DHR International Journal Of Biomedical and Life Sciences (DHR-IJBLS)
ISSN 2278-8301, Vol. 5(1), Special Issue 2014
Available online *http://www.doublehelixresearch.com/DHRIJBLS*

Original Article

Genetic Characterization of Malvi camel using Microsatellite markers

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Abstract

The single-humped camel (Camelus dromedaries) is bred in the western part of India. This breed is famous for high milk yield potential. In the present study, we evaluated the genetic diversity of a population of Malvi camel. Multilocus genotype data on 29 microsatellites were generated on 138 Malvi camel. A total of 240 alleles were observed. The mean observed heterozygosity in camel population for all the 29 loci was found to be 0.6041 ± 0.3256 while the mean expected heterozygosity was 0.5978 ± 0.1983 with the mean number of alleles (Na) over 29 loci was found to be 8.2759 ± 3.4938 while the mean effective number of alleles (Ne) was 3.1917 ± 1.7795 in Malvi camel population.

Key words: Malvi camel, Microsatellite marker, genetic diversity.

Introduction

Camel genetic resources in India are represented by the one humped camel *(Camelus dromedarius*, 2n=74). The camels in India are distributed in the North western India comprising states of Rajasthan, Haryana and Gujarat. The Bikaneri, Jaisalmeri, Kutchi and Mewari camel have been recognized as different breeds of dromedarian camel (http://www.nrccamel.com/camelbreed.php). The population of dromedaries in India is 0.52 million camel (Livestock census, 2007) (http://dahd.nic.in/statewiseReports.htm) depicting a sharp decline of 17.5% from the last livestock census in 2003 (0.63 million). Several ecotypes based on morphological appearance and socio-geographic origins have also been reported; Sindhi [1]; Marwari, Mewati [2], Shekhawati, Riverine strains [3] and Malvi camel [4]. Malvi camel breeding population is estimated to be 2500 -3000 and it is reared by Rebaris of Madhya Pradesh.

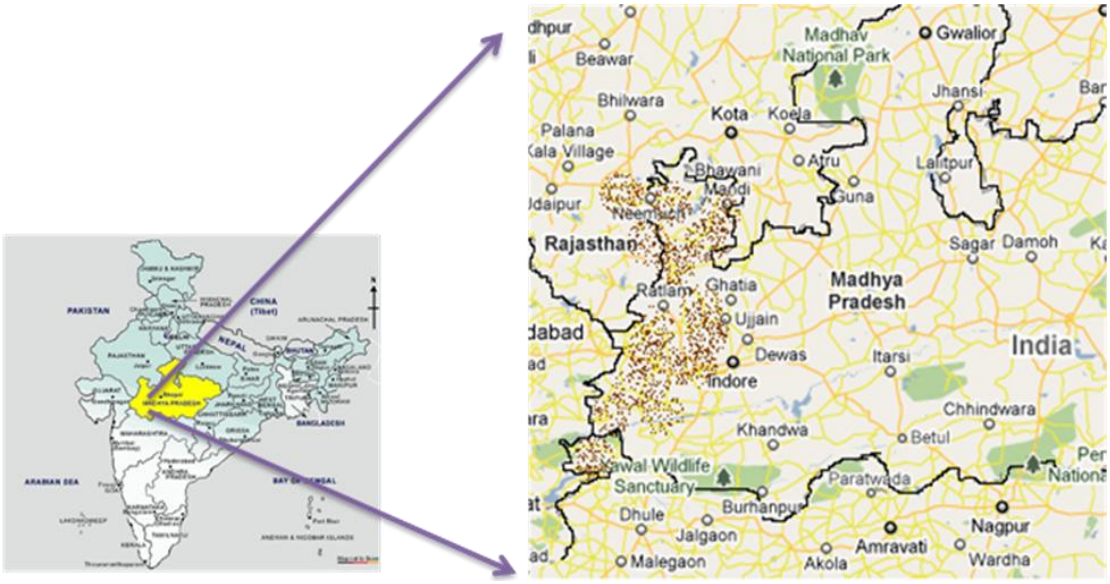

Fig. 1 Showing distribution area of Malvi camel in Madhya Pradesh

The Malvi camel are named after the Malwa (Malva) region, a distinct physiographic area in Central India (27° 70' - 25" 10'N and 73" 45' - 79" 14' E) comprising district of Mandsaur and Neemach district. Malva region receives annual rainfall between 800 to 1000 mm, representing an unusually humid habitat for camels. This breed is known for their high milk yield potential with top milk yield of 5-6 kg per day [4].

