Molecular genetic characterization of Kutchi breed of goat

S. P. Dixit[1,*], N. K. Verma[1], S. P. S. Ahlawat[1], R. A. K. Aggarwal[1], S. Kumar[1], R. Chander[1] and K. P. Singh[2]

[1]National Bureau of Animal Genetic Resources, Karnal 132 001, India
[2]Sardarkrushinagar Dantiwara Agricultural University, Sardarkrushinagar 385 506, India

Kutchi, an important dual-purpose (meat and milk) goat breed, is found in Banaskantha, Patan and Kutch districts of Gujarat, India. Genetic diversity within the breed was investigated using 25 microsatellite molecular markers. The PCR products were run on automated DNA sequencer for allelic differentiation at 25 microsatellite loci. The average number of alleles observed across the studied microsatellite loci was 12.0 ± 1.02. The average expected gene diversity within the population was 0.79 ± 0.02, whereas observed heterozygosity was 0.59 ± 0.06. Thirteen out of the total 25 studied loci showed significant deviations from Hardy–Weinberg equilibrium. The F_{is} (inbreeding) value was 0.23 ± 0.07. The genetic differentiation among sub-populations of this breed was low (F_{st} = 0.05 ± 0.01). The Sign and Wilcoxon tests detected significant departure from mutation drift equilibrium in the population at most of the studied loci. Appropriate breeding strategies for its conservation and improvement of its unique attributes like adaptability and fitness under harsh climatic conditions of the arid/semi-arid zone are warranted.

Keywords: Genetic variation, heterozygosity, Kutchi goat, microsatellite loci.

KUTCHI, an important dual-purpose (meat and milk) indigenous goat breed, is found in Banaskantha, Patan and Kutch districts of Gujarat, India. The breed derives its name from the Kutch region of Gujarat, which is its natural habitat. The Kutchi goats are medium-sized and black-coloured animals with white spots on the neck region (Figure 1). Natural service is method of breeding for this goat. Generally, a buck is maintained for every 20–100 does by the farmers on individual or community basis. The buck is usually replaced after 3–4 years of service. The average body weight of the adult male and female goat is 46.96 and 39.91 kg respectively[1]. The breed is well adapted to the inhospitable agro-climatic, and a wide range of vegetation and management conditions of the hot arid/hot humid region. The adaptive characteristics make this breed suitable for development and sustainability of the meat industry as well as for its own sustainable improvement under the peculiar characteristics of the arid region. Moreover, the breed is suitable for resource-poor farmers rearing these animals under fairly simple and extensive production system, with a relatively low level of managerial skills. In spite of their ecological and economic importance, the Kutchi goats are inadequately characterized, particularly at the DNA level. Currently, microsatellites are widely used to assess genetic variability at the DNA level since they are numerous, randomly distributed in the genome, highly polymorphic and show co-dominant inheritance. In the present study, an attempt has been made to find out within-breed genetic diversity using microsatellite molecular markers. The resulting information from this study may be useful for planning sustainable improvement, conservation and utilization of the breed.

Forty-six animals confirming to typical phenotypic characteristics of the Kutchi goat breed were chosen randomly from six different locations of the Kutch region (Figure 2). The farmers were interviewed about the social relationship among these animals so as to avoid relatedness of the individuals. Moreover, 1 or 2 samples were taken from each flock. The other two districts, viz. Banaskantha and Patan were not considered for blood sampling because of intermixing of Kutchi goats with two other goat breeds, Sirohi and Mehsana, in this region and also keeping in view the future strategy of working out the genetic relationship of this breed with 19 other goat breeds of India. The blood samples were collected from the 46 animals by puncturing the jugular vein in the vacutainer tubes (B.D. India Ltd) having EDTA as blood anticoagulant. The samples were brought to the laboratory in an ice-cooled box, where they were kept under –20°C in a deep freezer until DNA isolation.

Genomic DNA was isolated from the blood samples using a standard phenol : chloroform extraction method[2]. A battery of 25 heterologous polymorphic microsatellite markers (Table 1) were selected based on the guidelines of the International Society of Animal Genetics (ISAG), and Food and Agriculture Organization's Domestic Animal Diversity Information Service (FAO's DADIS) programme, to generate data in a panel of 46 animals. Most of the primers used were independent and belonged to

Figure 1. A Kutchi doe in the pasture.

