International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:11, No:7, 2017

Analysis of Genetic Variations in Camel Breeds (*Camelus dromedarius*)

Yasser M. Saad, Amr A. El Hanafy, Saleh A. Alkarim, Hussein A. Almehdar, Elrashdy M. Redwan

Abstract**—**Camels are substantial providers of transport, milk, sport, meat, shelter, security and capital in many countries, particularly in Saudi Arabia. Inter simple sequence repeat technique was used to detect the genetic variations among some camel breeds (Majaheim, Safra, Wadah, and Hamara). Actual number of alleles, effective number of alleles, gene diversity, Shannon's information index and polymorphic bands were calculated for each evaluated camel breed. Neighbor-joining tree that re-constructed for evaluated these camel breeds showed that, Hamara breed is distantly related from the other evaluated camels. In addition, the polymorphic sites, haplotypes and nucleotide diversity were identified for some camelidae *cox1* gene sequences (obtained from NCBI). The distance value between *C. bactrianus* and *C. dromedarius* (0.072) was relatively low. Analysis of genetic diversity is an important way for conserving *Camelus dromedarius* genetic resources.

Keywords**—**Camel, genetics, ISSR, *cox1,* neighbor-joining.

I. Introduction

THE camel's natural habitat is characterized as harsh conditions in the desert [1]. These animals can adapt for fluctuation in body temperature and water loss (for a relatively long time). The one-humped camels or *Camelus dromedarius* were domesticated in the Arabian Peninsula many years ago [2].

Despite the major importance of camels as locally adapted livestock in Saudi Arabia, little information is available about the characterization of these economic animals. There are several camel breeds that can be morphologically (especially based on coat color) identified in Saudi Arabia.

Few studies are concerned with genetic variations between camel breeds [2] in Saudi Arabia at DNA level. From this context it can be concluded that, molecular characterization of these camel breeds will be helpful for enhancing conservation processes for these valuable local genetic resources in Saudi Arabia. Many molecular methods such as ISSR, SSR, RFLP, AFLP, RAD and DNA sequencing were widely used for animal characterization and identification [3]-[8].

Calculation of some parameters such as gene diversity and Shannon's information index based on molecular marker analysis are recommended for exploring the genetic variations in many animal organisms [9]-[11].

This study aims to detect the molecular variations within and among some *Camelus dromedarius* for reconstructing the Neighbor-joining tree among applied camels. In addition, the study aims to explore the evolutionary variations between this species and other camelidae species based on some *cox1* gene sequences variations.

II. Material and Methods

Blood samples were obtained from (Molecular characterization of some milk proteins in Saudi camels for conservation of these local genetic resources) project's team. These samples (10 samples) were collected from four camel breeds namely: Majaheim (M), Safra (Y), Wadah (W) and Hamara (R). These breeds were originally domesticated in different Saudi locations. Genomic DNA samples were extracted from camel's blood using blood DNA QIAamp DNA extraction kit according to the manufacture's processing.

A. Analysis of Inter-Simple Sequence Repeats

Ten ISSR primers were originally selected to measure the genetic variability among applied camel breeds. For comparison, samples from each estimated camel breed were applied for each tested ISSR primer. Each PCR reaction was prepared in a 10 µL contained a 50 ng of DNA, a 0.3 µM of primer, a 0.2mM of dNTPs, a 25 mM of MgCl2, a 0.5 unit of GoTaq® polymerase (Promega) and 1 X buffer. Each ISSR primer code and sequence was presented in Table (I).

B. PCR Program

One cycle for 120 sec. at 95°C, 35 cycles for (30 sec. at 94°C, 45 sec. at 44°C and 60 sec. at 72°C) and one cycle for 15 min. at 72°C (Labnet MultiGene Gradient Thermal Cycler). The PCR products of the ISSRs were separated on agarose gel (1.5%) electrophoresis followed with ethidium bromide.

GelAnalyzer 3 software was used for analyzing Gel images. Some parameters [percentage of polymorphic loci, effective number of alleles (ne), actual number of alleles (na), Shannon's information index (i) and Nei's gene diversity (h)] were estimated to monitor the genetic diversity among applied camel breeds. POPGENE (version 1.32) was used for data analysis [12].

