In Vitro Cellular & Developmental Biology - Animal
https://doi.org/10.1007/s11626-018-0238-6

Identification of Bactrian camel cell lines using genetic markers

Abdolreza Daneshvar Amoli[1] · Seyed Abolhasan Shahzadeh Fazeli[2,3] · Mehdi Aminafshar[1] · Naser Emam Jomeh Kashan[1] · Hamidreza Khaledi[4]

Received: 24 November 2017 / Accepted: 28 January 2018 / Editor: Tetsuji Okamoto

Abstract

Iranian Bactrian camel population is less than 100 animals. Iranian biological resource center produced more than 50 Bactrian camel fibroblast cell lines as a somatic cell bank for conservation animal genetic resources. We compared two type markers performance, including 14 random amplified polymorphic DNA (RAPDs) (dominant) and eight microsatellite (co-dominant) for cell line identification, individual identification and investigation genetic structure of these samples. Based on clarity, polymorphism, and repeatability, four RAPD primers were selected for future analysis. Four RAPD primers and eight microsatellite markers have generated a total of 21 fragments and 45 alleles, respectively. RAPD primers revealed fragment size between 150 to 2000 bp and gene diversity since 0.27 (IBRD) to 0.46 (GC10), with an average of 0.37. Microsatellite markers generated number of alleles per locus ranged from 3 to 11, with an average of 5.62 alleles. The observed heterozygosity ranged from 0.359 (IBRC02) to 0.978 (YWLL08), and expected heterozygosity ranged from 0.449 (IBRC02) to 0.879 (YWLL08). Bottleneck analysis and curve showed that Bactrian camel population did not experience a low diversity. RAPD profiles were especially suitable for investigation population genetics. All primers generated novel and polymorphic fragments. Briefly, our results show that a multiplex PCR based on these markers can still be valuable and suitable for authentication of cell lines, investigating gene diversity and conservation genetic resources in Bactrian camel, while new technologies are continuously developed.

Keywords Bactrian camel · Identification cell line · RAPD · Microsatellite

Introduction

The conservation of genetic resources, especially for endangered species, either wild type or domestic, is an important issue in biological science and experimental research (Roosen et al. 2005; Li et al. 2009). There are various practical schemes to conserving genetic resources from devolution, including DNA banking, somatic cells, sexual gonads (sperm and ovule), and embryos banking and even the protection of individual animal (Gorji et al. 2016). These strategies serve different objectives and are also different in terms of costs and efforts required (Amoli et al. 2017b).

The establishment and banking of somatic cell lines are reported as an appropriate technology for conservation of endangered breeds and genetic screening (Makkar and Viljoen 2005). Somatic cells can be isolated from skin, tissue, or blood samples of animals. Additionally, collecting samples is low cost, fast, and easy, and it is also a possible animal cloning from every sample of somatic cell lines (Amoli et al. 2017a, b). Somatic cell banking has been reported to be an alternative technology for conservation of endangered breeds (Makkar and Viljoen 2005). In the future, induced pluripotent stem cells (iPSCs) will be generated from fibroblast cells and differentiation into sperm to provide us with animal restoration, reproduction, and transgenic production (Takahashi and Yamanaka 2006; Easley et al. 2012).

✉ Abdolreza Daneshvar Amoli
abdolrezadaneshvar@yahoo.com; daneshvar@ibrc.ir

Seyed Abolhasan Shahzadeh Fazeli
shfazeli@yahoo.com

[1] Department of Animal Sciences, Faculty of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran

[2] Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran

[3] Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran

[4] Department of Agriculture, Yadegar-e-Imam Khomeini (rah), Shahr-e- rey Branch, Islamic Azad university, Tehran, Iran

Published online: 01 March 2018

Bactrian camel (*Camelus bactrianus*) is better known as the camel with two humps. They are one of the most adaptable animals in the world, as they are able to withstand temperatures from 30 to −30°C (Wu et al. 2014). Many genes related to metabolism are under accelerated evolution in the camel, and their genome holds survival secrets. They need less feed and release less methane than other domestic ruminants (Dittmann et al. 2014). They were used as a means of transportation for many years ago and were really quite cost-effective. There is evidence about the importance of the Bactrian camel in the trade routes such as the Silk Road across Eurasia. But, development of mechanized transportation systems and network of roads reduced their efficiency (Kuz'mina 2008). As a result, their population declined year by year. Iranian Bactrian camel is an endangered species in Iran with fewer than 100 animals in their homeland. Therefore, preservation of the genetic resources using primary cell culture is crucial. The Iranian Biological Resource Center (IBRC) established more than 50 Bactrian camels fibroblast cell lines for conserving genetic resources, breed restoration, and future genetic researches. As a result, cells and individual identification are necessary for these new cell lines (Almeida et al. 2011).

