Analysis of DNA Polymorphisms in Arabic Camel (*Camelus dromedarius*) Using Random Amplified Polymorphic DNA Polymerase Chain Reaction Technique

Talib A. Jaayid, Muntaha Y. Yousief, Eftikhar H. Muhssen, Dhurgham K. Sager

Department of Animal Production, College of Agriculture, University of Basrah, Iraq
e-mail: taleb1968@yahoo.com

Abstract. Camel populations in Iraq are facing a severe decline which demands immediate actions to ensure its conservation. This study was carried out at molecular genetics laboratory, college of agriculture, university of Basrah. Twenty four blood samples were analyzed by random amplified polymorphic DNA (RAPD) to investigate genetic similarity and diversity among and within three Arabic camel flocks (*Camelus dromedarius*) represented in Basrah, Thy Qar and Muthanna provinces. Ten random primers were used to amplify DNA fragments in these three flocks. Among ten tested RAPD primers, 6 primers generated novel and polymorphic DNA fragments in all tested samples. A total of 381 bands were obtained, 168 of them were polymorphic. The number of polymorphic bands in each population was found to be different and was higher in the Muthanna than in Thy Qar and Basrah provinces (76, 50 and 42) bands, respectively. Number of polymorphic bands in Muthanna province population was approximately 1.5 times as diverse as that in Basrah and Thy Qar provinces populations. Comparison of banding patterns in Muthanna, Basrah and Thy Qar populations revealed substantial differences that the populations may have been subjected to a long period of geographical isolation from each other. Relationship was determined on the base of polymorphic products analysis and presented in the form of dendrogram (UPGMA percent method). There is a significant correlation between geographical distance and genetic distance between populations. RAPD analysis confirmed the presence of genetic variation within tested Arabic camel. The obtained data will help build conservation strategies to avoid the extinction of animals from their natural habitats in the future. RAPD technique makes it ideal for genetic mapping, plant and animal breeding programs and DNA fingerprinting, with particular utility in the field of population genetics. This study compared, for the first time, variation in DNA fingerprinting of Arabic camels in Iraq.

Key words: *Camelus dromedarius*, Genetic diversity, Population size, RAPD markers

Introduction

Camel is one of the most important animals in the desert of the Arab world, representing 20% of total livestock animal as numbering up to 160 million heads (6). Arabic camel (*Camelus dromedaries*) has one hump live in the Arabian peninsula and the desert areas of north Africa, which represents about 95% of the total camel in the world (8).

In Iraq, there are two main camel breeds, the first is Hkiwar spreading in the Northern Badia and Badia island between Iraq and Syria. The second breed is Joudi spreading in the Southern Badia between Iraq and Saudi Arabia (2), with a population of camel in Iraq about 58 thousand heads (6). This populations and spreads more intensively across the provinces of Wasit and Basrah, followed by Muthanna, Qadisiyah, Baghdad and Nineveh, less but significant number spreads over the provinces of Karbala and Anbar (14).

The Iraqi government launched recently concern for the development camel because they contribute to the production of meat and milk, role played in the development and maintenance of ecological balance, contribute to the stability of the citizen and reduce the immigration as well as the creation of

economic activity and social in arid and semi-arid (16). Genetic diversity in natural populations is being lost rapidly due to the process of climate change and deforestation. Both factors have contributed to reducing the size of natural populations, eliminated local populations, or fragmented earlier continuous populations into nonviable fragments. The preservation of genetic diversity both within and among natural populations is a fundamental goal of conservation biology (12).

Polymerase chain reaction-random amplified polymorphic DNA (PCR-RAPD) has the potential to distinguish between strains of almost any organism without prior knowledge of its DNA sequence utilizing short random primers of arbitrary sequences (17-18). This modern molecular biology diagnostic techniques allows to study the Iraqi camel genetics and biodiversity. Our main objective is to prepare a check list of camel farms from various parts of the country, through collaboration with local and national authorities. The potential outcomes regarding this paper would be the possible genetic verification of the origin of Iraqi camel and the establishment of framework genome maps for Iraqi camel.

