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Basavraj Inamdar
Livestock Research & Information Centre (Sheep), Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

R Nagaraja
Department of Animal Genetics & Breeding, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Ananthakrishna LR
Department of Veterinary Physiology and Biochemistry, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Naveen Kumar S
Department of Animal Genetics and Breeding, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Naveen Kumar GS
AICRP (Poultry), Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Satisha KB
Livestock Research & Information Centre (Sheep), Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

C Kotresh Prasad
Department of Livestock Farm Complex, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Vidyasagar
Department of Livestock Farm Complex, KVAFSU, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Corresponding Author:
Ananthakrishna LR
Department of Veterinary Physiology and Biochemistry, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka, India

Microsatellite marker-based genetic diversity and population structure analysis of Bidri goats

Basavraj Inamdar, R Nagaraja, Ananthakrishna LR, Naveen Kumar S, Naveen Kumar GS, Satisha KB, C Kotresh Prasad and Vidyasagar

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Abstract
The diversity status of Bidri goats of Karnataka was studied by using microsatellite marker analysis. The genomic DNA from unrelated Bidri goats were PCR-amplified with a panel of 10 microsatellite markers. Microsatellite PCR products were run on Metaphore agarose gel and the raw data obtained was analysed. A total of 131 alleles were detected, with allele numbers per locus ranging from 6 to 17, indicating high allelic richness compared to previous studies in Bidri and local goats. Observed heterozygosity ranged from 0 to 1, while expected heterozygosity exceeded 0.5 at all loci, confirming the markers' suitability for diversity assessment. Polymorphism information content (PIC) values were high (mean 0.865), and Shannon's index indicated substantial genetic variability. However, positive inbreeding coefficients (FIS) and significant deviations from Hardy-Weinberg equilibrium at most loci suggested possible inbreeding or population substructure. These findings highlight the considerable genetic diversity retained in Bidri goats, emphasizing the need for conservation and selective breeding strategies to sustain this indigenous meat resource critical to rural livelihoods and meat production in Karnataka. This comprehensive microsatellite-based characterization provides a valuable genetic baseline for future breed improvement and conservation efforts.

Keywords: Bidri goats, microsatellite markers, genetic diversity, allelic frequency, PIC

Introduction
In India, goats are primarily reared by small and marginal farmers, including landless agricultural labourers, and play a vital role in livelihood security, meat production and income generation in resource-poor rural areas. Goat meat contributes substantially to the national meat basket, yet per capita meat availability remains below recommended dietary levels, emphasizing the need to conserve and improve productive indigenous goat resources in a sustainable manner. Systematic characterization of native and region-specific goat populations is the first step in any scientific conservation and genetic improvement programme and can be achieved through phenotypic description, blood group and biochemical polymorphism studies, as well as molecular genetic approaches. Among the latter, microsatellite markers are widely used for preliminary diversity assessments because they are highly polymorphic, co-dominant, selectively neutral, abundant in the genome and largely unaffected by environmental variation, making them powerful tools to quantify genetic diversity, distinctiveness and population structure in goat populations. Microsatellites are simple sequence motifs of two to six bases that are tandemly repeated, arranged head to tail without interruption by other motifs, and usually located in non-coding regions (Litt and Luty, 1989) [2]; being selectively neutral, randomly distributed and showing a co-dominant inheritance pattern, they are particularly suitable for population diversity studies (Luty *et al.*, 1990) [3]. Against this background, the present study focuses on Bidri goats of Karnataka, building on earlier microsatellite-based work on Bidri and other local goats, with the objective of generating an updated and detailed assessment of genetic diversity and population structure to support their long-term conservation and sustainable utilization.

Materials and Methods
Microsatellite marker study was conducted using representative genomic DNA samples obtained from randomly selected flocks of Bidrigoats.

Ten pairs of microsatellite primers were used for PCR amplification of their respective sequences. Genomic DNA was isolated from venous blood samples of 50 goats using the high salt method described by Millers *et al.* (1988) [4] with minor modifications. The DNA quality was confirmed by spectrophotometer readings ranging from 1.7 to 2.0 at 260 and 280 nm, and by the presence of thick, clear, distinct bands on 0.8 per cent agarose gel electrophoresis.

