MOLECULAR GENETIC STUDIES ON NELLORE AND DECCANI SHEEP USING MICROSATELLITE MARKERS

By

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CERTIFICATE

This is to certify that **Pothumudi Amareswari** (RVD/2010-06) has satisfactorily prosecuted the course of research and that the thesis entitled **"Molecular Genetic Studies on Nellore and Deccani Sheep Using Microsatellite Markers"** submitted is the result of original work done and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by her for a degree of any university.

Date: 6.07.2015
Place: Hyderabad

(Dr M. GNANA PRAKASH)
Major Advisor

CERTIFICATE

This is to certify that the thesis entitled **"Molecular Genetic Studies on Nellore and Deccani Sheep Using Microsatellite Markers"** submitted in partial fulfilment of the requirements for the degree of the "**DOCTOR OF PHILOSOPHY"** of the **Sri Venkateswara Veterinary University,** Tirupati, is a record of the bonafide research work carried out by Pothumudi Amareswari**,** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma. The published part has been fully acknowledged. All the assistance and help received during the course of the investigation has been duly acknowledged by the author of the thesis.

(Dr M. GNANA PRAKASH)
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POTHUMUDI AMARESWARI

LIST OF ABBREVIATIONS

%	:	Per cent
±	:	Plus or Minus
°C	:	Degree Celcius
A	:	Absorbance
APS	:	Ammonium Per Sulfate
bp	:	Base Pair
cm	:	centimetre
DNA	:	Deoxyribo Nucleic acid
dNTP	:	Di Nucleotide Tri phosphate
EDTA	:	Ethylene Diamino Tetraacetic Acid
F_{IS}	:	Coefficient of Inbreeding
g	:	gram
H	:	heterozygosity
H_O	:	Observed heterozygosity
H_E	:	Expected heterozygosity
hr	:	hour
kb	:	Kilobase
kg	:	Kilogram
µg	:	Microgram
µl	:	Microlitre
µm	:	micrometre
mA	:	milli Ampere
mg	:	Milligram
ml	:	Millilitre
mm	:	Millimetre
mM	:	Milli Molar
MNA	:	Mean Number of Alleles
ng	:	Nanogram
nm	:	nanometre
P	:	Percentage of polymorphic loci
PAGE	:	Poly Acrylamide Gel Electrophoresis

PCR	:	Polymerase Chain Reaction
PIC	:	Polymorphism Information Content
rpm	:	Revolutions Per Minute
TBE	:	Tris Borate EDTA
TE	:	Tris EDTA
TEMED	:	N' N' N' N' - Tetra Methyl Ethylene Diamine
V	:	Volt
W	:	Watt
w/v	:	Weight by Volume

Declaration

I, **Pothumudi Amareswari**, hereby declare that the thesis entitled **"Molecular Genetic Studies on Nellore and Deccani Sheep Using Microsatellite Markers"** submitted to **Sri Venkateswara Veterinary University**, Tirupati for the degree of **DOCTOR OF PHILOSOPHY** is a result of original research work done by me. I also further declare that the thesis or any part thereof has not been published earlier elsewhere in any manner.

Place: Hyderabad

Date: 6.7.2015 **(POTHUMUDI AMARESWARI)**

Name of the author	:	**AMARESWARI POTHUMUDI**
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ABSTRACT

A total of 100 sheep, 50 each from Deccani and Nellore breeds were genotyped using 30 dinucleotide microsatellite marker primers. The genomic DNA was isolated using standard phenol-chloroform method. The mean optical absorbance ratio was 1.95 in both the breeds indicating good quality of genomic DNA.

A total of 254 and 260 alleles in Deccani and Nellore breeds were obtained with allele size range varying from 71 (OARCP20) to 288 (LSTS11) in both the breeds. The number of alleles ranged from 4 to 12 in Deccani with an average of 8.46 and 4 to 14 in Nellore with an average of 8.66. The effective number of alleles ranged from 2.135 to 9.580 in Deccani and 2.683 to 10.240 in Nellore whereas the overall mean effective number of alleles varied between 5.52 for Deccani and 5.62 for Nellore sheep. The allele frequency ranged from 1.0 to 65.6 per cent in Deccani and 1.0 to 51.1 per cent in Nellore sheep.

A total of 23 out of 254 and 37 out of 260 alleles amplified were found to be population specific for Deccani and Nellore sheep respectively. The overall mean PIC values ranged from 0.773 in Deccani to 0.777 in Nellore breed. Across the loci, the PIC values ranged from 0.587 to 0.886 in Deccani and 0.552 to 0.894 in Nellore breeds. The PIC values for all the loci were above 0.50 except for the loci OarHH35 (0.49) in Deccani.

The mean expected heterozygosity among the breeds ranged from 0.79 (Deccani) to 0.80 (Nellore) whereas the mean observed heterozygosity levels ranged from 0.77 in Deccani to 0.78 in Nellore sheep.

The overall mean inbreeding coefficient was 0.029 in Deccani and 0.031 in Nellore sheep. Of the 30 loci studied, 14 loci in Deccani and 16 loci in Nellore sheep showed negative inbreeding coefficient indicating the presence of outbreeding. Eight loci showed positive, mild to moderate inbreeding ranging from 0.007 to 0.25 in both the breeds studied. The F_{IS} values ranged from -0.372 to 0.74, F_{ST} values ranged from 0.001 to 0.172 and F_{IT} values ranged from -0.370 to 0.728 between the Deccani and Nellore sheep breeds studied. The unbiased expected heterozygosity in Deccani sheep ranged from 0.54 to 0.91 and in Nellore sheep, it ranged from 0.64 to 0.91.

The genetic distance was 0.225 and genetic identity was 0.799 between the two sheep studied. All the loci departed from the equilibrium frequency significantly in both the sheep breeds. This might be due to homozygote deficiency as the animals from both the breeds were selected from different regions across the states.

The results of this study contribute to the knowledge of genetic structure of Deccani and Nellore sheep. The results reveal high genetic variation within the breeds and hence the significant characters of the two breeds *Viz.*, the dual utility of Deccani sheep and tallest character of the Nellore sheep makes it imperative to strengthen the conservation measures. Also, integrating genetic improvement programme with marker oriented production strategies will raise the economy of the farmers of this region.

INTRODUCTION

CHAPTER I
INTRODUCTION

Sheep is an important livestock species for India. Biodiversity of sheep in India is reflected by its forty recognized breeds. India ranks second in sheep population and account for more than 6.13 per cent of world population (FAO STAT, 2012) with 75m sheep to its record. The sheep breeds used in the present study are Deccani and Nellore. Deccani are coarse wool sheep native to Deccan plateau, reared mainly for mutton production. Nellore, the tallest sheep of India is also a mutton producing breed distributed in Nellore, Prakasam and Ongole districts of Andhra Pradesh. Indiscriminate breeding and lack of scientific management has reduced the performance of these sheep breeds.

Genetic diversity plays a very important role in survival and adaptability of a species. Diversity is required to meet current production needs in various environments, to allow sustained genetic improvement and to facilitate rapid adaptation to changing breeding objectives (Notter, 1999). Genetic diversity refers to variation in nucleotides, genes, chromosomes or whole genomes of organisms (Wang *et. al*., 2009). Molecular markers based on sequence polymorphism are convenient and powerful tools for the analysis of genetic diversity.

Over the past decade, microsatellite markers have proven to be useful in genetic diversity studies in several livestock species including sheep (Cinkulov *et. al.*, 2008 and Alvarez *et. al*., 2009). Microsatellites are made up of simple tandem repeat sequence motif not more than six bases long, tandemly repeated di- and tri-nucleotide sequences of eukaryotic genome (Tautz, 1989) and are highly polymorphic, variable and densely

distributed in the genome at a frequency of one at every 6kb sequence (Bruford and Wayne, 1993). Because of their co-dominant nature and general availability in different allelic forms, they are extensively used in fingerprinting (Jeffreys and Pena, 1993 and Queller *et. al.*, 1993), linkage analysis (Todd *et. al.*, 1991; Dietrich *et. al.*, 1992 and Hillel, 2004) and reconstruction of phylogeny and demographic structuring of populations (Bowcock *et. al.*, 1994 and Hillel *et. al.*, 2003).

Diversity analysis using microsatellites markers allows the estimation of genetic diversity within each breed since the priority breeds for conservation should be the ones with largest within breed diversity. Genetic diversity measures provide additional basic information for the design and interpretation of breeding programmes by identification of the most heterozygous individuals in the population.

The amount of genetic divergence between populations is regarded as a major criterion for deciding their uniqueness and therefore prioritizing their conservation (Eding *et. al.*, 2002). Molecular genetic characterization will help characterize and clarify the relationships among existing breeds – a major prerequisite in deciding priorities for conservation and improvement programmes.

Hence, the present work on genetic variability and diversity in Deccani and Nellore sheep breeds using genome markers is undertaken with the following objectives:

1. To characterize Nellore and Deccani sheep breeds at molecular level.
2. To evaluate the genetic diversity between the two breeds.
3. To identify breed specific markers if any.
4. To establish phylogenetic relationship among the two sheep breeds.

REVIEW OF LITERATURE

CHAPTER – II

REVIEW OF LITERATURE

Animal genotyping using genetic markers is a powerful aid to animal breeding. Genetic markers provide information about allelic variation at a given locus which allows detailed analysis and evaluation of genetic diversity. They are good predictors of the overall genomic diversity of a population. Both within and between breed diversity parameters are classically measured using molecular genetic markers (Sunnucks, 2000). The bi-parental molecular loci are used to determine the population structure within and among populations (Wang *et. al.*, 2009). Mean number of alleles, observed and expected heterozygosity are most commonly calculated population genetic parameters for assessing within breed diversity whereas the genetic differentiation or fixation indices are the simplest parameters for assessing the distribution of diversity between the breeds. They also aid in the genetic management of small populations, to avoid excessive inbreeding.

2.1 SHEEP GENOME

Sheep possess many heritable traits that are of economic importance. The sheep genome is of similar size to the human genome, approximately 3×10^9 bp in size. The DNA is distributed over 54 chromosomes. Its length in terms of genetic distance was estimated to be 27 Morgans (Chapman and Bruere, 1977).

2.2 SHEEP MICROSATELLITES

Microsatellites, also called as simple sequence repeats are tandem repeats of one to six bp, which are interspersed throughout the DNA of animal genome (Litt and Luty, 1989; Tautz, 1989; Weber and May, 1989). Microsatellites as compared to other DNA

sequences are highly polymorphic which makes them attractive as genetic markers (Goldstein and Shlotterer, 1999). Assessment of genetic diversity using microsatellite markers has become an important tool for conservation programs and those involved in the breeding of animal herds in conservation nuclei.

