Analysis of the genetic variability of camel populations in Kazakhstan using ISSR-PCR markers

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Abstract. In this study, we analyzed the genetic characteristics of camel populations of Kazakhstan using ISSR-PCR markers. As a result, fragments of the following sizes were identified using the (AG)9C primer: 200, 320, 370, 430, 580, and 700 bp. Based on the (GA)9C primer, the length of the amplicons was 300, 350, and 430 bp. An analysis of the genetic differences of camels in the populations was carried out using ISSR-PCR markers. As a result, the number of allelic indices of polymorphic loci of the (AG)9C primer and related polymorphic loci in the population of the Shymkent population was equal to 77.78%, the allelic index of polymorphic loci of camels in the Taraz population - 33.3%, Kyzylorda and Atyrau populations - 44,44%. In camels selected for the study, the number of observed alleles (Na) averaged 1.27, the effective number of alleles (Ne) was equal to 1.37, the genetic diversity (h) according to Nei was equal to 0.21 and the Shannon index (I) was equal to 0.30. The average allelic score for polymorphic loci was 50.0% across four populations. Based on our results, the two markers used in the work were informative in assessing the genetic diversity of camel populations.

1 Introduction

The development of camel farming is more profitable than the breeding of other farm animals. After all, camel cattle can live freely in regions with a low grass, hot and dry climate, which many other farm animals cannot tolerate. Camel breeding is also economically profitable. As the main product of camel cattle, it receives nutritious meat and milk (shubat), which has beneficial healing properties for the human body. Through numerous research works, the positive effects of shubat drink on the human body have been shown. At the same time, camel hair and skin can also be used for many benefits. Currently, camel farms in Kazakhstan are supported by the state. One of the most important directions is the development of camel farming in the country. To carry out camel breeding work in Kazakhstan, it is necessary to conduct an appropriate analysis of local camel populations to search for Genetic Resources. Unfortunately, today in our country approximately.

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Unfortunately, today in our country, camel breeds have not been studied according to the SNP and the available molecular genetic markers [1].

The simplest and most convenient method for describing genomic polylocus is to assess the polymorphism of DNA fragments obtained as a result of PCR using a microsatellite region based on a single primer. Studies carried out by various scientific groups worldwide have shown that the use of this type of marker has a high information content [2]. Also, one of the most important areas of its application in breeding is the identification of intergroup differentiation relationships according to such polylocus spectra and by complexes of morphophysiological characteristics.

The polylocus nature of ISSR-PCR markers facilitates calculating the clarity of combinations of animal groups closely related to origin, sorting, and phenotypic differentiation, which requires reliable information on characteristics of molecular genetic markers applied to the group of animals under the study. This method results in a high yield and can be successfully used in determining interspecific and intraspecific genetic variability, describing groups of plants and animals belonging to different taxonomic levels, and in some cases for individual genotyping [3, 4].

The creation of ISSR-PCR markers has its advantages. For example, there is no need to know in advance the nucleotide sequence of the DNA molecule under study. In addition, the sequence of primers is specific and there is a high chance of reproducing the method. The use of these primers is cost-effective. Another thing is that it can be determined from any tissue [5].

The main goal of this work is to conduct studies of camel breeds Camelus bactrianus and Camelus dromedarius using ISSR-PCR markers. The study of the genetic diversity of camels in Kazakhstani populations, including Kyzylorda, Atyrau, Taraz, and Shymkent, is based on ISSR-PCR markers allowing us to identify differences between these populations [6]

2 Materials and Methods

For genetic analysis using ISSR-PCR markers, 10 heads of one, and two-humped camels from each of the camel populations of Kyzylorda, Atyrau, Taraz, and Shymkent were selected as research materials.

The genomic DNA was isolated from camel blood samples using a set of reagents in a commercial kit for cleaning genomic DNA. We estimated the quality of the genomic DNA using agarose gel electrophoresis and a concentration spectrophotometer. During the experiment, seven ISSR primers were tested, of which two primers were selected as they demonstrated higher quality results: AG-ISSR, i.e. 5'-AGA GAG AGA GAG AGA GAG C3' and GA-ISSR – 5'-GAG AGA GAG AGA C-3' [7]. PCR was performed in a classical format on a special commercial PCR Master Mix (Thermo Scientific, USA) with the enzyme Taq-polymerase. PCR was performed on the mastercycler nexus Gradient amplifier (Eppendorf, Germany). The PCR cycle was as follows: initial denaturation 5 min 95°C; denaturation 95°C – 40 sec, adhesion 55°C – 30 sec, synthesis 72°C – 1 min (35 cycles); final synthesis 72°C, 2 min. By the number of alleles obtained, a base was created in Microsoft Excel. Population and genetic parameters were assessed in the TFPGA PROGRAM [8]. Fragment frequencies, proportions of polymorphic loci, genetic diversity, and genetic distance indices were calculated using the PopGene 1.32 program [9-10].

3 Results and Discussion

The results obtained using ag-ISSR and GA-ISSR markers in PopGene v. 1.32 and Microsoft Excel to determine the genetic heterogeneity of camel species using ISSR-PCR markers allowed to determine the frequency of DNA fragments, the proportion of polymorphic loci, and the genetic diversity index for the populations studied.