*For correspondence. (e-mail: dixitsp@gmail.com)

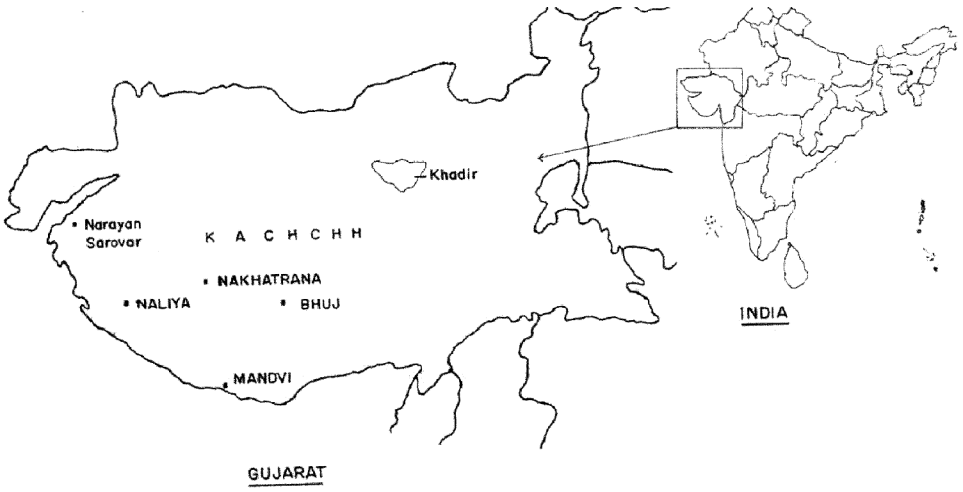

Figure 2. Map of Kutch District showing blood-sampling locations.

different chromosomes. Only forward primers at the 5′-end of each pair were labelled with one of the four flurophores, i.e. FAM-Blue, VIC-Green, NED-Yellow and Pet-Red. These dye-labelled primers were synthesized by Applied Biosystem (ABI, USA). Oligos supplied in lyophilized form was reconstituted in purified water (MiliQ) to the volume (in µl) equivalent to the mass (µg) of the primer and further diluted in MiliQ water to give a final concentration of 100 ng/µl.

Polymerase chain reaction (PCR) was carried out on about 50–100 ng genomic DNA in a 25 µl reaction volume. The reaction mixture consisted of 200 µM each of dATP, dCTP, dGTP and dTTP, 50 nM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 2.0 mM $MgCl_2$, 0.75 unit *Taq* DNA polymerase and 4 ng/µl of each primer using PTC-200 PCR machine (M. J. Research). The 'touchdown' PCR protocol was used with initial denaturation of 95°C for 1 min, 3 cycles of 95°C for 45 s and 60°C for 1 min, 3 cycles of 95°C for 45 s and 57°C for 1 min, 3 cycles of 95°C for 45 s and 54°C for 1 min, 3 cycles of 95°C for 45 s and 51°C for 1 min, and 20 cycles of 95°C for 45 s and 48°C for 1 min. At the end of the reaction, 5.0 µl of stop dye (95% formamide, 0.25% bromophenol blue and 0.25% xylene cyanol) was added and 6 µl of PCR products was loaded onto a 2% agarose gel, electrophoresed and visualized over UV light after ethidium bromide-staining to detect the amplification.

The PCR products were multiplexed and run on an automated DNA sequencer ABI 3100 Avant (Applied Biosystems). The electropherograms drawn through Gene Scan were used to extract DNA fragment sizing details using Gene Mapper software version 3.0 (Applied Biosystems).

For the 25 microsatellites loci analysed, observed and expected heterozygosity estimates were calculated after Levene[3] and Nei[4], as implemented in the POPGENE software[5] to determine genetic variation within the breed. Heterozygosity is defined as the probability that a given individual randomly selected from a population will be heterozygous at a given locus. The observed and effective number of alleles[6] was also calculated using POPGENE software. The tests for deviation from Hardy–Weinberg equilibrium were also derived using the exact tests of POPGENE.