2.3 ISOLATION OF DNA

DNA extraction methods follow some common procedures aimed to achieve effective disruption of cells, denaturation of nucleoprotein complexes, inactivation of nucleases and other enzymes, removal of biological and chemical contaminants, and finally DNA precipitation (Tan and Yiap, 2009).

There are various techniques of DNA isolation from blood cells. The traditional method of DNA isolation by Phenol Chloroform method (Sambrook *et. al*., 1989) is widely used which is a very effective yielding high quantity of pure DNA. This method was widely used by Cerit *et. al*. (2003), Mukesh *et. al.* (2006), Kumar *et. al*. (2007), Arora and Bhatia (2009), Chen *et. al.* (2009), Muigai *et. al*. (2009), Pramod *et. al*. (2009), Arora *et. al.* (2010), Jyotsana *et. al*. (2010), Rodrigo *et. al*. (2010), Radha *et. al*. (2011) and Hepshiba *et. al*. (2014) and Salting out method of DNA isolation (Miller *et. al*., 1988) was used by Farid *et. al*. (2000), Qanbari *et. al*. (2007), Karthikeyan *et. al*. (2008), Khan *et. al*. (2009) and Nanekarani *et. al*. (2011). Rapid non-enzymatic method (Lahiri and Nurnberger, 1991) was used by Girish *et. al*. (2007), Prema *et. al*. (2008a, 2008b) and Kumarasamy *et. al.*(2009).

DNA kits were used for the isolation of DNA by Pariset *et. al*. (2003), Ozerov *et. al*. (2008), Lasagna *et. al*. (2009), Kevorkian *et. al.* (2010), Dashab *et. al*. (2011), Ghazy *et. al*. (2013) and Salamon *et. al*. (2014).

2.4 MICROSATELLITE GENOTYPING

The polymerase chain reaction (PCR) is arguably the most powerful laboratory technique in molecular biology. It is an *in vitro* method for the amplification of DNA introduced in 1985. It has a variety of applications which include DNA cloning for sequencing, DNA-based phylogeny and functional analysis of genes, diagnosis of hereditary diseases, identification of genetic fingerprints (used in forensic sciences and paternity testing) and the detection and diagnosis of various infectious diseases.

Farid *et. al*. (2000), Girish *et. al*. (2007), Peter *et. al*. (2007), Ozerov *et. al*. (2008), Jyotsana *et. al*. (2010), Rodrigo *et. al*. (2010), Kevorkian *et. al*. (2010), Nanekarani *et. al*. (2011), Dashab *et. al*. (2011), Radha *et. al.* (2011) and Ghazy *et. al*. (2013) used the normal PCR protocol for the amplification of the microsatellite loci and the amplicons were scored by using 8 % polyacrylamide gel.

Diez-Tascon *et. al*. (2000), Mukesh *et. al* (2006), Kumar *et. al*. (2007), Arora and Bhatia (2009), Pramod *et. al*. (2009) and Arora *et. al*. (2010) used Common Touchdown PCR programme for the amplification of the markers and the amplified products were resolved on 6 % Urea-polyacrylamide gel electrophoresis denaturing sequencing gel.

Prema *et. al*. (2008a) carried out the normal PCR reaction and the amplified products were denatured at 95^0C and then electrophoresed on a standard 6% urea-PAGE denaturing sequencing gel.

El Nahas *et. al.* (2008) performed the PCR by overlaying the reaction mixture with sterile mineral oil and then the amplicons were subjected to electrophoresis on 10% non-denaturing polyacrylamide gel.

Chen *et. al.* (2009) used the normal PCR protocol and the amplified products were resolved on 10% non-denaturing polyacrylamide gel.

Salamon *et. al.* (2014) used four different PCR-multiplex reactions using fluorescent-labeled primers and hot-start polymerase and the diluted PCR–multiplex reactions were processed in a 16-capillary electrophoresis.

Pariset *et. al.* (2003) performed the PCR reaction using near-infrared (NIR) fluorescent dye end-labeled primers and the amplified products were electrophoresed on a 6.5% gel.

2.5 GENETIC CHARACTERIZATION

Microsatellites are tandem repeat motifs of variable lengths that are distributed throughout the eukaryotic nuclear genome in both coding and non-coding regions (Jarne & Lagoda, 1996). Molecular characterization using microsatellite markers allow detecting variation or polymorphism existing among individuals in the population for specific regions of the DNA which aids in the genetic management of small populations, to avoid excessive inbreeding and also to undertake conservation strategies.

At the level of molecular markers, genetic diversity is usually measured by the frequencies of genotypes and alleles, the proportion of polymorphic loci, the observed and expected heterozygosity, or the allelic diversity. The intra breed genetic variation in various sheep breeds reported by earlier research workers is presented below.

2.5.1 ALLELE DIVERSITY

The allelic diversity (number of alleles segregating in the population) is an alternative criterion to measure genetic diversity. Allelic diversity is also important from a long-term perspective, because the limit of selection response is determined by the initial number of alleles (Hill and Rasbash, 1986) and higher differentiation between populations can be observed for allelic diversity than for gene diversity (Foulley and Ollivier, 2006).

Indigenous Breeds

Arora and Bhatia (2004) assessed the genetic structure of Muzzaffarnagri sheep using 25 microsatellites. All the 126 alleles detected were found to be polymorphic. The number of observed alleles varied between two (BM6506 and HUJ616) and eight (TGLA137) with an average of 5.04. The effective number of alleles was less than the observed values ranging from 1.50 (HUJ616) to 5.84 (TGLA137) with a mean of 3.64.

Twenty five microsatellite markers were used by Arora and Bhatia (2006) to investigate genetic diversity of Magra sheep. A total of 144 distinct alleles were detected across all the loci studied. The number of alleles varied from three (BM6506, OarCP20) to ten (CSSM31) while the effective number of alleles per locus ranged from 1.5 (CSSM47) to 7.3 (BM1314).

Mukesh *et. al.* (2006) studied the genetic structure of Nali, Chokla and Garole sheep by using 11 microsatellite markers. A total of 85 alleles were detected. Of these, 69 were found in Chokla and 62 each in Nali and Garole sheep. Higher mean number of alleles was observed in Nali (6.27) than in Chokla (5.63) and Garole (5.63). The mean effective number of alleles was also highest for Nali (3.49) followed by Garole (3.24) and Chokla (3.05).

Eighteen microsatellite markers were used to analyze the genetic variation in Hassan sheep (Sharma *et. al.*, 2006). A total of 133 alleles were detected. All the loci were polymorphic and the number of observed alleles varied from four (OarAE129) to twelve (OarFCB48) with an overall mean of 7.40 ± 2.0. The effective number of alleles varied from 1.445 to 6.711 with a mean of 3.694.

Sodhi *et. al*. (2006) characterized Nali and Chokla sheep using 25 microsatellite markers. All the loci were polymorphic in both the sheep populations. The number of observed alleles ranged from three (BM6506, OarHH47) to ten (OarFCB48) in Nali and two (TGLA377) to eight (OarFCB48) in Chokla with an overall means of 5.52 and 5.32, respectively, while the effective number of alleles varied from 1.29 (BM6506) to 6.61 (OarFCB48) with an average of 3.34 in Nali sheep and 1.63 (CSSM47) to 5.80 (OarHH64) with a mean of 3.27 in Chokla sheep.

Girish *et. al.* (2007) studied the genetic variation in Nilgiri sheep using 25 microsatellite markers and observed a total of 125 alleles. The observed number of alleles ranged from three (CSSM47 and OarAE129) to eight (BM1314) with a mean of five across all loci which is greater than the effective number of alleles that ranged from 2.18 (OarAE129) to 6.49 (BM1314) with a mean of 3.84 across all loci.

Bhatia and Arora (2007) studied the genetic variability within and between Marwari and Sonadi breeds of sheep using 25 microsatellite markers. All the loci were found to be polymorphic. The mean number of alleles was observed as 6.24 for Marwari and 5.88 for Sonadi sheep.

Genetic variability in Bellary sheep was studied using 20 microsatellite loci. A total of 133 alleles were observed. The number of alleles observed across all the loci varied from two

(BM6506) to ten (OarHH35) with an overall mean of 6.7 which is more than the effective number of alleles that ranged from 1.69 to 7.25 (Kumar *et. al*., 2007)

Pandey *et. al.* (2007) analyzed the genetic variability in Bonpala sheep using 24 microsatellite markers and a total of 96 distinct alleles were identified. The number of observed alleles varied from two (BM6506, ILSTS005) to seven (OarHH47) with an overall mean number of alleles per locus of 4.0 ± 1.25. The observed number of alleles for all loci exceeded the effective number of alleles that varied from 1.21 (ILSTS005) to 5.64 (OarHH47) with a mean of 2.47 ± 0.96.

Arora and Bhatia (2008) studied the genetic diversity within and among Magra, Marwari and Sonadi sheep based on 13 microsatellite loci. A total of 106 alleles were detected across all the loci. The number of alleles per locus varied from three (BM8125, CSSM47, OarAE129) to nine (BM1314, OarHH35). The mean number of alleles was found to be 5.5, 6.1 and 5.6 respectively for Magra, Marwari and Sonadi sheep.

A total of 148 alleles across 25 microsatellites were found in Jaluani sheep and the actual number of observed alleles at each locus ranged from two (BM8125) to nine (CSSM31, OMHC1 and TGLA137) with a mean of 5.92. The effective number of alleles was distinctly lower than the observed number of alleles which ranged between 1.3 (OarCP20) to 6.3 (TGLA137) with an average of 3.7 (Arora and Bhatia 2008).

Prema *et. al.* (2008a) evaluated the genetic diversity in Mecheri sheep using 17 microsatellite primer sets. A total of 85 alleles were detected and the number of observed alleles ranged from three (BM6506) to seven (OarCP38) with a mean of 5.00 and the effective number of alleles ranged from 2.24 (BM6506) to 4.59 (OarHH41) with a mean of 3.61 across all loci. And the frequency of alleles ranged from 0.021 (OarCP38) to 0.625 (BM 827).

Molecular characterization of Madras Red sheep was done by Prema *et. al.* (2008b) using 17 microsatellite loci and all the 85 alleles observed were found to be polymorphic. The number of alleles observed across all loci ranged from three (BM6526) to eight (OarCP38) with a mean of 4.94 ± 0.14. The frequency of the alleles ranged from 0.02 (CSSM47) to 0.48 (OarCP20).

Arora and Bhatia (2009) studied the genetic diversity among Jalauni, Marwari and Sonadi sheep using 20 microsatellite markers. Mean number of observed alleles in Jalauni, Marwari and Sonadi sheep was 5.85, 6.25 and 5.75 respectively. The number of effective alleles per locus varied from 1.30 (Jalauni-OarCP20, Sonadi-CSSM47) to 6.7 (Sonadi-OarHH47). The mean effective number of alleles was highest in Marwari (4.02) followed by Sonadi (3.71) and Jalauni (3.69).