Cell line misidentification has been a continuous problem in research and industry for decades (Almeida et al. 2011). In order to validate a cell line, some parameters are important such as the identification and the origin of a cell line, free from all known forms of microbial contamination, performance accepted (growth properties, phenotypic expression), and use (Freshney 2005). Unfortunately, it is observed that many cell lines were contaminated with another cell line and eventually led to waste a significant amount of time, cost, activities, and laboratory supplies and potentially invalidating the results (Stacey 2000). Identification of cell line is a category test that gives validity to research papers and handicap of that is a great defect for any research activity. As a result, more scientific journals are now requiring proof of cell line authentication for manuscript submission (Almeida et al. 2011). Fortunately, a cell line authentication can be achieved by using molecular and genetic profile.

Dominant and co-dominant markers are recommended for cell line and individual identification, genetic population, and conservation studies. Dominant markers such as RAPD (random amplified polymorphic DNA) and AFLP (amplified fragment length polymorphism) are simple to use and do not require the use of radioactive materials. They were revealed multi-fragments that can be scored as present/absent (Vandewoestijne et al. 2008). So, they were limited to use in conservation studies which cannot distinguish homozygous from heterozygous and problems of reproducibility. RAPD's primers did not need genome sequence information and much DNA. However, they remained the least expensive population genetic markers for use on species. RAPD markers are a single primer with 10 nucleotides which are able to differentiate between genetically distinct individuals without genomic information (Turlure et al. 2014).

Co-dominant genetic markers such as RFLPs (restriction fragment length polymorphism), microsatellites or STRs (short tandem repeat), and SNP (single-nucleotide polymorphism) are nowadays the most widely used in cell line or individual identification, population genetics, and conservation studies (Finger and Klank 2010). Microsatellite occurs frequently in most eukaryote genomes and can be very informative, multi-allelic, reproducible, and distinguish heterozygous from homozygous (Al-Atiyat 2015). However, isolation and development of these markers through microsatellite enrichment followed by Sanger sequencing are time-consuming and costly (Turlure et al. 2014). The use of short tandem repeat markers has been recommended for cell line authentication, and these methods are currently being used to identify human cell lines (Almeida et al. 2011).

We chose the RAPDs from the dominant markers and microsatellite from co-dominant markers. Our objective was to compare the performance of two markers for authentication Bactrian camel cell lines and investigation, genetic diversity, and structure of these samples. We also discuss the impact of these results for the design of species-specific conservation measures.

Materials and Methods

Cell lines Fifty-one Bactrian camel cell lines were selected from human and animal cell Bank of the Iranian biological resource center. Bactrian camels were selected for sampling from Meshkin Shahr Research Complex (10 individuals), National Center of Conservation, and development of two-hump Camels (10 individuals) and private flocks (31 individuals) in Ardebil Province. The sex composition of samples included 20 males and 31 females. Bactrian camel photo, genus, age, and certificate of all cell lines were registered. These cell lines originated from ear margin tissue samples. Genomic DNA was isolated from each cell line using a commercially available DNA extraction kit (IBRC, MBK0021, Tehran, Iran). Genomic DNA was quantified using a Nano Dot Microspectrophotometer (Hercuvan, ND-3800, Tehran, Iran) at an absorbance of 260 nm.

Loci selection Fourteen RAPD primers were selected and initial screening was performed using 10 samples GC10, G2, GT10, OPB08, OPF05, OPA03, OPA04, OPA11, OPB02, OPB07, OPB07, OPB10, OPB14, and IBRD. Based on clarity, polymorphism, and repeatability of RAPD primers, four primers were selected out of 14 (IBRD, OPF05, GC10, GT10).