The number of sequences (x), number of polymorphic sites

Yasser M. Saad is with is with the Biology Department, Faculty of Science, King Abdulaziz University, and Genetics Lab, NIOF, Egypt (e-mail: yasser_saad19@yahoo.com).

Amr A EL Hanafy is with the Biology Department, Faculty of Science, King Abdulaziz University, P. O. Box.80203, Jeddah, 21589, Saudi Arabia and with the Department of Nucleic Acid Research, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research &Technology Applications, Borg El-Arab, P. O. box. 21934, Alexandria, Egypt.

Saleh A. Alkarim, Hussein A. Almehdar, and Elrashdy M. Redwan are with the Biology Department, Faculty of Science, King Abdulaziz University, P. O. Box.80203, Jeddah, 21589, Saudi Arabia.

Elrashdy M. Redwan is with the Biology Department, Faculty of Science, King Abdulaziz University, P. O. Box.80203, Jeddah, 21589, Saudi Arabia and Therapeutic and Protective Proteins Laboratory, Protein Research Department, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technology Applications, New Borg EL-Arab 21934, Alexandria, Egypt.

(SNP), number of haplotypes (h), nucleotide diversity (pi), average number of nucleotide differences (K) and sequence conservation (C) were calculated for some camelidae cox1 gene fragment sequences (obtained from NCBI). The accession numbers of analyzed fragment (593bp) sequences are: KU605080.1, KU605079.1, KU605078.1, KU605077.1, KU605076.1, KU605075.1, KU605074.1, KU605073.1, KU605072.1, KT750037.1, KT750036.1, AB753121.1, AB753118.1, AB753115.1, AB753113.1, AB753109.1, JN632608.1, EU159113.1, KX554934.1, KX554933.1, KX554932.1, KX554931.1, AP003423.1, EF507799.2, EF507798.2, EF212037.2, KX554930.1, KX554929.1, KX554928.1, KX554927.1, KX554926.1, KX554925.1, FJ456892.1, DQ534056.1, KU168760.1, LC143635.1, DQ534055.1, AJ566364.1, Y19184.1, DQ534054.1, AP003426.1, KX388534.1, KX388532.1 and EU681954.1

Sequences were aligned and the Neighbor Joining tree was constructed among evaluated species fragments using MEGA V6. Analysis of SNPs (single nucleotide polymorphisms) was carried out using DNAsp. (Ver.5.10.01).

TABLE I
ISSR PRIMER CODES AND SEQUENCES

Code	Sequence	Code	Sequence
17899A	5'[CA]6AC'3	SAS1	5'[GTG]4 GC'3
HB15	5' [GTG]3GC3'	IT1	5' [CACA]4 GT' 3
UBC827	5' [AC]8G 3'	TERRY	5'[GTG]4 C'3
UBC811	5'[GA]8C3'	SAS3	5'C [AGG]4 '3
MAO	5'[CTC]4 RC'3	IT3	5'[GAG]4AG' 3

III. RESULTS

A. General Genetic Variations among C. dromedarius Breeds Based on ISSR Markers

ISSR technique was used to differentiate among the applied camel breeds. In this study, 10 different ISSR primers were tested. All the ISSR primers (17899A, HB15, UBC 827, UBC 811, MAO, SAS1, IT1, TERRY, SAS3 and IT3) were succeeded in matching and amplifying genomic DNA of all applied camel breeds.

All the Electrophoretic patterns were scored, the band was considered as 1 if present and 0 if absent.

Numbers (N), average of band frequencies (ABF±SD) and relative front ranges of obtained ISSR bands (using the ISSR primers) were calculated (Table II). The number of detected bands was ranged from six (generated by SAS3) to 14 (generated by IT1). The lowest band frequency value (0.57±0.3) was calculated for primer SAS1.

B. Genetic Variations within Each Estimated C. dromedarius Breed Based on ISSR Markers

The genetic variations within each estimated camel breed was explored by calculating the mean ± SD (Standard deviation) of (ne), (na), (i) and (h) for each studied camel breed (Table III).

The percentages of polymorphic bands were 53.57%, 41.96%, 25.5% and 29.46% in R, Y, W and M samples, respectively.

