Original article

Genetic characterization and bottleneck analysis of Maghateer camel population in Saudi Arabia using microsatellite markers

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A B S T R A C T

In this study, twenty microsatellite markers were applied to study the genetic polymorphism in Maghateer Camels in the Kingdom of Saudi Arabia. About 50 unrelated Maghateer camels were selected and hair roots were subjected for the extraction of total genomic DNA. The selected microsatellite forward-, and revised-primers amplified 19 SSR markers from the Camels. In the experimental condition, CMS25 and VOLP67 markers did not yield any genomic amplification from the DNA of Camels. A total of nineteen microsatellite loci have been determined as polymorphic nature. In this experiment 155 alleles were obtained by the loci of 19 microsatellites, range of 4 to 16 alleles per locus and the mean of 8.158 alleles per locus. The observed heterozygosity (Ho) range varied from 0.280 to 1.000 and the mean value was determined as 0.717. The mean expected heterozygosity (He) was found to be 0.667 and the range varied between 0.282 and 0.871. In Maghateer population, the effective number (mean) was found to be 3.599. A total of 12 loci were determined in this study. In the selected Camel population no bottleneck in nearest past or no potent heterozygote excess was obtained based on standardized differences, sign and tests such as Wilcoxon tests and L shaped distribution of mode-shift test. The present findings showed the utility of these 19 microsatellite loci for analyzing genetic polymorphism in *Camelus dromedarius* (dromedary camel).

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1. Introduction

Camels are unique animals in various aspects and are very difficult to compare with other farm animals based on their adaptation and responses to the environment. Camels are diverse in nature and it is found in Arabian Peninsula, Afghanistan, African deserts and Somalia. Arabian Camels meet the food requirement in various countries than sheep and cattle, which are mainly affected by water scarcity and heat. In Kingdom of Saudi Arabia, Camel population is classified based on morphological characters and area of origin (Al-Hazmi et al., 1994). Generally coat colour is used to classify Camels in Saudi Arabia. Based on coat colour, Saudi Arabian Camels have classified into three types. These include Al-Sofr (brown), Maghateer (white), and Al-Homr and Magaheem (black). In a study, Elamin and Wilcox (1992a,b) classified Camels into four types, including, Omani (used for riding), Al-Hawara (survives in Al-Hawara area), Wadha (survies in Hejaz area) and Najdi or Magaheem. The camels in Saudi Arabia are one humped and dromedaries types. In Saudi Arabia, Camels have various roles in celebrations as the part of Kingdom heritage, and is the typical icon in Kingdom heritage. In Arabian Peninsula, a total of 1.6 million populations are found and this population is about 53% population of entire Saudi Arabia (FAO, 2012). The Camels, Wadha is dominant in Hejaz region and Al-Hawara type camels are predominant in Northern Saudi Arabia. In Najd and AL Dawaser valley, the populations of Maghateer are very high. Maghateer type Camels are white in colour and rigid in nature and is well known to yield milk than other types under range conditions. In Saudi Arabia, studies on genetic variability of Camels are very rare, it is very important to study the Camels using molecular markers to assess the population. This kind of genetic analysis is useful to improve camels and to develop various breeding mechanisms to improve Camels. In Saudi Arabia, the genetic variation of dromedary populations has been poorly documented. In a study, Spencer et al. (2010) studied the genetic variance in Camel population has been

studied using the genetic markers like, Randomly Amplified Polymorphic Dimorphism (RAPD) and DNA fingerprinting (DFP). Recently, Musthafa (2015) analyzed genetic diversity in the Camels from Tunisia, Egypt, India, Canary Islands, Saudi Arabia, Kenya and Australia. These Camels from these countries showed range of genetic diversity and analyzed using various microsatellite markers. The microsatellite markers such as, CMS50, CVRL07, CVRL06, CVRL05, CVRL01, LCA66, VOLP67, VOLP32, VOLP10, VOLP08, VOLP03, YWLL59, YWLL44, YWLL38, YWLL09 and YWLL08 were used to analyze the heterozygosity among Camel population. These microsatellite markers have rich of polymorphic information and degree of allelic richness (Musthafa, 2015). It is anticipated that the obtained information from this study can be used in conservation and genetic improvement plans of Maghateer Camel population in Saudi Arabia.

