemical analysis

Discontinuous nondenaturing (Native) gel electrophoresis

Native protein electrophoresis was carried out; stock solutions and gel were prepared according to Bollag and Edelstein (1994).

Enzymes Assay

The gels were stained after electrophoresis according to each enzyme system and incubated at 37°C in dark for complete staining after adding the appropriate substrate and staining solution. The constitutions of the staining solutions for different isozymes are listed in Table (1) according to Richardson *et.al.* (1980).

Molecular genetic study

Camel genomic DNA was isolated by a method described by Sambrook *et al.* (1989). After the DNA, extraction 10 μ l of DNA was taken from each sample. They were mixed very well, to form 6 bulked samples for both males and females of the three breeds. These bulked samples were used for DNA analysis.

Inter simple sequence repeat (ISSR)

Fourteen ISSR primers were used to characterize the three camel breeds. The names and sequences of these primers are shown in Table (2).

Polymerase Chain Reaction (PCR) conditions

PCR was performed in 20 μ l volume in 0.2 ml eppendorf PCR tubes according to Williams *et al.* (1990) with slight modifications, as follows: 2X prime Taq Premix^{*} (1 unit/10 μ l); 2 μ l Primer; 2 μ l Template DNA; and 6 μ l H₂O (d.w.). ^{*}2X prime Taq Premix solution: (20 mM Tris-HCl, 80 mM KCl, 4 mM MgCl₂, enzyme stabilizer, sediment, loading dye, pH 9.0, 0.5 mM of each dATP, dCTP, dGTP, dTTP).

PCR program

The amplification procedures were carried out in thermocycler (Techne Tc-

312) programmed as follows: 1 cycle at 94°C for 2 min; 35 cycles of 94°C for 30 sec; 44°C for 45 sec and 72°C for 1.5 min; 1 cycle at 72°C for 7 min; and a final step was kept at 4°C.

Gel electrophoresis and visualization of DNA bands

The ISSR-PCR and specific primers products were resolved by electrophoresis in 1% agarose gel at 100 volts for half an hour. Each gel was examined on ultraviolet transilluminator filter (302 nm wavelength), to detect the ethidium bromide/DNA complex. The DNA marker used with ISSR-PCR products was 1 kb DNA ladder, consisting of 8 fragments; i.e. 2.0, 1.5, 1.0, 0.8, 0.7, 0.6, 0.5 and 0.4 kb.

Densitometric scanning and analysis

All gels resulted from the different three genetic criteria, protein, isozymes, and DNA amplification, were scanned using Bio-Rad GelDoc (2000) and analyzed with the Quantity One Software package supplied from the manufacturer. The densitometric scanning of such band is based on its three dimensions characteristics. The similarity matrices were calculated and used to draw dendrograms using SPSS windows, Version 10 software (DICE method).

RESULTS AND DISCUSSION

Native plasma protein electrophoresis

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 (β -globulin), and α -globulins according to Mordacq and Roberta (1994).

The electrophoretic studies of plasma proteins revealed the presence of up to five different zones in each of the individual samples of the three camel breeds. Immunoglobulins (γ -globulin), transferrins (β -globulin), α -globulins were divided into slow α -globulin, fast α -globulin, albumin as shown in Fig. (1).

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

1. Immunoglobulins (y-globulin) zone

In Sudani and Falahi camel samples, three sets of bands were detected with relative mobility's (Rf) of 0.17, 0.41 and 0.44 respectively. While Maghrabi samples showed, four sets of bands, with Rf of 0.17, 0.33, 0.41 and 0.44. Therefore, only two groups could be recognized in all tested samples. The first group contains both Sudani and Falahi camel samples with three sets of bands and the second was confined to the Maghrabi samples with an extra band (4 bands). Where the band with Rf of 0.33 can be considered as marker for Maghrabi breed.

2. Transferrins (β-globulin) zone

In Sudani and Falahi camel samples, three sets of bands were detected with Rf of 0.49, 0.53 and 0.58, respectively. While in Maghrabi samples, only two sets of bands were seen, with Rf of 0.49 and 0.53. Therefore, the absence of the band with Rf of 0.58 is characteristic for Maghrabi breed.