C. Genetic Variations among Estimated Camel Breeds

Nei's genetic distance values (Table IV) were calculated among applied camel breeds based on ISSR polymorphism. The lowest genetic distance values (0.042 and 0.043) was calculated between (W and M) and (W & Y) camel's breeds, respectively. The lowest genetic identity value was detected between R and W camel breeds. The distance and similarity values were reflected in Fig. 1. R breed was distantly related from the other evaluated camel breeds.

The lengths between evaluated camel breeds and nodes on the neighbor-joining tree were presented in Table V. The gene flow value (0.95) was calculated from the analyzed data. The highest values of the previous parameters were detected in (R) samples. On the other hand, the lowest values were calculated in W samples.

TABLE II
NUMBERS (N), AVERAGE FREQUENCIES (ABF±SD) AND RELATIVE FRONT RANGES OF OBTAINED ISSR BANDS

Primer	N	ABF	RF
17899A	11	0.7±0.3	0.11-0.88
HB15	9	0.77±0.3	0.32-0.90
UBC827	12	0.64±0.3	0.16-0.75
UBC811	12	0.85±0.8	0.14-75
MAO	13	0.78±0.2	0.20-0.77
SAS1	11	0.57±0.3	0.15-0.67
IT1	14	0.66±0.3	0.16-0.70
TERRY	11	0.66±0.3	0.26-0.93
SAS3	6	0.60±0.3	0.20-0.62
IT3	13	0.74±0.2	0.18-0.83

TABLE III
MEAN ±STANDARD DEVIATION OF ACTUAL NUMBER OF ALLELES (NA), EFFECTIVE NUMBER OF ALLELES (NE), NEI'S GENE DIVERSITY (H) AND SHANNON'S INFORMATION INDEX (I) FOR EACH STUDIED CAMEL BREEDS

	na	ne	h	i
R	1.535±0.5	1.326±0.3	0.189±0.2	0.284±0.2
Y	1.419±0.4	1.307±0.4	0.165 ±0.2	0.242±0.3
W	1.25±0.43	1.142±0.2	0.08±0.16	0.128±0.23
M	1.296±0.45	1.209 ±0.35	0.117±0.19	0.178±0.27
All	1.750 ± 0.43	1.341±0.31	0.214 ±0.17	0.33±0.24

TABLE IV
GENETIC DISTANCE VALUES AMONG APPLIED CAMEL BREEDS BASED ON ISSR POLYMORPHISM

Breed	R	Y	W
Y	0.188		
W	0.224	0.043	
M	0.194	0.061	0.042

Fig. 1 Reconstruction of neighbor-joining tree among the applied camel breeds (R, Y, W and M) based on ISSR polymorphism.

TABLE V
THE LENGTHS BETWEEN EVALUATED CAMEL BREEDS AND NODES ON THE PHYLOGENETIC TREE

Between	And	Length
3	R	10.11
3	2	7.48
2	Y	2.62
2	1	0.525
1	W	2.097
1	M	2.097

D. Reconstruction of Neighbor-Joining Tree among Some C. dromedarius based on CoxI sequence Variations

The neighbor-joining tree among some *C. dromedarius* (obtained from NCBI) based on *CoxI* gene variations were reconstructed (Fig. 2) to explore the genetic variation within this species. The overall genetic distance within this species was calculated. Based on *CoxI* gene sequence variations, this value was very low (0.003).

E. Genetic Distance Values among Some Camelidae Genera based on CoxI sequence Variations

The number of sequences (x=44), number of sites (593), number of polymorphic sites (SNP= 129), number of haplotypes (h=14), nucleotide diversity (pi= 0.08), average number of nucleotide differences (k=48.58) and sequence conservation (C= 0.78) were calculated for some evaluated (Table VI) camelidae *cox1* gene fragments (obtained from NCBI). The same parameters were evaluated from the consensus sequences for each evaluated camelidae *cox1* gene fragments per each estimated camelidae species.

The lowest genetic distance value (based on *CoxI* variations) was calculated between *V. pacos* (Vp), and *L. glama* (Lg). The highest distance value was calculated between *C. bactrianus* (Cb), and *V. vicugna* (Vv). *C. dromedarius* (Cd) is distantly related from *V. pacos* (Vp), *V. vicugna* (Vv) and *L. glama* (Lg). In addition, the distance value between *C. bactrianus* (Cb), and *C. bactrianus* (Cb) is very low (0.072, relatively (Fig. 3 and Table VII).

