Original Research

Assessment of Genetic Diversity in Chokla Sheep Breed of Rajasthan Using Microsatellite Markers

Kritika Gahlot*, Mukul Purva, Sunil Maherchandani and Sudhir Kumar Kashyap

Department of Veterinary Microbiology and Biotechnology, College of Veterinary and Animal Sciences, Rajasthan University of Veterinary and Animal Sciences, Bikaner-334001, Rajasthan, India

***Corresponding author:** kritikagahlot86@gmail.com

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Abstract

Genetic diversity of Chokla sheep breed was investigated using 18 microsatellite markers suggested by FAO. Total of 150 alleles were detected across 18 microsatellite loci. The highly polymorphic locus was OarFCB48 with 13 alleles and the lowest number of allele was found in FCB128. All the loci were found highly polymorphic with 94% polymorphism in studied population, where mean polymorphic information content value was 0.751, indicating utility of these markers for genome mapping as well as population assignment. Various diversity estimates, mean observed heterozygosity (0.576), mean number of alleles (8.3), effective number of alleles (5.2) and gene diversity (0.783) showed significant within-breed diversity in studied population. However, positive F_{is} value (0.256) indicated considerable level of inbreeding in investigated sheep population. This study is important to understand the genetic diversity of studied population in present scenario for adjudication of demanded breeding programs as well as for creating useful conservation strategies.

Key words: Chokla, Genetic Diversity, Heterozygosity, Inbreeding Coefficient, PIC, Microsatellite Markers

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Introduction

Sheep is a keystone domestic livestock species with over forty indigenous breeds in India (Sharma *et al*., 2016). It plays a crucial role in the biodiversity and livelihood of small and marginal farmers and landless laborers. Chokla, breed of sheep is also known as Chapper and Shekhawati. The wool of this breed is used for manufacturing high quality carpet in Rajasthan. This important breed is found in bordering areas of Bikaner, Jaipur and Nagaur districts of Rajasthan along with Churu, Jhunjhunu and Sikar districts. Among the various molecular markers, microsatellites have shown excellence for breed characterization, paternity

detection, conservation genetics and evolutionary studies as they are found widely distributed throughout the genome (Shamjana *et al*., 2015). Microsatellite markers have been used for characterizing Indian cattle breeds (Sharma *et al*., 2015), goat breed (Yadav *et al*., 2015) as well in sheep breed (Sharma *et al*., 2016). Because of unpredictable crossbreeding, the extraordinary measure of intermixing and habitat destruction, Indian sheep breeds are as of now under risk (Sharma *et al*., 2016). There is requirement for estimating genetic diversity and inbreeding within specific breeds, since their observed declining trend in the population of such breeds (Sodhi *et al*., 2005).

Materials and Methods

Sampling and DNA Isolation

A total of 60 blood samples of genetically unrelated Chokla sheep were randomly collected, 20 samples from Nagaur district, 20 samples from the flock maintained on the farm at Central Sheep and Wool Research Institute, Bikaner and 20 samples from the bordering area of Bikaner district, Rajasthan. Genomic DNA was extracted from whole blood using the QIAamp® DNA Mini kit with slight modification. All DNA samples were analyzed on 1.5% agarose gel through horizontal electrophoresis. Furthermore, p36 Nanophotometer was used to measure the concentration and purity of the DNA sample.

Microsatellite DNA Typing

A total of 18 microsatellite marker loci were selected from the list as suggested by FAO's (FAO, 1996) for ovine, based on their allele size, the level of polymorphism and authenticity of alleles to characterize and uncover the level of genetic diversity in Chokla sheep breed. PCR was carried out in 25 µl reaction volume containing 1.5 mM $MgCl_2$, 200 µMdNTPs, .75µl of each primer, 3.5 µl of template DNA and 0.20 µl of Taq polymerase (Promega, Madison, USA) using Eppendorf master cycler gradient. PCR cycling conditions were 5 min at 95°C, after that 30 cycles of 1 min at 94°C, 1 min at annealing temperature (55–63°C) depending on the primer and PCR conditions standardized, 1 min at 72°C, and the final extension of 7 min at 72°C. The polymorphic typing of microsatellite marker was done on 8% native polyacrylamide gel electrophoresis (PAGE) at 80 V (Hoefer SE 600 series electrophoresis unit) and visualized by ethidium bromide staining. Allele size was estimated utilizing a 100 bp ladder (Promega, USA). The genotype of each individual animal at 18 different loci was recorded by manual counting and data was further analyzed using different statistical tools.

