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Microsatellite Markers as a Tool for Characterization of Small Ruminants: A Review

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A B S T R A C T

Microsatellites are short tandem nucleotide repeats usually 2-6 bp, highly polymorphic in nature, and are found scattered throughout the genome of eukaryotes. Microsatellites are also called short tandem repeats (STR) or simple sequence repeats (SSR), due to the nature of nucleotide repeats which can be mono, di, tri, penta and hexa. They are the most commonest markers used for genetic characterization in various farm animal, which is based on DNA sequence polymorphisms, which are identified by constructing PCR primers for the DNA flanking the microsatellite region. The genetic characterization of domestic animals is part and first step of the FAO for the Management of Farm Animal Genetic Resources. To conserve a population it is important to know how unique or how different it is from other populations. For molecular characterization of native livestock and poultry breeds using microsatellite markers a number of research programs have been undertaken. In order to study animal biodiversity, conservation and management of animal genetic resources the information regarding molecular characterization of indigenous animals very useful.

Introduction

On the basis of polymorphism occurring at different levels like morphological, biochemical, cellular and DNA level, genetic diversity can be measured. Earlier, blood groups and biochemical polymorphisms were mainly used to study evolutionary relationships in different livestock species (Khanna and Braend, 1968; Khanna, 1979; Schlotterer, 2004). However, with these classical markers it was difficult to measure genetic divergence and gene flow among closely related populations because they possess low discriminating power (VanZeveran *et al.,* 1995; Goldstein and Schlotterer, 1999) the reason behind that was extremely low mutation rates. In genome studies, with the advent of time much attention has been focused on the level of variability at the DNA level. Molecular markers capable of detecting the genetic variation both within coding as well as non-coding sequences of DNA have been developed and has became

possible to uncover a large number of genetic polymorphisms at the DNA level. The DNA polymorphism as molecular markers uncovered many gates in genetic characterization, improvement, conservation, and molecular evolution studies in different livestock species. For a particular species there are particular genetic markers which are used in estimation of evolutionary history, population subdivision, dispersal, gene flow, effective population size, extended pedigrees, levels of relatedness, breeding structure, etc. (Blot *et al.,* 1998; Goldstein and Schlotterer, 1999) Based on function, genetic markers have divided in two groups (O'Brien, 1992): Type I markers, which are gene based with low degree of polymorphism, but have extensive evolutionary conservation. And Type II markers which are highly, but they are not well conserved between species.

For detecting diversity and breed differentiation a number of techniques are available using molecular markers, including restriction fragment length polymorphisms (RFLP), variable number of tandem repeats (VNTR), denaturing gradient gel electrophoresis (DGGE), single strand conformational polymorphism (SSCP), random amplified polymorphic DNA (RAPD) and microsatellites. Microsatellite makes use of polymorphism of the short tandem repeats. Microsatellites are contributing the most in diversity studies because of their high polymorphic nature, ease of amplification by PCR and abundance in the genome (Dodgson *et al.,* 1997). FAO has recommended 30 microsatellite markers each for Sheep (Table 1) and Goat (Table 2) (FAO, 2011).

History in India

In India value and need for the characterization and conservation of different livestock genetic resources has been very well realized. National Bureau of Animal Genetic Resources (NBAGR) which was established in 1984 is considered as the beginning of the efforts for characterization and conservation of livestock genetic resources. At the beginning efforts made by NBAGR and other research groups in India were concentrated on the morphological (Joshi *et al.,* 1995; Nivsarkar *et al.,* 2000; Sahana, 2000; Pundir *et al.,* 2003; Pundir *et al.,* 2004; Verma *et al.,* 2005; Vij *et al.,* 2006) and biochemical marker based (Khanna and Singh, 1970; Singh and Bhat, 1980) characterization of the livestock. However, in 1997, at National Bureau of Plant genetic resources, New Delhi, it was decided that microsatellite markers would be used for molecular characterization of native livestock. In this decision Food and Agricultural Organization (FAO) of the United Nations, made a programme for the global management of genetic resources of various livestock species, in this Project DADIS, MoDAD was formulated, in which suggestion regarding the use of species specific microsatellite loci was made. In diversity analysis it was recommended to use 30 microsatellite loci recommended by FAO.

