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Genetic diversity evaluation of Sojat goat population of India

REKHA SHARMA[1]✉, RENUKA SEHRAWAT[1], SONIKA AHLAWAT[1], M L GURJAR[2] and M S TANTIA[1]

ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana 132 001 India

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ABSTRACT

Sojat goat forms the part and parcel of the lives of the farmers in the Barmer region of Rajasthan. These are large-sized goats, white in colour, and have dual utility. These goats fetch premium price during the Eid festival. Assessment of diversity is essential for germplasm characterization and management. Genomic microsatellite markers being a valuable tool for estimating genetic diversity were selected for exploring existing genetic variability in the Sojat goat population. The standard metrics of genomic diversity detected moderate variability with a total of 162 alleles across 22 loci in this lesser-known population. The expected number of alleles had a mean value of 3.40±0.39. Similarly, a moderate magnitude of diversity was recorded in the Sojat population as the mean observed heterozygosity was 0.54±0.05. Expected heterozygosity was higher than the observed (0.60±0.06), indicating a deviation from Hardy-Weinberg Equilibrium (HWE) and the possibility of inbreeding due to the non-random mating in the population. Accordingly, significant heterozygote deficiency was noticed (F=0.08±0.03). The population did not suffer a reduction in effective population size in the last few generations. Mutation drift equilibrium did not reveal significant heterozygosity excess under different models of microsatellite evolution and no shift was recorded in the frequency distribution of alleles. To conclude, the results provided the first insights into the genetic diversity of Sojat goats. A moderate genetic variability with heterozygote deficiency within the population warrants immediate attention for scientific management of this unique goat population to conserve the existing genetic variation and to avoid any escalation of inbreeding.

Keywords: Bottleneck, Characterization, Genetic diversity, Microsatellite markers, Rajasthan, Sojat

Goats have been associated with mankind since the dawn of agriculture and domestication of animals, making them socio-economically a very important animal providing products (meat, milk, fibre, hair) and service to the human race throughout the world, especially in the developing countries including India (Kumar *et al.* 2020). Indian livestock registered an increase between the last two censuses, rising from 512 million in 2012 to 535.78 million in 2019 (Anonymous 2019). This augmentation is largely (95%) constituted by a spike in the numbers of small ruminants such as sheep and goats. As per the livestock census of 2019, goats in the country registered an increase of 10.14% and the present number stands at 148.88 million (Sharma *et al.* 2021). India is bestowed with a rich repository of goat genetic resources reflected in the 34 gazettes notified registered goat breeds distributed in different parts of the country (https://nbagr.icar.gov.in/en/home/). These breeds have evolved through natural selection and selective breeding and are adapted to different agro-ecological conditions, socio-cultural conditions, and specific ecological niches. The adaptation to the vivid climatic and management conditions has endowed them with better disease resistance, heat tolerance, and feed conversion efficiency.

In addition to these well-defined breeds, several goat populations have not yet been studied or described as 59% of total indigenous goats are categorized as non-descript. Considering the prevailing scenario, there is a compelling need to take up the characterization of lesser-known goat populations to harness the full potential of our indigenous caprine germplasm and to ensure their sustainable development and conservation. These goats play a pivotal role in the economy of the weaker section due to the requirements of low or negligible input. Besides, goat keeping also acts as insurance to the poor farmers during natural hazards likes drought, famine, flood, etc. The Sojat goat is one such goat population in the Rajasthan, a state custodian of the largest goat population (20.84 million, Livestock census 2019) in India. It is distributed in the Marwar region of Rajasthan. The native belt of Sojat goat mainly includes Sojat and Jaitran tehsils of Pali, Bilara, and Pipar tehsils of Jodhpur and Nagaur tehsil of Nagaur districts of Rajasthan. The population is locally known as Totapuri and Red coat goat. Its animals can be

Present address: [1]ICAR-National Bureau of Animal Genetic Resources, Karnal, Haryana. [2] College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan (Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan). ✉Corresponding author email: rekha.sharma1@icar.gov.in

distinguished from other breeds by prominent physical characteristics, viz. large size and very heavy deep body, and thin and shiny hair coat. Typical-looking animals are of pure and mixed colours but they are generally white, and occasionally patches of tan or black are found on the body. The ears are very long, flat, and drooping. Both sexes are found mostly without horns with a short and thin tails. The bucks are larger than does and are very beautiful. Sojat goats provide remunerative employment to landless and marginal farmers. The large-sized meat-type animal produces excellent chevon and famous leather. They are considered as moving fertilizer plants and the fixed deposits for the poorest by the virtue of their ready market demand (Gurjar *et al.* 2021).

