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Genetic diversity analysis of Jayawadagi goats by microsatellite markers

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Abstract
Genetic diversity status of Jayawadagi goats was studied by using microsatellite markers. The genomic DNA from Jayawadagi goats of different flocks 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 141 alleles were detected across ten FAO-recommended microsatellite loci, with the observed number of alleles ranging from 7 to 24 (mean 14.1±0.63) and effective alleles from 3.44 to 18.2 (mean 9.30±0.55). The observed heterozygosity (Ho) varied widely within the population (0 to 1), while the expected heterozygosity (He) exceeded 0.5 at all loci, indicating high genetic variability and suitability of the markers for diversity analysis. The polymorphism information content (PIC) values ranged from 0.679 to 0.940, with an average of 0.842, underscoring the informativeness of these loci. Shannon's Information Index averaged 2.298, reflecting substantial genetic diversity, and inbreeding coefficients (F_{IS}) varied from-0.094 to 1, demonstrating different degrees of heterozygosity reduction across loci. All markers significantly deviated from Hardy-Weinberg equilibrium ($p<0.01$), likely due to the multi-allelic nature of microsatellites, gene flow, and sample size effects. These results collectively reveal rich genetic polymorphism within the Jayawadagi goat population, highlighting its potential for sustainable genetic improvement and conservation efforts as an important indigenous breed.

Keywords: Jayawadagi goats, genetic diversity, microsatellite markers, heterozygosity

Introduction
In India, goats are mainly 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 regions. Although goat meat forms a significant portion of the national meat basket, per capita availability remains below recommended dietary levels, highlighting the need for sustainable conservation and improvement of productive indigenous goat resources. Systematic characterization of native and region-specific goat populations is essential as a foundation for scientific conservation and genetic improvement programs. This can be achieved through phenotypic characterization, blood group and biochemical polymorphism studies, along with molecular genetic approaches. Among molecular markers, microsatellites are widely employed for preliminary diversity assessments due to their high polymorphism, co-dominant inheritance, selective neutrality, abundance in the genome, and minimal environmental influence. Microsatellites are tandemly repeated simple sequence motifs of two to six bases found mainly in non-coding regions (Litt and Luty, 1990) [3] and being selectively neutral and randomly distributed with co-dominant inheritance, are particularly suited for population diversity studies (Luty *et al.*, 1990) [3]. Against this backdrop, the present study focuses on microsatellite-based genetic diversity and population structure analysis of Jayawadagi goats to generate an updated and detailed assessment, thereby supporting their long-term conservation and sustainable utilization.

Materials and Methods
The microsatellite marker study was conducted on representative genomic DNA samples collected from randomly selected flocks of Jayawadagi goats. Genomic DNA was extracted from venous blood of 50 individuals using a modified high salt method as described by

Millers *et al.* (1988) [4]. DNA quality was confirmed by spectrophotometric readings at 260/280 nm ranging from 1.7 to 2.0 and by distinct bands on 0.8 per cent agarose gels.
Ten FAO-recommended microsatellite markers (ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS058, ILSTS059, ILSTS087, OarFCB48, and OMHC1) were selected for PCR amplification. The PCR reaction mix (15 µl) included 1 µl template DNA (50 ng/µl), 1.5 µl forward and reverse primers (1 pmol/µl each), 7.5 µl master mix (2.0 mM), and 3.5 µl nuclease-free water. PCR cycling was optimized for 35 cycles with initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 45 s, annealing at 60 °C for six markers and 63 °C for four markers for 30 s, followed by extension at 72 °C for 30 s, and a final extension at 72 °C for 10 min.
PCR products were visualized on 1.5 per cent agarose gel and resolved on 4 per cent MetaPhore® agarose gel alongside a 20 bp DNA ladder at 100 V for 5 hours. Visualization under UV light and documentation were performed using a Bio-Rad system, with allele sizes determined through AlphaDigiDoc 1201 software. Data on allele sizes and frequencies were compiled for all markers (Table 4). Genetic diversity parameters including observed and effective allele numbers (na, ne), observed and expected heterozygosities (Ho, He), Nei's heterozygosity, Shannon's Index (I), Polymorphism Information Content (PIC), inbreeding coefficient (F_{IS}), allelic diversity, and Hardy-Weinberg Equilibrium status were calculated using POPGENE software version 1.32 and summarized in Table 5.