3. Slow a-globulin zone

All investigated samples had two sets of bands with Rf of 0.63 and 0.67. However, the Maghrabi male samples did not exhibit the band with Rf of 0.67.

4. Fast α-globulin zone

All investigated samples had two bands with relative mobility's of 0.75 and 0.87, except for the Maghrabi male samples where the band with Rf of 0.87 was absent.

5. Albumin zone

In the studied breeds, albumin was resolved as a single band with Rf of 0.89. In all samples, the albumin band was broad and heavily stained.

Table (3) shows the similarity matrix for the three camel breeds based on native-PAGE of plasma protein analysis. It shows an average genetic distance ranging from 0.802 to 1.000 with a mean value of 0.903.

The highest similarity index (1.000) was recorded between each two of Falahi females, Falahi males, Sudani females and Sudani males. Which means the high similarity between males and females of the same breed. However, the

lowest similarity index (0.802) was observed between Maghrabi males and each of Falahi females, Falahi males, Sudani females and Sudani males.

The data obtained was used to draw the precise relationships among the three tested camel breeds. The resultant dendrogram shown in Fig. (5) illustrates the divergence between the used samples and suggests their tree branching. Cluster analysis of the DICE method revealed two main clusters. Cluster A consisted of Falahi females, Falahi males, Sudani females and Sudani males. Cluster B consisted of Maghrabi females, and Maghrabi males. Indicating the divergence of Maghrabi breed from the other two breeds.

Isozymes electrophoresis

Six isozyme system patterns (Carboxylesterase (EC 3.1.1.1), Acid phosphates (EC 3.1.3.2), malate dehydrogenase (EC 1.1.1.37), alcohol dehydrogenase (EC 1.1.1.1), glycerol-3-phosphat dehydrogenase (EC 1.1.1.8) and phosphogluconate dehydrogenase (EC 1.1.1.44)) were analyzed, (Figs. 2 and 3). Their results are presented as follows:

1-Esterase isozymes

In the present study, we focused on carboxylesterase (EC 3.1.1.1), which belongs to class hydrolases of enzymes. Two substrates have been applied, α naphthyl acetate and β - naphthyl acetate, (Fig. 2a and b). A total number of six bands were detected in plasma of the Sudani, Falahi and Maghrabi camels using α - naphthyl acetate and β - naphthyl acetate substrates. Bands were assigned to three main zones as follows:

Zone I (α -Est-1 and β -Est-1) two bands were detected in all tested individuals. Therefore, this locus has two alleles, one for the first band and the other for the second.

Zone II (α -Est-2 and β -Est-2) that involves bands number three and four which might be due to the activity of another esterase locus. Absence of this locus in some Sudani and Falahi individuals might be attributed to regulatory mechanisms affecting the quantitative expression of this locus. Hence, the amount of protein produced is not sufficient to be detected. The weak stain ability of band number four in Maghrabi individuals could be due to the low expression of the corresponding locus in these individuals.

Zone III (α -Est-3 and β -Est-3) that has been detected in all tested individuals (band no. 6) might be due to the activity of a genetic locus which is common in all studied camel samples. The appearance of an extra darkly stained small band (no.5) in only one individual of Falahi females might be due to post-translational modification of the formed enzymatic polypeptide chain. The expression of the previous locus in all investigated samples reflects its biological significance in camels. It may represent a housekeeping esterase locus that serves as general metabolic function in the three camel breeds.

The present results would lead to a conclusion that, the network of the genetic material, which control esterases, is probably polygenic. This means that more than one genetic locus are responsible for the biosynthesis of esterases in camels. In other words, the three zones of esterolytic activity detected in camels could be attributed to three structural genes. One gene controls esterases of zone I, another gene for zone II and the third gene is responsible for zone III.

2- Acid phosphatase (Acp)

Two groups of acid phosphatase isozymes (Acp I and Acp II) were electrophortically detected in all the studied samples (Fig. 2c). Both groups appeared as a single band. However, bands in Sudani and Maghrabi camel samples were faster than in Falahi.

3- Malate dehydrogenase (Mdh)

Based on similarity in electrophoretic mobility and concordance in variation, two distinct zones were seen, (Fig. 3a). All samples had one band in the first zone, except for the two males of Maghrabi which had two bands. The second zone appeared as having two sets of bands in all studied samples.