Thus, characterization of Sojat Goat was undertaken under the Network Project on Animal Genetic Resources (Indian Council of Agricultural Research, ICAR) during 2018-2021 at the College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan (Rajasthan University of Veterinary and Animal Sciences, Bikaner). The project was executed by the ICAR-National Bureau of Animal Genetic Resources, Karnal, the nodal agency for the registration of newly identified germplasm of livestock and poultry of the country. Phenotypic characteristics, management practices, and socio-economic profiles of owners were elucidated (Gurjar *et al.* 2021). Since, it is imperative to understand the existing genetic variability, as well as the heterozygote deficiency status of a population to design scientifically appropriate breeding plans for maintaining variability and purity of the population, genetic variation within the Sojat goat population, was explored using the nuclear microsatellites, also referred to as the simple sequence repeat markers (nSSR). Nuclear SSR has been widely accepted as a useful tool for measuring genetic diversity and divergence within and among livestock populations (FAO 2011).

MATERIALS AND METHODS

Blood sample collection: Sampling was done from the Pali, Jodhpur, and Nagaur districts encompassing the native breeding tract of the Sojat population. Care was taken to select the animals that were true to the Sojat phenotype (Fig.1) and guidelines of the Measurement of Domestic Animal Diversity program were followed in selecting animals (FAO 2011).

Blood samples were collected from unrelated animals belonging to multiple flocks, and across different villages. Farmers were interviewed in detail to ascertain the unrelatedness of collected samples. About 5 ml of blood was aseptically collected by the trained veterinarians from the jugular vein with the consent of the farmers. Samples were brought at 4°C in the laboratory and stored at -20°C until DNA extraction. Genomic DNA was extracted from blood using the standard phenol-chloroform protocol (Sambrook *et al.* 1989). The quantity of DNA was analyzed using a NanoDrop spectrophotometer.

Fig. 1. A representative animal of Sojat goat.

Molecular characterization: Twenty-two FAO (http://dad.fao.org/en/refer/ library/guideline/marker.pdf) and ISAG (International Society for Animal Genetics) recommended simple sequence repeat markers (nSSR) for goats were selected for the diversity analysis. These were highly polymorphic, spread all over the genome, and with the ability to co-amplify in PCR reactions. The forward primer of each marker was 5' labeled with a fluorescent dye (FAM, VIC, NED, and PET). PCR reaction mixture (10 μl) consisted of 10–20 ng of DNA, 0.2 μM of each primer, and PCR master mix (0.2 mM of each dNTP, 2 mM of $MgCl_2$). Negative control with all reaction constituents apart from the template DNA was incorporated to detect any likely contamination. Touchdown protocol was run consisting of initial denaturation of 95°C for 1 min; amplification cycle with steps of denaturation at 95°C for 45 sec, 60-51°C with a decrease of 3°C every third cycle for 1 min, 72°C for 45 sec and 20 cycles of denaturation 95°C for 45 sec, amplification at 48°C for 1 min, extension at 72°C for 45 sec followed by final extension step at 72°C for 5 min. Two loci (ILSTS049 and OarAE129) were amplified with a specific temperature protocol which consisted of initial denaturation of 95°C for 1 min; 32 cycles of 95°C for 30 sec, specific annealing temperature (58 and 60°C, respectively) for 45 sec, 72°C for 45 sec and final extension step at 72°C for 10 min. The amplified products were electrophoresed on a 1.8% agarose gel treated with ethidium bromide (0.5 mg/ml) for visualization of DNA bands under ultraviolet light. PCR products were multiplexed and genotyping was carried out on an automated DNA sequencer using LIZ 500 as the internal size standard. Allele sizing was done using GeneMapper software v3.7.