Materials and Methods

DNA extraction and PCR template preparation Blood samples were collected from 24 individuals from Basrah, Thy Qar and Muthanna provinces in Iraq. All the blood samples were from unrelated animals. In order to achieve reproducible results, DNA extraction should be performed with highest quality reagents. Samples of whole blood were taken into 10 ml heparinized vials. Genomic DNA was extracted from the whole blood by phenol-chloroform method described by John et al. (11) with minor modifications. The method is described as follows: portion of each blood sample, collected into an EDTA container was immediately stored at -5^{o}C. After thawing, an aliquot of 50 µl blood, 700 µl of lysis buffer (10 mM Tris–HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0, 0.5% SDS) and 60 µl of proteinase K (20 mg/ml) were added. The mixture was vortexed and incubated at 37°C overnight. DNA was extracted with equal volumes of phenol–chloroform (1:1) and chloroform: isoamylalcohol (24:1). DNA was precipitated by adding 0.1 (by volume) of 3 M sodium acetate and 2 volumes of chilled ethanol. The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in TE buffer (10 mM Tris–HCl, 1 mM EDTA). The concentration of DNA and its relative purity were determined using a spectrophotometer based on absorbance at 260 and 280 nm, respectively. The integrity of extracted genomic DNA was checked by electrophoresis in 0.2 % agarose gels using DNA as a molecular weight marker. The quality and concentration of extracted DNA was assessed by NanoDrop from Fisher scientific company, 2000 series, USA).

Primers and amplification conditions

The primers chosen arbitrarily for these experiments, were obtained from Eurofins MWG Operon, AL, USA. All of these decamer random primers had G+C content in the range 60-70%. Random amplified polymorphic DNA (RAPD) analysis was performed by using OPA (-01, -02, -03, -04, -08, -10, and OPB (-04, -07, -08) and OPF-05. The genomic DNA were amplified using PCR with Ten 10-base primers (5' to 3') in a DNA thermal cycler (Veriti™ Thermal Cycler, Applied Biosystems, USA).

Table 1: Primers name, sequence, GC% content of 10 selected random primers used in the RAPD technique.

Primers	Primer sequence 5'-3'	GC content %	Primers	Primer sequence 5'-3'	GC content %
OPA-08	GTG ACG TAG G	60	OPF-05	CCGAATTCCC	60
OPA-10	GTGATCGCAG	70	OPA-01	CAGGCCCTTC	70
OPB-04	GGACTGGAGT	60	OPA-02	TGCCGAGCTG	60
OPB-07	GGTGACGCAG	70	OPA-03	5AGTCAGCCAC	60
OPB-08	GTCCACACGG	70	OPA-04	AATCGGGCTG	60

Results and Discussion

The ability to detect polymorphisms at the DNA level has led to new approaches for the genetic analysis of livestock species. The RAPD assay has the potential to make useful contributions to genetic analysis of livestock, especially in terms of relatedness among either breeds or species (9). A major application has been to compare the genomes of closely related species in order to determine the extent of genetic divergence (7). RAPD technique based on numerous polymorphic bands have been used to investigate genetic similarity and diversity among and within three flocks. Ten primers were examined in the camel genomic. The largest amplified RAPD products observed were 1500 bp, while the smallest were 50 bp. The total number of bands generated were 381, 63.5 bands per primer as a mean (table 2 and figures 1-6).

Table 2: Primers name and frequency distribution of RAPD alleles in the polymorphic loci among different camel flocks.

Primers	Total amplified fragments				Polymorphic fragments				Polymorphic %
		Basrah	Thy Qar	Muthanna		Basrah	Thy Qar	Muthanna	
OPA-08	105	35	30	40	45	10	15	20	43
OPA-10	72	12	20	40	43	6	15	22	60
OPB-04	31	15	10	6	15	7	4	4	48
OPB-07	53	15	18	20	20	7	4	9	38
OPB-08	90	40	15	35	35	10	9	16	39
OPF-05	30	9	9	12	10	2	3	5	33
Total bands	381	126	102	153	168	42	50	76	44