Malate dehydrogenase plays a principle role as energy producing enzyme. It is found in all eukaryotic cells as two isozymes: mitochondrial (m-Mdh) and cytoplasmic (soluble, s-Mdh). The two isozymes consisting of two very similar subunits and having similar enzymatic activity that appear as different proteins (Bleile *et al.*, 1975).

Appearance of two widely spaced zones for (Mdh) activity indicates that its genetic background could be due to the activity of two loci, one for each zone. The first locus has two alleles; one was shared by all samples while the second was silent in almost all samples except for the two males of Maghrabi individuals, which might be considered as marker for them. The second locus has two alleles; that codes for the two bands found in zone II in all investigated samples.

4- Alcohol dehydrogenase (Adh)

Two distinct zones could be detected, (Fig. 3b). All investigated samples had one band in the first zone, except for the two males of Maghrabi that had two bands. The second zone of (Adh) appeared only in the female samples of the Falahi camel samples.

The possible genetic interpretation of these findings could be summarized in the following: The two zones indicate the control of two loci, one for each zone. The first locus has two alleles; one was shared by all samples while the second was silent in almost all samples except for two males of Maghrabi individuals. The second locus has one allele; that codes for one band found only in the female samples of the Falahi camel breed. The disappearance of this band in the rest of samples could be due to low gene expression of this locus.

Alcohol dehydrogenases are groups of dehydrogenase enzymes that occur in many organisms and facilitate the conversion between alcohols and aldehydes or ketones. In humans and many other animals, they serve to break down alcohols which could otherwise be toxic.

5- Glycerol-3-phosphate dehydrogenase (GPD)

Two distinct zones can be described as shown in (Fig. 3c):

- Zone I: which appeared in all samples, except for the three samples of Sudani (female no. 3, and males' nos. 4 and 5) which had no sign of this band.
- Zone II: was represented by different sets of bands. In Sudani camel breed, the female samples (1, 2, and 3) had two sets of bands, while the male samples (4 and 5) had only one set. In Falahi all the examined samples had one set of bands. Samples of Maghrabi camel samples had one set of bands, but this band did not appear in one female sample (no. 13).

There are two main forms of intracellular glycerol-3-phosphate dehydrogenase: mitochondrial glycerol 3phosphate dehydrogenase (mGPD) and cytoplasmic glycerol-3-phosphate dehydrogenase (cGPD). The electrophoretic pattern in the present study is for (cGPD).

6- Phosphogluconate dehydrogenase (6-PGD) (decarboxylating)

Two distinct zones were seen (Fig. 3d). Zone I appeared as a single band in all investigated Maghrabi camel breed. It was also seen in the two male samples of Falahi breed. This zone did not appear in Sudani and in Falahi female samples. Zone II, also showed one band, in all examined samples.

The Phosphogluconate dehydrogenase polymorphism is the product of two alleles, one for each zone: Allele 1 that is expressed only in Maghrabi and the two male samples of Falahi. Allele 2, which is restricted to the second zone and appeared in all investigated samples.

In the present study, during staining the gels of the four isozymes: malate dehydrogenase, alcohol dehydrogenase. glycerol-3-phosphat dehvdrogenase and phosphogluconate dehydrogenase, white bands of superoxide dismutase (SOD) appeared. Copper- and zinc-containing superoxide dismutase (Cu Zn SOD) is the form of superoxide dismutase enzyme that was detected. The fast oxidation process is the result of the appearance of (SOD), which was consistent in the studied samples. The appearance of this enzyme would indicate the capability of camels to overcome hard environmental stresses.

Table (4) shows the similarity matrix for the three camel breeds based on native-PAGE of plasma isozyme electrophoresis analysis. The similarity matrix between the females and males of the three camel breeds shows an average genetic distance ranging from 0.706 to 0.923 with a mean value of 0.815.

The highest similarity index (0.923) was recorded between Falahi females and males. However, the lowest similarity index (0.706) was observed between Maghrabi males and Sudani females.