To identify cell lines and estimate the genetic diversity, microsatellite loci were selected that had high allelic diversity. Eight microsatellite markers included IBRC01, IBRC02,

IBRC03, IBRC04, CMS50, LCA65, Volp32, and YWll08 selected.

PCR conditions and amplifications RAPD PCR amplifications were performed in 15 μL reaction volumes containing 1× PCR buffer, 2 mM magnesium chloride ($MgCl_2$), 1 U of Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA), 200 μM of dNTPs (Thermo Scientific, Waltham, MA), 20–100 ng of the template DNA, and 10 pM of each primer. Amplifications were conducted on a Bio-Rad My Cycler Thermal Cycler using the following conditions: initial denaturing at 94°C for 5 min; 45 cycles at 94°C for 50 s, 35–39°C annealing for 1 min, and 72°C for 1 min; 72°C for 10 min; and held on 4°C. We performed two separate amplifications for all samples to evaluate repeatability of fragments.

Microsatellite PCR amplifications were performed in 15 μL reaction volumes containing 1× PCR buffer, 2 mM M magnesium chloride ($MgCl_2$), 1 U of Taq DNA Polymerase (IBRC, MBE0100), 200 μM of dNTPs (Thermo Scientific), 20–100 ng of the template DNA, and 0.2 μM of each primer. Amplifications were conducted on a Bio-Rad My Cycler Thermal Cycler using the following conditions: 1 cycle 94°C for 5 min; 30 cycles 94°C for 30 s, 56–58°C for 1 min, and 72°C for 45 s; 72°C for 10 min; and held on 4°C (Table 1).

Data analysis The electrophoresis of RAPD and microsatellite PCR products was done on 1.5% agarose and 8% denaturing acrylamide gels, respectively. Agarose gels were visualized by Gel Doc System 2000 (Bio-Rad, Hercules, CA). The microsatellite and RAPD bands length and the allele frequencies were estimated using PyELph analyzer software (version 3.1) (Pavel and Vasile 2012).

All loci of both markers were used to calculate genetic parameter of samples. Based on microsatellite data, allele frequencies, number of alleles per locus (n), number of effective alleles (n_e), observed heterozygosity (H_o), expected heterozygosity (H_e), probability of identity (P_I), and Shannon index (I) were determined using GenAlEx 6 (Peakall and Smouse 2006). The heterozygosity (observed, expected) was calculated by dividing the number of heterozygotes at a locus into the total number of samples. The P_I was calculated by the summation of the square of the genotype frequencies. In the formulas below, n is the number of samples, P_I is the frequency of the ith allele, and x_i is the frequency of the ith genotype. Inbreeding coefficient (F-statistics) and deviations from Hardy-Weinberg equilibrium (HWE) were calculated by chi-square (χ^2) and likelihood ratio test using GenePop version 4.3 (Yeh et al. 1999).

$$\text{Heterozygosity} = \sum_{i}^{n} P_i^2$$

$$\text{Probability of identity } (PI) = \sum_{i=1}^{n} x_i^2$$

A binary matrix was generated by RAPD primers, reflecting the presence (1) or absence (0) of the DNA fragments. Polymorphic fragment percentage ($\%P$) was calculated by dividing the number of polymorphic fragments at the population by the total number of fragments surveyed. Nei's gene diversity and Shannon index for genetic variation were calculated using analysis of molecular variation (AMOVA) in GenAlEx 6 (Peakall and Smouse 2006).

To estimate the discriminatory power of the microsatellite loci, the polymorphic information content (PIC) for each locus was estimated by PIC = 1 − ∑Pi2, where Pi2 referred to the sum of the ith allelic frequency of each microsatellite locus for the genotypes using PIC Calc (Nagy et al. 2012). The CERVUS was also used for parentage analysis (Kalinowski et al. 2007).

Finally, bottleneck analysis was tested for a disproportional decrease in allelic diversity compared to heterozygosity due to founder effects following population using the software BOTTLENECK 1.2.02. The probability distribution was calculated using 1000 simulations under three models: Infinite Allele Model (IAM), Two-Phase Model of mutation (TPM), and Stepwise Mutation Model (SMM) (Cristescu et al. 2010).