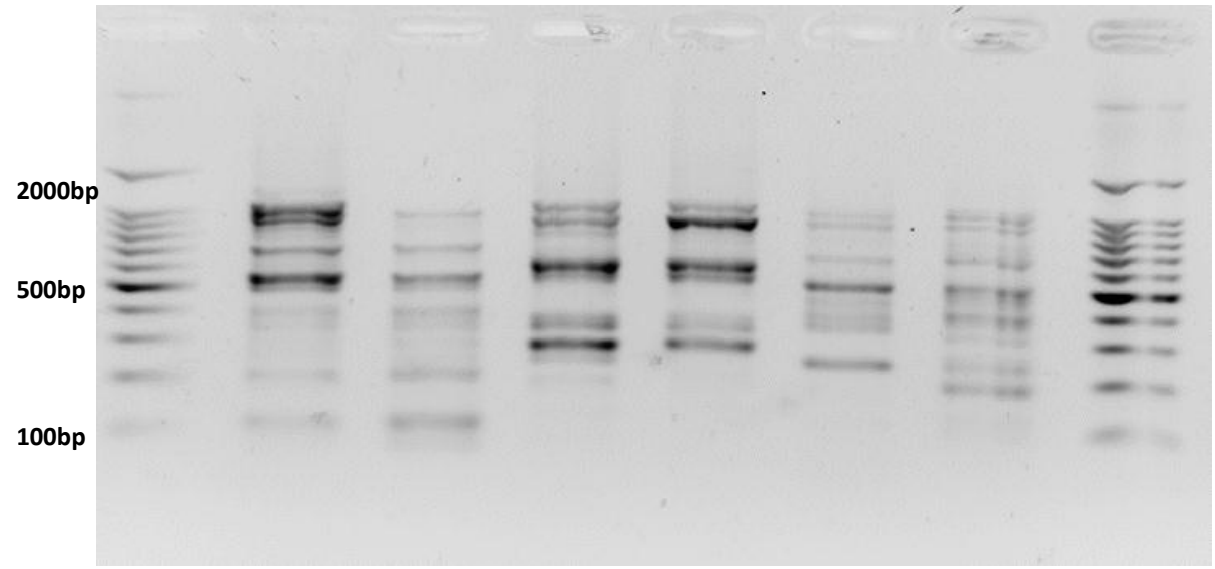

Figure 1.: RAPD profiles of camel (*Camelus dromedarius*) amplified by arbitrary primer OPA-10. Amplification products were electrophoresed on a 1.4% agarose gel with TBE (0.09 M Tris, pH 8.5, 0.09 M boric acid, 2.5 mM EDTA) and detected by staining with ethidium bromide. The gels were illuminated with UV light and taken photographs by Photonyx S140 direct copy system (Nyx Technik company, USA).

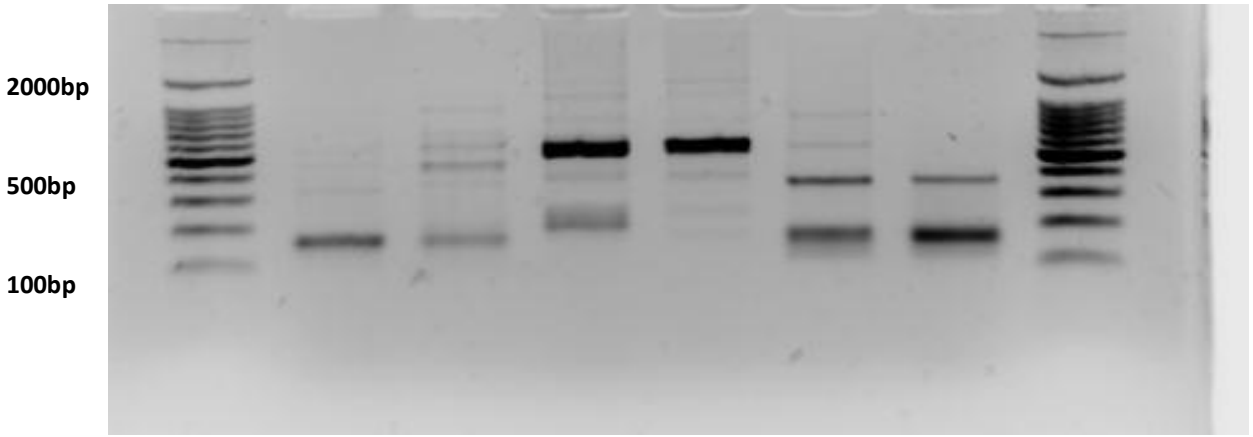

Figure 2.: RAPD profiles of camel (*Camelus dromedarius*) amplified by arbitrary primer OPA-08.