A panel of ten FAO-recommended microsatellite markers (ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS058, ILSTS059, ILSTS087, OarFCB48, and OMHC1) was selected for analysis. The PCR reaction mixture volume was 15.0 µl, containing 1.0 µl template DNA (50 ng/µl), 1.5 µl each of forward and reverse primers (1 pmol/µl), 7.5 µl PCR master mix (2.0 mM), and 3.5 µl nuclease-free water. PCR cycling was optimized with 35 cycles consisting of initial denaturation at 94 °C for 5 minutes, denaturation at 94 °C for 45 seconds, annealing at 60 °C or 63 °C for 30 seconds depending on the marker, and extension at 72 °C for 30 seconds, followed by a final extension at 72 °C for 10 minutes. Annealing temperature was optimized to 60 °C for six markers (ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, OMHC1) and to 63 °C for the remaining four markers (ILSTS058, ILSTS059, ILSTS087, OarFCB48). Specific PCR products were identified by clear band visualization on 1.5 per cent agarose gel electrophoresis.

Amplified products were resolved on 4 per centMetaPhore® agarose gel. Ten microliters of PCR products were run alongside a 20 bp DNA ladder at 100 volts for five hours. Gels were visualized under a UV transilluminator and documented using a Bio-Rad gel documentation system. Allele sizes were estimated using the 20 bp DNA ladder as a molecular size marker (Genei, Bengaluru), and allele sizes were determined using AlphaDigiDoc 1201 computer software.

Allele sizes generated from AlphaDigiDoc 1201 software were organized according to base pair length for all ten markers. Data on allele numbers and frequencies for each microsatellite marker are presented in the Table 4. Genetic diversity parameters including observed number of alleles (na), effective number of alleles (ne), observed heterozygosity (Ho), expected heterozygosity (He), Nei's heterozygosity, Shannon's index (I), polymorphism information content (PIC), inbreeding coefficient (FIS), allelic diversity, and Hardy-Weinberg equilibrium (HWE) for all microsatellite markers were estimated using POPGENE software version 1.32 and summarized in the results Table 5.

Results and Discussion

The different alleles obtained for 10 microsatellite primers were analyzed using POPGENE (Version 1.32) software to assess genetic diversity status in the studied goats. Different genetic diversity measures like allelic frequencies, observed number of alleles (n_a),effective number of alleles (n_e), observed (H_o) and expected heterozygosities (H_e), Nei heterozygocity, Shannon Index (I), Polymorphism Information Content (PIC), F_{IS}, allelic diversity and Hardy-Weinberg equilibrium (HWE) were estimated.

A total number of 131alleles were observed from 10 microsatellite markers for Bidri goats.The observed number of alleles were ranged from 6 to 17 with mean value of 13.1 ± 0.36 and the effective number of alleles were ranged from 4.37 to 12.78 with a mean value of 8.82 ± 0.26 and in the same breed, Tantia *et al.* (2018) [5] have reported average observed and effective number of alleles as 9.30 and 4.25, respectively. Jayshree *et al.* (2019) [7] have reported an average observed and effective number of alleles as 7.60 and 4.49, respectively in local goats of Karnataka.

In the ILSTS008 microsatellite marker, a total of 13 alleles were identified in Bidri goats. For the same marker, Tantia *et al.* (2018) [5] reported 7 alleles each in Bidri and Nandidurga goats, while Jayshree *et al.* (2019) [7] reported 6 alleles in local goats of Karnataka. This indicates a higher allelic richness at ILSTS008 in the present Bidri goat population compared to earlier reports on Bidri, Nandidurga and non-descript local goats.For the ILSTS019 marker, 11 alleles were identified in Bidri goats. In contrast, Tantia *et al.* (2018) [5] reported 7 alleles in Bidri and 8 alleles in Nandidurga goats, whereas Jayshree *et al.* (2019) [7] reported 10 alleles in local goats of Karnataka. The slightly higher number of alleles in the present study suggests relatively greater genetic variability at ILSTS019 in Bidri goats.