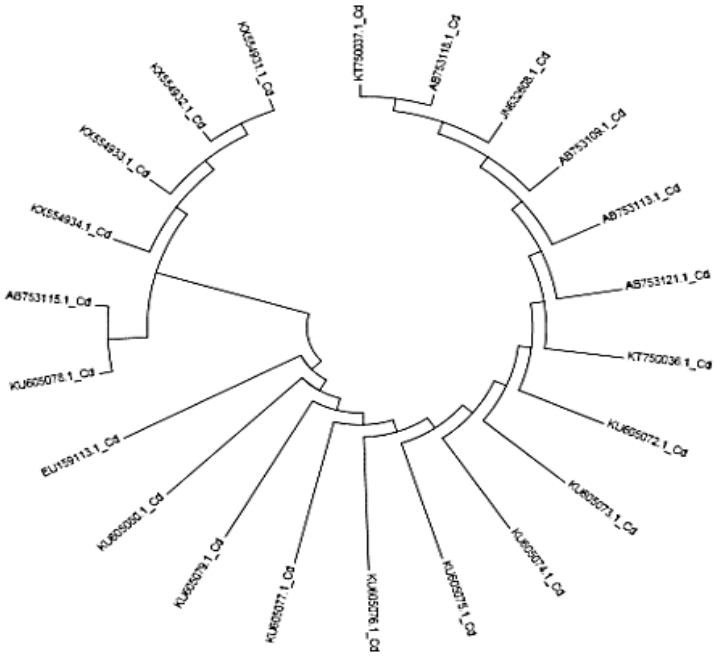

Fig. 2 Reconstruction of neighbor-joining tree among *C. dromedarius* based on *CoxI* variations

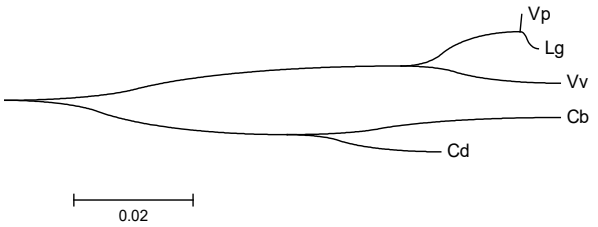

Fig. 3 Reconstruction of neighbor-joining tree among the estimated camelidae based on *CoxI* variations. *C. bactrianus*= Cb, *C. dromedarius*= Cd, *V. pacos*= Vp, *V. vicugna*= Vv and *L. glama* =Lg

IV. DISCUSSION

A lot of molecular methods such as ISSR, SSR, RFLP, AFLP, RAD and DNA sequencing were widely used for animal characterization and identification [3]-[7], [13]. Out of these methods, ISSR and *CoxI* gene system are considered an efficient and simple techniques that are widely used for characterizing many organisms [9], [14], [15].

ISSR technique was used in the present study for evaluating the genetic variations and calculating the genetic distance values among four Saudi camel breeds (R, Y, W and M). The

number of ISSR markers (generated by all the ten used primers) was calculated in all performed PCRs. In addition, these markers were analyzed for re-constructing the Neighbor joining tree among evaluating camel breeds.

TABLE VI
GENETIC DIFFERENCES IN THE ESTIMATED CAMELIDAE BASED ON *COXI* VARIATIONS

Parameters species	SNP	Hd	Pi	K	C	h	x
(Cb)	1	0.533	0.0009	0.53	0.99	2	10
(Cd)	5	0.571	0.00251	1.48	0.99	4	22
(Vp)	30	0.90	0.023	14	0.94	4	5
(Vv)	0	0	0	0	1	1	2
(Lg)	11	0.90	0.008	4.80	0.98	4	5
All	129	0.86	0.08	48.58	0.78	14	44
Consensus	123	1	0.11	66.5	0.79	5	5

C. bactrianus= Cb, *C. dromedarius*= Cd, *V. pacos*= Vp, *V. vicugna*= Vv and *L. glama*= Lg, number of sequences= x, number of polymorphic sites= SNP, number of haplotypes= h, nucleotide diversity=pi, average number of nucleotide differences= K and sequence conservation= C.