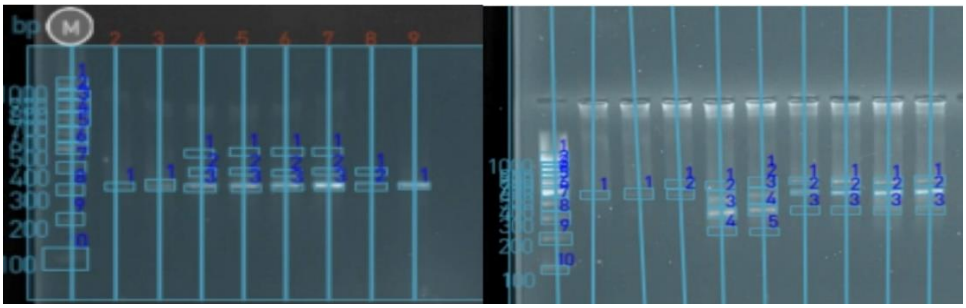

Fig.1. Variability of ISSR markers in camel populations in Kazakhstan. M - molecular marker.

The calculations were carried out individually according to the AG-ISSR and GA-ISSR markers. As a result, 6 fragments on GA-ISSR and 12 fragments with the AG-ISSR marker were identified for four populations. According to the AG-ISSR marker, 9 polymorphic fragments were identified in camel species in the populations from Atyrau, Kyzylorda, Shymkent, and Taraz. According to Nei calculations, the genetic diversity in the Shymkent population of camels was approximately equal to the ratio of 0.36 (Shannon Diversity Index 0.37) and 0.15 (Shannon Diversity Index 0.29) for the population of Taraz, 0.17 for Kyzylorda and Atyrau. According to this data, an analysis with the AG-ISSR marker shows that Shymkent population of camels has a higher heterogeneity than the other three populations. In addition, according to the GA-ISSR marker, three polymorphic loci were identified in individuals of all populations.

Table 1. The results of studies of the population of Atyrau, Kyzylorda, Taraz, and Shymkent camels bred in Kazakhstan using ag-ISSR and GA-ISSR markers

Locus	N	Number of observable alleles, (Na)	The effective number of alleles, (Ne)	Genetic diversity by NEI, (H)	Shannon index (I)
Camels in the population of Shymkent					
200(AG) 9c	10,000	0,000	1,000	0,000	0,000
320(AG) 9c	10,000	2,000	1,923	0,480	0,673
370(AG) 9c	10,000	2,000	1,923	0,480	0,673
430(AG) 9c	10,000	2,000	1,923	0,480	0,673
580(AG) 9c	10,000	2,000	2,000	0,500	0,693
700(AG) 9c	10,000	2,000	1,923	0,480	0,673
300(GA) 9c	10,000	1,000	1,000	0,000	0,000
350(GA) 9c	10,000	2,000	1,471	0,320	0,500
430(GA) 9c	10,000	2,000	1,923	0,480	0,673
Average	10,000	1,667	1,676	0,358	0,507
Note: the number of polymorphic loci is 9; the percentage of polymorphic loci is 77.78%.					
Camels in the population of Taraz					
200(AG) 9c	10,000	2,000	1,724	0,420	0,611
320(AG) 9c	10,000	2,000	1,923	0,480	0,673
370(AG) 9c	10,000	0,000	1,000	0,000	0,000
430(AG) 9c	10,000	1,000	1,000	0,000	0,000

Continuation of Table 1

580(AG) 9c	10,000	0,000	1,000	0,000	0,000
700(AG) 9c	10,000	1,000	1,000	0,000	0,000
300(GA) 9c	10,000	1,000	1,000	0,000	0,000
350(GA) 9c	10,000	1,000	1,000	0,000	0,000
430(GA) 9c	10,000	2,000	1,724	0,420	0,611
Average	10,000	1,111	1,263	0,147	0,211
Note: the number of polymorphic loci is 9; the percentage of polymorphic loci is 33,3%.					
Camels in the population of Atyrau					
200(AG) 9c	10,000	0,000	1,000	0,000	0,000
320(AG) 9c	10,000	1,000	1,000	0,000	0,000
370(AG) 9c	10,000	0,000	1,000	0,000	0,000
430(AG) 9c	10,000	2,000	1,220	0,180	0,325
580(AG) 9c	10,000	2,000	1,724	0,420	0,611
700(AG) 9c	10,000	2,000	1,923	0,480	0,673
300(GA) 9c	10,000	1,000	1,000	0,000	0,000
350(GA) 9c	10,000	1,000	1,000	0,000	0,000
430(GA) 9c	10,000	2,000	1,724	0,420	0,611
Average	10,000	1,222	1,288	0,167	0,247
Note: the number of polymorphic loci is 9; the percentage of polymorphic loci is 44,44%.					
Camels in the population of Kyzylorda					
200(AG) 9c	10,000	0,000	1,000	0,000	0,000
320(AG) 9c	10,000	0,000	1,000	0,000	0,000
370(AG) 9c	10,000	0,000	1,000	0,000	0,000
430(AG) 9c	10,000	1,000	1,000	0,000	0,000
580(AG) 9c	10,000	2,000	1,724	0,420	0,611
700(AG) 9c	10,000	2,000	1,923	0,480	0,673
300(GA) 9c	10,000	1,000	1,000	0,000	0,000
350(GA) 9c	10,000	2,000	1,220	0,180	0,325
430(GA) 9c	10,000	2,000	1,724	0,420	0,611
Average	10,000	1,111	1,288	0,167	0,247
Note: the number of polymorphic loci is 9; the percentage of polymorphic loci is 44,44%.					