Statistical analysis: Allelic polymorphisms at each SSR locus were calculated. The genotype data were analyzed using GenAlEx 6.5 software (Peakall and Smouse 2012) to calculate allele frequencies at each locus for each population, the average number of allele per population, observed (Na) and effective numbers of alleles (Ne) and heterozygosity values (observed, Ho and expected, He), Shannon information index (I) and heterozygote deficit (F_{IS}) per locus and across the population. Chi-square tests of deviations from Hardy-Weinberg equilibrium (HWE) were derived. Average values were expressed as

Mean±SE from values at each locus. Bottleneck v1.2.02 (http://www.ensam.inra.fr/URLB) software was utilized to assess bottleneck events in the population following two different approaches. The first approach involved three heterozygosity tests viz. (i) Sign test, (ii) Standardized differences test, and (iii) Wilcoxon sign-rank test developed by Cornuet and Luikart (1996). The probability distribution was established using 1,000 simulations under the Infinite allele model (IAM), step-wise mutation model (SMM), and two-phase model of mutation (TPM). The second method was the graphical representation of the mode-shift indicator (Luikart *et al.* 1998).

RESULTS AND DISCUSSION

Genetic diversity was assessed in a lesser-known Sojat goat population of Rajasthan using polymorphic microsatellite markers. The selected panel of markers effectively explained the genetic variability within the famous but not yet registered goat population of Rajasthan. All microsatellite loci selected for estimating diversity were polymorphic with a total of 162 alleles across 22 loci. The absence of any significant linkage disequilibrium among the 22 loci based on an exact test for genotypic linkage disequilibrium assured the Independent assortment of selected loci. A mean value of 1.29±0.132 for the Shannon's Index (I), which combines both evenness and richness in a single measure (Moges *et al.* 2016) reiterated the potential usefulness of selected microsatellite panel for varied genetic purposes embracing linkage mapping, parentage testing, and individual identification (Table 1). Furthermore, the utility of these markers had been previously suggested by the International Society of Animal Genetics (ISAG)-FAO working group (FAO 2004) and endorsed in 2011 for use in the genetic studies of goats (FAO 2011). These have also been validated for the indigenous goat diversity analysis (Dixit *et al*. 2009).

Genetic variability in Sojat goat population: The estimated values for the number of alleles (observed and effective), heterozygosity, expected heterozygosity, and fixation index based on nSSR markers in the Sojat goat population is given in Table 2. All the 22 microsatellites used in this study manifested sufficient polymorphism for evaluating genetic variation within a breed and exploring genetic differences between the breeds as FAO guidelines for molecular characterization of genetic resources that recommends a minimum of 4 distinct alleles per locus for proficient judgment of genetic diversity was satisfied (FAO 2011). OMHC1 showed the highest number of observed alleles per locus (17) while ETH225, ILSTS065, OarJMP29, ILSTS34 showed the lowest (4).

The use of microsatellites with a range of polymorphisms reduced the risk of overestimating genetic variability, which might occur with microsatellites exhibiting only high polymorphism. The mean observed number of alleles (7.36±0.79) pointed towards the moderate diversity existing in the population. The expected number of alleles varied from 1.12 (ILSTS34) to 7.75 (OMHC1) with a mean of 3.40±0.39. Much higher allelic diversity has been reported in the published literature on goat breeds of India including Changthangi goat of the Himalayan region (10.4±3.91; Mishra *et al.* 2010), Palamu goat of Jharkhand (9.14±2.0; Sharma *et al.* 2021), Black Bengal (8.53±0.26; Vijh *et al.* 2010), Bidri (8.48±0.88) and Nandidurga (8.22±0.66; Tantia *et al.* 2018). Similarly, Rout *et al.* (2008) reported the mean number of alleles in the range of 8.1 (Barbari) to 9.7 (Jakhrana) in the Indian goats. High allelic diversity is an indication of immense genetic variation that may be attributed to cross-breeding or admixture. While, the lesser value indicates low variation due to genetic isolation, historical population bottleneck, or founder effect.