In the beginning manual genotyping using urea-PAGE was used to study diversity in which allele sizes were determined by running a 10bp ladder along with the samples and by direct counting method genotype of individual was recorded. Sahiwal was first cattle breed among cattle which came in focus (Mukesh *et al.,* 2004). Various research groups in the country started the characterization of their regional breeds (Metta *et al.,* 2004) based on the footprints set by NBAGR. By this manual approach different breeds were characterized. However, manual genotyping being time consuming and cumbersome technique was not very successful. In genetic characterization studies microsatellite markers have become the most frequently used markers among all other markers. In India, almost all the descript breeds of different livestock have been

characterized by using microsatellite markers. The studies done by (Sodhi *et al.,* 2005 and 2010; Mukesh *et al.,* 2004 and 2006; Sharma *et al.,* 2006; Arora and Bhatia, 2009a and 2009b) have indicated the presence of adequate genetic diversity in the studied Indian native breeds.

Applications of microsatellite markers

In present days microsatellite markers are the markers of choice for a wide range of molecular genetic studies such as establishing genetic linkage maps (Arora *et al.,* 2004), population structures and mating system analysis.

They are used in the study of genetic variation in vertebrates (Ganai and Yadav, 2001) and in other livestock species viz., poultry, sheep, goats, buffaloes and bovines (Li and Valentini, 2004). These markers are neutral and co-dominant hence, helps in detection of homozygote and heterozygote genotypes. Gene duplication and deletions can be studied by these markers. They are also ideal for paternity determination, recombination mapping and population genetic studies.

Microsatellite markers in genetic characterization

Microsatellites are short tandem nucleotide repeats, highly polymorphic in nature and these are found scattered throughout the genome of eukaryotes (Ellegren, 2004). Microsatellites in different farm animals have been used to measure genetic diversity (Handley *et al.,* 2007).

In this the polymorphisms are identified by constructing PCR primers for the DNA flanking the microsatellite region (Hajeer *et al.,* 2000). Useful molecular information has been provided by microsatellite markers that could help in the future management of the small stock breeds (Boettcher *et al.,* 2010). A microsatellite locus varies in length between 5 and 40 repeats, however longer strings of repeats are also possible. For molecular genetic studies dinucleotide, trinucleotide and tetranucleotide repeats are common choices. Among these dinucleotide repeats account for the majority of microsatellites (Li *et al.,* 2002).

Trinucleotide and hexanucleotide repeats are likely repeat classes to appear in coding regions as they do not cause a frame shift (Toth *et al.,* 2000). Because of problems with amplification mononucleotide repeats are less reliable (Li *et al.,* 2002). For genetic diversity study microsatellites have become markers of choice (Sunnucks, 2000) and are recommended markers for characterizing various farm animal genetic resources, because of their co-dominant nature and ease of amplification. In the last few decades the development of molecular tools has made an important contribution to characterize variation within and between breeds (Toro *et al.,* 2009).

Useful information for assessing, among other factors, the amount of genetic diversity, the structure of diversity in samples and populations, the rates of genetic divergence among populations and the distribution of diversity in populations found in different locations has been provided by molecular characterization information (Hanotte and Jianlin, 2005; Toro *et al.,* 2009). Molecular characterization has a role in understanding of gene flow, the movement of alleles within and between populations of the same or related species, and its consequences (Toro *et al.,* 2009). Molecular characterization helps in genetic management of small populations, to avoid excessive inbreeding. Information from molecular markers is the basis for improving conservation approaches (Hanotte and Jianlin, 2005).

Studies carried on molecular characterization of small ruminants (Sheep and Goats) using microsatellites as DNA makers

Studies on sheep

Arranz *et al.,* (2001) using 18 microsatellites studied genetic variability in five Spanish breeds of sheep viz., Churra, Latxa, Castellana, Rasa-Aragonesa and Merino. About 7% of total variability of population subdivision was calculated between Spanish breeds from F_{ST} diversity indices. From 18 loci a high degree of reliability was obtained for individual-breed assignment using different approaches among which the Bayesian method was most efficient with an accuracy of 9 microsatellites of over 99%.