2. Materials and methods

2.1. Samples

In the present study, 50 Camels were selected and hair samples were collected in a clean container. Also registered the history of sample preparations and breed of Camel in all containers. The collected samples were stored at −80 °C for further use. DNA of the hair sample was extracted using QIAgen DNeasy blood and tissue kit according to the manufacture's instructions (Hildane, Germany). The purified DNA sample was stored immediately at −40 °C. The isolated DNA was checked for its purity and quantified by using a UV–Visible Spectrophotometer (Krackler Scientific Incorporation, USA).

2.2. Primers

In this study, 21 pair of microsatellites was applied to analyze the genotype of the selected 50 Camel (FAO, 2000). The list of primers were tabulated in Table 1.

2.3. Amplification of DNA and analyses

To analyze the use of SSR markers (22) in Camel samples, DNA from various Camel samples were applied. Optimum annealing temperature was evaluated using PCR gradient system for every pair of each SSR marker. Genomic DNA was amplified using a Polymerase Chain Reaction 9700 thermocycler machine. Initial denaturation step was set as 2 min at 94 °C, and then 94 °C was maintained for 30. Annealing temperature was set between 50 and 60 °C (30 s) based on the microsatellite primer-pair applied, followed by 72 °C for 30 s. Final extension was performed for 5 min at 72 °C. The purity of the amplified genomic DNA was tested using Agarose gel electrophoresis. Further, the amplified DNA was multiplexed based on their fluorescent dye and the amplified DNA was run in Genetic Analyzer. To the multiplexed PCR products (0.5 μl), 9.25 μl of HiDi formamide and 0.25 μl of GeneScan® LIZ standard have been incorporated. After that, the reaction mixture was denatured immediately at 95 °C (5 min) and kept on ice (5 min). Two microliters samples were used to analyze the DNA. The fluorescent dyes such as, PET, VIC, NED and FAM was used and the obtained results were analyzed using genetic analyzer (software version 3.0).

2.4. Statistical analysis

The important parameters such as, number of alleles (Na), locus, expected (He) heterozygosities, observed (Ho) heterozygosities and effective number of alleles (Ne) were evaluated as suggested by Kalinowski et al. (2007) using Cervus version 3.0.3. GenePop was used to find the deviations from Hardy-Weinburg Equilibrium (HWE). Bottlenecks in the locality of population and population size were evaluated using the bottleneck program (Pritchard et al., 2000) to evaluate population structure based on Bayesian multi-locus clustering. Simulations were run and the results from multiple runs for each K were concatenated by clumpp (Evanno et al., 2005).

3. Results and discussion

All the microsatellite primer-pairs successfully amplified 19 SSR markers from the selected markers from the selected Camels. The tested markers, CMS25 and VOLP67 did not yield any result after altering various PCR conditions. The other nineteen microsatellite loci were all polymorphic. The tested various genetic parameters for the population of Maghateer Camel have been described in Table 2. In the tested Camel population showed various alleles per locus and it varied from 4 to 16 which is a good indicator of genetic polymorphism. The allele range obtained in the result was similar with previous results (Nolte et al., 2005; Vijh et al., 2007). However, the range was higher than observations made previously by various research groups (Mehta et al., 2007; Nolte et al., 2005; Vijh et al., 2007) in the analyzed Camel population. This range was very much higher than the results reported by Al-Swailem et al. (2009) in Saudi Camel population and in Kachchhi camel population (Mehta et al., 2007). In a study, Schulz et al. (2010) found 2 to 22 alleles per locus in the Canarian Camels. Likewise, Spencer and Woolnough (2010) reported 5 to 23 alleles per locus in Australian dromedary Camels. It was previously reported that the microsatellite loci should have more than four alleles per locus to minimize standard error (SE) of distance estimates (Li et al., 2002). In general, the average of alleles in a particular population is mainly depend on size of the sample (n) and the availability of unique alleles of very low frequencies. The number of alleles increases gradually when the sample size increases. In a study, Al-Swailem et al. (2009) found 61 alleles with a mean number of alleles per locus (3.81) in Saudi Camels. In Australian Dromedary Camels, 224 alleles with mean 13.18 alleles per locus was reported by Spencer et al. (2010). In another study, Schulz et al. (2010) reported 10.7 alleles per locus in 139 alleles. Among Camel population, 10.3 alleles per locus in 224 alleles was reported by Nolte (2003). In our study revealed a mean of 8.158 alleles in 155 alleles and this is in between previous findings. The locus CVRL06 showed only four alleles and is the least polymorphic, in the case of locus CVRL01, about 16 alleles and showed the most polymorphic SSR marker. In the present study, the average number of alleles (Ne) was reported as 3.599 and the mean Shannon index value was reported as 1.442, the maximum value of Ne in YWLL08 locus was 7.764, and the lowest value of Ne was 1.392 in locus VOLP32.Australian camels displayed higher effective number of alleles of 3.44 whereas Bikaneri camels displayed 4.40 as the highest average number of alleles (Mehta and Sahani, 2007; Vijh et al., 2007). Also, heterozygosity value of the reported effective number of the alleles also found to be high. There is a positive relationship between number of alleles at a given locus and number of dinucleotide repeats (Bishop et al., 1994). Heterozygosities also represent a good indicator of genetic polymorphism within breeds. The hetrozygosity level of a microsatellite varies from species to species and sometimes between breeds of the same species (Fatima, 2006). In present study, the expected heterozygosity value of Maghateer Camels populations was 0.667 (Table 2). This value was very much comparable to that of observed values (0.604) in the South African camels which was reported by Nolte et al. (2005). It was 0.633 in the case of Arabian camels and 0.680 in