Similarly, a moderate magnitude of diversity was recorded in the Sojat population as the mean observed heterozygosity was 0.54±0.05. Ho values ranged from 0.11(ILSTS34) to 0.85 (ILSTS033). The average observed heterozygosity was less than the values reported for several other Indian goat breeds, viz. Chegu (0.80) and Gaddi (0.75; Singh *et al.* 2015), Berari (0.79; Kharkar *et al.* 2015), Sanagamneri (0.73; Nath *et al.* 2014), Osmanabadi (0.71; Bhat *et al.* 2013), Black Bengal (0.69; Vijh *et al.* 2010), Mahboobnagar (0.69; Raghavendra *et al.* 2017), Palamu (0.64±0.14; Sharma *et al.* 2021) and Nandidurga (0.60; Tantia *et al.* 2018) goats. Sah and Dixit (2021) recently reported a high level of conserved genetic diversity in an extensive study encompassing more than 20 Indian goat breeds. Parallel to our observation, authors also reported lower diversity and a higher inbreeding level in the new lesser-known goat populations compared to the registered breeds. Similarly, much lower values have been observed in the goat populations of the North-Eastern Hill (NEH) region such as Sumi-Ne goat of Nagaland (0.49; Verma *et al.* 2019) and Assam hill goat (0.48; Zaman *et al.* 2013). It means that the Sojat goat has many loci with homozygous alleles as compared to the goat breeds having higher heterozygosities.

The high level of homozygosity could be influenced by multiple factors, such as inbreeding, small population size, and demographic history (Cardoso *et al.* 2018). Expected heterozygosity was higher than the observed (0.60±0.06) which might be due to the low selection pressure, the introduction of new genetic material, and non-random mating in this population (Sah and Dixit 2021). It varied between 0.11 (ILSTS34) and 0.87 (OMHC1). Observed heterozygosity is lower than that expected for a population that is deviating from the Hardy-Weinberg equilibrium (HWE) and hence, the possibility of inbreeding. Significant deviation from HWE was indeed observed at 9 loci (Table 2).

Various factors in a population can lead to deviation from HWE which can be systematic forces such as selection, migration, and mutation, and dispersive forces such as genetic drift and inbreeding. Heterozygote deficiency in the population was also reflected in the positive F value (0.08±0.03) that ranged from -0.25 to 0.39. F values range between –1 (all heterozygote individuals)

Table 1. Microsatellite markers, type of repeat, primer sequences (5´to 3´), labeling dye, amplified product size in Sojat goat and Shannon's index (I)

Locus	Type of repeat	Primer sequence	Fluorescent dye	Allele size range (bp)	I
ETH225	(CA)18	F:gatcaccttgccactatttcct R:acatgacagccaagctgctact	VIC	138-148	0.293
ILSTS044	(GT)20	F:agtcacccaaaagtaactgg R: acatgttgtattccaagtgc	NED	137-173	0.595
ILSTS08	(CA)12	F:gaatcatggattttctgggg R: tagcagtgagtgaggttggc	FAM	169-195	1.116
OarHH64	--	F:cgttccctcactatggaaagttatatatgc R:cactctattgtaagaatttgaatgagagc	PET	124-154	1.419
ILSTS059	(CA)4 (GT)2	F:gctgaacaatgtgatatgttcagg R:gggacaatactgtcttagatgctgc	FAM	109-121	1.337
ILSTS065	(CA)22	F:gctgcaaagagttgaacacc R:aactattacaggaggctccc	PET	111-121	0.474
OarJMP29	(CA)21	F:gtatacacgtggacaccgctttgtac R:gaagtggcaagattcagaggggaag	NED	102-134	0.268
OMHC1	--	F:atctggtgggctacagtccatg R:gcaatgctttctaaattctgaggaa	NED	151-199	2.374
ILSTS033	(CA)12	F:tattagagtggctcagtgcc R:atgcagacagttttagaggg	PET	141-183	1.927
OarE129	(CA)14	F:aatccagtgtgtgaaagactaatccag R:gtagatcaagatatagaatatttttcaacacc	FAM	129-169	1.635
OarFCB48	(CT)10	F:gagttagtacaaggatgacaagaggcac R:gactctagaggatcgcaaagaaccag	VIC	129-171	2.117
ILSTS005	(nn)39	F:ggaagcaatgaaatctatagcc R:tgttctgtgagtttgtaagc	VIC	150-184	1.024
ILSTS019	(GT)10	F:aagggacctcatgtagaagc R;acttttggaccctgtagtgc	FAM	146-156	1.630
ILSTS058	(GT)15	F:gccttactaccatttccagc R:catcctgactttggctgtgg	PET	142-184	1.679
ILSTS87	(CA)14	F:agcagacatgatgactcagc R:ctgcctcttttcttgagagc	NED	140-156	1.104
ILSTS49	(CA)26	F:caattttcttgtctctcccc R:gctgaatcttgtcaaacagg	NED	158-172	1.452
ILSTS29	(CA)19	F:tgttttgatggaacacagcc R:tggatttagaccagggttgg	PET	147-169	1.515
ILSTS30	(CA)13	F:ctgcagttctgcatatgtgg R:cttagacaacaggggtttgg	FAM	163-175	1.816
ILSTS34	(GT)29	F:aagggtctaagtccactggc R:gacctggtttagcagagagc	VIC	157-159	0.208
ILSTS22	(GT)21	F:agtctgaaggcctgagaacc R:cttacagtccttggggttgc	PET	192-250	1.227
ILSTS82	(GT)17	F:ttcgttcctcatagtgctgg R:agaggattacaccaatcacc	PET	82-132	1.984
RM4	(CA)13	F:cagcaaaatatcagcaaacct R:ccacctgggaaggccttta	NED	111-117	1.199
Mean					1.291
SE					0.132