Kumarasamy *et. al*. (2009) observed a total of 143 alleles across 27 microsatellite loci in Coimbatore sheep. The number of observed alleles ranged from three (BM6506 and OMHC1) to eight (TGLA377 and OarCP20) with a mean of six across all loci. The effective number of alleles ranged from 3.72 (OarCP20) to 6.33 (OarJMP8) with a mean of 4.9325 ± 0.60 across all loci.

Pramod *et. al.* (2009) reported a total of 147 alleles for 25 microsatellite marker loci in Vembur sheep with a mean of 5.88 ± 0.29 alleles per locus. The number of alleles observed at each locus varied between two (OarHH47) and nine (HUJ616). The effective number of alleles over all loci ranged from 1.9406 (OarHH47) to 6.8368 (HUJ616) with a mean of 4.095 ± 0.23 alleles per locus.

Microsatellite profiles for 25 loci located on 19 chromosomes were recorded for Ganjam sheep by Arora *et. al.* (2010). The allele frequencies ranged from 0.022 to 0.863. A total of 138 alleles were detected across all loci and the actual number of observed alleles at

each locus ranged from three (BM757, BM6506, CSSM47, OarAE129, OarFCB128) to a maximum of nine (CSSM31 and OarHH35). The mean number of alleles was 5.48 across all loci. The effective number of alleles was lower than the observed number of alleles which ranged from 1.27 in CSSM47 to 7.87 in CSSM31.

Jyotsana *et. al.* (2010) studied the genetic structure of Patanwadi, Marwari and Dumba breeds using 20 microsatellite markers. A total of 207 alleles were detected across the pooled population with a mean number of 10.35 alleles. A total of 165 alleles were found in Patanwadi, 181 in Marwari and 160 in Dumba sheep and the mean number of alleles observed was 8.25, 9.05 and 8.00 in Patanwadi, Marwari and Dumba breeds, respectively.

A total of 219 alleles were found in Changthangi sheep by using 25 microsatellite markers. OarCP49 and MAF214 showed the highest number of observed alleles per locus (15), while BM6506 (4) showed the lowest with the mean number of alleles of 8.760. Expected number of alleles varied from 1.144 (CSSM47) to 10.509 (OarCP49) with a mean of 4.593 (Sharma *et. al.*, 2010).

Arora *et. al.* (2011a) estimated the genetic diversity among six sheep breeds of India (Deccani, Madgyal, Nellore, Ganjam, Chotanagpuri and Garole) and observed a total of 349 alleles across 25 microsatellite loci studied. The number of alleles per locus varied from seven (BM6506, BM8125) to twenty five (CSSM31), while the effective number of alleles varied from 2.462 (MAF214) to 12.774 (CSSM31). The least allele diversity was observed for Garole (6.40) while the most allele diversity was revealed for Nellore (7.92) breed.

Arora *et. al.* (2011b) studied the genetic diversity among six Indian sheep breeds - Marwari, Chokla, Jaisalmeri, Magra, Nali and Pugal with 25 microsatellite loci and detected a total of 366 alleles. The number of alleles per locus varied from seven (BM6506) to twenty four (CSSM31).

Radha *et. al.* (2011) used 25 microsatellite markers to estimate genetic variability in Kilakarsal sheep and observed a total of 190 alleles. The number of alleles at each locus varied from three (CSSM47) to thirteen (OarFCB128) with a mean of 7.6 across all loci. The effective number of alleles ranged from 1.53 (BM8125) to 6.95 (OMHC1) with a mean of 3.88 across all loci.

Yadav *et. al.* (2011) reported that the allele frequencies varied from 0.013 to 0.888 in Munjal sheep. The number of alleles observed across all the loci varied from four (BM6506) to fourteen (INRA63) with a mean of 8.64 ± 2.60, while the effective number of alleles ranged from 1.264 (CSSM47) to 8.399 (OarJMP29) with an average of 4.57 ± 1.64.

A total of 134 alleles were detected in Coimbatore sheep that ranged between two (CSSM31, CSSM47 and MAF214) to ten (OarHH47 and OarHH35) with a mean of 5.58 ± 0.05, while the effective number of alleles varied from 1.13(OarHH64) to 7.71 (OarHH47) (Hepsibha *et. al*., 2014)

Exotic Breeds

Farid *et. al*. (2000) genetically analyzed ten sheep breeds using 10 microsatellite primers. A total of 93 alleles were detected from all the loci. The average number of alleles per locus for the breeds varied from 5.4 to 6.0, except for Romanov and Red Masai which had smaller values (4.3 and 5.0). About 46.2% of alleles in the pooled data were smaller than 0.05 and 17 of the alleles were present only in one breed but all had low (≤ 0.13) frequencies. Twenty alleles were present at nine loci in every breed and all of them showed a wide range of frequencies across the breeds, suggesting large genetic differences among the breeds.

Pariset *et. al.* (2003) assessed the genetic variability in 17 flocks of Sarda sheep breed in Italy using 11 microsatellite markers. A total of 165 alleles were detected. The mean number of alleles varied from 10.5 to 5.5 with an overall mean value of 7.34.

Peter *et. al.* (2007) analyzed the genetic diversity of 57 European, Middle Eastern marginal and cosmopolitan sheep breeds by typing 31 microsatellite markers and detected a total of 564 alleles across the 31 loci. A total of 65 breed specific alleles were detected at 24 loci, but none of these alleles possessed frequencies above 5%.

Ozerov *et. al*. (2008) assessed four Kazak sheep breeds used 20 microsatellite loci and found a total of 233 alleles. The number of alleles at the locus varied from 6 (OarCP34) to 17 (BM4621, CSSM31 and OarFCB304) with an average value of 12.

Nahas *et. al.* (2008) assessed genetic diversity in three Egyptian indigenous sheep breeds using 14 microsatellite loci. The total number of alleles detected varied from six (CSSM47) to fourteen (TGLA377). The mean number of alleles per locus was 8.2, 5.7 and 7.2 in Barki, Ossimi and Rahmani breeds, respectively.

Tapio *et. al*. (2010) screened 52 sheep breeds from the Eurasian subcontinent with 20 microsatellite markers and detected a total of 342 alleles. The mean allelic richness between the breeds varied from 4.41 to 6.67.

Rodrigo *et. al.* (2010) studied the genetic structure of four Chilean sheep breeds using nine microsatellite markers. The allele number varied from 9 (INRA26) to 25 (CSSM31).

Kevorkian *et. al*. (2010) investigated genetic relationship in four autochthonous Romanian sheep breeds (Botosani Karakul, Karabash, Palas Milk Line and Palas meat Line) using 11 microsatellite markers and detected 197 alleles over all the loci. MAF70 showed the

highest number of alleles per locus (30) while OarCP20 the lowest (11) with a global mean of 17.9 ± 5.87 alleles.

The genetic diversity in two sub-populations of Baluchi sheep was analyzed using seven microsatellite loci. The total number of alleles was 39 and the number of identified alleles per locus ranged from four to seven. The mean number of alleles per sub-population 1, 2 and overall population for all loci were 4.86, 5.40 and 5.60, respectively (Dashab *et. al*., 2011).

Nanekarani *et. al*. (2011) used fifteen microsatellites to evaluate genetic diversity within Karakul sheep breed reared in Iran. A total of 121 alleles were detected across all the loci studied. The observed number of alleles per locus ranged from 4.00 (MAF64) to 12.00 (MCMA2) with a mean of 8.07 and reported a substantial variation in Karakul breed based on allele diversity.

Ahmed *et. al.* (2014) analyzed Kail sheep of Azad Jammu and Kashmir using 11 microsatellite markers. The mean number of alleles ranged from three (MM12, INRA32) to eight (MAF70). Mean of effective number of alleles 3.94 was less than the observed, which ranged from 2.03 (MM12) to 5.88 (MAF33).

2.5.2 HETEROZYGOSITY

Heterozygosity is an appropriate measure of genetic variability within a population when populations are expanding (Hanslik *et. al*., 2000). It is the most widely used parameter to measure diversity within populations, defined by Nei (1973) as the probability that two alleles chosen at random from the population are different.

Indigenous Breeds

The average observed and expected heterozygosity values in Muzzaffarnagri sheep were 0.652 and 0.697, respectively. This high mean heterozygosity was attributed to low level of inbreeding, low selection pressure and large number of alleles present in the population (Arora and Bhatia, 2004).

The observed and expected heterozygosity values of the microsatellite loci in Magra sheep ranged from 0.200 (BM6506) to 0.882 (BM1314) and 0.368 (CSSM47) to 0.864 (BM1314). The mean observed heterozygosity value was 0.597, while the mean expected heterozygosity value was 0.694 (Arora and Bhatia, 2006).

Mukesh *et. al.* (2006) found that the average observed and expected heterozygosity values were high in Nali (0.47 and 0.65) followed by Chokla (0.47 and 0.64) and Garole (0.44 and 0.59), respectively.

The average expected gene diversity within population of Hassan sheep ranged from 0.392 (OarAE129) to 0.851 (OarFCB48) with an overall mean of 0.678 ± 0.148 (Sharma *et. al*., 2006).

Sodhi *et. al.* (2006) showed that the expected heterozygosity values of Nali sheep ranged from 0.225 (BM6506) to 0.849 (OarFCB48), while in Chokla it was 0.385 (CSSM47) to 0.828 (OarHH64) with overall averages of 0.651 and 0.697, respectively.

Girish *et. al.* (2007) reported that the observed heterozygosity in the Nilagiri sheep ranged from 0.4222 (BM6506) to 1.000 (HUJ616) with a mean value of 0.7610 whereas the expected heterozygosity ranged from 0.5415 (OarAE129) to 0.8459 (BM1314) with a mean value of 0.7213. The high mean heterozygosity values were attributed to the low level of inbreeding.

The average observed heterozygosity (0.512) was less than the expected heterozygosity (0.684) in Bellary sheep. The average expected gene diversity within the population ranged from 0.411 (OarHH64) to 0.862 (OarHH35) with an overall mean of 0.676 (Kumar *et. al.*, 2007).

The observed heterozygosity value for Bonpala sheep was 0.53 ± 0.18 which was slightly lower than the expected heterozygosity value that ranged from 0.18 (ILSTS005) to 0.83 (OarHH47) with an overall mean of 0.55 ± 0.15 (Pandey *et. al.*, 2007).

The observed heterozygosity ranged from 0.32 (CSSM47) to 0.95 (OarHH35) for Magra, O.29 (OarHH64) to 1.0 (OarHH35) for Marwari and 0.18 (OarHH64) to 0.96 (OarHH35, RM4) for Sonadi. The expected heterozygosity per locus varied from 0.37 (CSSM47) to 0.86 (BM1314) in Magra, 0.31 (CSSM47) to 0.84 (OarHH35) in Marwari and 0.27 (CSSM47) to 0.83 (OarHH35) in Sonadi sheep (Arora and Bhatia, 2008).