TABLE VII
GENETIC DISTANCE VALUES AMONG THE ESTIMATED CAMELIDAE BASED ON COXI VARIATIONS

	(Cb)	(Cd)	(Vp)	(Lg)
(Cd)	0.072			
(Vp)	0.179	0.163		
(Vv)	0.191	0.163	0.047	
(Lg)	0.181	0.165	0.003	0.050

C. bactrianus = Cb, *C. dromedarius* = Cd, *V. pacos* = Vp, *V. vicugna* = Vv and *L. glama* = Lg.

All the 10 ISSR primers were succeeded in matching and amplifying genomic DNA fragments of all applied camel breeds. Some of these markers (bands) were polymorphic and informative for exploring the genetic variations within and among applied camel breeds. This information was used for calculating the mean ±SD of (na), (ne), (h), (i) and (%PL) for each studied camel's breed.

The percentages of polymorphic bands were 53.57%, 41.96%, 25.5% and 29.46% in R, Y, W and M samples, respectively. The gene flow value (0.95) was high among estimated camels.

The lowest genetic distance values (0.0422 and 0.043) was calculated between (W and M) and (W & Y) camel's breeds, respectively.

From analyzed ISSR data, the R breed was distantly related from the other evaluated camels. The high similarity among evaluated camel breeds may be due to the probable common origin of these economic animals in the Kingdom of Saudi Arabia. This observation is confirmed by the high calculated gene flow among the applied camel breeds.

Some evaluated Saudi camels shared in a common origin (due to close genetic relations among evaluated camel's breeds). Detection more of microsatellite markers were recommended by Mahmoud et al., [5] for exploring the true genetic variability values among Saudi camels.

Mahrous et al. [4] calculated the genetic distance values among some camel breeds (Baladi, Somali, Sudani, Maghrabi and Mowallad) using Microsatellites and Random amplified polymorphic DNA techniques. They found that, genetic distances values between Baladi, Somali, Sudani, Maghrabi and Mowallad camels were low. They concluded that, these camel breeds may have the same ancestor.

The neighbor-joining among the estimated (Cd) *C. dromedarius* (obtained from NCBI) based on *CoxI* gene variations were reconstructed to explore the genetic variation within this animal species. The analyzed *CoxI* fragment sequences were divided into two groups due to some detected SNPs (5) among evaluated sequences.

The overall genetic distance within this species was calculated. Based on CoxI variations this value was very low (0.003).

The calculated number of haplotypes (h), nucleotide diversity (pi), average number of nucleotide differences (K) and sequence conservation (C) values in *C. dromedarius* are higher than *C. bactrianus.* These parameters were affected by number of polymorphic sites (SNP).

In the present study, the number of sequences, number of polymorphic sites, number of haplotypes, nucleotide diversity, average number of nucleotide differences and sequence conservation were calculated for some camelidae *cox1* gene fragment sequences (obtained from NCBI) for exploring the evolutionary variations within this family.

Due to the efficiency of the *CoxI* gene [15] in animal barcoding and evaluation studies, this system was recommended for calculating the genetic distance values between *C. dromedarius* and other camelidae animals.

The highest distance value was calculated between *C. bactrianus*, and *V. vicugna*. *C. dromedarius* is distantly related to *V. pacos*, *V. vicugna* and *L. glama*. In addition, the distance value between *C. bactrianus* and *C. dromedarius* (0.072) is very low, relatively.

Fingerprinting using molecular markers [6] for domestic animals represents the most recent and important area of research, leading to the conserving of local genetic resources and genetic improvement of local breeds through breeding programs.

Molecular genetic variation data among camel breeds can be used for understanding *C. dromedarius* adaptation to changes in their environment.

New genetic variations can arise in a breed from either a spontaneous mutation of a gene or by breeding with genetically different individuals [16].

V. CONCLUSION

In conclusion, ISSR technique was easy and efficient tool for reconstructing Neighbor joining tree among applied *C. dromedarius* breeds (R, Y, W and M). The detected levels of genetic diversity among the applied camel breeds are needed for identifying probable speciation in camels.