Alvarez *et al.,* (2004) 14 microsatellites studied genetic relationship among sheep breeds from Northern Spain belonging to 6 sheep breeds determining the relative genetic contributions existing between populations and their historical relationships. In order to assess the existence of underlying genetic structure individual genotypes were analyzed.

Arora and Bhatia (2006) using 25 microsatellite markers studied genetic diversity of Magra sheep breed of India. All used microsatellites were polymorphic. The observed heterozygosity ranged from 0.200 (BM6506) to 0.947 (OarHH35) and the expected heterozygosity ranged from 0.368 (CSSM47) to 0.864 (BM1314). PIC value varied from 0.347 (CSSM47) to 0.849 (BM1314). Inbreeding was reflected by the high average inbreeding coefficient (F_{IS} = 0.159) which is due to the unequal sex ratio of the breeding animals.

Girish *et al.,* (2007) using 25 microsatellite markers studied Genetic variation in Nilagiri sheep. The mean number of observed alleles was 5 across all loci ranging from 3 to 8. A total of 125 alleles were observed at the 25 loci, all of 25 loci were highly polymorphic. The mean effective number of alleles was 3.84 ranging from 2.18 to 6.49. The mean PIC value was 0.6485 ranging from 0.4587 to 0.8277. 17 microsatellites were in Hardy-Weinberg Equilibrium out of all 25. The mean observed and expected heterozygosity was 0.7610 and 0.7213 respectively. Except 6 loci, all other loci showed negative inbreeding estimates (F_{IS}) indicating excess of heterozygotes in the population.

Pandey *et al.,* (2008) using 20 microsatellites studied genetic variability in Rampur Bushair sheep. The overall mean of observed number of alleles was 6.0±1.91 ranging from 2 to 9. The overall mean effective number of alleles was 3.47±1.29. The average observed and expected heterozygosity was O.515±0.18 and O.675±0.14. The average F_{IS} was O.227±O.05 indicated inbreeding in the population.

Pramod *et al.,* (2009) using 25 microsatellite markers studied molecular characterization of Vembur sheep of Tamil Nadu. A total of 147 alleles were found, with a mean of 5.88±0.29 alleles per locus. The mean effective number of alleles observed was 4.0950±0.23 alleles per locus and mean PIC value was 0.6905±0.02. The mean observed and expected heterozygosity (H_o) values were 0.5202±0.04 and 0.7339±0.02 respectively, varied between 0.1333 to 1.0000 and 0.4847 to 0.8537 respectively. Negative values of FIS for three loci indicated more heterozygous nature of the population.

Sharma *et al.,* (2010) using 25 microsatellite markers studied genetic diversity in Changthangi sheep. 219 alleles in total were detected. All the Microsatellite markers were highly polymorphic, with mean of observed number of alleles was 8.760±0.587 ranging from 4 to 15 per locus. The mean of expected

number of alleles was 4.539±0.412 ranging from 1.144 (CSSM47) to 10.509 (OarCP49). The mean observed heterozygosity was 0.691±0.039 ranging from 0.053 (CSSM47) to 0.912 (OarCP49) which indicates the presence of substantial amount of genetic variability in the sheep breed.

Zhong *et al.,* (2011) using 26 microsatellites studied the genetic diversity and structure of Mongolian sheep in China. The Bayesian clustering was done which indicated five clusters as the most probable genetic structure of the populations. In three populations distributed at large geographical scales a clear genetic structure was revealed, while the other cluster encompassed UQ and HLBR sheep that displayed no clear differentiation, which may be due to their close and small geographical distributions.

Hoda and Marsan (2012) using 31 microsatellite markers recommended by MoDAD/FAO studied genetic characterization, genetic diversity, genetic relationship and structure of local sheep in Albania. Raja *et al.,* (2012) using 24 Microsatellite markers studied genetic diversity in Ramnad White sheep. GENEMAPPER software was used for allele sizing.

Yadav and Arora (2014) based on microsatellite markers studied the genetic differentiation in Muzaffarnagri and Munjal sheep. Microsatellite markers data showed high level of allelic richness (>6.0) and genetic diversity (>0.7) for both breeds/populations. Bayesian cluster analysis was done which indicated distinct clusters with an average membership coefficient of 0.974 for Muzaffarnagri and 0.972 for Munjal sheep.