Results and Discussion

The allelic profiles generated from ten microsatellite primers were analysed using POPGENE software (Version 1.32) to evaluate the genetic diversity among the studied goat population. Various measures of genetic variability were estimated, including allelic frequencies, observed number of alleles (na), effective number of alleles (ne), observed heterozygosity (Ho), expected heterozygosity (He), Nei's heterozygosity, Shannon's information index (I), polymorphism information content (PIC), inbreeding coefficient (FIS), allelic diversity, and Hardy-Weinberg equilibrium (HWE).
A total of 141 alleles were identified across the ten microsatellite markers analysed in Jayawadagi goats. The observed number of alleles varied from 7 to 24, with a mean of 14.1±0.63, while the effective number of alleles ranged from 3.44 to 18.2, averaging 9.30±0.55. In comparison, Jayshree *et al.* (2019) [7] reported mean observed and effective allele numbers of 7.60 and 4.49, respectively, in local goat populations of Karnataka.
In the ILSTS008 microsatellite marker, ten alleles were detected in Jayawadagi goats. For the same marker, Tantia *et al.* (2018) [5] reported seven alleles each in Bidri and Nandidurga goats, whereas Jayshree *et al.* (2019) [7] documented six alleles in local goats of Karnataka. In the ILSTS019 locus, sixteen alleles were identified in present study. Tantia *et al.* (2018) [5] observed seven alleles in Bidri and eight alleles in Nandidurga goats, while Jayshree *et al.* (2019) [7] reported ten alleles in local goat populations of Karnataka. At the ILSTS030 marker, eighteen alleles were recorded in Jayawadagi goats compared to ten and seven alleles in Bidri and Nandidurga goats, respectively, as reported by Tantia *et al.* (2018) [5]. Jayshree *et al.* (2019) [7] documented only five alleles for this marker in local goats of Karnataka.
For the ILSTS034 marker, seven alleles were identified in Jayawadagi goats. Tantia et *al.* (2018) [5] reported four alleles in Bidri and eleven in Nandidurga goats, whereas Jayshree *et al.* (2019) [7] found nine alleles in local goats of Karnataka. In the ILSTS044 locus, fourteen alleles were detected in Jayawadagi goats. Reports by Tantia et *al.* (2018) [5] indicated five and eleven alleles in Bidri and Nandidurga goats, respectively, while Jayshree *et al.* (2019) [7] documented ten alleles in local goats. The ILSTS058 marker revealed thirteen alleles in Jayawadagi goats, whereas Tantia et *al.* (2018) [5] recorded fourteen alleles in Bidri and ten in Nandidurga goats; Jayshree *et al.* (2019) [7] observed eleven alleles in the Karnataka local goats.
For the ILSTS059 marker, eight alleles were identified in Jayawadagi goats. The same number was reported in Bidri goats by Tantia et *al.* (2018) [5], who also noticed seven alleles in Nandidurga goats, while Jayshree *et al.* (2019) [7] recorded seven alleles in local goats of Karnataka. In the ILSTS087 marker, nine alleles were observed in Jayawadagi goats. According to Tantia et *al.* (2018) [5], eleven and ten alleles were observed in Bidri and Nandidurgagoats, respectively, whereas Jayshree *et al.* (2019) [7] reported six alleles in Karnataka's local goats.
For the OarFCB48 marker, twenty-two alleles were noted in Jayawadagi goats. In contrast, Tantia et *al.* (2018) [5] reported twelve alleles in Bidri and eleven in Nandidurga goats, while Jayshree *et al.* (2019) [7] recorded only five alleles in local goats of Karnataka. Finally, at the OMHC1 marker, twenty-four alleles were detected in Jayawadagi goats. For this locus, Tantia *et al.* (2018) [5] reported fifteen alleles in Bidri goats and nine in Nandidurga goats, whereas Jayshree *et al.* (2019) [7] documented six alleles in local goats of Karnataka.
In Jayawadagi goats, the observed heterozygosity values ranged from 0 to 1, reflecting variability within the flock. The expected heterozygosity values exceeded 0.5 across all loci, demonstrating the effectiveness of these markers in assessing genetic diversity in this population. According to Takezaki and Nei (1996) [6], a genetic marker is considered suitable for population variation studies when the mean expected heterozygosity ranges between 0.3 and 0.8. The mean observed heterozygosity (Ho), expected heterozygosity (He), and Nei's heterozygosity in Jayawadagi goats were 0.41±0.03, 0.87±0.01, and 0.86±0.01, respectively. In comparison, Jayshree *et al.* (2019) [7] reported average Ho and He values of 0.535 and 0.775, respectively, in local goat populations of Karnataka. The higher He values exceeding 0.5 in Jayawadagi goats indicate a genetically diverse population maintained within its native breeding tract.
The Polymorphism Information Content (PIC) values in Jayawadagi goats ranged from 0.679 (ILSTS059) to 0.940 (OMHC1), with an overall mean of 0.842. Jayshree *et al.* (2019) [7] reported a mean PIC value of 0.755 in local goat populations of Karnataka. PIC represents the ability of a genetic marker to detect polymorphism within a population and serves as a general statistical indicator of its informativeness (Fatima *et al.*, 2008; Bruno-de-Souza *et al.*, 2011) [1, 8]. Microsatellite markers with higher PIC values are considered more informative and effective for assessing genetic diversity.