The data obtained from the analysis of native-PAGE of plasma isozyme electrophoresis was used to draw the precise relationships among the three tested camel breeds. The resultant dendrogram shown in Fig. (5) illustrates the divergence between the used samples and suggests their tree branching. Two main clusters were revealed, where cluster A consisted of two sub-clusters: sub-cluster I consisted of Sudani females and Sudani males. While, sub-cluster II consisted of Falahi females, and Falahi males. Cluster B consisted of Maghrabi females, and males.

Inter Simple Sequence Repeat (ISSR)

The molecular markers, which were detected for Sudani, Falahi and Maghrabi camel breeds are shown in Fig. (4) and Table (5). For Maghrabi breed, 9 positive markers can be detected since they were present in the bulked samples of either males or females of this breed, using different primers as follows: primer HB 10 with molecular size 1440 bp, primer HB 13 with molecular size 356 bp, primer HB 14 with molecular size 2690 bp, primer 15 with molecular size 398 bp, primer 814 A with molecular size 637 bp and primer 844 B with molecular sizes 2243 and 561 bp.

In Sudani breed, two primers succeeded to create two positive markers as follows: primer HB 11 and primer HB 15 with molecular sizes of 947 and 742 bp respectively. While in Falahi breed 3 positive markers were found using primer 17899 A with molecular sizes of 1461, 1196 and 919 bp.

Polymorphism percentages obtained by ISSR markers were variable among the used primers, as shown in Table (6). Primer HB 13 showed the lowest percentage of polymorphism (14 %.) On the other hand, primer 17899 A had the highest polymorphism percentage (75%). In general seven primers showed polymorphism with percentages lower than 50% (HB 08, HB 09, HB 10, HB 12, HB 13, 844 A and 17899 B). While the other primers had polymorphism percentage higher than 50%.

Table (7) shows total no. of bands, no. of monomorphic and polymorphic bands, homogeneity and polymorphism percentages for the studied camel breeds. Sudani breed had the lowest homogeneity ratio (89%), and the highest polymorphism ratio (10.9%) compared with other investigated camel breeds. On the other hand, Maghrabi recorded the highest homogeneity ratio (98.5%) and the lowest polymorphism.

Table (8) shows the similarity matrix for the three camel breeds based on ISSR-PCR analysis. The similarity matrix between the females and males of the three breeds shows an average genetic distance ranging from 0.730 to 0.967 with a mean value of 0.805. The highest similarity index (0.967) was recorded between Maghrabi females and males. However, the lowest similarity index (0.730) was observed between males of Maghrabi and Sudani.

The data obtained from the analysis of ISSR-PCR was computed to cluster the data and to draw the precise relationships among the three tested camel breeds. The resultant dendrogram shown in Fig. (5) illustrates the divergence between the used samples and suggests their tree branching. Two main clusters were revealed. Cluster A consisted of two sub-clusters: sub-cluster I consisted of Maghrabi females and males. While, sub-cluster II consisted of Sudani females. Cluster B consisted of two sub-clusters: sub-cluster I consisted of Falahi females and males Sub-cluster II had Sudani males.

Combined identification based on native plasma proteins, native isozymes, and ISSR-PCR analyses

Cluster analysis based on, native plasma proteins, isozymes and ISSR-PCR analyses is shown in Table (9). The similarity matrix between the females and males of the three camel breeds shows an average genetic distance ranging from 0.620 to 0.958.

The highest similarity index (0.958) was recorded between Maghrabi females and males. However, the lowest similarity index (0.620) was observed between males of Maghrabi and Sudani.

The data obtained from the analyses of the combined data was computed to cluster the data and to draw the precise relationships among the three tested camel breeds. The resultant dendrogram shown in Fig. (5) illustrates the divergence between the studied camel breeds and suggests two main clusters. Cluster A consisted of Maghrabi females and males. Cluster B is divided into two sub-clusters: sub-cluster I consisted of Falahi females and males, while sub-cluster II consisted of Sudani females and males.

The cluster analysis for the combined data of the three used techniques (Protein, isozyme and ISSR) separated the Maghrabi breed from the other two breeds. This obtained result is similar to the cluster revealed by native isozyme electrophoresis technique. That is to say, that isozyme electrophoresis can be considered as a suitable technique for studying the genetic relationships between camel breeds.