Table 1
Primers sequences and labels of the 21 primer pairs used to amplify microsatellite regions in the *Camelus Dromedarius* of the present study.

S.r.	Locus	Primer (5′ → 3′) F – Forward, R – Reverse	Annealing Temp. (°C)	Size range (bp)
1	YWLL08	F-ATCAAGTTTGAGGTGCTTTCC R-CCATGGCATTGTGTTGAAGAC	55 °C	133–180
2	YWLL09	F-AAGTCTAGGAACCGGAATGC R-AGTCAATCTACACTCCTTGC	55 °C	138–180
3	YWLL38	F-GGCCTAAATCCTACTAGAC R-CCTCTCACTCTTGTTCTCCTC	60 °C	174–192
4	YWLL44	F-CTCAACAATGCTAGACCTTGG R-GAGAACACCGCTGGTGAATA	60 °C	86–120
5	YWLL59	F-TGTGCAGCAGTTAGGTGTA R-CCATGTCTCTGAAGCTCTGGA	58 °C	96–136
6	VOLP03	F-AGACGGTTGGGAAGGTGGTA R-CGACAGCAAGGCACAGGA	60 °C	129–206
7	VOLP08	F-CCATTCACCCCATCTCTC R-TCGCCAGTGACCTTATTTAGA	55 °C	142–180
8	VOLP10	F-CTTTCTCCTTTCCTCCCTACT R-CGTCCACTTCCTTCATTTC	55 °C	231–268
9	VOLP32	F-GTGATCGGAATGGCTTGAAA R-CAGCGAGCACCTGAAAGAA	55 °C	192–262
10	VOLP67	F-TTAGAGGGTCTATCCAGTTTC R-TGGACCTAAAAGAGTGGAG	55 °C	142–203
11	LCA66	F-GTGCAGCGTCCAAATAGTCA R-CCAGCATCGTCCAGTATTCA	58 °C	212–262
12	CVRL01	F-GAAGAGGTTGGGGCACTAC R-CAGGCAGATATCCATTGAA	55 °C	188–253
13	CVRL05	F-CCTTGGACCTCCTTGCTCTG R-GCCACTGGTCCCTGTCATT	60 °C	148–174
14	CVRL06	F-TTTTAAAAATTCTGACCAGGAGTCTG R-CATAATAGCCAAAACATGGAAACAAC	60 °C	185–205
15	CVRL07	F-AATACCCTAGTTGAAGCTCTGTCCT R-GAGTGCCTTTATAAATATGGGTCTG	55 °C	255–306
16	CMS13	F-TAGCCTGACTCTATCCATTCTC R-ATTATTTGGAATTCAACTGTAAGG	55 °C	238–265
17	CMS17	F-TATAAAGGATCACTGCCTTC R-AAAATGAACCTCCATAAAGTTAG	55 °C	135–167
18	CMS18	F-GAACGACCCTTGAAGACGAA R-AGGAGCTGGTTTTAGGTCCA	60 °C	157–188
19	CMS25	F-GATCCTCCTGCGTTCTTATT R-CTAGCCTTTGATTGGAGCAT	58 °C	93–128
20	CMS50	F-TTTATAGTCAGAGAGAGTGCTG R-TGTAGGGTTCATTGTAACA	55 °C	129–190
21	CMS121	F-CAAGAGAACTGGTGAGGATTTTC R-TTGATAAAAATACAGCTGGAAAG	60 °C	128–166

Table 2
Number of alleles (Na), effective number of alleles (Ne), Shannon Index (I), Observed (HO) and Expected (He) heterozygosities for each locus of the Maghateer camel population in Saudi Arabia.