Results and Discussion

RAPD fragments characteristics RAPD revealed a multi-fragments banding pattern. Fourteen RAPD primer initial screening was performed using 10 samples. Based on clarity, polymorphism, and repeatability of RAPD primers, four primers were selected out of 14 with annealing temperature ranging from 36 to 38°C. Four informative and reproducible primers (IBRD, OPF05, GC10, and GT10) were applied for the analysis of 51 Bactrian Camel cell lines received from Iranian biological resource center. Several major and minor bands were shown.

These primers were generated fragments size between 150 and 2000 bp. The shortest fragment was amplified by OPF05, while the largest by GT10. Four RAPD primers generated a total of 21 fragments. Polymorphic loci percentage was 94.44% in this population. Gene diversity ranged from 0.27 (IBRD) to 0.46 (GC10), with an average of 0.37 (Nei 1978). The primer IBRD revealed the highest and lowest band frequency (0.95, 0.05). Band frequency was moderate in primer OPF05 and GC10. All values are presented in Table 2.

Microsatellite marker characteristics All microsatellite loci were analyzed. Amplified PCR products for all loci were observed in every cell line. These primers identified the 51 Bactrian camel cell lines received from Iranian biological resource center resulting in unique profiles for each sample analyzed. All microsatellite loci were polymorphic. Eight

Table 1. Microsatellite characterization such as primer sequence, accession number, size range, annealing temp. (°C), and number of alleles

Locus	Primer sequence (5′ to 3′)	Accession no.	Size range (bp)	Annealing temp. (°C)
IBRC01	F: GATGGACCTGGAGATCGTCA R: GTAGTTCATCCGTGTCCCCT	KX237506	130–142	60
IBRC02	F:AGGGGACACTCATCCATCCA R: TGCATAAGCAGGGAAGGTGG	KX237507	220–228	58
IBRC03	F: GGGTCAGATAGACCAGGGGT R: GCCTAAGGGCTGGTTTGACT	KX237508	280–290	58
IBRC04	F: GCTGTCTTCAGTGTCAGTATCC R: GTAGTTCATCCGTGTCCCCT	KU240014	140–155	57
CMS50	F: TTTATAGTCAGAGAGAGTGCT R: TGTAGGGTTCATTGTAACA	AF329142	150–190	58
LCA65	F: TTTTTCCCCTGTGGTTGAAT R: AACTCAGCTGTTGTCAGGGG	AF091124	165–190	58
VOLP32	F: GGAATGGCTTGAAAGGAATG R: CGAGCACCTGAAAGAAGACC	AF276038	252–270	56
YWLL08	F: ATCAAGTTTGAGGTGCTTTCC R: CCATGGCATTGTGTTGAAGAC	AF217608	150–180	56

microsatellite loci generated a total of 45 polymorphic alleles with 10 (23%) rare and private alleles (with frequency ≤ 0.05). The number of alleles per locus ranged from 3 to 11, with an average of 5.62 alleles (Fig. 1). The effective number of alleles ranged from 1.8 (IBRC02) to 7.7 (YWLL08), with an average of 3.97 alleles. The observed heterozygosity ranged from 0.359 (IBRC02) to 0.978 (YWLL08) with a mean value of 0.709. The expected heterozygosity ranged from 0.449 (IBRC02) to 0.879 (YWLL08) with a mean value of 0.697. The high mean heterozygosity (both observed and expected) values estimated in the present study indicate low inbreeding, low selection pressure, and large number of alleles in these samples. Since the highest heterozygosity value is maximum 1, Shannon index including higher levels 1 to 4 can be made a better comparison between loci with heterozygosity higher than 0.8. Based on the Shannon index, diversity varied from 0.785 (IBRC02) to 2.170 (YWLL08), with an average of 1.394 (Table 3).

The results of the χ^2 test revealed that the population was in HWE for IBRC02 locus ($P < 0.001$). The remaining seven loci revealed significant departure from HWE. The F_{IS} value ranged from − 0.435 (CMS50) to 0.307 (IBRC03). Four loci revealed negative F_{IS} values ($F_{IS} < 0$) with mean F_{IS} value (− 0.0476) indicating the absence of inbreeding at these loci and Bactrian camel population, respectively.

The PIC as an indicator for determining genetic diversity based on allele frequencies was calculated. PIC values varied from 0.400 (IBRC02) to 0.856 (YWLL08), with an average of 0.643. Six loci were highly informative (PIC ≥ 0.5), and two were reasonably informative (0.4 < PIC < 0.5).