Malvi camel is physically distinguishable from all other Indian camel breeds by external characteristic having very light or off- white colour, small body size and it is probably the smallest of all Indian camel breeds. The facial profile is also distinct, with a pronounced convex forehead, Roman nose and an extremely pendulous lower lip that is longer and protrudes further than the upper lip. Information on phenotypic characteristics of Malvi camel has been reported along with the body measurements [5].

Socio-economic Importance

The Malvi camel is a dual purpose breed. The male camels are utilized as work animals, usually for carrying loads. The female camels are used as milk animals. Owners of breeding herds thus have two sources of income. They sell their male calves and they market milk. The Malvi camel is famous in the region for its high milk yields and owners point out that it gives milk even if it is hungry. Malvi breeding camels are kept exclusively in extensive management systems and fed on natural graze only. The Malvi camel breeders usually sell their male offspring at the annual fare. These fairs are mostly attended by

buyers from Bihar, Bengal, and even South India who apparently prefer to purchase camels here. Being from a fairly humid area, the Malvi camel are better suited for export to the higher rainfall areas of India than the camels native to Rajasthan.

Although this breed has been reported as high milk yield potential breed with distinctive phenotypic characteristics but has not been described at genetic level in terms of variability and existence of population structure. We carried out genetic analysis of this breed using microsatellites markers to assess the genetic diversity, population genetic parameters and genetic structure of this breed/population. It can be used to upgrade the milk production potential of other Indian camel breeds.

Materials and methods

Sample collection

The blood samples of 138 Malvi Camel collected randomly from Neemach, Mandsaur and some adjoining areas of Madhya Pradesh. The samples were collected in EDTA vacationer tubes (Becton Dickinson, USA) and transported to lab at 0-5^{0}C.

DNA was extracted from whole blood using DNA extraction kit (Roche DNA extraction kit). The resulting DNA strands were spooled out and washed twice with ice cold 70% ethanol to remove excess salts. DNA was re-dissolved in 500-750µl of TAE buffer (pH 8.0). The quality and concentration of DNA were checked on 0.6% agarose gel.

Genotyping and data extraction

Twenty nine microsatellite loci were tested on 138 adult camels from Madhya Pradesh. The microsatellite primers used are given in Table 1. These 29 markers have previously demonstrated highly polymorphic in family Camelidae. The PCR amplification was carried out in 25 ml reaction volume consisting of 50 ng genomic DNA, 1X PCR buffer, 1.5mM $MgCl_2$, 200 mM dNTPs, 5 pmol of each primer and 0.5 u of Taq polymerase. Primers were fluorescently labelled with FAM, VIC, NED & PET dyes (Table 1).

Table 1 The characteristics of the primers from 29 microsatellite loci amplified in Malvi camel.