Locus	Na	Ne	I	Ho	He
CMS 121	9.000	4.088	1.710	0.720	0.755
CVRL 05	8.000	3.218	1.424	0.680	0.689
VOLP 08	6.000	1.735	0.763	0.480	0.424
YWLL 08	15.000	7.764	2.296	0.600	0.871
YWLL 38	6.000	2.483	1.181	0.580	0.597
CMS17	5.000	2.725	1.176	1.000	0.633
CMS13	7.000	2.317	1.070	0.480	0.568
CMS18	5.000	3.133	1.311	0.980	0.681
CVRL 06	4.000	2.225	1.039	0.700	0.551
LCA 66	9.000	3.283	1.539	0.620	0.695
VOLP 32	6.000	1.392	0.606	0.280	0.282
VOLP 03	9.000	3.113	1.443	0.840	0.679
CVRL07	11.000	6.935	2.080	0.920	0.856
CVRL 01	16.000	3.685	1.971	0.780	0.729
CMS50	11.000	6.098	1.999	0.840	0.836
YWLL44	8.000	2.707	1.295	0.640	0.631
YWLL59	5.000	3.934	1.429	0.840	0.746
YWLL09	6.000	2.960	1.336	0.920	0.662
VOLP10	9.000	4.583	1.722	0.720	0.782
Mean	8.158	3.599	1.442	0.717	0.667
SE	0.754	0.389	0.102	0.043	0.033

Table 3
Number of loci significantly deviating from Hardy–Weinberg equilibrium (HWE).

Locus	DF	ChiSq	Prob	Signif
CMS 121	36	31.165	0.698	ns
CVRL 05	28	66.334	0.000	***
VOLP 08	15	2.315	1.000	ns
YWLL 08	105	245.923	0.000	***
YWLL 38	15	79.522	0.000	***
CMS17	10	50.000	0.000	***
CMS13	21	9.890	0.980	ns
CMS18	10	55.457	0.000	***
CVRL 06	6	10.313	0.112	ns
LCA 66	36	96.375	0.000	***
VOLP 32	15	51.389	0.000	***
VOLP 03	36	204.861	0.000	***
CVRL07	55	205.480	0.000	***
CVRL 01	120	77.680	0.999	ns
CMS50	55	49.987	0.666	ns
YWLL44	28	11.623	0.997	ns
YWLL59	10	51.827	0.000	***
YWLL09	15	32.282	0.006	**
VOLP10	36	103.992	0.000	***

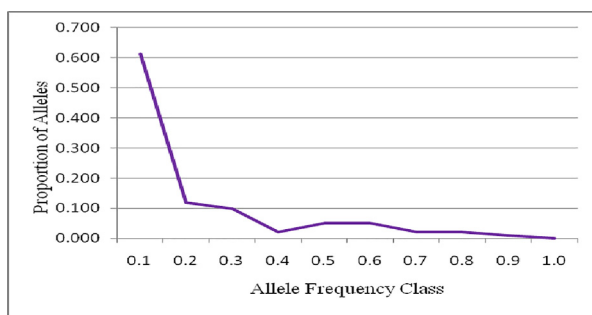ns = not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 1. Proportion of alleles and their frequencies in Maghateer population.

Table 4
Bottleneck analysis for Maghateer camel population.