Bottleneck analysis using sign, standardized differences, and Wilcoxon test was utilized in each of three mutations models IAM, TPM, and SMM. The bottleneck was analyzed based on allele frequencies and heterozygosity. All the observed heterozygosity was larger than expected equilibrium heterozygosity. The observed heterozygosity and expected equilibrium heterozygosity were calculated in three models (Table 4). When the population was reduced during a bottleneck, the allele number is reduced faster than the heterozygosity. The rare alleles were lost rapidly and have little effect on heterozygosity, normally, therefore generating a transient excess in heterozygosity comparable to that expected in a population of stable size with the same allele number (Cornuet and Luikart 1996; Cornuet et al. 1999).

Table 2. RAPD primer genetic parameters and characterization such as sequences, number, and size of bands

Primers	Sequence (5′ to 3′)	Annealing temp. (°C)	Number of bands	Size of band (bp)	*q*	*H*	*I*	Uh
GC-10	GCC GTC CGA G	38	3	650–1700	0.405	0.4595	0.6519	0.485
GT-10	GTG ATC GCA G	37	5	600–2000	0.544	0.3014	0.4703	0.335
OPF-05	CCG AAT TCC C	37	7	150–1500	0.369	0.3481	0.4863	0.333
IBRD	AAC CGC CTG A	36	6	400–1200	0.662	0.2766	0.4278	0.354
Mean			5.25			0.3754	0.5496	0.404
SD						0.1003	0.1174	0.071

q mean band frequency, *H* gene diversity, *I* Shannon index, *Uh* unbiased diversity

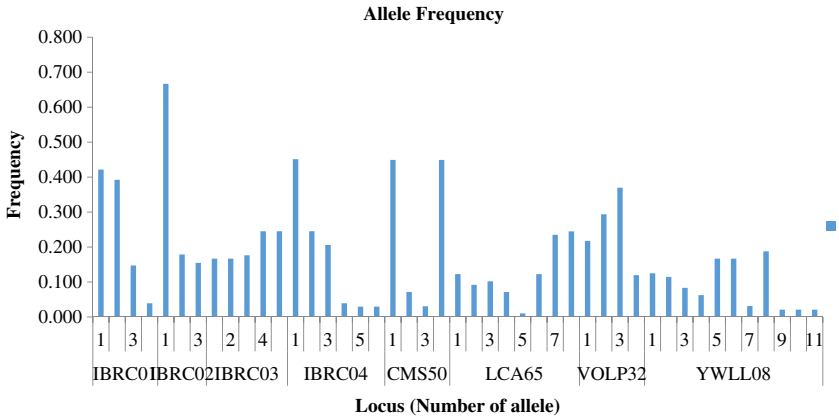

Fig. 1. Allele frequency for eight microsatellite loci.

Three tests for each mutation model revealed no deviation from mutation drift equilibrium. However, in the case of the sign test on the SMM model, one locus was heterozygosity deficient. In other tests, all loci were with heterozygosity excess in different models. The standardized difference test needed minimum 20 loci but, in our study with 8 loci, was showing positive and significant T_2 values under three models (Table 5).

The mode shift test as a qualitative test was used to visualize the allele frequency variation. The allele frequency distribution was nearly L-shaped form in Bactrian camel samples, but not very normal (Fig. 2). This observed distribution and curve showed that this population did not experience a recent bottleneck. The L-shape curve form was shown despite decreasing the Bactrian camel population in recent years, but there is a suitable genetic diversity for reviving the breed from extinction.

RAPDs or microsatellites In the current study, we compared RAPD and microsatellite marker results for investigation genetic structure and identification Bactrian camel cell lines. RAPD marker used a primer set and for primer design did not need sequence information. If we had no information about the organism, RAPD produced many fragments that can be scored, and unique allele may be determined. RAPD-PCR reaction is sensitive and results may vary across different experiments and labs (Mahrous et al. 2011).