Kavitha *et al.,* (2015) using 25 microsatellite markers studied genetic characterisation of Tiruchy Black sheep of Tamil Nadu. A total of 203 alleles were observed. The mean number of alleles observed was 8.12±0.45 ranging from 4 (OarHH41) to 13 (OarHH35). The mean effective number of alleles was 4.48±0.27 ranging from 1.59 (OarHH41) to 7.51 (OarHH35). The mean PIC was 0.8441±0.012 ranging from 0.7029 (OarHH41) to 0.9164 (OarHH35). As per $\chi 2$ test only 10 among the 25 microsatellites studied were in Hardy-Weinberg Equilibrium proportions.

Sassi-Zaidy *et al.,* (2016) using microsatellite markers studied genetic diversity and genetic relationship of the two groups of African sheep, thin-tailed and fat-tailed sheep, represented by the indigenous Tunisian sheep breeds Barbarine (BAR, fat-tailed) and Queue Fine de Ouest (QFO, thin-tailed). The mean of observed heterozygosity was 0.719 and mean of expected heterozygosity was 0.789. A notable level of inbreeding within the whole population was seen described by average F_{IS} (0.112) and F_{IT} (0.118) values.

Studies on goat

Ganai and Yadav (2001) using 16 microsatellite markers studied the genetic dsistance and variation in three Indian goat breeds. The mean value of heterozygosity was 0.54±0.2 and PIC value was 0.4±0.20. The study showed the closer relationship between Jamnapari and Barbari goat breeds.

Kim *et al.,* (2002) using microsatellites analysed the genetic diversity of the three domestic goat breeds Korean goat, Chinese goat and Saanen found in Korea and China. The expected mean heterozygosity, were H_e=0.381 (Korean goat), and H_e=0.669 (Chinese goat). The results showed genetic differentiation between Korean goat and the other two breeds, indicating that these breeds have been genetically subdivided.

Table.1 Recommended microsatellite markers for sheep

Name(s)	Chromosome	Primer sequence (5' -> 3') Forward Reverse	Annealing temperature (°C)
OarFCB128	OAR2	ATTAAAGCATCTTCTCTTTATTTCCTCGC CAGCTGAGCAACTAAGACATACATGCG	55
OarCP34	OAR 3	GCTGAACAATGTGATATGTTCAGG GGGACAATACTGTCTTAGATGCTGC	50
OarCP38	OAR 10	CAACTTTGGTGCATATTCAAGGTTGC GCAGTCGCAGCAGGCTGAAGAGG	52
OarHH47	OAR 18	TTTATTGACAAACTCTCTTCCTAACTCCACC GTAGTTATTTAAAAAAATATCATACCTCTTAAGG	58
OarVH72	OAR 25	GGCCTCTCAAGGGGCAAGAGCAGG CTCTAGAGGATCTGGAATGCAAAGCTC	57
OarAE129	OAR 5	AATCCAGTGTGTGAAAGACTAATCCAG GTAGATCAAGATATAGAATATTTTTCAACACC	54
BM1329	OAR 6	TTGTTTAGGCAAGTCCAAAGTC AACACCGCAGCTTCATCC	50
BM8125	OAR 17	CTCTATCTGTGGAAAAGGTGGG GGGGGTTAGACTTCAACATACG	50
HUJ616	OAR 13	TTCAAACTACACATTGACAGGG GGACCTTTGGCAATGGAAGG	54
DYMS1	OAR 20	AACAACATCAAACAGTAAGAG CATAGTAACAGATCTTCCTACA	59
SRCRSP9	CHI12	AGAGGATCTGGAAATGGAATC GCACTCTTTTCAGCCCTAATG	55
OarCB226	OAR 2	CTATATGTTGCCTTTCCCTTCCTGC GTGAGTCCCATAGAGCATAAGCTC	60
ILSTS5	OAR 7	GGAAGCAATGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	55
ILSTS11	OAR 9	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	55
ILSTS28	OAR 3	TCCAGATTTTGTACCAGACC GTCATGTCATACCTTTGAGC	53
SRCRSP5	OAR 18	GGACTCTACCAACTGAGCTACAAG GTTTCTTTGAAATGAAGCTAAAGCAATGC	56
MAF214	OAR 16	GGGTGATCTTAGGGAGGTTTTGGAGG AATGCAGGAGATCTGAGGCAGGGACG	58
SRCRSP1	CHI13	TGCAAGAAGTTTTTCCAGAGC ACCCTGGTTTCACAAAAGG	54
MAF33	OAR 9	GATCTTTGTTTCAATCTATTCCAATTTC GATCATCTGAGTGTGAGTATATACAG	60
MCM140	OAR 6	GTTCGTACTTCTGGGTACTGGTCTC GTCCATGGATTTGCAGAGTCAG	60
OarFCB20	OAR 2	AAATGTGTTTAAGATTCCATACAGTG GGAAAACCCCCATATATACCTATAC	56
OarFCB193	OAR 11	TTCATCTCAGACTGGGATTCAGAAAGGC GCTTGGAAATAACCCTCCTGCATCCC	54
OarFCB304	OAR 19	CCCTAGGAGCTTTCAATAAAGAATCGG CGCTGCTGTCAACTGGGTCAGGG	56
OarJMP29	OAR 24	GTATACACGTGGACACCGCTTTGTAC GAAGTGGCAAGATTCAGAGGGGAAG	56
OarJMP58	OAR 26	GAAGTCATTGAGGGGTCGCTAACC CTTCATGTTCACAGGACTTTCTCTG	58
MAF65	OAR 15	AAAGGCCAGAGTATGCAATTAGGAG CCACTCCTCCTGAGAATATAACATG	60
MAF70	OAR 4	CACGGAGTCACAAAGAGTCAGACC GCAGGACTCTACGGGGCCTTTGC	60
MAF209	OAR 17	GATCACAAAAAGTTGGATACAACCGTGG TCATGCACTTAAGTATGTAGGATGCTG	63
BM1824	OAR 1	GAGCAAGGTGTTTTTCCAATC CATTCTCCAACTGCTTCCTTG	58
INRA063	OAR 14	ATTTGCACAAGCTAAATCTAACC AAACCACAGAAATGCTTGGAAG	58