At the ILSTS030 marker, 14 alleles were observed in Bidri goats. For this marker, Tantia *et al.* (2018) [5] reported 10 alleles in Bidri and 7 alleles in Nandidurga goats, and Jayshree *et al.* (2019) [7] reported 5 alleles in local goats of Karnataka. The substantially higher allele count in the present Bidri population reflects considerable allelic diversity at ILSTS030 when compared with both recognized breeds and local goats.For the ILSTS034 marker, 6 alleles were identified in Bidri goats. Tantia *et al.* (2018) [5] reported 4 alleles in Bidri and 11 alleles in Nandidurga goats, while Jayshree *et al.* (2019) [7] reported 9 alleles in local goats of Karnataka. In this case, Bidri goats showed a lower number of alleles than Nandidurga and local goats, indicating relatively reduced variability at this locus.

In the ILSTS044 marker, 14 alleles were detected in Bidri goats. For the same marker, Tantia *et al.* (2018) [5] reported 5 alleles in Bidri and 11 alleles in Nandidurga goats, and Jayshree *et al.* (2019) [7] reported 10 alleles in local goats of Karnataka. The higher allelic number in the present study points to enhanced genetic diversity at ILSTS044 in the Bidri population compared with earlier reports.For the ILSTS058 marker, 17 alleles were identified in Bidri goats. Tantia *et al.* (2018) [5] reported 14 alleles in Bidri and 10 alleles in Nandidurga goats, whereas Jayshree *et al.* (2019) [7] reported 11 alleles in local goats of Karnataka. The increased allele number in the present study suggests that ILSTS058 is a highly polymorphic locus in Bidri goats, reflecting rich genetic variability.

At the ILSTS059 marker, 15 alleles were recorded in Bidri goats. For this marker, Tantia *et al.* (2018) [5] reported 8 alleles in Bidri and 7 alleles in Nandidurga goats, and Jayshree *et al.* (2019) [7] reported 7 alleles in local goats of Karnataka. The nearly two-fold increase in allele number over earlier Bidri data again indicates a higher level of polymorphism in the current Bidri population.In the ILSTS087 marker, 17 alleles were observed in Bidri goats. For this marker, Tantia *et al.* (2018) [5] reported 11 alleles in Bidri and 10 alleles in Nandidurga goats, while Jayshree *et al.* (2019) [7] reported 6 alleles in local goats of Karnataka. The markedly higher allelic diversity in the present Bidri sample highlights ILSTS087 as another highly informative locus.

For the OarFCB48 marker, 10 alleles were identified in Bidri goats. In comparison, Tantia *et al.* (2018) [5] reported

12 alleles in Bidri and 11 alleles in Nandidurga goats, and Jayshree *et al.* (2019) [7] reported 5 alleles in local goats of Karnataka. Here, the present Bidri population shows slightly fewer alleles than previously reported for Bidri and Nandidurga, but greater allelic diversity than the local goat population.In the OMHC1 marker, 14 alleles were detected in Bidri goats. For this marker, Tantia *et al.* (2018) [5] reported 15 alleles in Bidri and 9 alleles in Nandidurga goats, and Jayshree *et al.* (2019) [7] reported 6 alleles in local goats of Karnataka. These findings indicate that OMHC1 remains highly polymorphic in Bidri goats, with a level of allelic diversity broadly comparable to that reported earlier and clearly higher than that of local goats.

The observed heterozygosity in Bidri goats ranged from 0 to 1, confirming substantial within-flock genetic variability, while the expected heterozygosity was greater than 0.5 at all loci, indicating that these markers are suitable for assessing genetic diversity in this population. A marker is considered useful for measuring genetic variation in a population when the mean expected heterozygosity lies between 0.3 and 0.8 (Takezaki and Nei, 1996) [6]. In Bidri goats, the average observed heterozygosity (Ho), expected heterozygosity (He) and Nei's heterozygosity were 0.39 ± 0.25, 0.89 ± 0.005 and 0.88 ± 0.005, respectively. For comparison, Tantia *et al.* (2018) [5] reported mean Ho and He values of 0.602 and 0.649, respectively, in Bidri goats, whereas Jayshree *et al.* (2019) [7] reported corresponding values of 0.535 and 0.775 in local goats of Karnataka. In all these populations, He exceeded 0.5, reflecting the diverse nature of these goat populations within their respective breeding tracts.