RAPD markers were especially suitable for investigation population genetics. The marker analysis indicated that four markers were considered informative in the analysis of characterization and genetic diversity of Bactrian camel, since they exhibited more than three different bands. All primers generated novel and polymorphic fragments. Primer GC-10 revealed most gene diversity 0.45 and IBRD revealed minimum gene diversity 0.27 with mean 0.37. In another study, GC-10 primer had maximum gene diversity between five markers in Indian camel (Mehta et al. 2006). In the current study, RAPD result revealed enough genetic diversity within Bactrian camel. These results are in agreement with previous studies (Mehta et al. 2006; Mahrous et al. 2011).

Based on microsatellite data, the mean number of alleles per loci was 5.62, which can be considered suitable when

Table 3. Genetic diversity parameters of Bactrian camel population

Locus	No. of allele	n_e	H_o	H_e	I	HWE (χ^2)	F_{IS}	PIC
IBRC01	4	2.76	0.823	0.644	1.11	28.82**	−0.290	0.565
IBRC02	3	1.8	0.359	0.449	0.78	6.84^{NS}	0.190	0.4
IBRC03	5	4.83	0.549	0.801	1.59	70.73**	0.307	0.760
IBRC04	6	2.82	0.784	0.652	1.29	35.97**	−0.215	0.6
CMS50	4	2.40	0.836	0.588	0.99	47.19**	−0.435	0.494
LCA65	8	5.91	0.490	0.839	1.88	153.55**	0.410	0.809
VOLP32	4	3.51	0.978	0.723	1.31	64.29**	−0.366	0.663
YWLL08	11	7.73	0.854	0.879	2.17	240.19**	0.018	0.857
Mean	5.62	3.97	0.709	0.697	1.39		−0.0476	0.643
St. dev	2.66	2.03	0.215	0.143	0.46		0.3238	0.158

n_e number of effective alleles, H_o observed heterozygosity, H_e expected heterozygosity, I Shannon index, *HWE* Hardy-Weinberg equilibrium by chi-square, F_{IS} inbreeding coefficient (F-statistics), *PIC* polymorphic information content, *NS* not significant

**Significant at $p < 0.001$

Table 4. Observed (H_o) and expected (H_e) heterozygosity in three mutation models

Locus	Observed	Expected equilibrium (H_e)		
	H_o	IAM	TPM	SMM
IBRC01	0.652	0.428	0.508	0.582
IBRC02	0.505	0.329	0.398	0.470
IBRC03	0.801	0.513	0.590	0.675
IBRC04	0.698	0.574	0.653	0.728
CMS50	0.597	0.430	0.506	0.584
LCA65	0.840	0.666	0.738	0.801
VOLP32	0.724	0.439	0.513	0.589
YWLL08	0.876	0.761	0.818	0.862
Mean	0.712	0.518	0.591	0.661
St. Dev	0.126	0.142	0.139	0.130

IAM Infinite Allele Model, *TPM* Two-Phase Model, *SMM* Stepwise Mutation Model

compared with other studies that assessed these markers in this breed (Mahrous et al. 2011; Shahkarami et al. 2012). The averages of H_o (0.709) were higher than those of H_e (0.697), which indicates a lower quantity of homozygote, strengthening the need for studies of genetic management in this breed. High observed heterozygosity (greater than 0.65) is suitable in selecting microsatellite marker for cell line identification (Almeida et al. 2011). In the current study, out of eight primers, five primers had high heterozygosity greater than 0.7 and three primer had less than 0.7. The three loci IBRC03 (0.80), LCA65 (0.83), and YWLL08 (0.87) were highest expected heterozygosity values. IBRC03 and IBRC04 were shown high alleles and heterozygosity in other study (Amoli et al. 2017a, b). The microsatellite markers with the lowest expected heterozygosity values were IBRC02 (0.44) and CMS50 (0.58). The expected heterozygosity value for YWLL08 calculated in another study was lower than our study (Shahkarami et al. 2012). Other loci did not use Bactrian camel, and our study is the first report about these loci in this breed. This finding demonstrates that there is a suitable variability in the population of Bactrian camel. Our sampling was from different space, and more than half

Table 5. Bottleneck analysis using three tests for mutation drift equilibrium in IAM, TPM, and SMM models

Test	IAM	TPM	SMM
Sign test	4.52	4.73	4.76
Standardized differences test (T_2 value)	3.63	2.90	1.64
Wilcoxon test			
P value (one tail for H excess)	0.00195	0.00195	0.00097