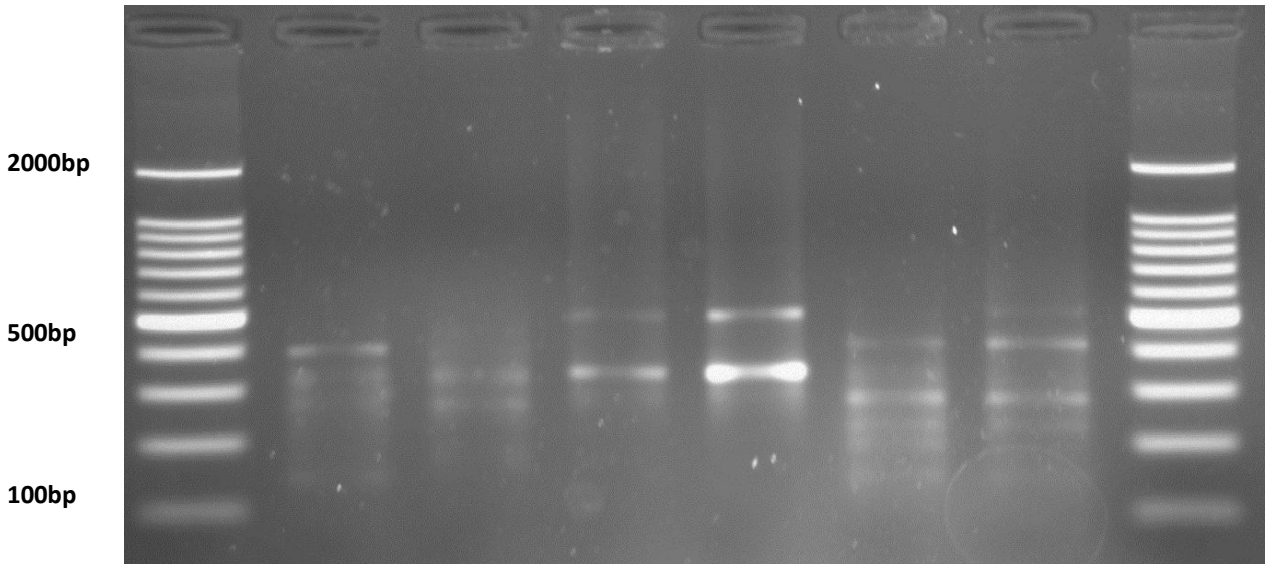

Figure 3.: RAPD profiles of camel (*Camelus dromedarius*) amplified by arbitrary primer OPB-04.

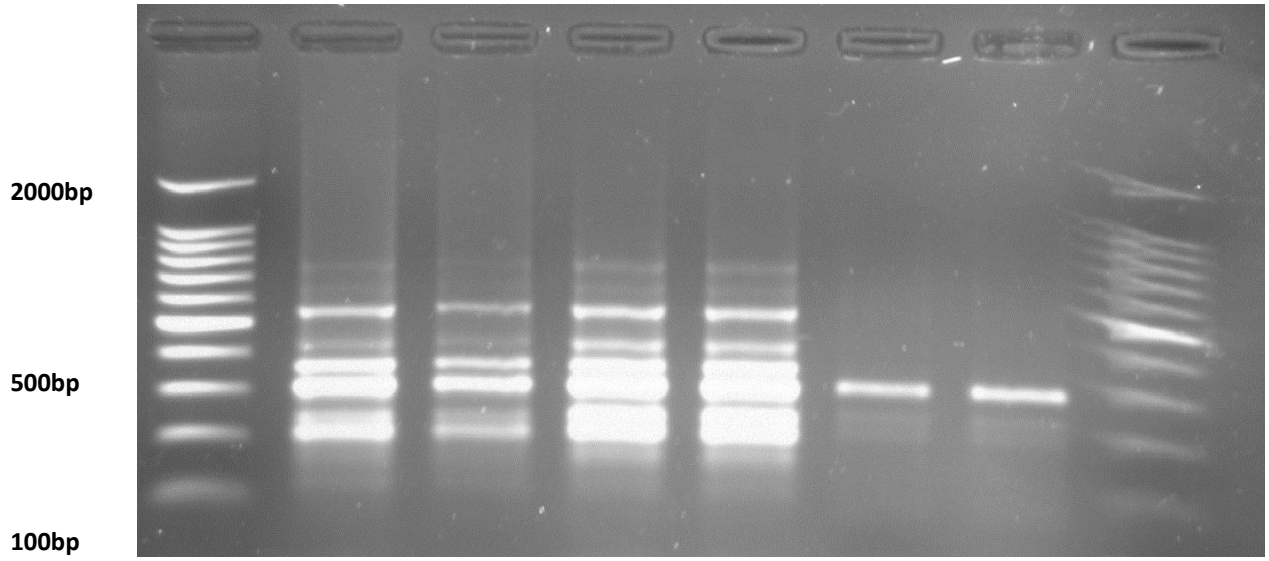

Figure 4.: RAPD profiles of camel (*Camelus dromedarius*) amplified by arbitrary primer OPB-07.