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 [11]
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 [11]
9.	OarFCB48	F R	GATTAGTACAAGGATGACAAGAGGC ACGACTCTAGAGGATCGCAAAGAACCAG	27 26	$(CT)_{10}$	149-181	17	M82875 Luikart *et al.*, 1999 [10]
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 °C for remaining 4 markers (ILSTS058, ILSTS059, ILSTS087 and OarFCB48).

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

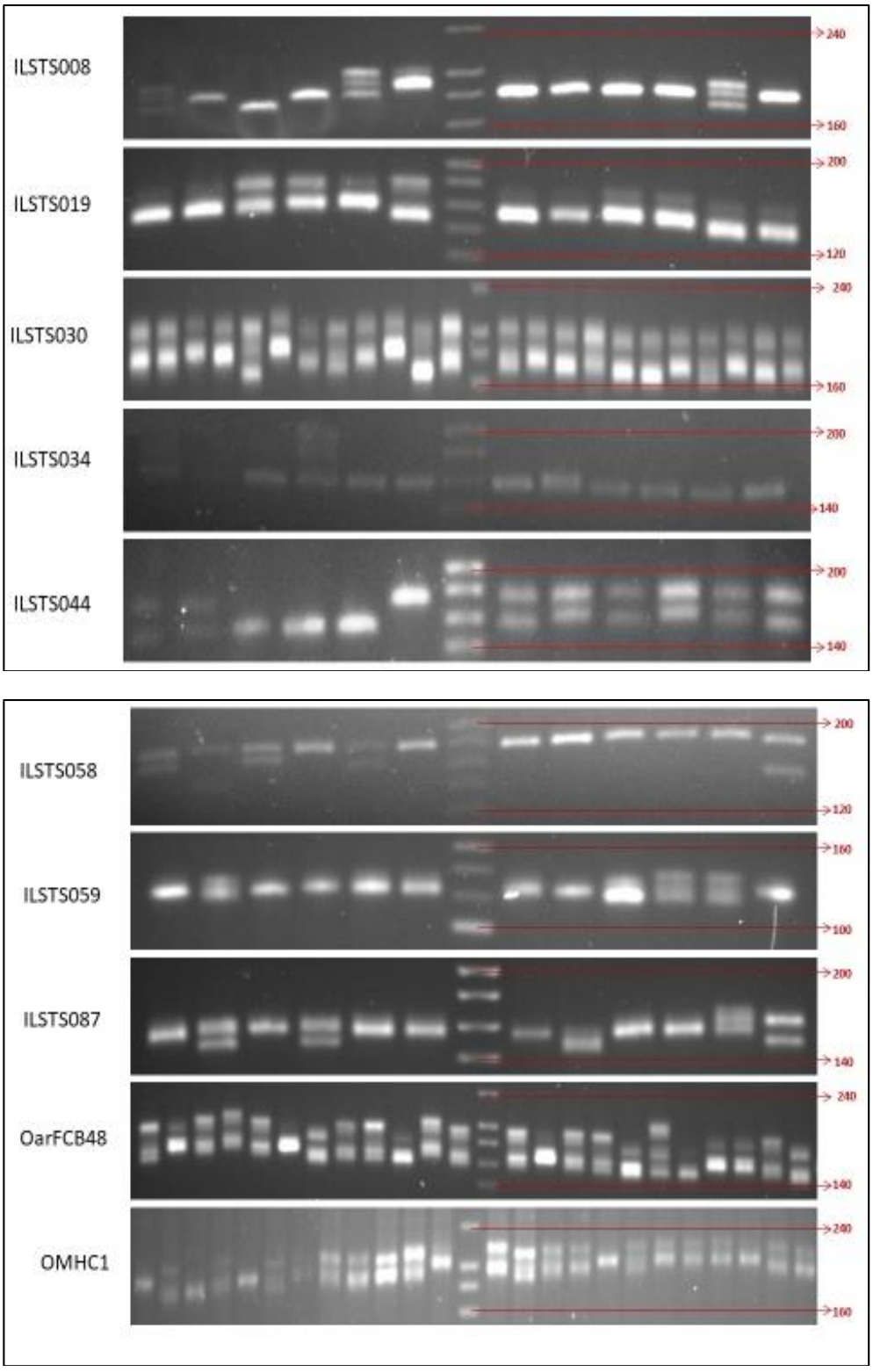
ILSTS008		ILSTS019		ILSTS030		ILSTS034		ILSTS044		ILSTS058		ILSTS059		ILSTS087		OarFCB48		OMHC1	
172	0.075	138	0.083	166	0.050	152	0.091	142	0.159	140	0.021	119	0.024	146	0.130	147	0.024	165	0.023
176	0.150	148	0.042	168	0.050	154	0.136	146	0.023	152	0.063	121	0.119	148	0.065	149	0.095	171	0.068
178	0.250	150	0.188	170	0.050	156	0.136	148	0.159	156	0.021	123	0.476	150	0.087	151	0.024	173	0.023
180	0.025	154	0.042	172	0.075	158	0.227	150	0.046	160	0.021	125	0.191	152	0.087	153	0.095	177	0.046
182	0.200	156	0.021	174	0.150	160	0.091	152	0.136	166	0.021	127	0.095	154	0.196	157	0.024	181	0.091
184	0.050	160	0.063	176	0.050	162	0.273	154	0.046	170	0.021	129	0.024	156	0.217	161	0.024	185	0.046
186	0.100	168	0.042	178	0.050	164	0.046	158	0.068	172	0.021	131	0.048	158	0.109	163	0.048	187	0.068
188	0.100	172	0.042	182	0.025			160	0.023	174	0.063	133	0.024	160	0.065	165	0.095	189	0.023
190	0.025	174	0.021	186	0.025			164	0.046	176	0.042			162	0.044	167	0.071	191	0.023
200	0.025	176	0.021	188	0.025			166	0.046	180	0.042					169	0.048	193	0.023
		178	0.167	190	0.050			170	0.068	182	0.083					173	0.071	197	0.046
		180	0.104	192	0.025			174	0.068	186	0.500					175	0.048	199	0.091