Table.2 Recommended microsatellite markers for goat

Name(s)	Chromosome	Primer sequence (5' -> 3') Forward Reverse	Annealing Temperature (°C)
SRCRSP5	CHI21	GGACTCTACCAACTGAGCTACAAG TGAAATGAAGCTAAAGCAATGC	55
MAF065	OAR15	AAAGGCCAGAGTATGCAATTAGGAG CCACTCCTCCTGAGAATATAACATG	58
MAF70	BTA4	CACGGAGTCACAAAGAGTCAGACC GCAGGACTCTACGGGGCCTTTGC	65
SRCRSP23	unknown	TGAACGGGTAAAGATGTG TGTTTTTAATGGCTGAGTAG	58
OarFCB48	OAR17	GAGTTAGTACAAGGATGACAAGAGGCAC GACTCTAGAGGATCGCAAAGAACCAG	58
INRA023	BTA3	GAGTAGAGCTACAAGATAAACTTC TAACTACAGGGTGTTAGATGAACT	58
SRCRSP9	CHI12	AGAGGATCTGGAAATGGAATC GCACTCTTTTCAGCCCTAATG	58
OarAE54	OAR25	TACTAAAGAAACATGAAGCTCCCA GGAAACATTTATTCTTATTCCTCAGTG	58
SRCRSP8	Unknown	TGCGGTCTGGTTCTGATTTCAC GTTTCTTCCTGCATGAGAAAGTCGATGCTTAG	55
SPS113	BTA10	CCTCCACACAGGCTTCTCTGACTT CCTAACTTGCTTGAGTTATTGCCC	58
INRABERN172	BTA26	CCACTTCCCTGTATCCTCCT GGTGCTCCCATTGTGTAGAC	58
OarFCB20	OAR2	GGAAAACCCCCATATATACCTATAC AAATGTGTTTAAGATTCCATACATGTG	58
CSRD247	OAR14	GGACTTGCCAGAACTCTGCAAT CACTGTGGTTTGTATTAGTCAGG	58
McM527	OAR5	GTCCATTGCCTCAAATCAATTC AAACCACTTGACTACTCCCCAA	58
ILSTS087	BTA6	AGCAGACATGATGACTCAGC CTGCCTCTTTTCTTGAGAG	58
INRA063	CHI18	GACCACAAAGGGATTTGCACAAGC AAACCACAGAAATGCTTGGAAG	58
ILSTS011	BTA14	GCTTGCTACATGGAAAGTGC CTAAAATGCAGAGCCCTACC	58
LSTS005	BTA10	GGAAGCAATTGAAATCTATAGCC TGTTCTGTGAGTTTGTAAGC	55
SRCRSP15	Unknown	CTTTACTTCTGACATGGTATTTCC TGCCACTCAATTTAGCAAGC	55
SRCRSP3	CHI10	CGGGGATCTGTTCTATGAAC TGATTAGCTGGCTGAATGTCC	55
ILSTS029	BTA3	TGTTTTGATGGAACACAG TGGATTTAGACCAGGGTTGG	55
TGLA53	BTA16	GCTTTCAGAAATAGTTTGCATTCA ATCTTCACATGATATTACAGCAGA	55
ETH10	CHI5	GTTCAGGACTGGCCCTGCTAACA CCTCCAGCCCACTTTCTCTTCTC	55
MAF209	CHI17	GATCACAAAAAGTTGGATACAACCGTG TCATGCACTTAAGTATGTAGGATGCTG	55
INRABERN185	CHI18	CAATCTTGCTCCCACTATGC CTCCTAAAACACTCCCACACTA	55
P19 (DYA)	Unknown	AACACCATCAAACAGTAAGAG CATAGTAACAGATCTTCCTACA	55
TCRVB6	BTA10	GAGTCCTCAGCAAGCAGGTC CCAGGAATTGGATCACACCT	55
SRCRSP7	CHI6	TCTCAGCACCTTAATTGCTCT GGTCAACACTCCAATGGTGAG	55
BM6444	BTA2	CTCTGGGTACAACACTGAGTCC TAGAGAGTTTCCCTGTCCATCC	65
DRBP1	BTA23	ATGGTGCAGCAGCAAGGTGAGCA GGGACTCAGTCTCTCTATCTCTTTG	58