Marker loci	Primer (5'→3') forward	Primer (3'→5') Reverse	labeled dye	Repeat motif	Size range (bp)
CDR03	CTCCCTGATCCTGCATCTCT	TTGACGGAGTAACCACACACA	FAM	(TG)12	117-155
CDR04	GCGAGCTGCACTTGTGTTT	GCATTTTATCACATTGATTTTACACA	VIC	(CA)7	80-102
CMS10	TGGACATTCATCACACACACA	TTCCTTTCATGCTTAATTGAACT	HEX	(CA)7	78-134
CMS104	CACTTAGGTCCCTGGGCTTT	GCATTCTCTTGCATCGTGTG	NED	(CA)7	75-101
CMS113	TCCTCTGCTGTCTGTCTGTCTC	CTTGGCTCTCACTTTTTCCA	FAM	(GT)21	86-138
CMS121	CAAGAGAACTGGTGAGGATTTTC	AGTTGATAAAAATACAGCTGGAAAG	NED	(TG)11/ (GA)9	139-173
CMS15	AAAACTAAAGCCAGAAAGGCAAA	TTTTTCCAGATCTTGCACCAC	FEM	(TG)19	81-121
CMS18	GAACGACCCTTGAAGACGAA	AGCAGCTGGTTTTAGGTCCA	FAM	(GT)14	144-166
CMS58	AATATACATCCTCCCAACTGGT	TTATTTCTCTTAACCCCTCTCTAA	NED	(AC)18	96-122
LCA70	TGTATGGCATAGCGATTCAGTT	TGGGGGTAAGAGCAGGATAA	PET	(CA)11	169-193
LCA71	CCGTATCTATCTATACACACACACACA	TCACCCTCCTCCTATTTTGG	VIC	(AC)7	127-167
LCA82	CGTGACACCAGGCTAAGTGA	TTTCAGATGGTAGCTTTAAAAATTG	PET	(CA)10	85-129
LCA83	TGCAGTTCCTGGTGCATTTA	GACTCCAAGCAGGACGAGAC	PET	(AC)6	162-184
LCA85	ACACACACACACACACACACAC	TGGTGCTTACAGTTTTAGAAGGTTT	VIC	(AC)17	82-130
LCA94	TTTGTCCATTCATCCAGCAC	TGTTTGGCTGTCACACTGGT	FAM	(CA)13	165-183
VOLP03	GCCAAAATAGGCTTACCCTTG	CCCGCTTCATCTATTGGAAA	FEM	(TG)13	129-163
VOLP12	TCTCAACAGGGACTGCACTG	GGAAGACCAGACAGGGTCCTA	NED	(CA)7	163-183
VOLP50	AAAATAAGGGCACTTCTTCTGTC	CCACAAAACTCTGTACCCACTG	HEX	(TA)6	135-173
VOLP55	TGTGTGTGTGTGTGTGTGTTT	TGCTTTAATATATCTCCCTTAGCTTT	HEX	(TG)14	78-112
VOLP72	AGCACCAGGAAACCCAACTA	GGGCAGGATGTACGAGCTTA	VIC	(AC)14	144-184
VOLP77	AGGAAACAAAGGGGTGTGTG	CCAGAAACAGGGCTTGGTAA	FAM	(GT)20	129-141
VOLP81	TGACAGACTTACAGCCAACATTT	ACACACACACACACACACACAC	NED	(TG)13	87-103
YWLL29	GAAGGCAGGAGAAAAGGTAG	CAGAGGCTTAATAACTTGCAG	FEM		200-228
YWLL44	CTCAACAATGCTAGACCTTGG	GAGAACACAGGCTGGTGAATA	FAM	(TG)18	95-111
YWLL40	CACATGACCATGTCCCCTTAT	CCAGTGACAGTGTGACTAAGA	HEX		149-189
CVRL02	TGTCACAAATGGCAAGAT	AGTGTACGTAGCAGCATTATTT	VIC		205-215
CVRL07	AATACCCTAGTTGAAGCTCTGTCCT	GAGTGCCTTTATAAATATGGGTCTG	FAM		279-299
CMS36	TGCTTTCCAGTTGTTTGCTG	GCAAGGTGGTGTTGGAGATT	HEX	(CA)9	195-227
YWLL36	CAAATGTTGTAAAATTGCACAGAAC	TTCAGGTTTGCCTAGTTTTAACTGT	FAM	(AC)23	115-155