In Shymkent population of camels, the number of polymorphic loci was 9, and the allelic index of polymorphic loci was 77.78%. The allelic index of polymorphic loci in Taraz population of camels was 33.3%, and 44.44% in Kyzylorda and Atyrau. Overall, the number of alleles observed across all populations (Na) averaged 1.27, the effective number of alleles (Ne) was equal to 1.37, the genetic diversity (H) for Nei was equal to 0.21, and the Shannon index (I) was equal to 0.30. The allelic index of polymorphic loci averaged 50.0% (Table 1). The next stage of analysis used principal component (PCA) analysis of genetic diversity, population structure, similarity, and differences in gene pool, which allowed defining the degree of reliability of the data obtained as a result of the study and the interspecific differences in camel populations. This program analyzes the structure of the population using Multi-Focus genotype data consisting of unrelated genetic markers. Using this program allows us to determine the number of individual cluster populations, the distribution of individuals between cluster populations, and hybrid individuals. Each of the clusters is an allele of certain loci characterized by the number of frequencies. Within the cluster, the loci are thought to be in equilibrium according to the Hardy-Weinberg's law.

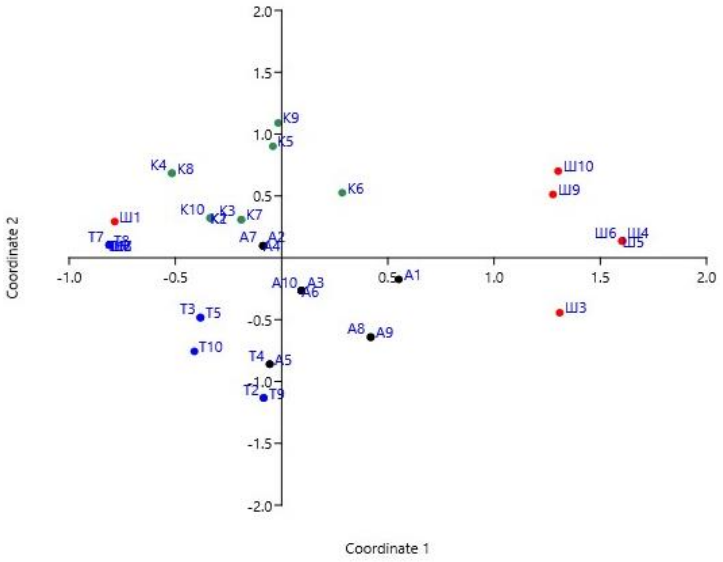

Fig. 2. Clustering by the RSA method: sh-Shymkent, T-Taraz, K-Kyzylorda, a-Atyrau populations

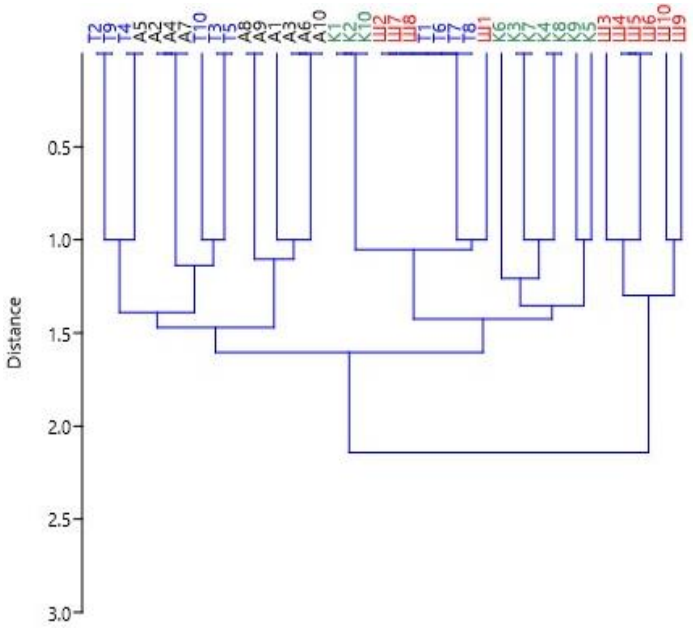

Fig. 3. Phylogenetic analysis of individuals taken for study based on the Neighbor-joining method

The Neighbor-joining method showed that the individuals in both populations were completely separated from each other as separate clusters (Fig. 3).

4 Conclusion

The results obtained allowed to identify interbreeding and interbreeding relationships, as camels in each population were classified into several subclasses.

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