Arora *et. al.* (2008) studied genetic diversity in Jalauni sheep and reported that the observed heterozygosity ranged from 0.26 (OarHH47) to 0.95 (TGLA137) and the expected heterozygosity varied from 0.24 (OarCP20) to 0.84 (TGLA137). The mean observed and expected heterozygosity values were 0.58 and 0.68, respectively.

The observed heterozygosity in Mecheri sheep population ranged from 0.510 (BM757) to 0.939 (CSSM47) with a mean of 0.669, while the expected heterozygosity ranged from 0.562 (BM827) to 0.816 (OarCP38) with a mean of 0.706 (Prema *et. al.*, 2008a).

In studies on genetic variability in Madras Red sheep, Prema *et. al.* (2008b) reported that the observed heterozygosity ranged from 0.551 (OMHC1) to 0.776 (OarCP38) with a mean of 0.697 and the expected heterozygosity ranged from 0.554 (BM6506) to 0.796 (CSSM47) with a mean of 0.706.

The observed heterozygosity per locus as reported by Arora and Bhatia (2009) ranged from 0.227 (Sonadi-OarAE129) to 1.000 (Marwari-OarHH35) while the expected heterozygosity per locus varied from 0.248 (Jalauni-OarCP20) to 0.852 (Sonadi-OarHH47). The highest average heterozygosity was seen in Sonadi sheep (0.611) followed by Marwari (0.598) and Jalauni sheep (0.570). Mean expected heterozygosity was observed to be largest in Marwari (0.689) and lowest in Sonadi (0.670) and Jalauni (0.673).

Kumarasamy *et. al*. (2009) studied genetic diversity in Coimbatore sheep and reported that the observed heterozygosity ranged from 0.6250 (OarHH41) to 0.8462 (CSSM31) with a mean of 0.7404 ± 0.06, while the expected heterozygosity ranged from 0.7211 (OarCP20) to 0.8422 (OarJMP8) with a mean of 0.8106 ± 0.03.

The observed heterozygosity values in Vembur sheep varied between 0.1333 (BM1314) to 1.000 (OarCP20), while the expected values ranged from 0.4847 (OarHH47) to 0.8537 (HUJ616). The mean values were 0.5202 ± 0.04 and 0.7339 ± 0.02 for observed and expected heterozygosities, respectively (Pramod *et. al*. 2009).

The intra population observed heterozygosity ranged from 0.105 (OarAE129) to 0.909 (OMHC1) in Ganjam sheep. The expected heterozygosity per locus varied from 0.214 (CSSM47) to 0.873 (CSSM31). The values of the mean observed heterozygosity and gene diversity were 0.623 and 0.685, respectively (Arora *et. al*., 2010).

Jyotsana *et. al*. (2010) reported that the observed heterozygosity was high, approaching unity, indicating high genetic variability in Patanwadi, Marwari and Dumba breeds of sheep. The highest average observed heterozygosity was seen in Marwari (0.6380) followed by Dumba (0.6360) and Patanwadi sheep (0.6058). The expected heterozygosity values were 12, 12 and 11 for Patanwadi, Marwari and Dumba breeds, respectively.

Sharma *et. al*. (2010) stated that the expected heterozygosity was higher than the observed heterozygosity indicating high level of information of the chosen microsatellite set in Changthangi sheep. The observed and expected heterozygosity values ranged from 0.053 (CSSM47) to 0.912 (OarCP49) and from 0.126 (CSSM47) to 0.905 (OarCP49) with an overall mean of 0.691 ± 0.039 and 0.716 ± 0.036, respectively.

Arora *et. al.* (2011b) reported that the observed heterozygosity per locus varied from 0.179 (CSSM47) to 0.878 (OarCP49) whereas the expected heterozygosity per locus varied from 0.293 (CSSM47) to 0.909 (BM1314). The range of observed heterozygosity was 0.505 (Marwari) to 0.672 (Magra) and mean expected heterozygosity was 0.686 (Marwari) to 0.766 (Magra).

Radha *et. al.* (2011) studied the population structure of Kilakarsal sheep and recorded the observed heterozygosity values from 0.180 (CSSM47) to 0.881 (OarFCB48) with a mean value of 0.618, while the expected heterozygosity value ranged from 0.349 (BM8125) to 0.865 (OMHC1) with a mean value of 0.725.

Yadav *et. al.* (2011) reported that the observed heterozygosity in Munjal sheep ranged from 0.175 (CSSM47) to 0.925 (OarCP20) while the expected heterozygosity ranged from 0.209 (CSSM47) to 0.881 (OarJMP29). The mean observed heterozygosity (0.712) was lower than the mean expected heterozygosity (0.744).

Hepsibha *et. al*. (2014) reported that the mean observed and expected heterozygosity values in Coimbatore sheep breed were 0.6005 and 0.6046, respectively

Exotic Breeds

The average H_O over all breeds and loci was 0.59 and was lower ($P < 0.001$) than the corresponding estimate for H_E (0.74). Estimates of H_O among the breeds varied between 0.50

in Red Masai and 0.67 in Cheviot. Red Masai, Romanov and Suffolk had the lowest H_O values (0.50 to 0.67) and H_O of the other breeds was intermediate and varied within 0.02 points (0.59 to 0.61). Pair wise comparisons of the breeds showed that H_E of Romanov breed was significantly lower than those in all other breeds except that of Red Masai and H_E of Red Masai (0.57) was significantly lower than those of North Country Cheviot, Cheviot and Scottish Blackface, which showed the largest values (0.66 to 0.67).The estimates of H_E were greater than or equal to H_O in every breed, but Suffolk with the largest difference (0.10) was the only breed for which difference was significant (Farid *et. al*., 2000).

Diez-Tascon *et. al*. (2000) reported that all the six populations of the Merino sheep exhibited similar genetic diversity. The observed heterozygosity ranged from 0.679 (FM) to 0.763 (PW) and the gene diversity values ranged from 0.686 (FM) to 0.774 (S).

Pariset *et. al*. (2003) reported that the observed and expected heterozygosity per flock in all the 17 flocks of Sarda sheep breed ranged from 0.44 to 0.76 and from 0.66 to 0.82 respectively. The mean expected heterozygosity over all flock was 0.75 and the mean observed heterozygosity over all flock was 0.60.

The allelic variation ranged from 5.00 to 7.52 with an average of 6.42 alleles per breed within the 57 European and Middle Eastern sheep breeds. The average expected heterozygosity over all loci ranged from 0.63 to 0.77, while observed heterozygosity varied from 0.58 to 0.75. Mean estimates of expected and observed heterozygosities over all loci and breeds were 0.72 and 0.67 respectively (Peter *et. al.* 2007).

The indices of the observed heterozygosity per locus in all the four Kazak breeds (Ozerov *et. al*. 2008) varied from 0.62 (BM 6506) to 0.90 (INRA23) with an average value of 0.75. The results of the expected heterozygosity differed little from the observed heterozygosity.

The average direct count of observed heterozygosity values in Barki, Ossimi and Rahmani breeds of Egypt were 0.590, 0.547 and 0.615, respectively. The average expected heterozygosity value at overall loci in these breeds was 0.860, 0.811 and 0.855, respectively (Nahas *et. al*., 2008).

The total genetic diversity in 52 sheep breeds from Eurasis as estimated by Tapio *et. al*. (2010) varied from 0.651 to 0.807 in the Danish and the Ukrainian groups, respectively. Among breeds, the unbiased expected heterozygosity ranged from 0.613 (Norwegian Cheviot) to 0.806 (Russian Karakul) with an average value of 0.759.

Rodrigo *et. al*. (2010) reported that the observed heterozygosity in the four Chilean breeds varied from 0.595 (Romney Marsh) to 0.809 (Suffolk Down) whereas the expected heterozygosity varied from 0.695 (Romney Marsh) to 0.755 (Chilota). This value shows that Chilota is the most genetically diverse breed, while Romney Marsh is the least.

The mean observed heterozygosity overall loci in Gray, Zandi and Karakul breeds are 0.984, 0.986 and 0.988 respectively whereas the mean expected heterozygosity overall loci for the above three breeds are 0.815, 0.807 and 0.808 respectively (Nanekarani *et. al*. 2010).

Kevorkian *et. al*. (2010) reported that in the four Romanian sheep breeds, the overall mean heterozygosity value as 0.733 ± 0.09, while the mean observed heterozygosity value as 0.611 ± 0.17. The value for expected heterozygosity was highest for HSC (0.87) and lowest for OarCP20 (0.52).

The genetic architecture of two sub-populations of Baluchi sheep revealed that the overall expected heterozygosity was 0.66 ranging from 0.47 (BM1853) to 0.76 (MCM200). The mean expected heterozygosities in sub-population 1 and 2 were 0.63 and 0.65, respectively. The observed heterozygosity values varied between 0.39 (BM1853) and 0.99

(MCM200). Expected heterozygosity value was higher than its corresponding observed values for BM1853, BM6465, BM7247 and BMS1714 loci (Dashab *et. al*., 2011).

The expected heterozygosity value in Karakul sheep ranged from 0.691 (MAF64) to 0.910 (MCMA2). The observed heterozygosity value varied from 0.968 (BMS460) to 1.00 (OARFCB20, LSCV38 and MCMA26) (Nanekarani *et. al*., 2011).

Ahmed *et. al*. (2014) reported the mean values of observed and expected heterozygosities in Kail sheep as 0.76 and 0.72 respectively. Eight markers out of 11 showed higher observed heterozygosity than expected. ILSTS011 and OarFCB48 showed high observed heterozygosity (1.00) while, INRA32 showed lowest (0.20) and expected heterozygosity ranged from 0.51 (MM12) to 0.84 (MAF33).

2.5.3 INBREEDING ESTIMATES

With pedigrees, the usual way to estimate diversity is through inbreeding coefficient and kinship coefficient. They are the probabilities that two genes taken at random from the same or different individuals, respectively, are identical by descent. They form the key parameters in monitoring conservation programmes.

Indigenous Breeds

The within population inbreeding estimate (F_{IS}) in Muzzafarnagri sheep was 0.058. This low rate of inbreeding was attributed to absence of directional selection (Arora and Bhatia, 2004).

Magra population showed a high within population inbreeding estimate (0.159), which revealed a heterozygote deficiency (Arora and Bhatia, 2006).