This result seems to be reliable one since it goes with the expectation of clustering males with females of the same breed. Gathering both Sudani and Falahi in one cluster could be due to that, Sudani comes from the south and Falahi spread through the Delta and Nile valley. They almost share the same habitats. On the other side, Maghrabi breed scatters in the Northern coast of Egypt, having different breeding methods and different genetic origin. The results of the present study showed high percentage of homogeneity inside each camel breed.

SUMMARY

The main objective of the present study is to characterize the three camel breeds (Sudani, Falahi and Maghrabi), using different genetic criteria. Native protein profiles of plasma showed slight differences between the examined animals. Six isozyme system patterns; Carboxylesterase, Acid phosphates, Malate dehydrogenase, Alcohol dehydrogenase, Glycerol-3-phosphat dehydrogenase and Phosphogluconate dehydrogenase were used. The isozymes analysis revealed that, Falahi females and males showed the highest similarity followed by Maghrabi. While, the lowest similarity was observed for Sudani breed. ISSR-PCR gave different DNA markers using fourteen primers. The presence of different amplified DNA segments in the studied breeds could be used as genetic markers for each breed.

REFERENCES

Bleile, D., M. Foster, J. Brady and J. Harrison (1975). Identification of essential arginyl residues in Cytoplasmic Malate Dehydrogenase with Butanedione. J. Biol. Chem., 250: 6222.

- Bollag, M. and J. Edelstein (1994). Protein Methods. Fourth Edition. Wiley-Liss, Inc., 605 Third Avenue, New York.
- FAO (2003). Production Yearbook. Vol. 56, Rome.
- Mordacq John C. and Roberta W. Ellington (1994). Polyacrylamide Gel Electrophoresis (PAGE) of Blood Proteins. Association for Biology Laboratory Education (ABLE) http://www.zoo.utoronto.ca/able.
- Richardson, B. J., P. M. Rogers and G. M. Hewitt, (1980). Ecological genetics of the Wild Rabbit in Australia. Aust. J. Biol. Sci., 33: 371-83.

- Sambrook, J., E. F. Fritsh and T. Maniatis (1989) Molecular cloning, a laboratory manual. Cold spring harbor laboratory press, New York.
- Wardeh, M. F., A. A. Zaied, H. S. Horier and R. T. Wilson (1990). Camel breeds in Arab Africa. Proc. Of the International Conference on Camel Production and Improvement, 10-13 December, 1990, Tobruk, Libya, 78-86.
- Williams, L. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nuc. Aci. Res., 18: 6531-6535.

Enzyme	Staining conditions	
	Phosphate buffer*	100 ml
Carbourlastaras	Fast blue RR	25 mg
Carboxylesterase	(1:1) Acetone + $d.H_2O$	3 ml
(EC 3.1.1.1)	α - naphthyl acetate	25 mg
	β - naphthyl acetate	25 mg
	Acid phosphatase buffer*	100 ml
	Fast blue BB	0.1 g
Acid phosphates	1- Naphthyl phosphate salt mono-	25 mg
(EC 3.1.3.2)	sodium monohydrate	-
	Magnesium chloride (MgCl ₂)	0.1 g
	Manganese chloride (MnCl ₂)	0.1 g
	0.1 M Tris-HCl, pH 8	100 ml
Malata dahudraganaga	NAD*	25 mg
Malate dehydrogenase (EC 1.1.1.37)	Malic acid	25 mg
(EC 1.1.1.57)	NBT*	3 mg
	PMS [*]	1 mg
	0.1 M Tris-HCl, pH 7.4	100 ml
Alcohol dehydrogenase	NAD	25 mg
(EC 1.1.1.1)	Ethanol	10 ml
(EC 1.1.1.1)	NBT	3 mg
	PMS	1 mg
	0.1 M Tris-HCl, pH 7.8	100 ml
Glycerol-3- phosphate	NAD	25 mg
dehydrogenase (NAD ⁺)	α- glycerophosphate	25 mg
(EC 1.1.1.8)	NBT	3 mg
	PMS	1 mg
Phosphogluconate	0.1 M Tris-HCl, pH 8	100 ml
Phosphogluconate dehydrogenase	NADP	25 mg
(decarboxylating)	6-phosphogluconate	25 mg
(EC 1.1.1.44)	NBT	3 mg
(EC 1.1.1.44)	$PMS = \frac{1}{2} \left(\frac{1}{2} \left(\frac{1}{2} \right) + \frac{1}{2} \left($	1 mg

Table (1): Staining conditions for the studied enzymes.