The calculated gene flow value was high. Due to the efficiency of the *CoxI* gene in animal barcoding and evaluation studies, this system was recommended for calculating the genetic distance values among evaluated camelidae. Developing more genetic markers is needed for conserving camel genetic resources in Saudi Arabia. This

knowledge is considered a basic principal for the management of camel breeds.

ACKNOWLEDGMENT

This work was supported by a grant from the King Abdulaziz City for Science and Technology (A.B-35-195).

REFERENCES

[1] Schmidt-Nielsen K (1979). Desert Animals, Physiological Problems of Heat and Water. New York: Dover Publications Inc.
[2] Uerpmann, H. P. and M. Uerpmann (2002). The appearance of the domestic camel in Saudi Arabia. J. Oman Studies 12:235-260.
[3] Frankham, R., Ballou J. D., and Briscoe D.A., (2002). Introduction to conservation genetics. Cambridge University.
[4] Mahrous Karima F., Hassan A. I. Ramadan, Sekena H. Abdel-Aziem, Mohamed Abd-El Mordy and Dalia M. Hemdan (2011). Genetic variations between camel breeds using microsatellite markers and RAPD techniques. J. Appl. Biosci. (39) 2626 – 2634.
[5] Mahmoud A. H., M. A. Alshaikh, R. S. Aljumaah and O. B. Mohammed (2012). Genetic variability of camel (*Camelus dromedarius*) breeds in Saudi Arabia based on microsatellites analysis. African Journal of Biotechnology. 11(51) 11173-11180.
[6] Saad Y. M., and ELShikh, Omar A. M. (2015). Analysis of molecular variations in some Sox14 gene fragments in some ray-finned fishes.Wulfenia Journal. Vol 22 (12) 80-92.
[7] EL Hanafy Amr A, Yasser M. Saad, Saleh A. Alkarim, Hussein A. Almehdar, Elrashdy M. Redwan (2016). Camel genetic resources conservation in Saudi Arabia via molecular markers. Wulfenia Journal.23 (11) 88-103.
[8] Al-Swailem AM, Al-Busadah KA, Shehata MM and Askari E, (2008). The role of parentage studies in Arabian and Bactrian camel's pedigree verification. J. Food, Agric. Environ. 6, 280-285.
[9] Saad Y. M., Abu Zinadah, O. A. H., and El-Domyati, F. M. (2013). Monitoring of genetic diversity in some parrotfish species based on inter simple sequence repeat polymorphism. Life SciJ.10(4):1841-1846.
[10] Correa Luz A, Biol Cindy Reyes E, Biol Enrique Pardo P and Teodora Cavadia M, (2015). Genetic diversity detection of the domestic horse (*Equus caballus*) by genes associated with coat color. Rev. MVZ Córdoba (20)3: 4779-4789.
[11] Ashraf Hafiz Muhammad, Muhammad Kashif Zahoor, Shabab Nasir, Humara Naz Majeed, Sarwat Zahoor (2016). Genetic Analysis of *Aedes aegypti* Using Random Amplified Polymorphic DNA (RAPD) Markers from Dengue Outbreaks in Pakistan.J. Arthropod Borne Dis. (10):4: 546–559.
[12] Yeh, F. C and T. B. J. Boyle, (1997). Population genetic analysis of co-dominant and dominant markers and quantitative traits. Belgian Journal of Botany 129: 157.
[13] Simmons Mark P., Li-Bing Zhang, Colleen T. Webb, Kai Müller (2007). A penalty of using anonymous dominant markers (AFLPs, ISSRs, and RAPDs) for phylogenetic inference. Molecular Phylogenetics and Evolution 42: 528–542
[14] Saad Y. M., AbuZinadah O. A. H., El-Domyati F. M. and Sabir J. M. (2012). Analysis of Genetic signature for some *Plectropomus* species based on some dominant DNA markers. LifeSci J. 9(4) 2370-2375.
[15] Ward Robert D.; Tyler S. Zemlak; Bronwyn H. Innes1; Peter R. Last; & Paul D. N. Hebert (2005). DNA barcoding Australia's fish species. Phil. Trans. R. Soc. B. 360:1847–1857.
[16] Hoffmann Irene (2010). Climate change and the characterization, breeding and conservation of animal genetic resources. *Animal Genetics, 41 (Suppl. 1), 32–46.*