Statistical Analysis

Allele frequency, observed number of alleles (Na), effective number of alleles (N_e), observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated by GenAlEx 6.5 version (Peakall and Smouse, 2006, 2012). Hardy-Weinberg equilibrium (HWE) was estimated using GENEPOP 1.2 version

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(Raymond and Rousset, 1995). Fixation index (F_{is}) was estimated using FSTAT version 2.9.3 (Goudet, 2001). Polymorphic Information Content (PIC) for each locus was estimated according to Botstein *et al.* (1980).

Table 1: Details of microsatellite markers used in sheep of Chokla breed (Sodhi *et al.*, 2005)

Locus	Primer sequence	Allele Size	Chr. No.	Ann. Temp. (in°C)
BM6526	F-CAT GCC AAA CAA TAT CCA GC R-TGA AGG TAG AGA GCA AGC AGC	146-172	26	60
BM757	F-TGG AAA CAA TGT AAA CCT GGG R- TTG AGC CAC CAA GGA ACC	178-198	9	55
BM8125	F- CTC TAT CTG TGG AAA AGG TGG G R- GGG GGT TAG ACT TCA ACA TAC G	107-135	17	55
BM827	F- GGG CTG GTC GTA TGC TGA G R- GTT GGA CTT GCT GAA GTG ACC	210-222	3	55
CSSM31	F- CCA AGT TTA GTA CTT GTA AGT AGA R- GAC TCT CTA GCA CTT TAT CTG TGT	130-170	23	55
OarAE129	F- AAT CCA GTG TGT GAA AGA CTA ATC CAG R- GTA GAT CAA GAT ATA GAA TAT TTT TCA ACA CC	140-164	5	60
OarCP34	F- GCT GAA CAA TGT GAT ATG TTC AGG R- GGG ACA ATA CTG TCT TAG ATG CTG C	110-128	3	63
OarFCB128	F- CAG CTG AGC AAC TAA GAC ATA CAT GCG R- ATT AAA GCA TCT TCT CTT TAT TTC CTC GC	108-134	2	63
OarFCB48	F- GAG TTA TGT ACA AGG ATG ACA AGA GGC AC R- GAC TCT AGA GGA TCG CAA AGA ACC AG	138-166	17	55
OarHH35	F- AAT TGC ATT CAG TAT CTT TAA CAT CTG GC R- ATG AAA ATA TAA AGA GAA TGA ACC ACA CGG	128-160	4	63
OarHH41	F- TCC ACA GGC TTA AAT CTA TAT AGC AAC C R- CCA GCT AAA GAT AAA AGA TGA TGT GGG AG	96-120	6	63
OarHH64	F- CGT TCC CTC ACT ATG GAA AGT TAT ATA TGC R- CAC TCT ATT GTA AGA ATT TGA ATG AGA GC	116-132	4	55
OarJMP29	F- GTA TAC ACG TGG ACA CCG CTT TGT AC R- GAA GTG GCA AGA TTC AGA GGG GAA G	130-150	24	55
OarJMP8	F- CGG GAT GAT CTT CTG TCC AAA TAT GC R- CAT TTG CTT TGG CTT CAG AAC CAG AG	119-131	6	63
OarVH72	F- CTC TAG AGG ATC TGG AAT GCA AAG CTC R- GGC CTC TCA AGG GGC AAG AGC AGG	113-137	25	63
RM4	F- CAG CAA AAT ATC AGC AAA CCT R- CCA CCT GGG AAG GCC TTT A	135-143	15	55
TGLA137	F- GTT GAC TTG TTA ATC ACT GAC AGC C R- CCT TAG ACA CAC GTG AAG TCC AC	119-161	5	55
TGLA377	F- GAC TGT CAT TAT CTT CCA GCG GAG R- GAT CTC TGG TTG AAA TGG CCA GCA G	86-122	2	55

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Result and Discussion

A total of 18 microsatellite markers were successfully amplified and produced clear banding patterns, so that individual genotype can be easily accessed. Numerous genetic diversity measures were utilized to evaluate allele frequency, observed number of allele, effective number of allele, observed heterozygosity, expected heterozygosity and fixation index for each locus. Total 150 alleles were found across all 18 microsatellite loci and allele frequency of selected markers is shown in a Table 2.