*Phosphate buffer (pH 7): 39 ml of (1.56 g /100 ml of Na H PO₄) + 41 ml of (1.41 g/100ml of NaH₂PO₄).

*Acid phosphatase buffer (pH 5): 5.7 g NaOH + 10.3 ml glacial acetic acid up to 1liter. *EC : Enzyme Code. *NAD: β- Nicotinamide adenine dinucleotide sodium salt (from yeast). *NBT: Nitro blue tetrazolium chloride. *PMS: Phenazine methosulfate.

Primer	Sequence
HB 08	5`- GAG AGA GAG AGA GG -3`
HB 09	5`- GTG TGT GTG TGT GG -3`
HB10	5`- GAG AGA GAG AGA CC-3`
HB 11	5` - GTG TGT GTG TGT CC - 3`
HB12	5`- CAC CAC CAC GC-3`
HB13	5`- GAG GAG GAG GC-3`
HB14	5`- CTC CTC CTC GC-3`
HB 15	5` - GTG GTG GTG GC- 3`
814 A	5° - CTC TCT CTC TCT CTC TTG -3°
844 A	5` - CTC TCT CTC TCT CTC TAC -3`
844 B	5` - CTC TCT CTC TCT CTC TGC -3`
17899A	5`- CAC ACA CAC ACA AG-3`
17898B	5°- CAC ACA CAC CAC GT-3°
17899B	5`- CAC ACA CAC ACA GG-3`

Table (2): List of ISSR primers.

Table (3): Similarity matrix for the three camel breeds based on native-PAGE of plasma protein analysis.

Breed	♀ Sudani	් Sudani	♀ Falahi	👌 Falahi	$\stackrel{\bigcirc}{_{+}}$ Maghrabi
♂ Sudani	1.000				
♀ Falahi	1.000	1.000			
🕈 Falahi	1.000	1.000	1.000		
♀ Maghrabi	0.859	0.859	0.859	0.859	
් Maghrabi	0.802	0.802	0.802	0.802	0.912

Table (4): Similarity matrix for the three camel breeds based on isozyme electrophoretic analysis.

Breed	\bigcirc Sudani	් Sudani	♀ Falahi	👌 Falahi	♀ Maghrabi
♂ Sudani	0.913				
♀ Falahi	0.862	0.874			
🕈 Falahi	0.839	0.842	0.923		
♀ Maghrabi	0.786	0.805	0.782	0.801	
👌 Maghrabi	0.706	0.709	0.765	0.781	0.842

Primer	MS (bp)	Sudani female	Sudani male	Falahi female	Falahi male	Maghrabi female	Maghrabi male
HB 08	1111	-	+	+	+	+	+
	845	-	-	-	-	+	+
HB 09	575	+	+	+	+	-	-
HB 10	1440	-	-	-	-	+	+
	1346	-	-	-	+	-	-
HB 11	947	+	+	-	-	-	-
	557	-	-	-	-	-	+
HB 12	485	-	-	+	+	+	+
HB 12	434	-	-	+	+	+	+
HB 13	356	-	-	-	-	+	+
HB 14	2690	-	-	-	-	+	+
	742	+	+	-	-	-	-
UD 15	724	-	-	+	+	+	+
HB 15	511	-	-	+	+	+	+
	398	-	-	-	-	+	+
	1679	-	-	-	+	-	-
814 A	1490	-	-	-	+	-	-
014 A	644	-	-	+	+	-	-
	637	-	-	-	-	+	+
844 A	1408	-	-	+	+	+	+
	2243	-	-	-	-	+	+
	1627	+	-	-	-	+	+
844 B	1572	+	-	-	-	+	+
	1371	-	-	+	+	+	+
	561	-	-	-	-	+	+
	1461	-	-	+	+	-	-
17899 A	1196	-	-	+	+	-	-
	919	-	-	+	+	-	-
	1269	-	-	+	+	+	+
17898 B	881	-	-	+	+	+	+
	787	-	-	+	+	+	+
17899 B	894	+	+	+	+	-	-

Table (5): Molecular genetic markers for Sudani, Falahi and Maghrabi Camel breeds based on ISSR-PCR analysis.