Tests for pairwise linkage (genotypic) disequilibrium among the microsatellite loci were done using F-STAT[7] version 2.9.3 for the 25 microsatellite loci whose genotypes were determined directly. To study population structure, *F*-statistics was determined according to Weir and Cocheran[8] as used in the F-STAT software with jackknifing procedure applied over loci in deriving significance levels. F_{is} is an estimate of variation within population that measures the reduction in heterozygosity in an individual due to non-random mating within its subpopulations. F_{st} and R_{st} measure the degree of genetic differentiation among sub-populations[9]. F_{it} is the overall reduction in heterozygosity in an individual relative to the total population. This includes contribution due to non-random mating within sub-populations (F_{is}) and that due to population sub-division (F_{st}). The average relatedness of individuals within sub-populations due to mating system among them was estimated after Hamilton[10] using F-STAT.

Finally the bottleneck hypothesis[11] was investigated using BOTTLENECK 1.2.01. The BOTTLENECK tests for the departure from mutation drift equilibrium were based on heterozygosity (not heterozygotes), 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 to 20 polymorphic loci in a sample of approximately 20–30 individuals. The BOTTLENECK test compares heterozygo-

Table 1. Microsatellite markers, their sequences, type of repeat, size range, location and accession numbers

Locus	Primer sequence	Type of repeat	Size range	Chr. no.*	GenBank accession number
ILST008	gaatcatggattttctgggg tagcagtgagtgaggttggc	$(CA)_{12}$	167–195	14	L23483
ILSTS059	gctgaacaatgtgatatgttcagg gggacaatactgtcttagatgctgc	$(CA)_4(GT)_2$	105–135	13	L37266
ETH225	gatcaccttgccactatttcct acatgacagccagctgctact	$(CA)_{18}$	146–160	14	Z14043
ILST044	agtcacccaaaagtaactgg acatgttgtattccaagtgc	$(GT)_{20}$	145–177	Ann	L37259
ILSTS002	tctatacacatgtgctgtgc cttaggggtgaagtgacacg	$(CA)_{17}$	113–135	Ann	L23479
OarFCB304	ccctaggagctttcaataaagaatcgg cgctgctgtcaactgggtcaggg	$(CT)_{11}(CT)_{15}$	119–169	Ann	L01535
OarFCB48	gagttagtacaaggatgacaagaggcac gactctagaggatcgcaaagaaccag	$(CT)_{10}$	149–181	17	M82875
OarHH64	cgttccctcactatggaaagttatatatgc cactctattgtaagaatttgaatgagagc	–	120–138	4	212[a]
OarJMP29	gtatacacgtggacaccgctttgtac gaagtggcaagattcagaggggaag	$(CA)_{21}$	120–140	Ann	U30893
ILSTS005	ggaagcaatgaaatctatagcc tgttctgtgagtttgtaagc	$(nn)_{39}$	174–190	10	L23481
ILSTS019	aagggacctcatgtagaagc acttttggaccctgtagtgc	$(TG)_{10}$	142–162	Ann	L23492
OMHC1	atctggtgggctacagtccatg gcaatgctttctaaattctgaggaa	–	179–209	Ann	228[a]
ILSTS087	agcagacatgatgactcagc ctgcctcttttcttgagagc	$(CA)_{14}$	142–164	Ann	L37279
ILSTS30	ctgcagttctgcatatgtgg cttagacaacaggggtttgg	$(CA)_{13}$	159–179	2	L37212
ILSTS34	aagggtctaagtccactggc gacctggtttagcagagagc	$(GT)_{29}$	153–185	5	L37254
ILSTS033	tattagagtggctcagtgcc atgcagacagttttagaggg	$(CA)_{12}$	151–187	12	L37213
ILSTS049	caattttcttgtctctcccc gctgaatcttgtcaaacagg	$(CA)_{26}$	160–184	11	L37261
ILSTS065	gctgcaaagagttgaacacc aactattacaggaggctccc	$(CA)_{22}$	105–135	24	L37269
ILSTS058	gccttactaccatttccagc catcctgactttggctgtgg	$(GT)_{15}$	136–188	17	L37225
ILSTS029	tgttttgatggaacacagcc tggatttagaccagggttgg	$(CA)_{19}$	148–191	3	L37252
RM088	gatcctcttctgggaaaaagagac cctgttgaagtgaaccttcagaa	$(CA)_{14}$	109–147	4	U10392
ILSTS022	agtctgaaggcctgagaacc cttacagtccttggggttgc	$(GT)_{21}$	186–202	Ann	L37208
OARE129	aatccagtgtgtgaaagactaatccag gtagatcaagatatagaatatttttcaacacc	$(CA)_{14}$	130–175	7	L11051
ILSTS082	ttcgttcctcatagtgctgg agaggattacaccaatcacc	$(GT)_{17}$	100–136	2	L37236
RM4	cagcaaaatatcagcaaacct ccacctgggaaggccttta	$(CA)_{13}$	104–127	15	U32910