The polymorphism information content (PIC) in this study ranged from 0.736 (ILSTS034) to 0.916 (ILSTS059), with a mean value of 0.865 in Bidri goats. Jayshree *et al.* (2019) [7] reported a mean PIC value of 0.755 in local goats of Karnataka. PIC represents the ability of a marker to detect polymorphism within a population and is widely used as a general statistical measure of how informative a marker is (Fatima *et al.*, 2008; Bruno-de-Souza *et al.*, 2011) [1, 8]. Microsatellite markers with higher PIC values are considered more informative for genetic diversity studies.

In Bidri goats, Shannon's information index (I) ranged from 1.592 (ILSTS034) to 2.618 (ILSTS059), with a mean value of 2.316, whereas Tantia *et al.* (2018) [5] reported a lower mean value of 1.521 in Bidri goats, and Jayshree *et al.* (2019) [7] reported an average value of 1.666 in local goats of Karnataka. These results indicate a higher level of genetic diversity in the present Bidri population, as Shannon's index quantifies the extent of allelic richness and evenness, and the generally high values across most loci reflect substantial polymorphism and the suitability of these markers for further genetic diversity studies in goat populations of Karnataka.

The inbreeding coefficient (F_{IS}) in Bidri goats ranged from 0.277 (ILSTS059) to 1.000 (ILSTS034), while Tantia *et al.* (2018) [5] reported a mean F_{IS} of 0.039 in Bidri goats with values ranging from-1.475 to 1.000, and Jayshree *et al.* (2019) [7] recorded a mean F_{IS} of 0.353 in local Karnataka goats, ranging from 0.0074 (OARHH64) to 1.000 (ETH225). F_{IS} measures the average reduction in heterozygosity of individuals due to inbreeding within a population, with values ranging from-1 (all individuals are heterozygous) to +1 (no observed heterozygotes), and the relatively high positive values at several loci in the present study suggest a heterozygote deficit that may be associated with inbreeding, population substructure or non-random mating.

All the microsatellite markers studied (ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS058, ILSTS059, ILSTS087, OarFCB48 and OMHC1) showed significant deviation from Hardy-Weinberg equilibrium ($p<0.01$) in Bidri goats, whereas Jayshree *et al.* (2019) [7] reported departure from equilibrium at most loci (14) but not at ETH10, ILSTS065, ILSTS087, OarHH64, OarJMP29, OMHC1, SRCRSP5, SRCRSP8 and SRCRSP23. In multi-allelic microsatellite systems, attainment of Hardy-Weinberg equilibrium may take longer, and the observed disequilibrium in these goat populations could be attributed to factors such as population heterogeneity, gene flow (immigration and emigration of alleles), non-random mating and the relatively small sample size used in the study, all of which can contribute to deviations from equilibrium expectations.

The present study reveals that Bidri goats possess substantial genetic diversity as demonstrated by high allelic richness and polymorphism across all ten microsatellite markers analyzed. The observed heterozygosities confirmed significant within-population variability and expected heterozygosities above 0.5 indicate the usefulness of these markers for genetic diversity studies. The high polymorphism information content (PIC) and Shannon's index further underscore the genetic informativeness and richness in the Bidri population compared to previous reports. However, the positive FIS values and departure from Hardy-Weinberg equilibrium at most loci suggest possible inbreeding, population substructure or non-random mating, which should be considered in future conservation and breeding strategies. The elevated genetic diversity combined with signs of genetic structuring highlight both the uniqueness and vulnerability of Bidri goats, affirming the necessity for targeted conservation programmes. These findings provide a foundational genetic baseline that can guide the sustainable management, improved utilization, and preservation of Bidri goats as a valuable indigenous meat resource in Karnataka and contribute to India's broader goat livestock sector, which remains crucial for food security and rural livelihoods amid shifting production and market dynamics.