For more information visit Arkdb database (http://www.thearkdb.org); I, Shannon's information index = -1* Sum (pi*Ln (pi); Where pi is the frequency of the i^{th} allele for the population.

to +1 (no observed heterozygotes) quantifying the mean reduction in heterozygosity of an individual due to the non-random mating within a population (Tantia *et al*. 2018). Moreover, the F value reflects the degree of inbreeding and potential of endangerment and thus is regarded as a key tool to evaluate the conservation priority (Simon *et al.* 1993). Therefore, a breed is considered as not endangered (F<0.05), potentially endangered (F, 0.05 to 0.15), minimally endangered (F, 0.25 to 0.40), and critically endangered (F>0.40). A positive value of F indicated the need for scientific management of Sojat goat breeding to avoid further increase in the magnitude of inbreeding. It is

Table 2. Diversity indices and deviation of loci from Hardy-Weinberg Equilibrium in Sojat goat population

Locus	N	Na	Ne	Ho	He	F	ChiSq	Significance
ETH225	45	4	1.30	0.21	0.23	0.07	0.207	ns
ILSTS044	37	6	1.33	0.19	0.25	0.24	17.380	ns
ILSTS08	42	5	2.53	0.50	0.60	0.17	79.534	***
OarHH64	40	7	3.40	0.43	0.71	0.39	26.343	ns
ILSTS059	47	6	3.14	0.55	0.68	0.19	15.241	ns
ILSTS065	47	4	1.30	0.21	0.23	0.07	1.237	ns
OarJMP29	46	4	1.12	0.11	0.11	-0.04	0.152	ns
OMHC1	43	17	7.75	0.81	0.87	0.07	207.003	***
ILSTS033	46	12	5.29	0.85	0.81	-0.05	56.990	ns
OarE129	48	9	3.70	0.65	0.73	0.12	29.403	ns
OarFCB48	48	14	6.29	0.79	0.84	0.06	131.163	**
ILSTS005	44	6	1.92	0.48	0.48	0.00	28.926	*
ILSTS019	44	6	4.55	0.68	0.78	0.13	39.678	***
ILSTS058	44	9	3.96	0.57	0.75	0.24	74.459	***
ILSTS087	44	6	2.34	0.52	0.57	0.09	4.785	ns
ILSTS049	48	6	3.58	0.65	0.72	0.10	66.678	***
ILSTS29	33	8	3.35	0.67	0.70	0.05	78.328	***
ILSTS30	48	7	5.65	0.56	0.82	0.32	57.220	***
ILSTS34	47	4	1.12	0.11	0.11	-0.04	0.148	ns
ILSTS22	44	6	2.81	0.79	0.64	-0.23	12.896	ns
ILSTS82	42	12	5.43	0.79	0.82	0.04	99.084	**
RM4	42	4	3.01	0.83	0.67	-0.25	11.050	ns
Mean	44.05	7.36	3.40	0.54	0.60	0.08		
SE	1.13	0.79	0.39	0.05	0.06	0.03		

N, Sample size; Na, alleles; Ne, effective alleles; Ho, observed heterozygosity; He, expected heterozygosity; F, fixation Index; *P<0.05; **P<0.01; ***P<0.001; ns, non-significant.

important to note that inbreeding leading to genetic erosion may be marked by the loss of fertility, viability, disease resistance, and the recurrent incidence of recessive genetic diseases (Taberlet *et al.* 2008). An overall heterozygote deficiency of 8% in the Sojat goat population is much lower than many Indian breeds including Sumi-Ne (25.8%; Verma *et al.* 2019), Sikkim Singharey (22.5%; Shivahare *et al.* 2017), Changthangi (17.7%; Mishra *et al.* 2010), Bidri (13.6%) and Nandidurga (13.7%) (Tantia *et al.* 2018), Chegu (11.2; Vijh *et al.* 2010), and Palamu (0.09±0.02; Sharma *et al.* 2021).