*Chr. no., Chromosome number; [a]Accession number of Arkdb database (http://www.thearkdb.org); Ann, Anonymous microsatellite from other species.

sity expected (H_E) at Hardy–Weinberg equilibrium (H_E) with the heterozygosity expected at mutation drift equilibrium (H_{eq}) in the same sample, that has the same size and the same number of alleles. All the three models of mutation were used to calculate H_{eq}: the strict one stepwise mutation model[12], the infinite allele model[6] and the two-phase model[13].

Various measures of genetic variation are presented in Table 2. The number of alleles observed across the studied microsatellite loci varied from seven (OarJMP 29, RM 088, ILSTS 022, RM 4) to 26 (ILSTS 058), with an overall mean of 12.0 ± 1.02. The observed number of alleles across the loci was more than the effective number of alleles (2.29 to 12.63) according to expectations. All

Table 2. Measures of genetic variation in Kutchi breed of goat

Locus	Sample size	Observed number of alleles	Effective number of alleles	Heterozygosity		
				Observed	Expected	Nei's
ILST008	46	9	2.66	0.15***	0.63	0.62
ILSTS059	44	14	4.36	0.81	0.78	0.77
ETH225	24	9	3.28	0.20***	0.71	0.69
ILST044	46	17	8.46	0.91	0.89	0.88
ILSTS002	44	12	4.35	0.56***	0.78	0.77
OarFCB304	35	25	12.63	0.97	0.93	0.92
OarFCB48	35	16	9.28	0.91	0.90	0.89
OarHH64	49	12	4.65	0.46***	0.79	0.78
OarJMP29	28	7	5.11	0.14***	0.81	0.80
ILSTS005	46	9	5.24	0.26***	0.82	0.81
ILSTS019	45	13	7.76	0.87	0.88	0.87
OMHC1	35	17	11.50	0.86	0.93	0.91
ILSTS087	45	16	9.35	0.29***	0.90	0.89
ILSTS30	45	11	2.53	0.29***	0.61	0.60
ILSTS34	46	9	2.96	0.15**	0.67	0.66
ILSTS033	35	16	6.34	0.77	0.85	0.84
ILSTS049	44	14	5.23	0.82	0.81	0.81
ILSTS065	44	8	2.29	0.61	0.57	0.56
ILSTS058	45	26	12.12	0.75***	0.93	0.92
ILSTS029	46	10	3.42	0.80	0.71	0.71
RM088	45	7	5.05	0.98	0.81	0.80
ILSTS022	45	7	3.92	0.46**	0.75	0.74
OARE129	46	14	5.26	0.84	0.82	0.81
ILSTS082	45	15	8.77	0.75*	0.89	0.88
RM4	46	7	3.95	0.13***	0.75	0.75
Mean	42	12	6.02	0.59***	0.80	0.79
S.E.		1.02	0.62	0.06	0.02	0.02

Effective number of alleles was calculated after Kimura and Crow[6]. Expected heterozygosity was computed using Levene[3] and Nei[4].
*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

the studied microsatellite loci were polymorphic, which indicates that the microsatellites used were suitable for genetic diversity analysis. The average observed heterozygosity was lesser than the expected value (Table 2). The average expected gene diversity[4] within the population ranged from 0.56 (ILSTS065) to 0.92 (Oar FCB304, ILSTS 058) with an overall mean of 0.79 ± 0.02, whereas the observed heterozygosity ranged from 0.13 (RM 4) to 0.98 (RM 088) with an average of 0.59 ± 0.06. Thirteen out of the total 25 studied loci showed significant deviations from the Hardy–Weinberg equilibrium. All these loci showed significant heterozygote deficiency (positive F_{is} values) in the Kutchi goat population. Significant linkage disequilibrium was detected in the overall microsatellite data for 18 out of the 300 loci pairs.