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Table 2: Allele frequency across 18 microsatellite loci

Allele/n	BM8125	FCB48	FCB128	HH35	HH41	HH64	JMP8	JMP29	TGLA137	CP34	AE129	BM827	CSSM31	BM6526	BM757	TGLA377	VH72	RM4
1	0.23	0.07	0.38	0.033	0.083	0.1	0.05	0.15	0.017	0.017	0.133	0.05	0.183	0.03	0.133	0.1	0.183	0.017
2	0.15	0.02	0.12	0.217	0.133	0.033	0.283	0.183	0.133	0.2	0.367	0.37	0.067	0.08	0.566	0.033	0.5	0.067
3	0.25	0.17	0.47	0.25	0.15	0.117	0.2	0.033	0.166	0.332	0.017	0.05	0.05	0.13	0.017	0.367	0.083	
4	0.07	0.12	0.03	0.034	0.2	0.2	0.134	0.1	0.2	0.166	0.166	0.3	0.1	0.15	0.25	0.1	0.017	0.033
5	0.07	0.17		0.033	0.267	0.2	0.05	0.1	0.017	0.017	0.017	0.1	0.3	0.33	0.017	0.1	0.017	0.116
6	0.12	0.13		0.05	0.15	0.067	0.2	0.183	0.067	0.067	0.3	0.1	0.117	0.07	0.017	0.3	0.017	
7	0.1	0.1		0.35	0.017	0.017	0.083	0.034	0.133	0.05		0.03	0.067	0.02			0.15	0.016
8	0.02	0.02		0.033		0.183		0.068	0.133	0.117			0.033	0.1			0.033	0.167
9		0.05				0.083		0.033	0.117	0.017			0.017	0.02				0.167
10		0.1						0.083	0.017	0.017			0.067	0.03				0.317
11		0.03						0.033						0.03				0.1
12		0.02																
13		0.02																

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The most polymorphic marker was OarFCB48 with a total of 13 alleles and least polymorphic loci were FCB128 with 4 alleles. The total number of alleles is closer to previously studied Magra sheep breed (144). The overall allele diversity, considered to be a reasonable indicator of genetic variation within the population, displayed high genetic variation (8.3) in Chokla sheep in comparison to previous studied Indian sheep breeds (Sodhi *et al*., 2006; Mukesh *et al*., 2006; Arora and Bhatia, 2006; Arora *et al*., 2011). The value of the effective number of allele (N_e) ranged from 2.486 (BM757) to 8.612 (FCB48) with mean value of 5.2. The effective number of allele (N_e) at each locus was less than the observed number of allele and comparable to earlier reported Magra sheep breed (Arora and Bhatia, 2006). Allelic diversity in terms of observed number of alleles per locus and mean number of alleles across all the loci along with the effective number of alleles is greater in comparison to earlier reported studies (Sodhi *et al., 2006;* Mukesh *et al*., 2006; Sodhi *et al*., 2003; Sodhi *et al*., 2004).

All the loci were highly polymorphic with 94% value of polymorphism in studied population. Polymorphic information content (PIC) value ranged between 0.543 (BM757) and 0.857(FCB48) with a mean value of 0.751. The mean PIC value (0.751) in present study is higher than earlier reported studies on Chokla, 0.605 (Sodhi *et al*., 2006*;* Mukesh *et al*., 2006), Magra, 0.648 (Arora and Bhatia, 2006), Tibetan 0.632 (Sharma *et al., 2016*) and Nali, 0.613 (Sodhi *et al*., 2006*;* Mukesh *et al*., 2006). All loci had high PIC value (>0.5), indicating utility of these markers for population assignment (Tolone *et al*., 2012) as well as genome mapping (Kawka, 2012). All the markers considered for this study are highly informative to characterize Chokla sheep breed population and showed potential to detect genetic diversity in this population. The heterozygosity estimates genetic variability within a population in a considerable manner. Observed and expected heterozygosity at individual loci are present in Table 3. The expected gene diversity (H_{exp}) ranged from 0.598 (BM757) to 0.884 (FCB48) with an overall mean of 0.783. The highest heterozygosity (0.584) was found in samples collected from CSWRI farm, Bikaner followed by samples collected from bordering area of Bikaner district (0.577) and least value of heterozygosity was found in samples obtained from Nagaur district (0.566). The overall observed heterozygosity (H_o) across 18 loci ranged between 0.433 (BM6526) and 0.867 (HH35, FCB128) with a mean value of 0.576. The mean H_o detected in the present study is higher than that reported earlier for Chokla (0.47), Garole (0.44), Nali (0.47) sheep breeds (Mukesh *et al*., 2006) with sample size of 48, 48 and 41 respectively and utilized 11 markers which were common to the markers used in the present study. In some other studies on sheep breeds of northern temperate region namely: Rampur bushair, 0.515 (Pandey *et al*., 2008), Gurej, 0.490 (Gupta *et al*., 2007), Karnah, 0.530 (Gupta and Gannai, 2007) and Tibetan, 0.473 (Sharma *et al*., 2016) also showed comparatively lower heterozygosity as compared to our study and the possible reason could be small sample size, although same set of microsatellite markers was used.