Table 1: Details of microsatellite primers utilized for molecular characterization

	Marker name		Sequence	(bp)	Type of repeat	Size range	Chr. no.	Gene Bank Acc. No
1.	ILSTS008	F R	GAATCATGGATTTTCTGGGG TAGCAGTGAGTGAGGTTGGC	20 20	$(CA)_{12}$	167-195	14	L23483 Kemp *et al.*, 1995 [10]
2.	ILSTS019	F R	AAGGGACCTCATGTAGAAGC ACTTTTGGACCCTGTAGTGC	20 20	$(TG)_{10}$	142-162	Ann	L23492 Kumar *et al.*, 2009 [9]
3.	ILSTS030	F R	CTGCAGTTCTGCATATGTGG CTTAGACAACAGGGGTTTGG	20 20	$(CA)_{13}$	159-179	2	L37212 Kumar *et al.*, 2009 [9]
4.	ILSTS034	F R	AAGGGTCTAATGCCACTGGC GACCTGGTTTAGCAGAGAGC	20 20	$(GT)_{29}$	153-185	5	L37254 Kumar *et al.*, 2009 [9]
5.	ILSTS044	F R	AGTCACCCAAAAGTAACTGG ACATGTTGTATTCCAAGTGC	20 20	$(GT)_{20}$	145-177	Ann	L37259 Kumar *et al.*, 2009 [9]
6.	ILSTS058	F R	GCCTTACTACCATTTCCAGC CATCCTGACTTTGGCTGTGG	20 20	$(GT)_{15}$	136-188	17	L37225 Kumar *et al.*, 2009 [9]
7.	ILSTS059	F R	GCTGAACAATGTGATATGTTCAGG GGGACAATACTGTCTTCGATGCTGC	20 20	$(CA)_4$ $(GT)_2$	105-135	13	L37266 Kumar *et al.*, 2009 [9]
8.	ILSTS087	F R	AGCAGACATGATGACTCAGC CTGCCTCTTTTCTTGAGAG	20 19	$(CA)_{14}$	142-164	6	L37279 Kemp *et al.*, 1995 [10]
9.	OarFCB48	F R	GATTAGTACAAGGATGACAAGAGGCA CGACTCTAGAGGATCGCAAAGAACCAG	27 26	$(CT)_{10}$	149-181	17	M82875 Luikart *et al.*, 1999 [11]
10.	OMHC1	F R	ATCTGGTGGGCTACAGTCCAT G GCAATGCTTTCTAAATTCTGAGGAA	22 25	-	179-209	Ann	228 (Ark data base)

F-Forward, R-Reverse, bp-base pair, Ann-Anonymous

Table 2: Optimized PCR reaction mixture used for amplification of different alleles of various microsatellite markers

Sl. No.	PCR Component Quantity	Quantity
1.	2X PCR Master Mix	7.5 µl
2.	Nuclease Free Water	3.5 µl
3.	Forward Primer (1p.mol/µl)	1.5µl
4.	Reverse Primer (1p.mol/µl)	1.5µl
5.	DNA Template	1.0 µl
6.	Total	15.0 µl

Table 3: Optimized thermal profile for amplification of different alleles of various microsatellite markers

Step	Process	Temperature (°C)	Time
1.	Initial denaturation	94	10 min
2.	Cyclic denaturation	94	45 sec
3.	Cyclic annealing	60/63	30 sec
4.	Cyclic extension	72	30 sec
5.	Steps 2 to 4 were repeated 35 times		
6.	Final extension	72	10 min
7.	Refrigeration	4	Forever
8.	End		

The annealing temperature was optimized to 60 °C for 6 microsatellite markers (ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044 and OMHC1) and to 63 ºC for remaining 4 markers (ILSTS058, ILSTS059, ILSTS087 and OarFCB48).