Temperature optimisation between 50 and 65 °C was performed for all primers, with all performing well at 55 °C. The PCR reaction was carried out in eppendrof thermo cycler. The thermo cycling conditions utilized were initial denaturation at 94ºC for 5 min, followed by 40 cycles of 60 s at 94ºC, 45 s at annealing temperature, and 60 s at 72ºC. The final extension at 72ºC was prolonged for 10 min. Then, fragments were combined with loading mix, containing Hi-Di Formamide and Genescan Liz-500 size standard (Applied Biosystems). Fluorescently-labelled DNA fragments were run on Applied Biosystems 3130XL Genetic Analyzer. The loci were amplified and the fragment size was estimated using GeneScan

analysis software (Applied Biosystems, USA). The Gene Mapper over 3.0 software was used for extraction of allele size in Malvi camel population. The extracted data was run on several softwares to estimate genetic diversity.

Statistical Analysis

For 29 microsatellites loci analyzed, observed and expected heterozygosity estimates were calculated as implemented in POPGENE software [6]. The observed and effective numbers of alleles [7] were also calculated using POPGENE software. The tests for deviation from Hardy-Weinberg equilibrium were derived. k & g test was performed to evaluate population expansion [8]. Finally the bottleneck hypothesis was investigated using BOTTLENECK software [9]. The bottleneck tests for the departure from mutation drift equilibrium based on heterozygosity excess or deficiency. This does not require information on historical population sizes or level of genetic variations. It requires only measurement of allele frequencies from 5-20 polymorphic loci. The bottleneck compares heterozygosity expected at Hardy-Weinberg equilibrium to the heterozygosity expected at mutation drift equilibrium. All the three models of mutation were used, the strict one stepwise mutation model (SMM) [10], the infinite allele model (IAM) [7] and two-phase model (TPM).

Result and discussion

Various measures of genetic variation were estimated are presented in the Table 2. A total 240 allele were detected the mean number of alleles (Na) over 29 loci was found to be 8.2759 ± 3.4938 while the mean effective number of alleles (Ne) was 3.1917 ± 1.7795 in Malvi camel population (Table 2). The observed heterozygosity and the expected heterozygosity on the basis of allele frequency are also given. The mean observed heterozygosity in camel population for all the 29 loci was found to be 0.6041 ± 0.3256 while the mean expected heterozygosity was 0.5978 ± 0.1983. Alleles observed across the microsatellite loci studied varied from 3 (LCA94 and VOLP77) to 17 (VOLP50). LCA94 and VOLP77 with 3 alleles were least polymorphic while VOLP 50 observed to be highly polymorphic with 17 alleles. The genetic variation in terms of number of individuals examined and a total of 240 alleles were observed with the observed number of alleles (3-17) across the loci was more than the effective number of alleles (1.06 to 7.05) as per expectations. The Fis estimates across 18 out of 29 studied loci were negative based on table wide randomizations ($p<0.05$). It reflects that there is no population substructure, presumably resulting from the unplanned and indiscriminate mating prevalent in the breeding tract. Most probably camel owner use male camel of Mewati or Mewari breed for breeding purpose.

Table 2 Measures of genetic variation in Malvi camel population

Locus	Obs_Het	Exp_Het	Na	Ne	Fis
CDR03	0.522	0.645	5	2.798	0.188
CDR04	0.616	0.471	6	1.884	-0.313
CMS10	1.000	0.643	8	2.780	-0.562
CMS104	0.942	0.659	6	2.909	-0.435
CMS113	0.746	0.744	10	3.857	-0.008
CMS121	0.826	0.858	16	6.885	0.033
CMS15	0.775	0.664	10	2.955	-0.172
CMS18	0.268	0.342	8	1.517	0.213
CMS58	0.717	0.812	8	5.233	0.113
LCA70	0.993	0.632	8	2.697	-0.577
LCA71	0.196	0.475	8	1.900	0.587
LCA82	0.920	0.861	13	7.057	-0.072
LCA83	0.935	0.609	6	2.547	-0.539
LCA85	0.007	0.719	7	3.532	0.989
LCA94	0.362	0.313	3	1.453	-0.162
VOLP03	0.265	0.302	8	1.430	0.118
VOLP12	0.710	0.502	7	2.000	-0.419
VOLP50	0.899	0.777	17	4.435	-0.160
VOLP55	0.913	0.861	15	7.051	-0.064
VOLP72	0.109	0.567	7	2.296	0.808
VOLP77	0.058	0.057	3	1.060	-0.023
VOLP81	0.869	0.633	8	2.705	-0.379
YWLL29	0.754	0.607	8	2.528	-0.247
YWLL40	0.341	0.427	10	1.739	0.199
YWLL44	0.906	0.593	7	2.444	-0.533
CVRL02	0.581	0.759	5	4.106	0.232
CVRL07	0.058	0.636	5	2.729	0.908
CMS36	0.369	0.319	6	1.468	-0.159
YWLL36	0.860	0.850	12	6.561	-0.015
Mean	0.604	0.598	8.276	3.192	-0.014
St. Dev	0.326	0.198	3.494	1.779	