Primer	Total no. of bands	Monomorphic bands	Polymorphic bands	Polymorphism percentage
HB 08	5	4	1	20.0%
HB 09	6	4	2	33.0%
HB 10	5	4	1	20.0%
HB 11	6	3	3	50.0%
HB 12	5	3	2	48.0%
HB 13	7	6	1	14.0%
HB 14	7	3	4	57.0%
HB 15	6	2	4	66.0%
814 A	6	2	4	66.0%
844 A	4	3	1	25.0%
844 B	9	4	5	55.0%
17899A	4	1	3	75.0%
17898B	7	3	4	57.0%
17899B	4	3	1	25.0%
Average	5.7	3.2	2.5	43.6%

Table (6): Total no. of bands, no. of monomorphic and polymorphic bands and polymorphism percentage by 14 ISSR primers.

Table (7): Homogeneity and polymorphism percentages for the studied camel breeds.

Breed	Total no. of bands	Monomorphic bands	Polymorphic bands	Homogeneity percentage	Polymorphism percentage
Sudani	55	49	6	89.0%	10.9%
Falahi	68	63	5	92.6%	7.3%
Maghrabi	69	68	1	98.5%	1.4%

Table (8): Similarity matrix for the three camel breeds based on ISSR-PCR analysis.

Breed	\bigcirc Sudani	👌 Sudani	♀ Falahi	👌 Falahi	$\stackrel{\bigcirc}{_{+}}$ Maghrabi
👌 Sudani	0.766				
♀ Falahi	0.772	0.851			
👌 Falahi	0.770	0.839	0.946		
♀ Maghrabi	0.843	0.731	0.749	0.768	
👌 Maghrabi	0.842	0.730	0.751	0.756	0.967

Table (9): Similarity matrix for the three camel herds based on native plasma proteins, native isozymes and ISSR-PCR analyses.

Breed	\bigcirc Sudani	් Sudani	♀ Falahi	👌 Falahi	♀ Maghrabi
♂ Sudani	0.885				
♀ Falahi	0.786	0.784			
🕈 Falahi	0.746	0.743	0.912		
♀ Maghrabi	0.677	0.623	0.692	0.706	
🕈 Maghrabi	0.674	0.620	0.687	0.713	0.958

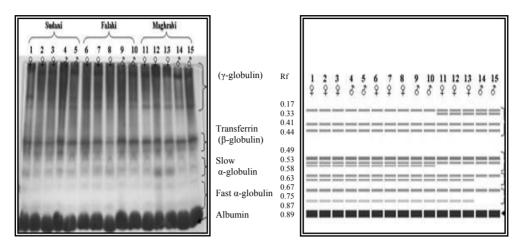


Fig. (1): Electrophoretic pattern for plasma proteins of Sudani (1-5) Falahi (6-10) and Maghrabi (11-15) camel breeds using Native-PAGE.

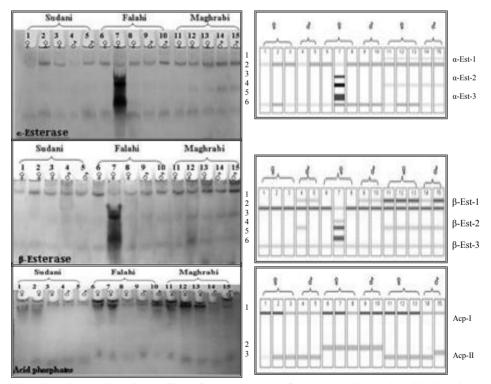


Fig. (2): Electrophoretic profiles of α-Esterase (a), β-Esterase (b) and Acid phosphates
(c) from plasma of Sudani (1-5), Falahi (6-10) and Maghrabi (11-15) camel breeds using Native-PAGE.