The overall means of F-statistics obtained from jackknifing over loci were significantly different from zero (Table 3). The significant positive values of F_{is} ranged from 0.13 ± 0.06 (ILSTS 082) to 0.84 (RM 4) with an overall mean of 0.23 ± 0.07. The overall global deficit of heterozygotes across sub-populations (F_{it}) amounted to 27.0% and genetic differentiation among sub-populations (F_{st}) was low (5.0%). R_{st}, an other measure of the genetic differentiation also revealed low differential ($R_{st} = 0.02$) among the studied samples. Estimates of F_{st} between each sub-population pair varied from 0.004 to 0.084. However, these estimates were either non-significant or level of significance could not be worked out after applying Bonferroni corrections. The average relatedness of individuals within sub-populations compared to whole population was 0.08 (Table 3).

The Sign and Wilcoxon tests detected significant departure from mutation drift equilibrium in the population at most of the studied loci (data not shown). This deviation was significant under two-phase and single-step mutation models. The standardized differences test also revealed significant departure from mutation drift equilibrium. The deviation was negative under two-phase and single-step mutation models ($T_2 = -3.71$ and -11.97 respectively; $P < 0.05$). These tests detected heterozygosity deficiency in the population from the observed heterozygosity at Hardy–Weinberg equilibrium. This deficiency could be attributed to population expansion or due to immigration of new alleles into the population.

All measures of genetic diversity revealed that there was substantial genetic variation within the Kutchi goat population. The observed number of alleles/locus (7–26) demonstrates that all the microsatellite loci were sufficiently polymorphic in the studied goat population. Thus, the markers used were appropriate because the number of

Table 3. *F*-statistics and relatedness analysis for the studied microsatellite loci in Kutchi breed of goat

Locus	F_{it}	F_{st}	F_{is}	Relat
ILST008	0.75 ± 0.07***	0.07 ± 0.10***	0.74 ± 0.08***	0.08 ± 0.11
ILSTS059	–0.05 ± 0.04	–0.01 ± 0.02	–0.04 ± 0.05	–0.03 ± 0.03
ETH225	0.71 ± 0.22***	0.27 ± 0.19***	0.56 ± 0.25***	0.33 ± 0.18
ILST044	–0.02 ± 0.05	0.04 ± 0.03	–0.06 ± 0.05	0.09 ± 0.06
ILSTS002	0.27 ± 0.09***	–0.03 ± 0.02	0.29 ± 0.10***	–0.06 ± 0.02
OarFCB304	–0.04 ± 0.04	0.02 ± 0.02	–0.06 ± 0.05	0.05 ± 0.05
OarFCB48	–0.01 ± 0.07	0.01 ± 0.02	–0.01 ± 0.08	0.02 ± 0.04
OarHH64	0.44 ± 0.14***	0.13 ± 0.09***	0.35 ± 0.13***	0.19 ± 0.11
OarJMP29	0.83 ± 0.09***	0.02 ± 0.04***	0.83 ± 0.10***	0.02 ± 0.04
ILSTS005	0.73 ± 0.12***	0.13 ± 0.06***	0.67 ± 0.14***	0.15 ± 0.07
ILSTS019	0.04 ± 0.04	–0.00 ± 0.02	0.04 ± 0.06	–0.00 ± 0.04
OMHC1	0.07 ± 0.07	–0.01 ± 0.02	0.08 ± 0.06	–0.01 ± 0.03
ILSTS087	0.69 ± 0.10***	0.00 ± 0.02***	0.69 ± 0.10***	0.00 ± 0.02
ILSTS30	0.50 ± 0.16***	0.14 ± 0.13***	0.42 ± 0.14***	0.19 ± 0.17
ILSTS34	0.74 ± 0.07***	–0.02 ± 0.04**	0.75 ± 0.07***	–0.02 ± 0.04
ILSTS033	0.11 ± 0.04*	0.09 ± 0.06***	0.03 ± 0.05	0.17 ± 0.11
ILSTS049	0.00 ± 0.04	0.00 ± 0.02	–0.00 ± 0.04	0.01 ± 0.04
ILSTS065	–0.06 ± 0.15	–0.00 ± 0.03	–0.05 ± 0.16	–0.01 ± 0.06
ILSTS058	0.19 ± 0.07***	–0.00 ± 0.01	0.19 ± 0.07***	–0.01 ± 0.02
ILSTS029	–0.12 ± 0.10	0.00 ± 0.01	–0.12 ± 0.10	0.00 ± 0.04
RM048	–0.21 ± 0.03	0.01 ± 0.03	–0.22 ± 0.05	0.03 ± 0.07
ILSTS022	0.40 ± 0.06***	0.22 ± 0.08***	0.25 ± 0.12**	0.31 ± 0.12
OARE129	–0.03 ± 0.08	0.03 ± 0.02***	–0.07 ± 0.09	0.07 ± 0.04
ILSTS082	0.16 ± 0.06***	0.04 ± 0.02***	0.13 ± 0.06*	0.06 ± 0.03
RM4	0.86 ± 0.06***	0.11 ± 0.07***	0.84 ± 0.06***	0.12 ± 0.07
Over all loci	0.27 ± 0.07***	0.05 ± 0.01***	0.23 ± 0.07***	0.08 ± 0.02