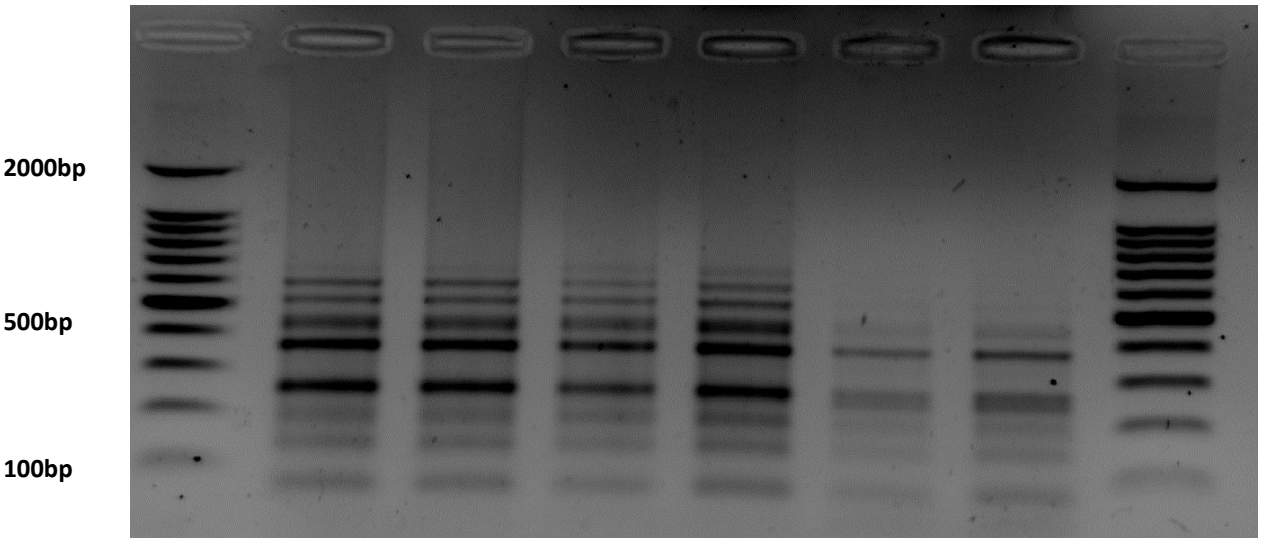

Figure 5.: RAPD profiles of camel (*Camelus dromedarius*) amplified by arbitrary primer OPB-08.