Expected heterozygosity were computed using [12] and [13]expected heterozygosity.

Effective number of alleles [7].

The microsatellite data on Malvi camel was also subjected to population bottleneck & population expansion studies. We utilized k & g test for testing if the Malvi camel population has undergone population expansion. We utilized the within locus k-test which is based on simple stepwise model as well as on expectations about the systematic differences in the shapes of the allele length distribution for constant sized or expanding population. The statistical values obtained for the Malvi camel was 18

loci with negative values with p-value of 0.01498 which signifies population expansion. The inter locus g-test revealed a value of 2.3557 which is much higher than the 5th percentile cut offs for interlocus test table value of 0.30 [8]. The test also reveals that Malvi camel population has undergone population expansion.

We utilized three tests viz; Sign test (Nonparametric), Standardized Differences test (Parameteric) and Wilcoxon test to test if the Malvi population is in mutation drift equilibrium. Populations which have experienced a recent reduction of their effective population size exhibit a correlative reduction of the allele numbers and heterozygosity at polymorphic loci. But the allelic diversity is reduced faster than the heterozygosity, i.e. the observed heterozygosity is larger than the heterozygosity expected from the observed allele number were the locus at mutation-drift equilibrium. Strictly speaking, this has been demonstrated only for loci evolving under the Infinite Allele Model (IAM) [11]. If the locus evolves under the strict Stepwise Mutation Model (SMM), there can be situations where this heterozygosity excess is not observed. However, few loci follow the strict SMM, and as soon as they depart slightly from this mutation model towards the IAM, they will exhibit a heterozygosity excess as a consequence of a genetic bottleneck.

Table 3 Test for null hypothesis for mutation drift equilibrium under three mutation models (IAM, TPM and SMM) using Sign rank, Standardized Differences and Wilcoxon tests in Malvi camel.

	Sign test		
	IAM	**TPM**	**SMM**
No.of loci with heterozygosity excess	16.91	16.91	17.12
Observed no.of loci with H excess	20	10	2
Probabilty	0.1642	0.00820	0.00000***
	Standardized Difference test		
	IAM	**TPM**	**SMM**
T2 Value	0.814	-4.420	-16.892
Probabilty	0.20772	0.00000***	0.00000***
	Wilcoxon test		
	IAM	**TPM**	**SMM**
Probability (two tails for H excess or deficiency)	0.26521	0.01476	0.00000***