IAM Infinite Allele Model, *TPM* Two-Phase Model, *SMM* Stepwise Mutation Model

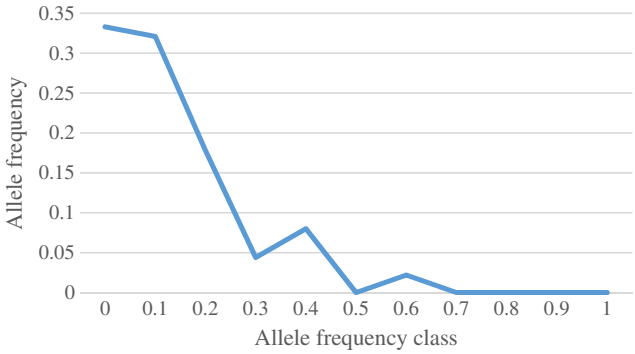

Fig. 2. Allele frequency of Bactrian camel in mode-shift with nearly L-shape.

samples were from people flocks. Thus, despite the decreasing number of the Bactrian camel in recent years, we were shown suitable gene diversity in this population.

In conclusion, our results show that a multiplex PCR based on eight microsatellite markers with heterozygosity values ranges from 0.44 to 0.87 and four RAPD primers are suitable for authentication cell lines and investigate gene diversity in Bactrian camel, respectively. So, genetic stability of microsatellite markers was generated by high passage cell line in previous studies (Almeida et al. 2011). Here, we have shown that RAPD markers may still be important to consider in organisms with no genomic information. The use of RAPD data remains, however, limited to investigating inbreeding and genetic structure at small spatial scales. Also, RAPD suffers from needy reproducibility and their use is limited in genetic studies, nowadays. New technologies for strong and cheap genetic polymorphism detection are continuously developed such as next-generation sequencing to complete genome sequencing. However, their price and accessibility are not compatible with cost, storage, and analysis of data of sequences produced (Turlure et al. 2014). Therefore, RAPD and microsatellite markers may still be valuable to the authentication cell line, investigation genetic diversity, and conservation genetic resources.

Consequently, the results of this study indicate that attention should be paid to the genetic management of Iranian Bactrian camel population. This population has modest allele richness. These results indicate that the population needs a reproductive management program and the exchange or introduction of new individuals. Particularly, these points should be made at the breeding stations so that these values do not decrease over the years. In addition, we propose to perform analysis of the data from the genotype separately in camels kept on conservation sites. These processes help to maintain and improve the genetic basis of these valuable genetic resources and make it easier to protect this population with a suitable genetic diversity in Iran.

Acknowledgements The authors express their gratitude to Parvaneh Farzaneh for providing technical advice and access to required equipment and all colleagues in Human and Animal cell bank.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Al-Atiyat RM (2015) The power of 28 microsatellite markers for parentage testing in sheep. Electron J Biotechnol 18(2):116–121. https://doi.org/10.1016/j.ejbt.2015.01.001

Almeida JL, Hill CR, Cole KD (2011) Authentication of African green monkey cell lines using human short tandem repeat markers. BMC Biotechnol 11(1):102. https://doi.org/10.1186/1472-6750-11-102

Amoli AD, Aminafshar M, Fazeli SS, Emam N, Kashan J, Khaledi KJ (2017a) Isolation and characterization of microsatellite markers from endangered species (*Camelus bactrianus*). Iran J Appl Anim Sci 7(4):693–698

Amoli AD, Mohebali N, Farzaneh P, Fazeli SAS, Nikfarjam L, Movasagh SA, Moradmand Z, Ganjibakhsh M, Nasimian A, Izadpanah M (2017b) Establishment and characterization of Caspian horse fibroblast cell bank in Iran. In Vitro Cell Dev Biol Anim 53(4):337–343. https://doi.org/10.1007/s11626-016-0120-3

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144(4):2001–2014

Cornuet J-M, Piry S, Luikart G, Estoup A, Solignac M (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153(4):1989–2000

Cristescu R, Sherwin WB, Handasyde K, Cahill V, Cooper DW (2010) Detecting bottlenecks using BOTTLENECK 1.2.02 in wild populations: the importance of the microsatellite structure. Conserv Genet 11(3):1043–1049