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Table 3: Details on Observed Heterozygosity (H_o), Expected Heterozygosity (H_e), Number of alleles (Na, Observed and N_e, Effective), Polymorphism information content (PIC) and Fixation Index (F_{is})

Locus	H_o (Observed)	H_e (Expected)	Na (Observed)	Ne (effective)	F_{is} (Fixation Index)	PIC (overall)
BM8125	0.733	0.828	8	5.806	0.114	0.806
FCB48	0.467	0.884	13	8.612	0.472	0.857
FCB128	0.867	0.621	4	2.635	-0.397	0.546
HH35	0.867	0.761	8	4.186	-0.139	0.725
HH41	0.833	0.819	7	5.521	-0.018	0.794
HH64	0.7	0.85	9	6.667	0.176	0.832
JMP8	0.667	0.81	7	5.263	0.177	0.784
JMP29	0.767	0.874	11	7.965	0.123	0.853
TGLA137	0.567	0.86	10	7.143	0.341	0.844
CP34	0.567	0.799	10	4.986	0.291	0.774
AE129	0.6	0.729	6	3.696	0.177	0.684
BM827	0.033	0.749	7	3.991	0.956	0.713
CSSM31	0.633	0.836	10	6.081	0.242	0.818
BM6526	0.433	0.823	11	5.66	0.474	0.803
BM757	0.6	0.598	6	2.486	-0.004	0.543
TGLA377	0	0.744	6	3.913	1	0.706
VH72	0.467	0.685	8	3.175	0.319	0.65
RM4	0.567	0.814	9	5.389	0.304	0.792
Mean±SE	0.576±0.057	0.783±0.019	8.3±0.530	5.2±0.409	0.256±0.079	0.751

However, heterozygosity in Magra sheep (0.597) was near to that observed in the present study with 48 samples and 25 markers (Arora and Bhatia, 2006). Moreover, pelt sheep namely; Gray (0.984), Zandi (0.985) and Karakul (0.988) (Nanekarani *et al*., 2015), showed higher heterozygosity in comparison to Chokla sheep where 360 samples and 15 different set of microsatellite markers were used. The expected heterozygosity (0.783) obtained in present study is higher than earlier reported studies (Sodhi *et al., 2006;* Mukesh *et al*., 2006; Arora *et al*., 2011; Sharma *et al*., 2016) but lower than that of Pelt sheep (Nanekarani *et al*., 2015). The present study also revealed that overall expected heterozygosity (H_e) is higher than observed heterozygosity (H_o) hence, showing departure from Hardy-Weinberg equilibrium (HWE) and possibility of inbreeding. This was also reflected in positive F_{is} value (0.256±0.079), which ranged from -0.397 to 1.000. The mean F_{is} value (0.256) is in similar range with earlier reported study of same breed (Sodhi *et al., 2006;* Mukesh *et al*., 2006) and found higher than Magra (0.159), Sahabadi (0.215) and Rampur-Bushair (0.227). However, F_{is} value found high in Tibetan sheep (0.302) than Chokla, mainly due to confinement of Tibetan sheep breed in specific area causing more inbreeding.

Conclusion

The present study of genetic variability in Chokla sheep breed of North-western arid region of Rajasthan was estimated using 18 microsatellite markers. All the loci were highly polymorphic with 94% value of polymorphism observed in Chokla sheep breed. The high PIC value indicates informativeness of markers

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used to characterize Chokla sheep population. In spite of decline in the animals of this breed; population is showing reasonable genetic variability within the breed in terms of allelic variation. However, the differences between mean observed and expected heterozygosity suggested, tendency of markers towards heterozygote deficiency and same is reflected by positive inbreeding estimation (F_{is}) value. The probable causes of heterozygote deficiency may be because of segregation of non-amplifying (null) alleles, Wahlund effect (population substructure) or other reasons.

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