Genetic bottleneck analysis: Any recent reduction in population size influences the distribution of genetic variation within the population. As a consequence in recently bottlenecked populations, the majority of loci will exhibit an excess of heterozygotes, over and above the heterozygosity expected in a population at mutation drift equilibrium. To estimate the excess of such heterozygosity Sign, Standardized differences and Wilcoxon sign rank tests were utilized for the three models of evolution followed by microsatellites; Infinite allele model (IAM), stepwise mutation model (SMM), and two-phase model of mutation (TPM). Non-significant heterozygote excess based on all the three different models (P>0.05) was revealed by the Wilcoxon rank test.

It indicated that the populations were in mutation drift equilibrium and there was no reduction in population size. However, the Sign test and Standardized difference test did not support the absence of bottleneck event under TPM and SMM models (Table 3). Therefore, a second approach, the Mode-shift indicator test was also utilized as a method to detect potential bottlenecks. The non-bottleneck populations that are near mutation-drift equilibrium are expected to have a large proportion of alleles with low

Table 3. Population bottleneck analysis of Sojat goats

Test/ Model		I.A.M.	T.P.M.	S.M.M.
Sign test (Number of loci with heterozygosity excess)	Exp	12.73	12.93	12.82
	Obs	15	6	4
	P value	0.22365	0.00272*	0.0001400*
Standardized differences test	T2 value	0.478	-2.707	-8.743
	P value	0.31640	0.00339*	0.0*
Wilcoxon rank test (one tail for heterozygosity excess)	P value	0.22170	0.97875	0.99998

*Rejection of null hypothesis, Bottleneck frequency. A graphical representation utilizing allelic class and proportion of alleles showed a normal 'L' shaped distribution (Fig. 2).

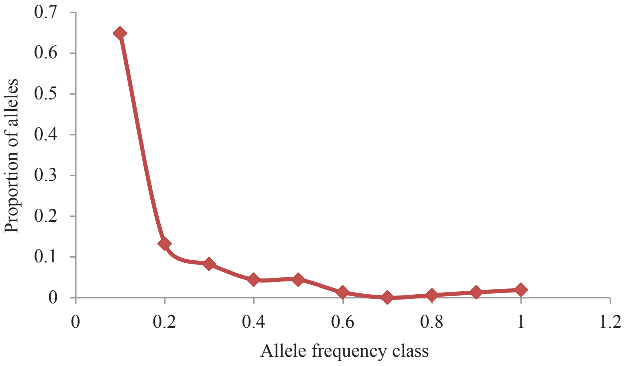

Fig. 2. Mode shift curve depicting lack of bottleneck in the Sojat goat population.

The L-shaped curve indicated the abundance of low frequency (<0.10) alleles. This finding suggested the absence of any detectably large, recent genetic bottleneck (last 40-80 generations) in the existing Sojat goat population.

In conclusion, within-population diversity estimates calculated based on genomic microsatellite markers accomplished that the Sojat goat population has moderate genetic variability. Sojat goat provides nutrition, protection, support service, and above all association to their keepers. They, in lien, require almost nothing from their keepers. The majority of the Rajasthan goat farmers belong to the weakest section of the society. Insufficient crop production due to the inadequate rainfall in the Marwar region makes people dependent on livestock for their livelihoods. Traditional production practice for the Sojat goat did not require extensive resources thus; it is becoming popular as a source of supplementary income for the rural masses. Heavy animals of Sojat goat are increasingly sought now-a-days as the goat meat farming business is growing day-by-day in India. This unique goat is adapted to a wide range of climatic and feeding conditions making it ideal for commercial and stall-fed goat farming. Population diversity parameters generated in the present study will provide baseline data crucial to designing breeding programs for Sojat goats to ensure their effective conservation and proper management.

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