Kotze *et al.,* (2004) using 18 microsatellite markers analyzed genetic diversity of Kalahari goat breed from South Africa and studying dilution of the Kalahari Red with Red Boer goats. The genetic characterization information formed the basis for future management of Kalahari Red goat.

Kumar *et al.,* (2005) using 25 microsatellite loci studied population structure and genetic bottleneck hypothesis on Marwari goats in Rajasthan. The genetic variation was seen in terms of effective number of alleles and gene diversities observed. The average polymorphism across the studied loci was 1.295 and the expected gene diversity in the population was 0.623 ± 0.041. The population, showed a good level of inbreeding (F_{IS}= 0.264 ± 0.046) and was observed to be significantly differentiated into groups.

Gour *et al.,* (2006) using 23 microsatellite loci studied the genetic distance of Jamunapari goats. The overall mean number of alleles observed was 4.913 ± 1.905 ranging from 2 to 10. Average polymorphism and expected gene diversity were 1.066 ± 0.510 and 0.528 ± 0.237 respectively. The population structure and genetic bottleneck hypothesis were examined. Population showed fairly high level of inbreeding (F_{IS}= 0.189 ± 0.049) and heterozygote deficit.

Oliveira *et al.,* (2007) based on microsatellites studied genetic distance, structure and relationships between Brazilian naturalized and exotic purebred domestic goat breeds (*Capra hircus*). By using 13 microsatellites genetic relationships and structure of 14 goat (*Capra hircus*) populations were estimated. All 13 loci were polymorphic with an average of 15.6 alleles per locus.

Dixit *et al.,* (2008) using 25 microsatellite markers studied genetic distance on Kutchi, Mehsana and Sirohi goat breeds of India. Among the 3 breeds, the Mehsana breed showed lowest genetic diversity. An overall significant heterozygote deficit was present in all the populations. The FIS values for Kutchi, Mehsana and Sirohi goat breeds were 0.26, 0.14 and 0.36 respectively.