The within population inbreeding estimates were observed to be high in all the three breeds Chokla (0.286), Nali (0.284) and Garole (0.227), which reflected the deficit of heterozygotes. Based on the pair wise F_{ST} and N_m between the breeds, Nali and Chokla (6.62% and 4.80) were observed to be closest followed by Garole and Nali (20.9% and 1.80) and Garole and Chokla (21.4% and 1.71) (Mukesh *et. al*., 2006).

The within population inbreeding estimate in Hassan sheep ranged from 0.075 to 0.808 with an average of 0.218 ± 0.060 (Sharma *et. al*., 2006).

Sodhi *et. al*. (2006) stated that both Nali and Chokla sheep populations showed significant heterozygote deficit, being 39.7% in Nali and 29.9% in Chokla sheep. The average F_{IS} value for most of the loci in both the breeds is significantly different ($p < 0.05$) from zero.

The overall within population inbreeding estimates or heterozygotes deficiency within-population calculated in Nilgiri sheep (Girish *et. al*., 2007) was -0.0551, indicating excess of heterozygotes in the population. The excess of heterozygotes in 80 percent of the loci reflects out-breeding and wide genetic variability in the population.

The overall positive F_{IS} value (0.129) and F_{IT} (0.179) > F_{ST} (0.058) indicated inbreeding to be one of the main cause for high genetic homogeneity. Also, the low genetic distance measures (Nei's unbiased = 0.189) and high gene flow (N_m = 4.1) values implied low level of genetic differentiation between Marwari and Sonadi breeds (Bhatia and Arora, 2007).

Pandey *et. al*. (2007) estimated the within-population inbreeding estimate in Bonpala sheep as 0.028 and the values for 24 microsatellite loci ranged from -0.339 to 0.627. On an average of 2.8% shortfall of heterozygotes is observed in Bonpala sheep population.

Arora and Bhatia (2008) observed that the value of genetic differentiation (F_{ST}) ranged from 6.2% for Marwari-Sonadi breed pair to 15% for Magra- Sonadi breed pair. The gene

differentiation values ranged from 6.3% (Marwari – Sonadi) to 15.1% (Magra – Sonadi). A considerable higher level of gene flow was observed between Marwari and Sonadi (N_m = 3.8) in comparision to Magra – Marwari (N_m = 2.4) or Magra – Sonadi (N_m = 2.1). The close relationship between Marwari and Sonadi was confirmed by Nei's genetic distance (0.27) whereas the distances between Magra - Sonadi (0.47) and Magra – Marwari (0.42) were comparatively greater. These values supported highest degree of divergence between Magra-Sonadi and Magra-Marwari and substantial genetic similarity between Marwari and Sonadi breeds.

Arora *et. al*. (2008) observed a population inbreeding estimate of 0.12 with a range from -0.32 (BM8125) to 0.61 (OarHH47) in Jalauni sheep. Positive F_{IS} value suggested inbreeding to be one of the main causes for lack of heterozygotes in Jalauni sheep.

The studies on Mecheri sheep revealed negative F_{IS} values in six of the loci while positive values were observed across 11 loci, the values ranged from -0.009 to 0.362 and the inbreeding estimate for within-population was 0.004 (Prema *et. al*., 2008a).

The within-population inbreeding estimates calculated in Madras Red sheep ranged from -0.074 to 0.298 with a mean of 0.021. Out of the seventeen loci studied, eight markers revealed negative F_{IS} value, while the remaining nine loci revealed positive values (Prema *et. al*., 2008b).

The genetic differentiation values ranged from 6.166% for Marwari-Sonadi breed pair to 14.8% for Jalauni-Sonadi breed pair. And the gene differentiation values ranged from 6.2% (Marwari – Sonadi) to 14.9% (Jalauni – Sonadi). A considerable higher level of gene flow was observed between Marwari and Sonadi (3.6), in comparision to Jalauni-Marwari (2.1) or Jalauni – Sonadi (1.7) The Nei's genetic distance revealed a close relationship between Marwari – Sonadi breeds (0.261), while the distances between Jalauni – Sonadi (0.468) and

Jalauni – Marwari (0.412). These results substantiated the high level of genetic similarity between Marwari and Sonadi breeds. (Arora and Bhatia, 2009).

The within population inbreeding estimate was found to be 0.066 with a range from -0.006 (OarHH72) to 0.223 (OarJMP29) in Coimbatore sheep (Kumarasamy *et. al*., 2009).

Pramod *et. al*. (2009) reported negative F_{IS} values for three loci (BM827, OarCP20 and OarJMP29) in Vembur sheep, while the values for other loci ranged from 0.1005 in BM1314 to 0.7882 in BM8125. The mean F_{IS} value observed was 0.2954 ± 0.31 indicating the inbreeding status of the population.

Arora *et. al*. (2010) reported an F_{IS} value of 0.087 with a range of -0.241 (OarVH72) to 0.821 (OarAE129) in Ganjam sheep. The positive F_{IS} value observed was not significant ($p > 0.05$) which indicated a very low rate of inbreeding in Ganjam sheep population.

The mean F_{IS} value was found to be 0.113, F_{IT} as 0.131 and F_{ST} as 0.020 in Patanwadi, Marwari and Dumba sheep breeds (Jyotsana *et. al*. 2010). The high F_{IS} and F_{IT} values indicate high level of inbreeding within and among the populations studied. Multilocus F_{ST} values indicated that only 2.0% of the total genetic variation was explained by a breed difference, the remaining 98% corresponds to differences among individuals.

Arora *et. al*. (2011b) estimated the range of pairwise differentiation between breeds from 1.1% for the Magra–Pugal and 5.9% for the Nali–Marwari pair. According to the global F_{ST} values, breed differences contribute 6.1% of the genetic variation and variation among individuals accounts for 93.9%. The genetic distance (D_A) revealed close relationships between the Magra-Pugal breeds (0.072) and the Nali-Jaisalmeri breeds (0.079) while the value for the Nali-Marwari pair (0.249) was comparatively greater.

Radha *et. al*. (2011) investigated 25 microsatellite loci in Kilakarsal sheep and observed a heterozygote deficiency at 16 loci. The positive F_{IS} value ranged from 0.016 (BM8125) to 0.648 (CSSM47). Nine loci revealed negative values ($F_{IS} < 0$) indicating absence of inbreeding at these loci. The mean F_{IS} value was 0.147.

The within population inbreeding estimates (F_{IS}) for 25 microsatellite loci in Munjal sheep ranged from -0.513 (BM6506) to 0.637 (OarHH64) with a mean value of 0.034. Seven of the loci studied showed significant heterozygote deficiency which has been attributed to segregation of non-amplifying alleles, Walhund effect, scoring biases, assortative mating, linkage with loci under selection, population heterogeneity or inbreeding (Yadav *et. al*., 2011)

Exotic Breeds

Farid *et. al*. (2000) computed F_{ST} over all the ten sheep breeds and loci and the value was 0.163 ± 0.040 indicating a significant level of differentiation among the breeds. Nei's genetic distances between the Bristish breeds(North Carolina Cheviot, Cheviot, Suffolk, Dorset and Scottish Blackface) were small ranging from 0.213 (NCC and DOR) to 0.383 (NCC and SBF). The North European breeds (Romanov, Icelandic and Finnish Landrace) were also genetically close to each other with genetic distance ranging from 0.256 (ICE and FIN) to 0.436 (ICE and ROM). MAS was close to the North European breeds and Texel did not show any clear patterns of genetic relationships with the breeds in any of these two groups.

Nei's genetic distance was smallest (0.086) between Spanish and Portuguese Black Merino. The French and German Merino are relatively close (Nei = 0.139). The distance between French Mutton and New Zealand is estimated to be 0.356 and German Mutton and New Zealand is 0.328. All the results confirmed closest association among Spanish and

Portuguese populations, followed by French Mutton and German Mutton with the New Zealand population most distantly related (Diez-Tascon *et. al*. 2000).

The mean F_{IS} value among all the 19 Sarda flocks was reported as 0.19 (Pariset *et. al*, 2003). Among the 17 flocks, 9 flocks did not vary significantly, five flocks showed significantly high F_{IS} value, while the other three showed a significant inbreeding.

Peter *et. al*. (2007) reported that there was high level of inbreeding in the 57 breeds of Europe and Middle-Eastern sheep populations which was confirmed by positive inbreeding estimates for all breeds ranging from 0.007 to 0.149. The average genetic differentiation between all breeds was 5.7% which was significantly different from zero ($P < 0.001$).

The values of the degree of inbreeding within the population of the Kazakh breeds (Ozerov *et. al*. 2008) did not deviate statistically from zero across all investigated loci which indicates the absence of intrabreed subdivision of the investigated populations.

El-Nahas *et. al*. (2008) found the highest within population inbreeding coefficient for the three Egyptian breeds (Barki, Ossimi and Rahmani) at BM827 locus, while the highest F_{ST} and F_{IT} values were observed at TGLA377 and BM827 loci. The global F_{IS}, F_{ST} and F_{IT} values were 0.308, 0.037 and 0.333 respectively.

The overall estimate of inbreeeding was 0.011 for the 52 sheep breeds of Eurasia (Tapio *et. al*. 2010). The breed wise inbreeding estimates were significantly ($P < 0.05$) greater than zero only for the Norwegian Rygia sheep and the Swedish Rya sheep suggesting that most breeds are quite uniform.

Nanekarani *et. al*. (2010) reported the global F_{IS}, F_{ST} and F_{IT} values as -0.19, 0.018 and -0.168, respectively for the three Iranian pelt sheep. All the markers had negative values of F_{IS}, showing an excess of heterozygote. F_{ST} values ranged from 0.003 (BMS332) to 0.042

(OarAE129). Per pair estimator of F_{ST}, which is a measure of differentiation among population is 0.013 for Gray-Zandi, 0.013 for Gray-Karakul and 0.015 for Zandi-Karakul. The smallest genetic distance (0.139) was estimated between Gray and Karakul.

The mean fixation indices F_{IT}, F_{IS} and F_{ST} as observed by Kevorkian *et. al*. (2010) in the four Romanian sheep are 0.247, 0.182 and 0.082 respectively. The genetic differentiation among the analyzed breeds (F_{ST}) was 8.2%. The Nei's genetic distances between breeds ranged from 0.263 for Milk Line Palas and Meat Line Palas to 0.606 for Karabash and Meat Line Palas.

Most of the markers used by Dashab *et. al*. (2011) in Baluchi sheep had positive values for F_{IS} (BM1853, BM6465, BM7247 and BM1714) while negative values were observed for MCM200, RM0006 and BM0741 markers. The overall inbreeding coefficients (F_{IS} = 0.003 and F_{IT} = 0.027) indicated a decrease of heterozygotes, thus the population was in the risk of inbreeding depression. The F_{ST} value (0.024) showed the low differentiation between the two populations.

The F_{IS} estimates as observed by Ahmed *et. al*. (2014) in Kail sheep ranged from -0.327 (MM12) to 0.655 ((INRA32).