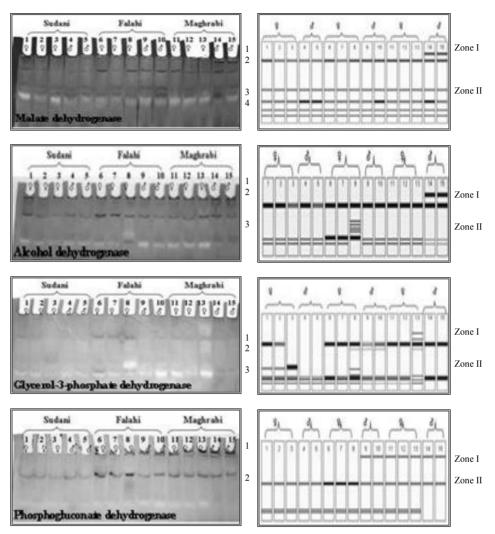


Fig. (3): Electrophoretic profiles of Malate dehydrogenase (a), Alcohol dehydrogenase (b), Glycerol-3-phosphate dehydrogenase (c) and Phosphogluconate dehydrogenase (decarboxylating) (d) from plasma of Sudani (1-5), Falahi (6-10) and Maghrabi (11-15) camel breeds using Native-PAGE.

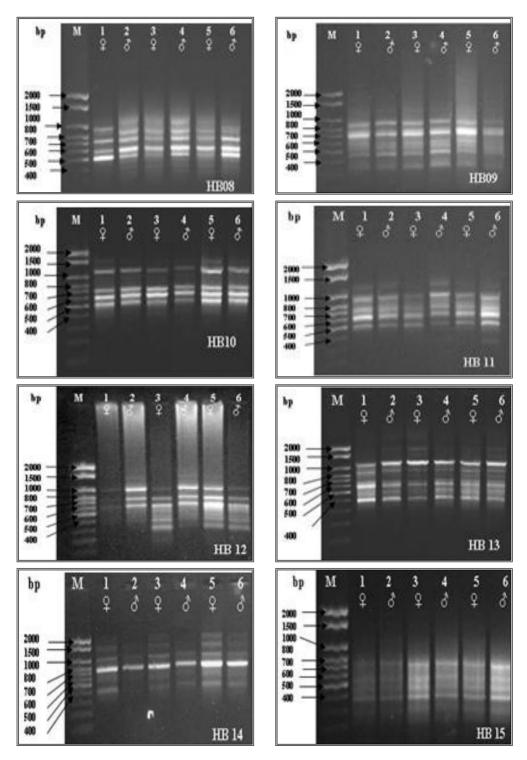


Fig. (4): Photographs showing ISSR-PCR products of the three camel breeds Sudani (lanes 1-2), Falahi (lanes 3-4) and Maghrabi (lanes 5-6) using fourteen primers.

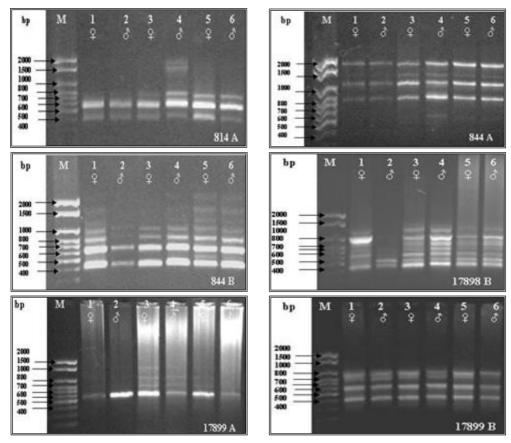


Fig. (4): continue.

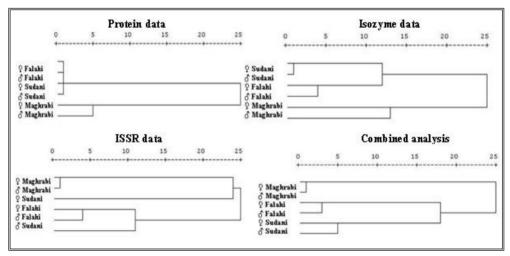


Fig. (5): Dendrograms of the three camel breeds based on similarity matrix of the three different genetic techniques and the combined analysis.