Dittmann MT, Runge U, Lang RA, Moser D, Galeffi C, Kreuzer M, Clauss M (2014) Methane emission by camelids. PLoS One 9(4): e94363

Easley CA 4th, Phillips BT, McGuire MM, Barringer JM, Valli H, Hermann BP, Simerly CR, Rajkovic A, Miki T, Orwig KE, GP S (2012) Direct differentiation of human pluripotent stem cells into haploid spermatogenic cells. Cell Rep 2(3):440–446. https://doi.org/10.1016/j.celrep.2012.07.015

Finger A, Klank C (2010). Review molecular methods: blessing or curse? Relict Species, Springer**:** 309–320, DOI: https://doi.org/10.1007/978-3-540-92160-8_18

Freshney RI (2005) Culture of specific cell types. Wiley Online Library

Gorji ZE, Khaledi KJ, Amoli AD, Ganjibakhsh M, Nasimian A, Gohari NS, Izadpanah M, Vakhshiteh F, Farghadan M, Moghanjoghi SM (2016) Establishment and characteristics of Iranian Sistani cattle fibroblast bank: a way to genetic conservation. Conserv Genet Resour 9(2):305–312

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16(5):1099–1106

Kuz'mina E E (2008) The prehistory of the silk road. University of Pennsylvania Press

Li LF, Guan WJ, Li H, Zhou XZ, Bai XJ, Ma YH (2009) Establishment and characterization of a fibroblast cell line derived from Texel sheep. Biochem Cell Biol 87(3):485–492. https://doi.org/10.1139/O09-005

Mahrous KF, Ramadan HA, Abdel-Aziem SH, Abd-El Mordy M, Hemdan DM (2011) Genetic variations between camel breeds using microsatellite markers and RAPD techniques. J. Appl. Biosci 39: 2626–2634

Makkar HP, Viljoen GJ (2005) Applications of gene-based technologies for improving animal production and health in developing countries. Springer. doi: https://doi.org/10.1007/b105256

Mehta S, Mishra B, Sahani M (2006) Genetic differentiation of Indian camel (*Camelus dromedarius*) breeds using random oligonucleotide primers. Animal Genetic Resources/Resources génétiques animales/Recursos genéticos animales 39(77–88

Nagy S, Poczai P, Cernák I, Gorji AM, Hegedűs G, Taller J (2012) PICcalc: an online program to calculate polymorphic information content for molecular genetic studies. Biochem Genet 50(9–10): 670–672. https://doi.org/10.1007/s10528-012-9509-1

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89(3):583–590

Pavel AB, Vasile CI (2012) PyElph-a software tool for gel images analysis and phylogenetics. BMC Bioinformatics 13(1):9. https://doi.org/10.1186/1471-2105-13-9

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. Mol Ecol Notes 6(1):288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Roosen J, Fadlaoui A, Bertaglia M (2005) Economic evaluation for conservation of farm animal genetic resources. J Anim Breed Genet 122(4):217–228

Shahkarami S, Afraz F, Sayed MZ, Banabazi M, Asadzadeh N, Asadi N, Hemmati B, Ghanbari A, Razavi K (2012) Genetic diversity in Iranian Bactrian camels (*Camelus batrianus*) using, microsatellite markers. Mod Genet J 7(330):249–258

Stacey G (2000) Cell contamination leads to inaccurate data: we must take action now. Nature 403(6768):356–356. https://doi.org/10.1038/35000394

Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676. https://doi.org/10.1016/j.cell.2006.07.024

Turlure C, Vandewoestijne S, Baguette M (2014) Conservation genetics of a threatened butterfly: comparison of allozymes, RAPDs and microsatellites. BMC Genet 15(1):114

Vandewoestijne S, Schtickzelle N, Baguette M (2008) Positive correlation between genetic diversity and fitness in a large, well-connected metapopulation. BMC Biol 6(1):46

Wu H, Guang X, Al-Fageeh MB, Cao J, Pan S, Zhou H, Zhang L, Abutarboush MH, Xing Y, Xie Z (2014) Camelid genomes reveal evolution and adaptation to desert environments. Nat Commun 5: 5188. https://doi.org/10.1038/ncomms6188

Yeh FC, Yang R-C, Boyle T (1999) POPGENE version 1.31. Microsoft window-based freeware for population genetic analysis. University of Alberta, Edmonton