*** *Significant value*

In Malvi camel under the sign test expected numbers of loci with heterozygosity excess were 16.91, 16.91 and 17.12 respectively whereas the observed numbers of loci with heterozygosity excess were 20, 10 and 2 for IAM, TPM and SMM respectively. The difference between expected and observed heterozygosity excess was no significant in IAM and TPM, whereas is significant for heterozygote deficiency in SMM. The sign test under Infinite Allele model revealed the null hypothesis that the population is in Mutation drift equilibrium is accepted. The Standardized difference test (statistic T_2) is equal to 0.814, -4.420 and -16.892 respectively for IAM, TPM and SMM models in Malvi camel. All the values are less than 1.645 (value from table of normal distribution) and thus accept the null hypothesis of mutation-drift equilibrium. Wilcoxon Rank test which is a nonparametric test gave probability values of 0.265, 0.014 and 0.000 respectively for IAM, TPM and SMM in Malvi camel. The values are more than 0.05 for IAM and thus accept the null hypothesis of mutation-drift equilibrium, where this hypothesis is rejected for TPM (0.1476) and SMM (0.00000), which is most stringent and the deviation is in the favour of heterozygosity deficiency. The lower magnitude of expected heterozygosity excess with their respective in all the cases reflects absence of genetic bottleneck (Table 3). Mode Shift test allow to check whether the distribution followed the normal L-shaped curve depicted in the graph given below (Fig. 2). Qualitative graphical method based on the allele frequency spectra detected no shift in the frequency distribution of alleles, where the alleles with the lowest frequencies (0.01–0.1) were found to be most abundant .This reflects that the population had not undergone bottleneck.