2.5.4 HARDY – WEINBERG EQUILIBRIUM

A population with constant gene and genotype frequencies is said to be in Hardy-Weinberg Equilibrium (HWE). The natural processes of mutation, migration, non-random mating, genetic drift and both artificial and natural selection are factors that are known to cause deviations from HWE. Ideal HWE populations do not actually occur in nature owing to various factors, which can shift the equilibrium and disrupt the stability of the population, giving rise to change in genetic structure.

Indigenous Breeds

Heterozygote deficiency analysis of Nali, Chokla and Garole breeds (Mukesh *et. al*., 2006) revealed that all the three populations exhibited significant deviations from HWE ($p < 0.05$) at several loci and the presence of low frequency null alleles segregating at these loci may be the possible cause for this deviation.

Fourteen of the 18 loci studied by Sharma *et. al*. (2006) in Hassan sheep showed significant deviations from Hardy-Weinberg equilibrium. Tests for heterogeneity of deviations from Hardy-Weinberg equilibrium for all the loci showed heterogeneity among loci.

The results of the χ^2 test of goodness of fit revealed that the Nilgiri sheep population was in HWE proportions for 17 out of 25 microsatellite loci. The remaining 8 loci departed from HWE which might be either due to the presence of unobserved null alleles (or) due to the consequence of several years of intensive selection practiced in the population of Nilgiri sheep (Girish *et. al*., 2007).

The Jalauni breed showed significant ($P < 0.05$) deviation from HWE at six loci, which might represent sub-structure present in the form of localized heterozygote deficiencies suggestive of localized inbreeding. Arora *et. al*. (2008) attributed these departures were due to the presence of null alleles.

The results of the χ^2 test of goodness of fit in Mecheri sheep revealed that the population was not in Hardy-Weinberg equilibrium ($p < 0.01$) at 11 loci, out of 17 loci screened. Systematic and dispersive processes operating in the population, selection pressure and decrease in effective number of breedable individuals as a result of less number of males siring the flock were the reasons suggested by Prema *et. al*. (2008a).

Prema *et. al*. (2008b) analysed the results of χ^2 test for goodness in Madras Red sheep and revealed that the population was in Hardy-Wienberg equilibrium proportions at BM1314, BM6526, BM757, OarCP38, OarHH41 and OMHC1 loci and the other disequilibria in the remaining loci which were attributed to the systematic and dispersive process operating in the population.

Only eight out of the 27 loci studied in Coimbatore sheep were in Hardy Weinberg Equilibrium and the remaining 19 loci significantly departed from Hardy Weinberg Equilibrium (Kumarasamy *et. al*., 2009).

Nineteen out of 25 loci investigated in Vembur sheep showed highly significant chi square values suggesting departure from HWE (Pramod *et. al*., 2009). The loci OarHH47, OarJMP29 and TGLA377 revealed statistically significant departure from HWE, while the loci BM1314, OarCP34 and OarFCB20 were found to be in HWE.

Arora *et. al.* (2010) reported that the Ganjam breed showed a significant ($p < 0.05$) departure from Hardy-Weinberg equilibrium at four loci (BM757, BM6506, BM6526 and OarVH72) and this deviation might represent non-random mating, selection or the presence of null alleles.

Sharma *et. al*. (2010) reported that most of the loci studied in Changthangi sheep were in Hardy-Weinberg equilibrium and significant departure from HWE had been observed only in 5 out of 25 loci ($P < 0.01$). The χ^2 value ranged from 2.816 to 168.278.

Arora *et. al.* (2011b) reported that the loci BM1314, CSSM47 and OarHH64 deviated from Hardy Weinberg Equilibrium in five populations and CSSM31 deviated in six populations ($P < 0.001$).

Radha *et. al*. (2011) observed that the Kilakarsal population was in Hardy-Weinberg equilibrium proportions for seven microsatellite loci (Oar FCB48, OarHH47, BM8125, CSSM31, CSRD247, HSC and INRA63) and the remaining 18 loci showed significant departure from HWE.

Hepsibha *et. al*. (2014) reported that out of 24 microsatellites studied in Coimbatore sheep, only 12 loci were in Hardy Weinberg Equilibrium, while the remaining 12 loci departed from Hardy Weinberg Equilibrium.

Exotic Breeds

Nanekarani *et. al*. (2010) reported that the tests of genotype frequencies for deviation from HWE at each locus in three Iranian pelt sheep breeds, reveal significant departure from HWE ($P > 0.001$).

Kevorkian *et. al*. (2010) reported that the deviations from the HWE in the four Romanian breeds were found to be significant ($p < 0.05$) in OarCP20, MAF70, MAF214, MAF33 and highly significant ($p < 0.001$) in OarFCB11.

All the seven loci investigated in Baluchi sheep deviated significantly ($p < 0.01$) from HWE (Dashab *et. al*., 2011).

All loci studied in Karakul sheep deviated from HWE ($p < 0.001$) which was attributed to heterozygote excess, migration, high mutation rate in microsatellite and artificial selection in breeds (Nanekarani *et. al*., 2011).

2.5.5 POLYMORPHIC INFORMATION CONTENT

Another measure of genetic diversity within breeds is Polymorphic Information content (PIC). The PIC is a parameter indicative of the degree of informativeness of a marker.

The PIC value may range from 0 to 1. Loci with many alleles and a PIC value of 1 are most desirable (Botstien *et. al*., 1980). Hence the degree of informativeness of a marker reveals its usefulness in diversity analysis of a breed. A higher value of PIC means more alleles and greater polymorphism at that locus.

Indigenous Breeds

The PIC values for the microsatellites BM6505, HUJ616, OarCO38 and TGLA377 were 0.360, 0.277, 0.467 and 0.458 apart from these the PIC values ranged from 0.533 (BM6526) to 0.808 (TGLA137) with a mean value of 0.636 which reflected high level of genetic heterogeneity in Muzzafarnagri sheep (Arora and Bhatia, 2004).

The PIC values of Magra sheep varied from 0.347 (CSSM47) to 0.849 (BM1314) with a mean value of 0.648. The study exhibited that about 88% of the markers used showed higher PIC values (Arora and Bhatia, 2006).

Nali breed displayed the highest average PIC (0.61) followed by Chokla (0.60) and Garole (0.56) sheep (Mukesh *et. al*., 2006).

All the 18 loci in Hassan sheep were polymorphic and highly informative with a mean PIC value of 0.644 ± 0.146 except for BM8125 and OarAE129 whose PIC values were 0.295 and 0.356, respectively (Sharma *et. al*., 2006).

Based on the PIC values, nearly 92% of the markers were found to be highly informative (PIC > 0.50) and only 8% of the markers were reasonably informative in Nali and Chokla sheep. The PIC values ranged from 0.210 (BM6506) to 0.849 (OarFCB48) with a mean value of 0.613 across all 25 loci studied (Sodhi *et. al*., 2006).

Girish *et. al.* (2007) reported that the PIC values in Nilgiri sheep varied from 0.458 (OarAE129) to 0.827 (BM1314) across 25 microsatellite loci. The mean PIC value for all loci

was 0.6485. And about 96% of the markers showed a PIC value more than 0.5, indicating the efficacy of the microsatellite markers.

The PIC values in Bellary sheep as reported by Kumar *et. al.* (2007) ranged from 0.328 (BM6506) to 0.848 (OarHH35) with a mean value of 0.636.

Arora *et. al.* (2008) reported that the PIC values ranged from 0.24 (OarCP20) to 0.82 (TGLA137) with a mean value of 0.64 in Jalauni sheep.

The PIC values across 17 microsatellite loci in Mecheri sheep varied from 0.523 (BM827) to 0.791 (OarCP38) with a mean value of 0.660 (Prema *et. al*., 2008a).

The study of 17 microsatellite loci in Madras Red sheep showed higher PIC values indicating suitability of these loci for genetic studies in sheep. The PIC values in this study ranged from 0.454 (BM6506) to 0.785 (oarFCB48) (Prema *et. al*., 2008b).

The PIC values for 27 microsatellite loci in Coimbatore sheep ranged from 0.396 (BM6506) to 0.809 (TGLA377), indicating the efficacy of these markers for genetic diversity studies in sheep (Kumarasamy *et. al*., 2009).

Pramod *et. al*. (2009) estimated that the PIC values in Vembur sheep ranged from 0.371 (OarHH47) to 0.836 (HUJ616) with a mean of 0.6905 ± 0.02. About 96 percent of the markers used in this study had PIC values greater than 0.5 and OarHH47 was found to be reasonably informative.

Arora *et. al.* (2010) reported that the PIC values in Ganjam sheep ranged from 0.199 (CSSM47) to 0.859 (CSSM31). About 84 percent of the markers used in this study were highly informative with PIC value more than 0.5, while 12% are moderately informative with PIC value ranging from 0.25 to 0.5 and the remaining 4% had a PIC value less than 0.25.

All the microsatellites studied, except BM6506 (0.46) and CSSM47 (0.15),had a PIC value more than 0.5 in Patanwadi, Marwari and Dumba breeds with mean PIC value of 0.66, 0.70 and 0.66, respectively (Jyostana *et. al*., 2010).

The study of 25 microsatellite markers by Radha *et. al*., 2011 in Kilakarsal sheep breed revealed that the PIC values ranged from 0.591 (CSSM47) to 0.917 (OarFCB128) with a mean value of 0.831.

The high mean PIC (0.721) value, for most of the microsatellite loci used in Munjal sheep indicated that they were highly informative and the values ranged from 0.210 (CSSM47) to 0.994 (OarJMP29) (Yadav *et. al*., 2011).

All the 24 microsatellite loci studied in Coimbatore sheep breed revealed high polymorphism and the PIC values ranged from 0.114 (OarHH64) to 0.835 (OarHH47) with a mean value of 0.5851 ± 0.04 (Hepsibha *et. al*., 2014).

Exotic Breeds

Nanekarani *et. al*. (2010) reported the mean PIC overall loci in Gray, Zandi and Karakl breeds as 0.815, 0.807 and 0.808, respectively. And fairly high level of genetic heterogeneity was further reflected within three breeds by a mean PIC value of 0.828.

The PIC values for the four autochthonous Romanian sheep breeds (Kevorkian *et. al*., 2010) ranged from 0.621 (OarCP20) to 0.86 (HSC).

Dashab *et. al*. (2011) reported relatively high PIC values (0.45 in BM1853 to 0.76 in MCM200) in Baluchi sheep breed.

Molecular characterization of Karakul sheep breed (Nanekarani *et. al*., 2011) revealed that the PIC values were within a range of 0.634 (MAF64) and 0.903 (MCMA2) with an overall mean of 0.808.