		182	0.063	194	0.025			176	0.046	188	0.083					177	0.048	201	0.046
		200	0.042	196	0.050			178	0.068							179	0.048	203	0.023
		204	0.021	198	0.025											183	0.024	205	0.091
		206	0.042	200	0.175											185	0.024	207	0.023
				202	0.025											187	0.024	211	0.046
				204	0.075											189	0.024	213	0.023
																193	0.024	215	0.023
																197	0.048	217	0.046
																199	0.048	221	0.046
																207	0.024	225	0.023
																		227	0.023
																		229	0.023

The observed numbers of alleles were 10, 16, 18, 7, 14, 13, 8, 9, 22 and 24 in ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS058, ILSTS059, ILSTS087, OarFCB48 and OMHC1, respectively.

Table 5: Measures of genetic variation in Jayawadagi goats

Locus	Sample Size	n_a	n_e	H_o	H_e	Nei	I	PIC	F_{IS}	Allelic diversity	HWE
ILSTS008	80	10	6.452	0.100	0.856	0.845	2.034	0.827	0.882	0.845	*
ILSTS019	96	16	9.931	0.333	0.909	0.899	2.519	0.891	0.629	0.899	*
ILSTS030	80	18	11.594	1.000	0.925	0.914	2.672	0.908	-0.094	0.914	*
ILSTS034	88	7	5.500	0.000	0.828	0.818	1.811	0.794	1.000	0.818	*
ILSTS044	88	14	10.083	0.500	0.911	0.901	2.464	0.893	0.445	0.901	*
ILSTS058	96	13	3.600	0.250	0.730	0.722	1.856	0.708	0.654	0.722	*
ILSTS059	84	8	3.445	0.191	0.718	0.710	1.558	0.679	0.732	0.710	*
ILSTS087	92	9	7.149	0.391	0.870	0.860	2.075	0.845	0.545	0.860	*
OarFCB48	84	22	16.962	0.667	0.952	0.941	2.954	0.938	0.292	0.941	*
OMHC1	88	24	18.264	0.636	0.956	0.945	3.036	0.940	0.327	0.943	*
Total	876	141									
Mean	88	14.100	9.298	0.407	0.866	0.856	2.298	0.842	0.541	0.855	
Std Dev		5.878	5.156	0.301	0.085	0.084	0.504				