*$P \leq 0.05$; **$P \leq 0.01$ and ***$P \leq 0.001$; F_{is}, Heterozygotes deficit within sub-population; F_{it}, Global deficit of heterozygotes; F_{st}, Differentiation among sub-populations, and Relat, relatedness of individuals within sub-populations when compared to the whole population.

alleles for each marker was higher than the minimum number of alleles (at least four alleles) recommended for microsatellite markers to be used in the estimation of genetic distance[14]. The observed number of alleles/loci in the Kutchi breed of goat was higher than that reported in various other goat breeds (Table 4). A more appropriate measure of genetic variation within a population is gene diversity[15]. The gene diversity (0.79) in this breed of goat was also higher than that observed in other breeds mentioned in Table 4. Higher genetic diversity observed in the Kutchi breed may be because of their large effective number and low selection pressure.

The observed heterozygosity was significantly lower than the expected heterozygosity at 13 out of the 25 studied loci, indicating departure from random mating, and suggested that some of studied loci were homozygous in the population. Some of these loci could be undergoing natural selection or linked to other loci affecting morphological, productive or adaptive traits undergoing natural selection. The heterozygote deficiency observed at these loci may be due to a variety of causes: population subdivision owing to genetic drift, null alleles, selection against heterozygotes or inbreeding. However, distinguishing among them is generally difficult[16]. The fairly high value of F_{is} indicated that some of the loci in this breed of goat were homozygous, presumably resulting from the mating between relatives and consequent genetic drift. This point is also supported by observed relatedness of the individuals in this breed. The non-random association of alleles (genotypic disequilibrium) across the loci was also compatible with the observed F_{is} and relatedness values. The measures of population subdivision (F_{st}, R_{st}) also revealed significant population structure in this breed of goat. However, null alleles are most unlikely to be segregating at all the loci. Therefore, homozygous loci, relatedness and population structure of the Kutchi breed of goat could be the causes of heterozygote deficit. Significant heterozygote deficiencies have also been reported in some studies on goats[17–21].

The different tests of BOTTLENECK hypothesis under TPM and SMM models indicated significant deficiency of heterozygosity, possibly caused by introduction of unique/rare alleles by immigrants.

The population of the Kutchi breed in its breeding tract was also assessed based on two-stages stratified sampling techniques. The sample estimates from 26 villages across four districts were superimposed on census data of goats available for each district (Livestock Census – 2003; Gujarat State, India) to work out the total population of the breed. There were around 453,209 animals in the entire tract based on sample estimates. In spite of this large size population, the breed is getting diluted mainly due to loss