Ahmed *et. al*. (2014) reported the mean PIC value as 0.69 across all loci. The BM1314 showed high PIC value (0.82) and MM12 showed the lowest (0.42).

MATERIAL & METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 EXPERIMENTAL MATERIAL

A total of 100 unrelated animals, 50 each from Deccani and Nellore breeds of sheep were utilized for the present study. Deccani is a medium sized animal, predominantly black or black with white markings on the body. The body weight of adult Ram is 38.48 ± 1.06 kg and that of Ewe is 28.58 ± 0.11 kg. Males are horned but ewes are polled. Nellore is relatively taller breed with adult body weights of Ram and Ewe being 36.69 ± 2.56 kg and 30.00 ± 0.27 kg respectively. Rams are horned and ewes are almost always polled.

Of the 50 blood samples of Deccani, 20 were collected from animals maintained at Sheep Farm, ILFC, College of Veterinary Science, Rajendranagar while the remaining 30 were collected from Livestock Research Station, Mahabubnagar. In case of Nellore sheep, 20 samples were obtained from Livestock Research Station, Siddiramapuram, Anantapur district; 14 from Livestock Research Station, Mamnoor, Warangal and the rest 16 from three different flocks of animals maintained by the farmers in Warangal district.

Fig 1. Deccani Ram

Fig 2. Deccani Ewe

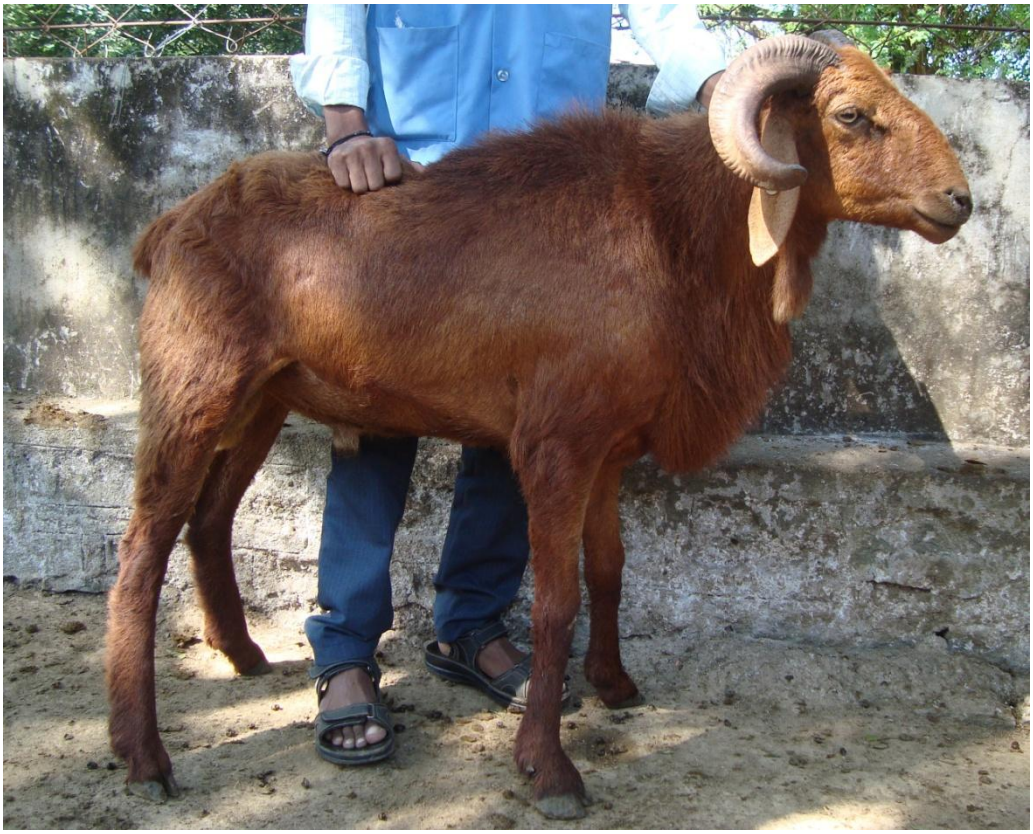

Fig 3. Nellore Ram

Fig 4. Nellore Ewe

Fig 5. Farmers flock of Nellore sheep

3.2 MICROSATELLITE MARKERS

A total of thirty microsatellite marker primers chosen from the list recommended by FAO were utilized in the present study. The markers comprised mostly dinucleotides. The sequences of 30 microsatellite marker primers utilized in the study along with the repeat motifs, annealing temperatures, allele size (bp) and chromosome location are detailed in Table 1.

3.3 ISOLATION OF GENOMIC DNA

Blood samples (5-6 ml per animal) were collected into vacutainers containing EDTA from the jugular vein, mixed gently and stored at -20^{0}C until further processing. DNA was extracted from blood samples following the phenol-chloroform method (Sambrook *et. al*., 1989).

3.3.1 Preparation of stock/working solutions for DNA isolation

0.1M TrisHcl (pH-7.5)

Tris base (1M)	-	1.21g
Distilled water	-	100 ml

1.6M Sucrose

Sucrose	-	87.54 g
Distilled water	-	100 ml

0.5M $MgCl_2$

$MgCl_2$ (1M)	-	2.38 g
Distilled water	-	50 ml

1M Tris (pH 8.0)

Tris base (1M)	-	12.11 g
Distilled water	-	100 ml

5M NaCl

NaCl	-	14.6 g
Distilled water	-	50 ml

0.5M EDTA

EDTA	-	18.61 g
Distilled water	-	100 ml

10% SDS(pH-7.2)

SDS	-	10g
Distilled water	-	100 ml

3M Sodium acetate (pH 5.2)

Sodium acetate	-	20.4 g
Distilled water	-	50 ml

TE buffer

1M TrisHcl (pH 8.0)	-	0.5 ml
0.5M EDTA (pH8.0)	-	100 µl

3.3.2 Isolation of DNA by Phenol - Chloroform method

- The blood samples were transferred from vacutainers into 15 ml centrifuge tubes.
- Chilled RBC lysis buffer, double the quantity of blood was added and mixed end to end and incubated on ice for about 10 min.
- The samples were then centrifuged at 4000 rpm for 10 min.
- The supernatant containing plasma and lysed RBC was discarded by pipetting and the step of adding lysis buffer, centrifugation followed b discarding supernatant was repeated for 3-4 times till the WBC pellet became free from red tinge.

- DNA extraction buffer was added @ 3ml/10 ml of blood and incubated at 37^0C for 30 min in water bath.
- 20 µl of 10% SDS was added and mixed gently by inversion
- 40µl of proteinase K (25 mg/µl) was added and incubated overnight at 50^0C in water bath
- Next morning an equal volume of Tris-saturated Phenol (pH > 8) was added and mixed by inverting the tubes for about 10 min.
- The tubes were then centrifuged at 4000 rpm for 10 min and the upper aqueous phase containing DNA was collected and transferred to 15 ml capacity centrifuge tubes
- The step was repeated once with Phenol:Chloroform:Isoamyl alcohol (25:24:1) and once with Chloroform:Isoamyl alcohol (24:1) and again the upper aqueous phase was transferred to fresh 15 ml capacity centrifuge tubes
- 3M sodium acetate ($1/10^{th}$ volume) was added and mixed gently
- Two volumes of isopropanol was added and mixed by gentle inversion and kept at room temperature for precipitation of DNA
- The precipitated DNA along with 500 µl of isopropanol was taken into 1 ml eppendorf tubes. The tubes were then centrifuged at 10000 rpm for 10 min and the supernatant was discarded by inversion
- The DNA pellet was washed in 70% ethanol twice and centrifuged at 10000 rpm for 10 min by discarding the supernatant by inversion

- Finally, the DNA pellet was air dried by inverting the tube on to blotting paper and then the pellet was dissolved in 200 µl of TE buffer.

3.4 QUALITY AND QUANTITY OF GENOMIC DNA ISOLATED

The quantity of genomic DNA was measured by UV spectrophotometer and quality by electrophoresis on 0.8% agarose gels. The ratio of optical absorbance (A) at 260 and 280 nm was used as an indicator of DNA purity. The A260/A280 ratio ranging from 1.7 to 1.9 was considered as relatively pure DNA and only such samples were used for PCR amplification. The concentration of the DNA was estimated by using the formula developed by Sambrook and Russell (2001).

$$\text{DNA in } {}^{\mu g}/_{\mu l} = A_{260} \text{ X } 50 \text{ X Dilution Factor}$$

Table 1. Details of microsatellite marker primers used in the study

S. No.	Locus	Repeat motif	Primer sequence	Allele size (bp)	Chromosome location	Accession number	Reference
1	BM1314	--	F: TTCCTCCTCTTCTCTCCAAAC R: ATCTCAAACGCCAGTGTGG	149-179	22	G18433	Bishop *et al*. (1994)
2	BM6506	$(CA)_{23}$	F: GCACGTGGTAAAGAGATGGC R: AGCAACTTGAGCATGGCAC	85-123	1	GI8455	Bishop *et al*. (1994)
3	BM6526	$(CA)_{22}$	F: CATGCCAAACAATATCCAGC R: TGAAGGTAGAGAGCAAGCAGC	161-175	26	G818454	Bishop *et al*. (1994)
4	BM757	$(GT)_{14}$	F: TGGAAACAATGTAAACCTGGG R: TTGAGCCACCAAGGAACC	--	9	BE603866	Bishop *et al*. (1994)
5	BM8125	$(CA)_{16}$	F: CTCTATCTGTGGAAAAGGTGGG R: GGGGGTTAGACTTCAACATACG	110-130	17	G18475	Bishop *et. al.* (1994)
6	CSSM31	$(AC)_{35}$	F: CCAAGTTTAGTACTTGTAAGTAGA R: GACTCTCTAGCACTTTATCTGTGT	--	23	U03838	Moore *et. al.* (1994)
7	ETH121	$(GT)_{22}$	F: CCAACTCCTTACAGGAAATGTC R: ATTTAGAGCTGGCTGGTAAGTG	182-198	Bovine 2	Z14037	Steffen et al. (1992)
8	HUJ616	$(GT)_{21}$	F: TTCAAACTACACATTGACAGGG R: GGACCTITGGCAATGGAAGG	114-160	13	M88250	Barendse *et. al.* (1995)
9	ILSTS030	--	F: CTG CAG TTC TGC ATA TGT GG R: CTT AGA CAA CAG GGG TTT GG	146-158	2	L37212	Kemp *et. al.* (1995)
10	ILSTS11	--	F: GCTTGCTACATGGAAAGTGC R: CTAAAATGCAGAGCCCTACC	256-294	9	L23485	Brezinsky *et. al.* (1993)
11	ILSTS28	--	F: TCCAGATTTTGTACCAGACC R: GTCATGTCATACCTTTGAGC	105-177	3	L37211	Kemp *et. al.* (1995)
12	MAF209	--	F: GATCACAAAAAGTTGGATACAACCGTGG R: TCATGCACTTAAGTATGTAGGATGCTG	--	17	--	Buchanan and Crawford (1992)
13	MAF214	$(GT)_{56}$	F: AATGCAGGAGATCTGAGGCAGGGACG R: GGGTGATCTTAGGGAGGTTTTGGAGG	174-282	16	M88160	Buchanan and Crawford (1992)
14	MAF33	$(CA)_{19}$	F: GATCTTTGTTTCAATCTATTCCAATTTC R: GATCATCTGAGTGTGAGTATATACAG	121-141	9	M77200	Buchanan and Crawford (1992)
15	MAF70	$(CA)_{39}$	F: CACGGAGTCACAAAGAGTCAGACC R: GCAGGACTCTACGGGGCCTTTGC	124-166	4	M77199	Buchanan and Crawford (1992)