Maghateer			
Models	Sign test	Standarized	Wilcoxon Test
IAM	Hee = 8.71	T2 = 0.240	P (One tail for H deficiency) 0.80530
	Hd = 6	P = 0.40509	P (One tail for H excess) 0.21060
	He = 9		P (Two Tail for H excess and difiency) 0.42120
	P = 0.54817		
TPM	Hee = 8.88	T2 = −2.848	P (One tail for H deficiency) 0.08441
	Hd = 11	P = 0.0000	P (One tail for H excess) 0.92429
	He = 4		P (One tail for H excess and defiency) 0.16882
	P = 0.01109		
SMM	Hee = 8.85	T2 = -8.550	P(One tail for H deficiency) 0.00021
	Hd = 13	P = 0.0000	P(One tail for H excess) 0.99985
	He = 2		P(Two tail for H excess and deficiency) 0.00043
	P = 0.00037		

Parameters for T.P.M.: Variance = 30.00 of SMM = 70%, Estimation based on 1000 replications, Hee: Heterozygosity excess expected; Hd: Heterozygosity deficiency; P: Probability; IAM: Infinite Allele Model: Two Phase Model; SMM: Stepwise Mutation Model.

Sudanese Camels (Schulz et al., 2010; Nolte et al., 2005). In Kenyan Camels, it was 0.530, however reduced value was reported in the case of United Arab Emirates camels (0.51) (Mburu et al., 2003). It was marginally increased in the case of *Camelus dromedaries* and the reported value was 0.544 (Spencer et al., 2010; Spencer and Woolnough, 2010). Table 2 shows the mean observed heterozygosity of the present study (0.717). This observed heterozygosity value was found to be higher than in Australian camel (0.455) and Tunisian camel (0.460) (Spencer and woolnough, 2010; Ould Ahmed et al., 2010). Moreover, in an earlier report the heterozygosity value was found to be 0.552 in Arabian Camel (Schulz et al., 2010). In Mewari, Kutchi, Jaisalmeri and Bikaneri populations, the observed heterozygosity values were found to be 0.600, 0.560, 0.570 and 0.580, respectively (Vijh et al., 2007). The observed and expected heterozygosities reported by Evdotchenko et al. (2003) and Mariasegaram et al. (2002) in dromedary are quite similar to those of present findings. These values of heterozygosity clearly show a significant level of variability in genetic level among Camel population from four populations from Saudi Arabia. Hardy-Weinberg Equilibrium (HWE) has been frequently used to analyze the genetic variation. HWE is defined as a breed with constant genotype and gene frequencies (Falconer and Mackay, 1996). The inbreeding estimate within the population of Camel, which basically reflects the deviation from HWE and the nonrandom union of gametes, showed that there were various genotypes with many loci that followed HWE ($P < 0.05$). In this investigation, the number of HWE was found to be 12 loci (Table 3). These markers critically indicated an excess and deficiency of
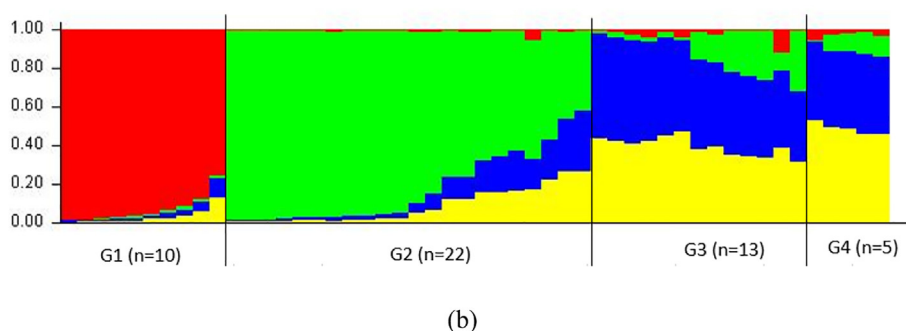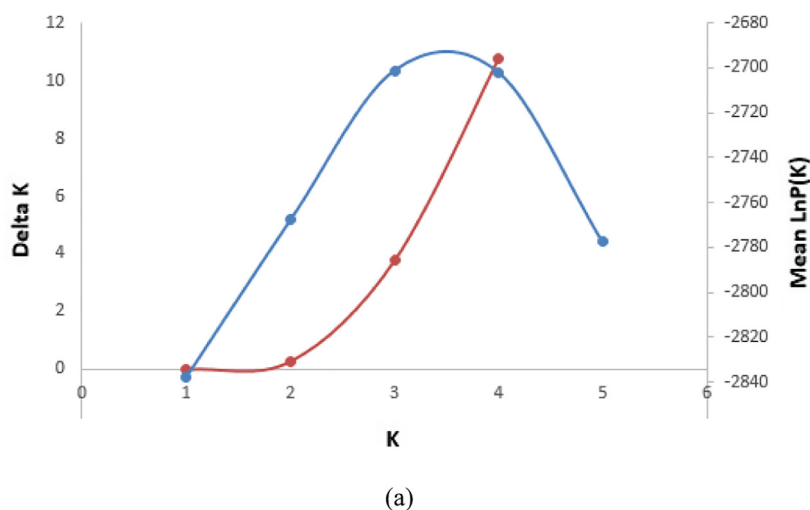
heterozygotes, this does not show the variation from HWE. It is well known fact that the migration of various natural processes of nonrandom mating, mutation, genetic drift and both natural selection and artificial selection are critical factors that are well known to induce variations from HWE. It is reported that some loci were usually derived from Hardy-Weinberg Equilibrium and showed that the loci 6, 6, 5 and 11 were not in HWE in the case of Mewari, Kutchi, Jaisalmeri and Bikaneri and statistically significant ($P < 0.01$). However, the loci 17, 17, 18 and 12 were followed JWE in Mewaricamel, Kutchi, Jaisalmeri and Bikaneri and the reported results were statistically significant (Vijh et al., 2007). A previous study in SaudiArabia using 16 microsatellite markers in Saudi Camel population indicated that half of the loci in the three-