Table 4: Allele size (bp) and frequencies for different microsatellite loci in Bidri goats

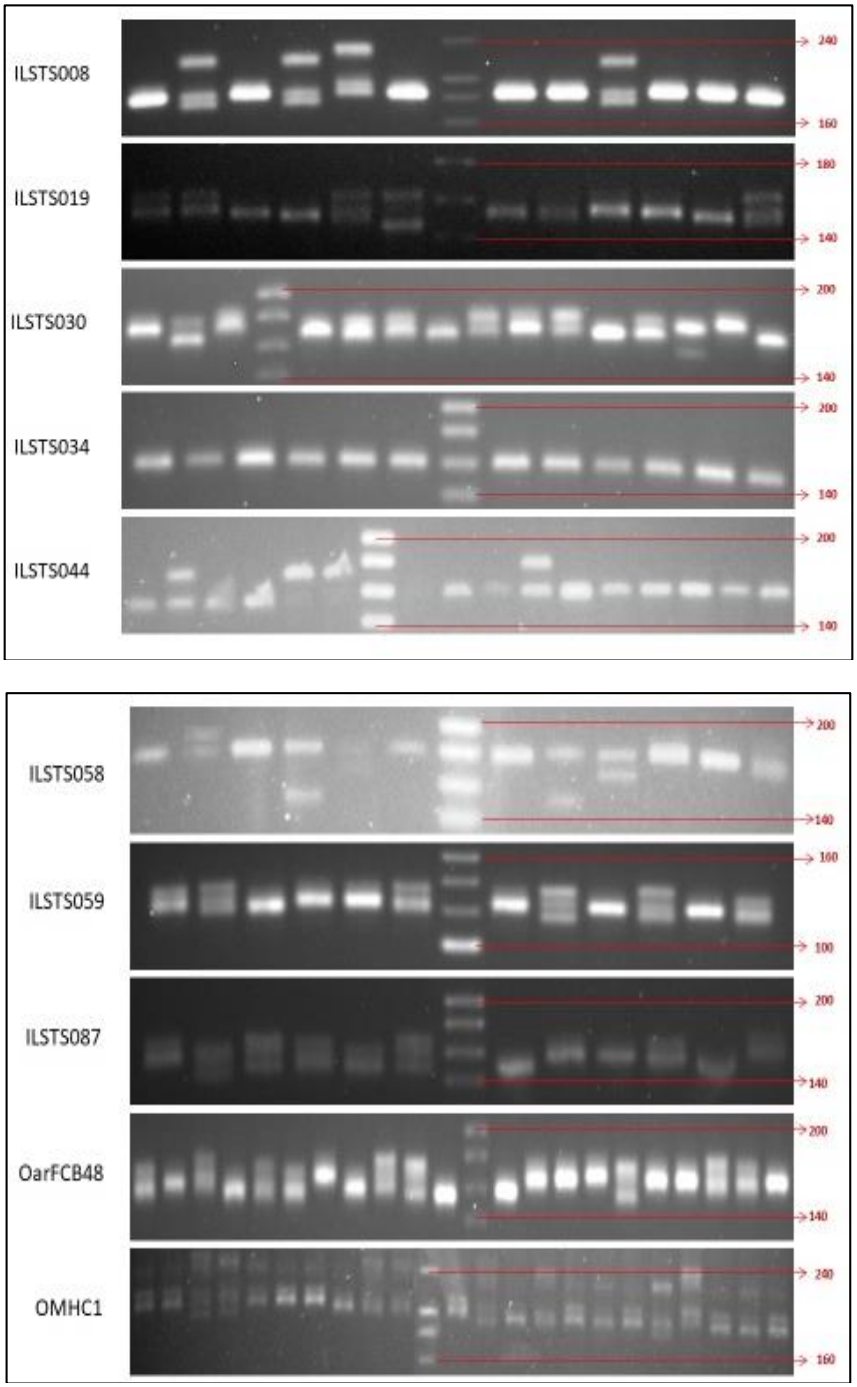
ILSTS008		ILSTS019		ILSTS030		ILSTS034		ILSTS044		ILSTS058		ILSTS059		ILSTS087		OarFCB48		OMHC1	
166	0.100	140	0.048	154	0.152	148	0.048	142	0.087	140	0.042	117	0.024	144	0.024	155	0.125	181	0.048
168	0.050	144	0.071	156	0.044	154	0.048	144	0.087	148	0.021	119	0.095	148	0.048	157	0.200	183	0.024
170	0.050	146	0.024	158	0.130	156	0.191	146	0.065	152	0.021	123	0.095	150	0.048	159	0.100	187	0.048
172	0.050	148	0.095	160	0.022	158	0.238	148	0.065	160	0.042	125	0.048	152	0.048	163	0.225	189	0.238
174	0.050	150	0.119	164	0.087	160	0.333	150	0.044	162	0.042	127	0.071	154	0.167	165	0.100	191	0.048
178	0.075	152	0.310	166	0.065	162	0.143	152	0.044	164	0.042	129	0.048	156	0.143	167	0.125	193	0.024
180	0.175	154	0.119	168	0.109			156	0.044	166	0.021	131	0.024	158	0.191	169	0.025	197	0.071
184	0.075	156	0.048	170	0.130			158	0.044	168	0.042	133	0.071	160	0.048	171	0.050	199	0.119
188	0.100	158	0.024	174	0.065			160	0.196	170	0.042	135	0.095	164	0.024	173	0.025	201	0.119
190	0.100	160	0.095	176	0.044			162	0.174	172	0.063	137	0.095	170	0.024	177	0.025	203	0.024
192	0.125	162	0.048	178	0.022			164	0.022	174	0.125	139	0.048	174	0.048			207	0.119
194	0.025			180	0.065			168	0.022	176	0.083	141	0.071	178	0.048			211	0.024
198	0.025			182	0.022			172	0.087	178	0.208	143	0.119	180	0.024			213	0.048
				184	0.044			180	0.022	180	0.042	145	0.048	182	0.024			217	0.048
										182	0.125	149	0.048	184	0.048				
										188	0.021			186	0.024				
										190	0.021			188	0.024				