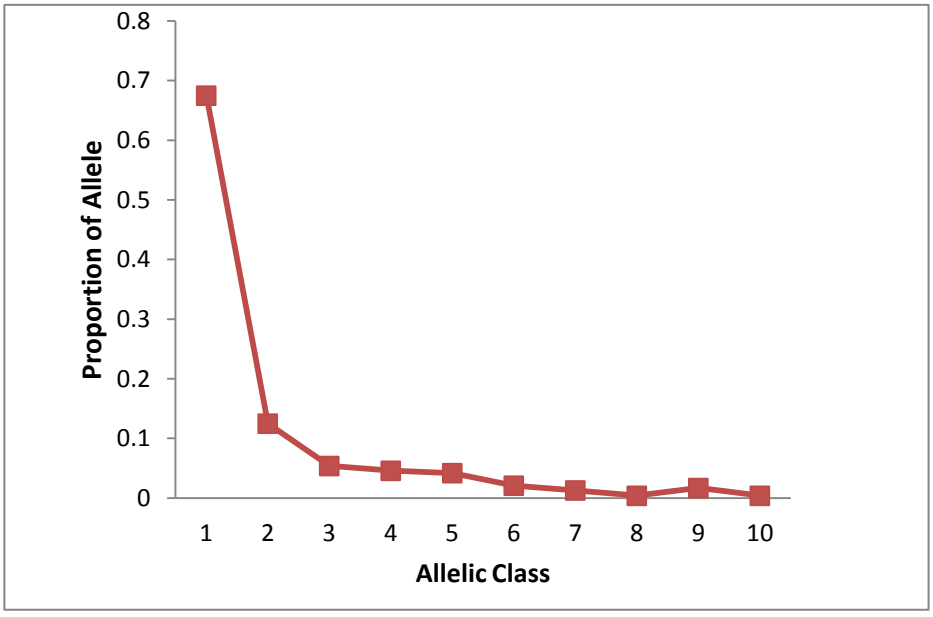

Fig. 2 Mode-shift test for bottleneck analysis in Malvi camel

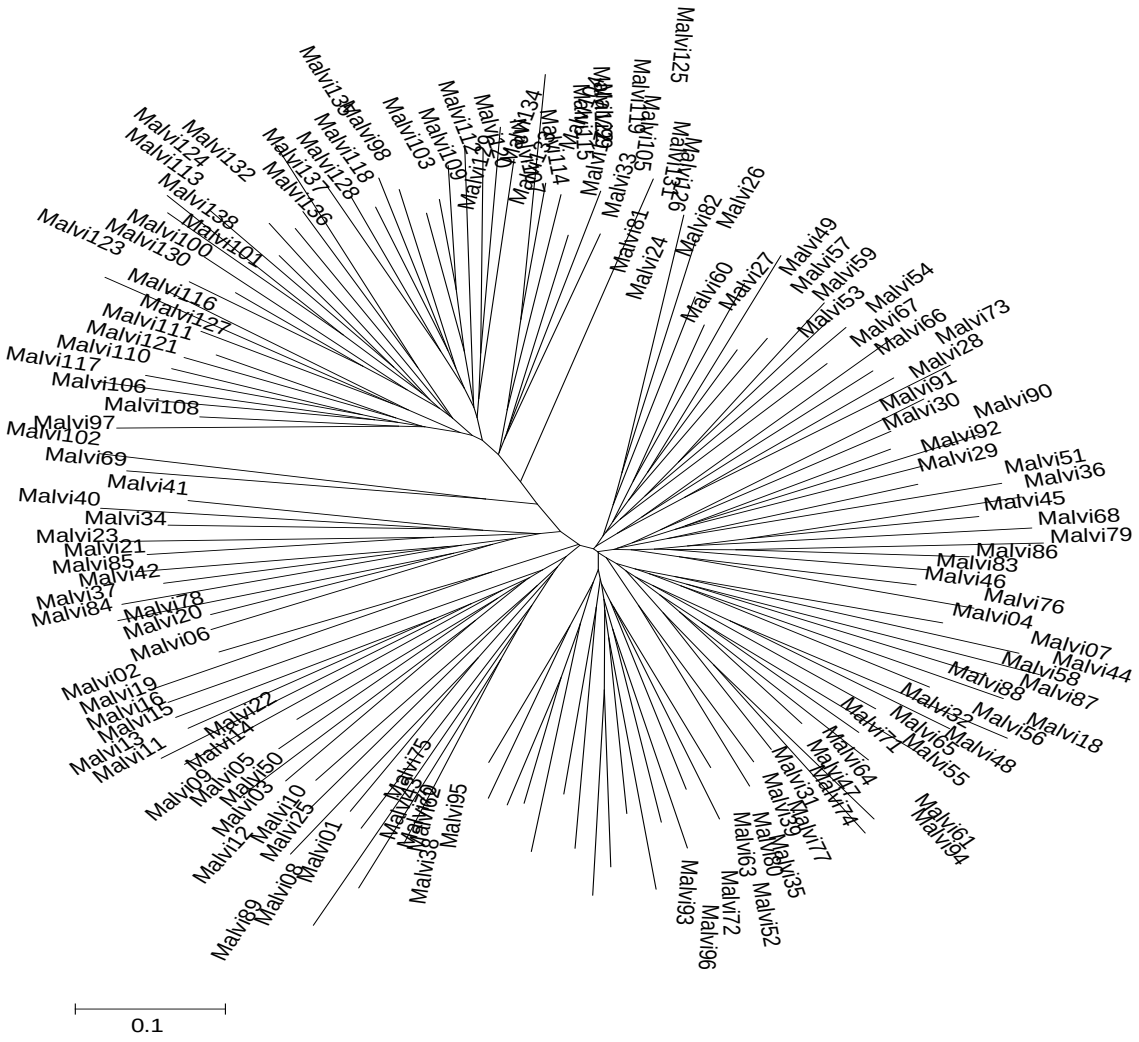

Fig. 3 Radiation tree constructed based on Nei's standard inter-individual genetic distances using neighbour joining method.

The phylogenetic analysis based on Nei's DA gentic distance [14], reaveled that distinct clustering of individuals of Neemach region of Madhya Pradesh and and few samples were collected from Mandasaur ans contiguous area of Mandasaur regions of Madhya Pradesh so they are clustering together. Phylogenetic tree based on Nei's DA depicted in Fig. 3.

Conclusion

The single humped camels (*Camelus dromedarius*) are denizen of hot arid land but Malvi camel is a distinct breed which exists in humid conditions as well. And most importantly this camel breed has high milk yield potential as compared to other breeds. The characterization and conservation of Malvi camel

assume prime importance as they provide food security and to the rural folk. This locally adapted camel is important biodiversity resources. The Malvi camel requires knowledge of genetic variations that can be effectively measured within and between populations. There has been no systematic study of Malvi camel in India however few reports have appeared (18th FAO report). In this present investigation an attempt has been made to genetically characterize Malvi camel population. The information generated from the present study can be useful in breeding and improvement programs for Malvi camel.

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