Ramamoorthi *et al.,* (2009) using 21 microsatellite markers studied genetic characterization and conservation on Barbari goats. The number of alleles ranged from 4 to 11 and allele frequencies was between 0.0104 and 0.5208. PIC values ranged from 0.5563 to 0.8348. Except for two microsatellite loci (ILSTS044 and ILSTS060), for all others the population was not in Hardy-Weinberg equilibrium. The observed and expected heterozygosities ranged from 0.8478 to 1.0000 and 0.6208 to 0.8509 respectively.

Verma *et al.,* (2010) using 25 microsatellite markers studied the genetic variability by phenotypic and genetic characterization of Sangamneri goat breed. The overall mean of number of alleles observed was 9.0 ranging from 3 (ILSTS 022) to 21 (OarFCB304). The average PIC value was 0.711 ranging from 0.271 (OarJMP29) to 0.878 (ILSTS 082). The mean expected and observed gene diversity within the population was 0.6970±0.033 and 0.5399±0.0549 respectively. Out of 25 loci 16 showed significant heterozygote deficiency.

Dixit *et al.,* (2011) using 25 microsatellite studied genetic variation in Kanniadu goats of Tamil Nadu. The average of observed number of alleles was 8.64±0.48 ranging from 5 (RM4) to 13 (RM088, OarE129).

The overall mean effective number of alleles was 4.22±0.34 ranging from 1.45 (ILSTS34) to 7.89 (ILSTS033 and OMHC1). The mean observed heterozygosity was 0.53±0.03 and expected heterozygosity was 0.73±0.02. High PIC values (0.30 to 0.86) indicated higher polymorphism in the breed.

Rout *et al.,* (2012) using 17 microsatellite loci studied genetic diversity on Jamunapari goat. The mean number of alleles per locus and the mean heterozygosity was 9.0 and 0.769 respectively, indicating high allelic variation. On comparison to 10 other Indian goat breeds the gene diversity and effective number of alleles were higher. In this study the genetic diversity of Y-chromosome genes was low.

Zaman *et al.,* (2013) using 23 microsatellite markers studied genetic diversity and population structure in Assam hill goats. All the loci under study were polymorphic. The overall mean of observed number of alleles (Na) was 4.9±2.220 ranging from 2 to 10. The mean effective number of alleles (Ne) was 2.68±1.590 ranging from 1.035 to 7.127. The overall mean observed heterozygosity (H_o) was 0.43 and expected heterozygosity (H_e) was 0.48.

Singh *et al.,* (2014) using 15 microsatellite markers studied genetic characterization of Gaddi goat. A total of 135 distinct allele were observed. The mean observed and effective number of alleles was 9.0000±0.82 and 6.5874±0.56 respectively. The mean observed (HO) heterozygosity was 0.7484±0.02 and mean expected (HE) heterozygosity was 0.8431±0.01.

Kharkar *et al.,* (2015) using micro-satellite markers studied the genetic variability in Berari goat breed The average observed number of alleles was 15.560±0.947 ranging from 9 (ILSTS059, RM004) to 25 (ILSTS005, ILSTS029). The mean effective number of alleles was 10.214±0.602 ranging from 6.036 (RM088) to 16.736 (ILSTS029). The average expected gene diversity within the population was 0.895±0.006 ranging from 0.834 (RM004) to 0.940 (ILSTS029). All the loci under study showed the polymorphic information content value greater than 0.5 indicating higher polymorphism in the breed.

Seilsuth *et al.,* (2016) using 12 microsatellite markers analysed the genetic relationships between different populations and breeds of exotic dairy goats in Thailand. Out of 12 microsatellite markers 5 were found to be polymorphic. A total of 37 alleles were present in all breeds with a mean of 7.40 alleles per locus, ranging from 5 (SPS115 and ETH225) to 11 (TGLA122). Mean expected heterozygosity value was 0.539 and the average observed heterozygosity was 0.529 showing genetic variability within the breeds was moderate.

Microsatellites markers are highly polymorphic DNA markers, they are highly abundant, very simply to analyze and easy to score, but nevertheless these types of markers have disadvantages such as null alleles, or size homoplasy (Schlotterer, 2004). Microsatellites are the most informative molecular marker with the advantage of easy and low-cost detection by PCR, because of high mutation rate. Another great advantage of microsatellites is their co-dominant feature and hence detect both homozygote and heterozygote genotypes, unlike RAPD and AFLP markers, which are dominant markers detecting only the presence or absence of a locus.

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