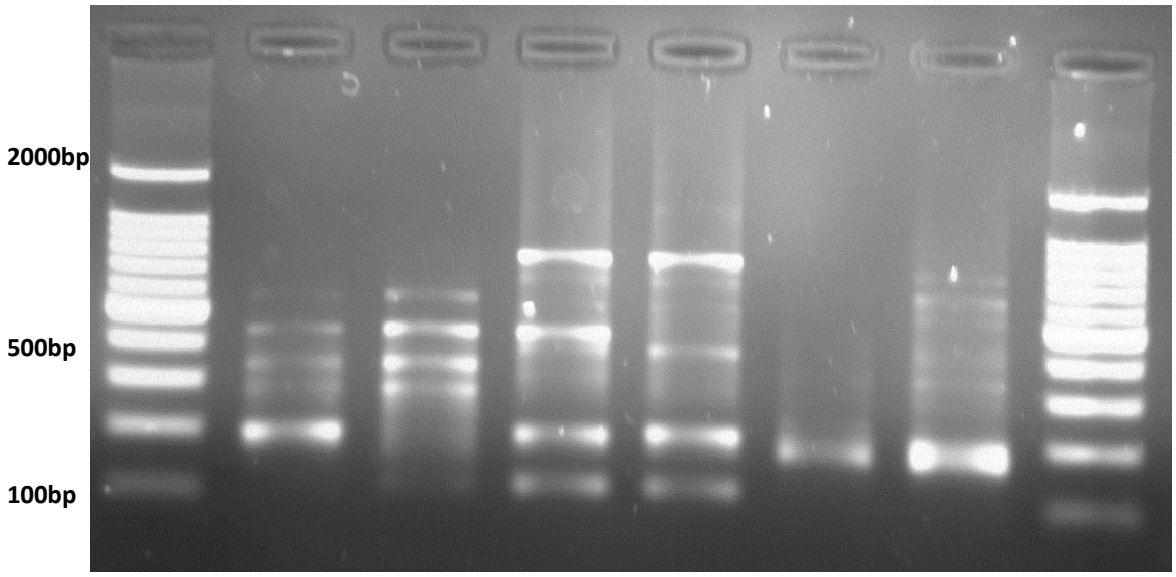

Figure 6.: RAPD profiles of camel (*Camelus dromedarius*) amplified by arbitrary primer OPF-05.

The specific minor bands patterns produced by random primer OPB-08, which showed DNA polymorphism, were in the range of 50 to 100 pb (figure 5). The results showed that the OPA-08 and OPA-10 primers produced multiple bands profiles and variation, these primers showed a few dense bands with a number of amplified DNA fragments averaging 105 and 72 scorable bands, respectively, and 45 and 43 polymorphic bands were scored per primer, respectively. On the contrary, OPA-0PB-04, 0PB-07, 0PB-08 and OPF-05 primers, with the exception of the OPB-08 primer, gave many more bands which were mostly similar.

The primers OPA-08 and OPA-10 produced maximum number of

polymorphic bands (54 and 43) respectively, while OPF-05 primer produced only 10 polymorphic bands. The highest polymorphism was 60% (OPA-10) followed by 48% (OPB-04), whereas the lowest was 33% (OPF-05). This proportion of polymorphism was higher as compared to some previous reported RAPD analysis in camel e.g., 35% for OPA-08 (4) and only 16 bands for OPB-08 (3).

Understanding of the genetic structure of a species is essential for defining strategies and actions for its management in a sustainable use, the establishment and management of plantations, genetic improvement and conservation in and ex situ (13). The UPGMA dendrogram based on Nei's (15), genetic distance between flocks was found to be correlated (figure 7). Thus, RAPD can be a useful markers for the analysis of genetic relationship among camel flocks. This current work reveals that genetic diversity exists among the three camel populations studied. In taxonomic, molecular systematic and species-specific, RAPD markers could be an invaluable tool for species variation and establishing the status of organisms and its evolution (3, 5, 10).

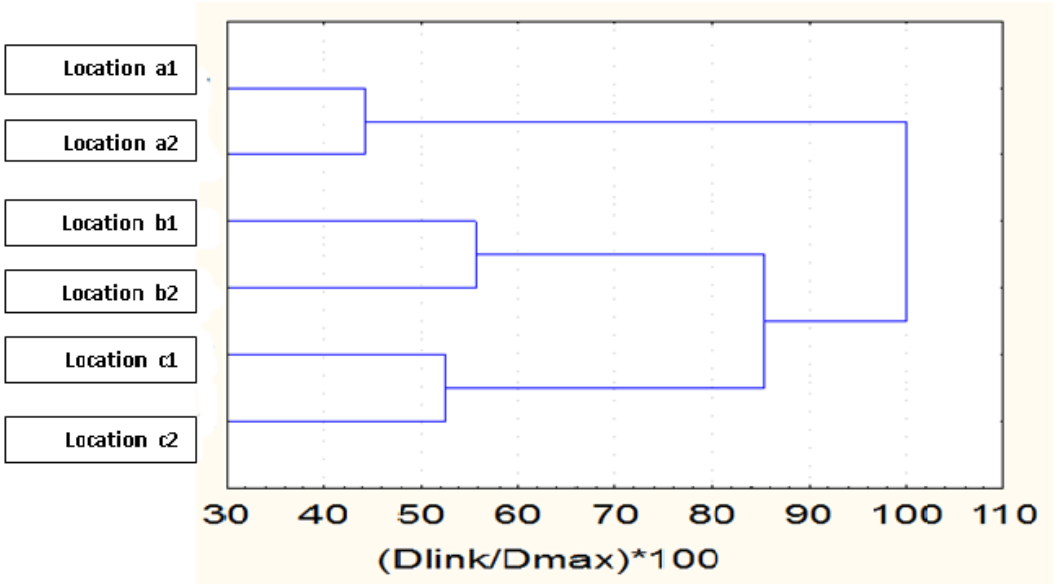

Figure 7.: UPGMA dendrogram of genetic relationships among Basrah 1 and 2, Thy Qar 1 and 2 and Muthanna 1 and 2 locations based on RAPD technique calculated from data of 6 selected arbitrary primers.