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 metaphore agarose gel showing different alleles obtained for microsatellite markers in Jayawadagi goats

In Jayawadagi goats, the Shannon's Information Index (I) ranged from 1.558 (ILSTS059) to 2.954 (OarFCB48), with a mean value of 2.298. Jayshree *et al.* (2019) [7] reported an average Shannon's Index of 1.666 in local goat populations of Karnataka. The Shannon's Information Index reflects the extent of genetic diversity within a population. In the present study, most markers exhibited high Shannon's Index values, indicating substantial polymorphism among the loci analysed. These findings highlight the effectiveness of the selected microsatellite markers for genetic diversity assessment in goat populations of Karnataka.

In Jayawadagi goats, the F_{IS} values ranged from-0.094 (ILSTS030) to 1 (ILSTS034). Jayshree *et al.* (2019) [7] reported a mean F_{IS} value of 0.353 in local goat populations of Karnataka, with estimates varying from 0.0074 (OARHH64) to 1 (ETH225). The F_{IS} statistic reflects the average reduction in heterozygosity of individuals resulting from inbreeding within a population. Its values can range from-1, indicating all individuals are heterozygous, to +1, indicating complete absence of heterozygotes.

All the microsatellite markers analysed (ILSTS008, ILSTS019, ILSTS030, ILSTS034, ILSTS044, ILSTS058,

ILSTS059, ILSTS087, OarFCB48, and OMHC1) showed significant deviation from Hardy-Weinberg equilibrium ($p<0.01$) in the Jayawadagi goat population. Similarly, Jayshree *et al.* (2019) [7] reported deviations from HWE ($p<0.01$) in most loci (14), except for ETH10, ILSTS065, ILSTS087, OarHH64, OarJMP29, OMHC1, SRCRSP5, SRCRSP8, and SRCRSP23 markers. Unlike diallelic systems, equilibrium is attained more slowly in microsatellite loci due to their multi-allelic nature of inheritance. The general deviation from equilibrium observed across goat populations may be attributed to genetic heterogeneity arising from the migration of alleles between populations through immigration and emigration. Additionally, the relatively small sample size used in this study may have contributed to the observed departure from Hardy-Weinberg equilibrium.

The genetic diversity assessment of Jayawadagi goats using ten microsatellite markers revealed substantial polymorphism with a total of 141 alleles identified and a high mean number of observed (14.1±0.63) and effective alleles (9.30±0.55). Observed heterozygosity showed variability within the flock (ranging from 0 to 1), and the high expected heterozygosity values (>0.5) across loci confirmed the markers' suitability for genetic studies. Polymorphism information content (PIC) values were notably high (mean 0.842), indicating informativeness of the markers, while Shannon's Information Index averaged 2.298, reflecting considerable genetic diversity. The inbreeding coefficient (F_{IS}) varied considerably (-0.094 to 1), showing mixed effects of inbreeding and outbreeding across loci. All markers significantly deviated from Hardy-Weinberg equilibrium, likely due to the multiallelic nature of microsatellites, population heterogeneity caused by gene flow, and the relatively small sample size. Collectively, these findings demonstrate that the Jayawadagi goat population maintains rich genetic variability, which is crucial for the conservation and sustainable genetic improvement of this indigenous breed.

Conclusion

The genetic diversity analysis of Jayawadagi goats using ten microsatellite markers revealed high polymorphism, with 141 alleles and substantial mean observed and effective allele numbers. High expected heterozygosity and PIC values confirmed the informativeness of the markers, while Shannon's Index indicated considerable genetic variability. Wide variation in FIS values reflected differing levels of inbreeding across loci. All markers significantly deviated from Hardy–Weinberg equilibrium, likely due to population heterogeneity and small sample size. Overall, the study highlights a genetically diverse Jayawadagi goat population, providing a strong foundation for conservation strategies and future genetic improvement programmes for this indigenous breed.

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