(a)

(b)

Fig. 2. (a) Plot of two graphical methods allowing detection of the true value for K. Blue represent the mean of LnP(k) over 8 runs for each K value analyzed the increase of the variance associated to LnP(D) across different K values tested. Red represents the values of ΔK calculated, based on the methodology proposed by Evanno et al. (14). (b) Bar plot of estimation of the membership coefficient (Q) for each individual of Maghateer camels. Each individual is represented by a thin vertical line, which is partitioned into K colored segments that represent the individual's estimated membership fractions in K clusters. Black lines separate individuals of different clusters based on structure analysis. Population groups are labeled below the figure, with their geographical affiliations above it. The figure shown for K = 5 is based on the highest probability run at that K.

populations were in Hardy-Weinberg Equilibrium (Al-Swailem et al., 2009). Statistical analysis was made on microsatellite to test the Maghateer population having undergone recent genetic bottleneck. The level of genetic variation, population size and historical population sizes are well known and this is used to detect bottlenecks in the absence of historical data. Maghateer population shows number of loci with gene potent diversity, which includes three tests, such as, "Wilcoxons sign-rank test", "standardized differences test" and "sign test". The microsatellite model evaluation methods such as, Two Phase Model (TPM), Stepwise Mutation Model (SPM) and InfiniteAllele Model (IAM) were used. In this study, no significant heterozygote excel was found in the Maghateer Camel population by using InfiniteAllele Model, Wilcoxon tests (TPM), standardized differences (SMM) along with the normal L shaped (Fig. 1) distribution of mode-shifttest. Moreover, the previous tests showed no bottleneck inrecent past in Maghateer population (Table 4).

Structure revealed, for the 50 individuals of Maghateer camel with no prior distribution, that ΔK has a strong peak at K = 4, suggesting that the optimal number of clusters was 4 (Fig. 2a). In the Fig. 2b, the colors represent four clusters. Each bar in the graph represents an animal and its inferred proportion of genome admixture. The proportion of membership in each of the 4 clusters was 20%, 44%, 26% and 10% for the shoun red, green, blue and yellow cluster, respectively. Within the bar plot, the coefficient for each individual indicates clearly unique ancestral genomes for each of the two first cluster. However, the two last ones appear sharing

allele frequencies which indicate low of genetic differentiation and extensive gene flow between these two clusters. The values of nucleotide sequences based on frequencies of allele divergence among the selected populations, confirm these observations. In fact, the highest values were recorded between the first cluster and the others (d1-2 = 0.6%, d1-3 = 0.38%, d1-4 = 0.35%). However, the lowest values were recorded between the two last clusters (d3-4 = 0.002%).

4. Conclusion

Nevertheless, these fifteen microsatellite markers proved useful to detect genetic variability in Majaheem camel populations in Saudi Arabia, thus can be effectively used to analyze the diversity in genetic level in Saudi Arabian Camels. Also, very large sampling of all Camels in Kingdom of Saudi Arabia are required to establish a state of art genetic analysis for genotyping and analyzing the Saudi Arabian Camel population.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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