Conclusion

Findings of this study are important for Iraqi institutes to help design a more sustainable national camel policy in Iraq. Update Iraq's data on camel genetic resources in FAO's domestic animal information system (DADIS) and building capacity in the area of genetic parameter estimation for Iraq's Animal Genetic Resources (AnGR) and improve Iraq's collaboration with animal breeding institutes worldwide, this study will be developed and validate a fast method for typing the main mutations of camel blood proteins genes by using RAPD technique.

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تحليل التشكل الوراثي للحامض النووي الرايبوزي منقوص الاوكسجين في الإبل العربية (*Camelus dromedarius*) باستعمال تقنية التضخيم العشوائي متعدد الاشكال لقطع DNA

طالب احمد جايد، منتهى يعقوب يوسف، إفتخار حسن محسن وضرغام كامل صكر

قسم الثروة الحيوانية، كلية الزراعة، جامعة البصرة، العراق
e-mail: taleb1968@yahoo.com

الخلاصة. تواجه تجمعات الإبل في العراق تراجع شديد الامر الذي يتطلب اتخاذ إجراءات فورية لضمان المحافظة عليها. قد أجريت هذه الدراسة في مختبر الوراثة الجزيئية، كلية الزراعة، جامعة البصرة. تم تحليل أربع وعشرين عينة بتقنية اطوال تشكلات قطع الحامض النووي الرايبوزي منقوص الاوكسجين للتحقيق من التشابه الوراثي والتنوع بين وداخل ثلاثة قطعان من الإبل العربية (*Camelus dromedarius*) ممثلة في محافظات البصرة، ذي قار والمثنى. استخدمت عشر بادئات عشوائية لتضخيم قطع الحامض النووي الرايبوزي في هذه القطعان الثلاثة. بين عشرة اختبار الاشعالRAPD ، 6الاشعال ولدت شظايا الحامض النووي رواية ومتعددة الأشكال في جميع العينات التي تم فحصها وشرائح استنساخه. وتم الحصول على ما مجموعه 381 حزمة، كانت 168 منها متعددة الأشكال الوراثية. تم العثور على عدد من الحزم متعددة الأشكال الوراثية في كل التجمعات في أن تكون مختلفة، وكان أعلى حزم متشكلة في المثنى مقارنة مما كان عليه في محافظات ذي قار و البصرة (76 و 50 و 42) حزمة على التوالي. وكان عدد الحزم متعددة الأشكال الوراثية في تجمعات محافظة المثنى اكثر بحوالي 1.5 مرة مقارنة مما كان عليه في محافظات ذي قار و البصرة. وكشفت المقارنة بين أنماط الحزم في تجمعات المثنى، البصرة و ذي قار اختلافات جوهرية إن هذه التجمعات قد تعرضت لفترة طويلة من العزلة الجغرافية عن بعضها البعض. لوحظ الحصول على بصمات متطابقة، إذ كانت أقل في البادئ OPA-03 وجدت علاقة ارتباط معنوي بين المسافة الجغرافية والمسافة الجينية بين التجمعات. أكد تحليل RAPD وجود الاختلافات الجيني داخل الابل العربية المختبرة. إن البيانات التي تم الحصول عليها تساعد على بناء استراتيجيات الحفظ لتفادي انقراض الحيوانات من المواطن الطبيعية في المستقبل. تجعل تقنية RAPD مثالية لرسم الخرائط، في النبات، الحيوان، برامج تربية وتحسين الحيوان والبصمة DNA، مع فائدة خاصة في مجال علم الوراثة العشائر. قارنت هذه الدراسة، لأول مرة التباين في بصمة DNA في الإبل العربية في العراق.

مفتاح الكلمات: الابل العربية ذات السنام الواحد، التنوع الوراثي، حجم العشيرة، واسمات RAPD.