Table 4. Genetic variability among different breeds of goat

Breed	Habitat	Observed no. of alleles	Observed heterozygosity	Expected heterozygosity	Reference
Asian goat population	Southeast Asia	4.3–5.9	0.30–0.48	0.46–0.0.60	17
Barbari	Etawah, Agra and Aligarh districts, UP; Bharatpur District, Rajasthan	2–10	0.47 ± 0.17	0.59 ± 0.19	23
Black Bengal	West Bengal and NE India; part of Bihar and Orissa	4–13	0.69 ± 0.11	0.81 ± 0.08	24
Brown, short-haired	Czech Republic	4–8		0.68	25
Chegu	Spiti, Yaksar and Kashmir Valley	6–11	0.66 ± 0.07	0.81 ± 0.04	24
Chinese goat breeds (9)	Different provinces of China	4–13	0.57 ± 0.23	0.71 ± 0.18	26
Chinese goat	China	5.8	0.67	0.67	27
Italian goat breeds (9)	Italy	4.9–9.2	0.61–0.76	0.61–0.76	28
Korean goat	Korea	3.4	0.36	0.38	27
Marwari	Jodhpur, Barmer, Bikaner, Nagaur, Jalore, Jaisalmer and Pali districts, Rajasthan	3–8	0.13–0.88	0.29–0.82	18
Mehsana	Patan, Bonaskantha, Mehsana, Gandhinagar and Ahmedabad districts, Gujarat	3–24	0.65 ± 0.26	0.76 ± 0.20	19
Saanen goat	Australia	5.3	0.59	0.62	27
Sirohi	Sirohi, Udaipur and Ajmer districts, Rajasthan	5–25	0.50 ± 0.05	0.79 ± 0.03	20
Sub-Saharan African breeds	Sub-Saharan Africa	5.26–7.05	0.34–0.47	0.54–0.67	29
White, short-haired	Czech Republic	7–13	–	0.77	25

of genetic variation at some of the loci and immigration of new alleles into the population. These results strongly contrast with those of Nivsarkar and Bhat[22], who pointed out that a goat breed with over 30,000 animals with no serious declining trend, can be considered as normal. Hence, the procedure for the identification and categorization of endangered breeds of livestock needs refinement. The results of this study clearly pinpoint the need for suitable genetic management so as to retain the founder alleles to the extent possible in the population.

In conclusion, there is substantial genetic variation and polymorphism across the studied loci in the Kutchi breed of goat and the population was neither in Hardy–Weinberg equilibrium nor in mutation drift equilibrium. Heterozygote deficit was also observed at some of the loci. The breed has also been receiving new genetic material through introduction of immigrants. Appropriate breeding strategies should, therefore, be designed under field conditions for its conservation and improvement of its unique attributes like adaptability and fitness under harsh climatic conditions of the arid/semi-arid zone.

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Monoclonal antibodies to the recombinant nucleocapsid protein of a groundnut bud necrosis virus infecting tomato in Karnataka and their use in profiling the epitopes of Indian tospovirus isolates

Hemalatha Venkat[1], Pradnya Gangatirkar[1], Anjali A. Karande[1], M. Krishnareddy[2] and H. S. Savithri[1,*]

[1]Department of Biochemistry, Indian Institute of Science, Bangalore 560 012, India
[2]Division of Plant Pathology, Indian Institute of Horticultural Research, Hesaraghatta Lake PO, Bangalore 560 089, India

A tospovirus infecting tomato in the fields of Karnataka, India, was propagated in greenhouse-grown *Nicotiana benthamiana* plants by mechanical inoculation. The viral RNA was extracted from purified virus and used for amplification of *N* and *NSs* genes by RT–PCR using appropriate primers. The *N* and *NSs* PCR products were cloned into a pRSET vector and sequenced. The *N* gene of tomato tospovirus showed 98% identity with that of *Groundnut bud necrosis virus* (GBNV), alternate name *Peanut bud necrosis virus* (PBNV). Interestingly, though the virus was isolated from tomato plants, it showed only 82% identity with the *N* gene of GBNV-To isolate from Taiwan. The *NSs* gene of the virus under study showed 98% identity with GBNV. These results suggest that the tomato tospovirus in Karnataka is a strain of GBNV and is henceforth designated as GBNV-To (K). The *N* gene was overexpressed in *Escherichia coli* and the recombinant N protein was purified using Ni–NTA agarose affinity chromatography. The purified protein was used for the generation of poly- and monoclonal antibodies (mAbs). The polyclonal antiserum thus obtained had a dilution end-point >1 : 32,000 and nine unique mAbs were also obtained. These mAbs were used for epitope profiling of the tospovirus isolates from South India and for developing detection methods. The results showed that there are distinct GBNV strains in South India. A simple dot-blot assay was developed for detection of GBNV from infected field samples.

Keywords: *Groundnut bud necrosis virus*, monoclonal antibodies, tomato, tospovirus.

IN recent years, thrips and tospoviruses have become a serious problem in various Leguminosae, Solanaceae and Cucurbitaceae crops all over India. The genus *Tospovirus*, family Bunyaviridae, includes viruses with enveloped, quasi-spherical particles of 80–120 nm diameter and a

*For correspondence. (e-mail: bchss@biochem.iisc.ernet.in)