The observed numbers of alleles were 13,11,14,6,14,17,15,17,10 and 14 in ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS058, ILSTS059, ILSTS087, OarFCB48 and OMHC1, respectively.

Table 5: Measures of genetic variation in Bidri goats

Locus	Sample Size	n_a	n_e	H_o	H_e	Nei	I	PIC	F_{IS}	Allelic diversity	HWE
ILSTS008	80	13	10.127	0.150	0.913	0.901	2.428	0.893	0.834	0.901	*
ILSTS019	84	11	6.438	0.476	0.855	0.845	2.119	0.830	0.436	0.845	*
ILSTS030	92	14	10.373	0.609	0.914	0.904	2.464	0.894	0.326	0.902	*
ILSTS034	84	6	4.366	0.000	0.780	0.771	1.592	0.736	1.000	0.771	*
ILSTS044	92	14	9.200	0.130	0.901	0.891	2.412	0.882	0.854	0.891	*
ILSTS058	96	17	10.017	0.417	0.910	0.900	2.557	0.904	0.537	0.911	*
ILSTS059	84	15	12.783	0.667	0.933	0.922	2.618	0.916	0.277	0.922	*
ILSTS087	84	17	9.587	0.571	0.907	0.896	2.530	0.887	0.362	0.896	*
OarFCB48	80	10	6.838	0.300	0.865	0.854	2.064	0.837	0.649	0.854	*
OMHC1	84	14	8.481	0.571	0.893	0.882	2.371	0.872	0.352	0.882	*
Total	860	131									
Mean	86	13.1	8.821	0.389	0.887	0.877	2.316	0.865	0.563	0.878	
Std Dev		3.348	2.393	0.232	0.044	0.044	0.311				

n_a-Number of alleles observed; n_e-Effective number of alleles; H_o-Observed heterozygosities; H_e-Expected heterozygosities; I-Shannon Index; PIC-Polymorphism information content; F_{IS}-F statistics; HWE-Hardy Weinberg equilibrium



Photograph of metaphoreagarose gel showing different alleles obtained for microsatellite markers in Bidri goats.

Conclusion

The study demonstrates that Bidri goats exhibit high genetic diversity, reflected through substantial allelic richness, strong polymorphism and elevated heterozygosity across ten microsatellite markers. Comparative analysis with earlier reports further confirms enhanced variability in the present population. High PIC and Shannon's index values indicate the markers' strong informativeness for diversity assessment. However, positive FIS values and consistent deviation from Hardy–Weinberg equilibrium suggest heterozygote deficiency, possibly due to inbreeding or population structuring. These results highlight both the genetic strength and potential vulnerability of Bidri goats, emphasizing the need for informed conservation and breeding strategies to sustain this valuable indigenous resource.

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