16	MCM140	$(GT)_{19}$	F: GTT CGT ACT TCT GGG TAC TGG TCT C R: GTC CAT GGA TTT GCA GAG TCA G	167-193	6	L38979	Hulme *et. al.* (1995)
17	OarAE129	$(CA)_{14}$	F: AATCCAGTGTGTGAAAGACTAATCCAG R: AATCCAGTGTGTGAAAGACTAATCCAG	133-159	5	L11051	Penty *et. al.* (1993)
18	OarCB226	$(CA)_{14}$	F: CTATATGTTGCCTTTCCCTTCCTGC R: GTGAGTCCCATAGAGCATAAGCTC	119-153	2	L20006	Buchanan *et. al.* (1994)
19	OarCP20	$(GT)_{14}$	F: GATCCCCTGGAGGAGGAAACGG R: GGCATCTCATGGCTTTAGCAGG	--	21	U15695	Ede *et. al.* (1995)
20	OarCP34	$(GT)_{16}$	F: GCTGAACAATGTGATATGTTCAGG R: GGGACAATACTGTCTTAGATGCTGC	112-130	3	U15699	Ede *et. al.* (1995b)
21	OarFCB 128	$(GT)_{15}$	F: ATTAAAGCATCTTCTCTTTATTTCCTCGC R: CAGCTGAGCAACTAAGACATACATGCG	96-130	2	L01532	Buchanan *et. al.* (1994)
22	OarFCB20	$(GT)_{15}$	F: AAATGTGTITAAGATTCCATACAGTG R: GGAAAACCCCCATATATACCTATAC	95-120	2	L20004	Buchanan *et. al.* (1994)
23	OarFCB48	$(GT)_{10}$	F : GACTCTAGAGGATCGCAAAGAACCAG R: GAGTTAGTACAAGGATGACAAGAGGCAC	146-166	17	M82875	Buchanan *et. al.* (1994)
24	OarHH35	$(CA)_{17}$	F : AATTGCATTCAGTATCTTTAAACATCTGGC R: ATGAAAATATAAAGAGAATGAACCACACGG	121-137	4	AF394447	Henry *et. al.* (1993)
25	OarHH41	$(CA)_{23}$	F : TCCACAGGCTTAAATCTATATAGCAA R: GAGCGGTGTAGTAGAAAATAGAAATCGACC	96-120	10	L12555	Henry *et. al.* (1993)
26	OarHH47	$(CA)_{22}$	F : TATATTGACAAACTCTCTTCCTAACTCCACC R: GTAGTTATI'TAMAAAATATCATACCTCTTAAGG	130-152	18	L12558	Henry *et. al.* (1993)
27	OarJMP29	$(CA)_{21}$	F : GTATACACGTGGACACCGCTTTGTAC R: GAAGTGGCAAGA'ITCAGAGGGGAAG	150-188	24	U30893	Penty *et. al*. (1993)
28	OarVH72	$(GT)_{14}$	F : GGCCTCTCAAGGGGCAAGAGCAGG R: CTCTAGAGGATCTGGAATGCAAAGCTC	121-145	25	L12548	Pierson *et. al.* (1993)
29	SRCRSP1	--	F : TGC AAG AAG TTT TTC CAG AGC R: ACC CTG GTT TCA CAA AAG G	116-148	13	L22192	Arevalo *et. al.* (1994)
30	SRCRSP5	--	F : GGA CTC TAC CAA CTG AGC TAC AAG R: GTT TCT TTG AAA TGA AGC TAA AGC AAT GC	126-158	18	L22192	Arevalo *et. al.* (1994)

3.4.1 Preparation of solutions/reagents for quality check

TBE Electrophoresis buffer

Tris base	-	54 g
Boric acid	-	27.5 g
EDTA	-	4.15 g
Distilled water	-	upto 1000 ml

Agarose (0.8%)

Agarose	-	0.8 g
TBE (1x)	-	100 ml

Ethidium Bromide

Ethidium Bromide	-	100 mg
Distilled water	-	1 ml

Appropriate amount of agarose was weighed and dissolved to make a final concentration of 0.8% in 1x TBE buffer. The agarose was melted in a microwave oven. The solution was allowed to cool and ethidium bromide was added at a concentration of 0.5 µg/ml of agarose gel solution. The melted agarose solution was poured into the gel tray with comb carefully avoiding air bubbles. Once the gel was sufficiently solidified, the comb was removed carefully and the tray was kept in an electrophoresis tank and 1x TBE buffer was poured to submerge the gel in the tank. The isolated DNA was mixed with $1/6^{th}$ volume of 6x gel loading buffer and loaded into the wells using pipette. The electrophoresis was carried out at 80 Volts for about half an hour. Then the gel was visualized under UV light and photographed using gel documentation system (Syngene). After checking, the isolated DNA was diluted to 100 ng/µl concentration for further analysis.

3.5 POLYMERASE CHAIN REACTION (PCR)

The PCR reactions were performed using thermal cycler (Eppendorf). A master mix was prepared for required number of samples in 0.2 ml tube as presented in Table 2 and vortexed.

Table 2.Composition of master mix for PCR amplification

Components	Volume (µl)	Final concentration
10 x Taq buffer	1.25	1 x
dNTPs (10mM)	0.25	2 mM
Primer forward (100 pM)	0.6	60 pM
Primer reverse (100 pM)	0.6	60 pM
$MgCl_2$ (25 mM)	0.75	1.5 mM
Taq Polymerase (1 unit/µl)	0.5	0.5 unit/ml
Autoclaved MilliQ water	7.55	

An aliquot of 11.5 µl of master mix per sample was drawn into thin-walled PCR tubes and 1µl (100 ng) of template DNA was added and the reaction was carried out using the following conditions.

Table 3. PCR reaction Conditions

Step	Process	Temperature (^{0}C)	Time
1	Initial denaturation	95	5 min
2	Cyclic denaturation	94	1 min
3	Primer annealing	Depends on primer	30 s
4	Cyclic extension	72	30 s
5	Repeated steps 2 to 4 for 34 cycles		
6	Final extension	72	5 min
7	Hold	4	--

The annealing temperatures ranged from 45^0C to 65^0C depending on the primer used. PCR tubes were kept in thermal cycler and the programme run. Each PCR amplification took about 2:30 hours. At the end of the PCR, the tubes were taken out and stored in refrigerator until further use.

3.5.1 Checking the amplicons

After PCR amplification, the amplified products were electrophoresed on 2% agarose to resolve the bands. Appropriate amount of agarose was dissolved in 1X TBE and melted in microwave oven. The solution was allowed to cool sufficiently and ethidium bromide (0.5 µg/ml) was added. The PCR product was mixed with 6X gel loading dye and loaded into the wells of the gel. Along with the samples, a 50 bp DNA ladder (Fermentas) was also loaded into one of the wells as a size indicator. Electrophoresis was carried out @ 5-6 Volts/cm, bands were visualized and documented in Gel documentation system (Syngene)

3.6 POLY ACRYLAMIDE GEL ELECTROPHORESIS (PAGE)

Polyacrylamide gels are chemically cross-linked gels formed by polymerization of acrylamide with a cross-linking agent, usually N, N' methylene bisacrylamide. Polymerization is initiated by free radical formation usually carried out with Ammonium per Sulphate as the initiator and N, N, N, N' tetramethylenediamide (TEMED) as a catalyst.

3.6.1 Preparation of solutions/reagents for PAGE (8%)

Acrylamide:Bisacrlamide (29:1) solution

Acrylamide	-	29 g
Bisacrlamide	-	1 g
Distilled water	-	100 ml

Ammonium per Sulphate, APS (10%)

Ammonium per Sulphate	-	0.1 g
Distilled water	-	1 ml

Polyacrlyamide gel

Acrylamide:Bisacrlamide (29:1) solution	-	8 ml
5 x TBE	-	8 ml
Ammonium per Sulphate	-	150 µl
TEMED	-	80 µl
Distilled water	-	upto 40 ml

3.6.2 Preparation and casting of the gel

The glass plates were cleaned thoroughly with water and detergent, rinsed with double distilled water and finally cleaned with 70% ethanol. The two plates separated with the spacers were well aligned and were mounted in the gel casting unit.

The PAGE gel mix was prepared by mixing 8 ml of 5X TBE, 8 ml Acrylamide:Bisacrylamide (29:1) solution and 24 ml of distilled water. Finally, 150 µl of APS and 80 µl of TEMED were added and the solution was poured directly between the two glass plates. Flat side of the comb was inserted at the top and the assembly was left undisturbed for complete polymerization. After polymerization, the comb was removed

and the gel assembly was placed in the PAGE apparatus partly filled with 1X TBE with the larger glass plate facing outside. The air bubbles were removed, if any trapped beneath the bottom of the gel. The wells were flushed with TBE

3.6.3 Sample loading

The samples were prepared by mixing 1 µl loading dye and 4 µl amplicons and then loaded into the wells. Along with the amplicons, a 50 bp ladder (Fermentas) was also loaded into a lane to compare the size of the amplicons generated. The mixture was carefully loaded into the wells with long tipped micropipette. The electrodes were connected to a power pack. The gel was run at 1-8 V/cm. the gel was run until the marker dyes migrated the desired distance. Then the electric power was turned off and disconnected the lead. The glass plates were taken out of the tank and laid on the bench. By using a thin spatula, the corner of the upper glass plate was lifted gently. After confirming that the gel remained attached to the lower plate, the upper plate was pulled out gently, without causing any damage to the gel.

3.7 SILVER STAINING

The gel was stained by silver nitrate as described by Cominicini *et. al.* (1995) with minor modifications.

3.7.1 Preparation of solutions/reagents for Silver-staining

Fixative / stop solution

Ethanol (10%)	-	50 ml
Acetic acid (10%)	-	50 ml
Distilled water	-	upto 500 ml

Oxidative Solution (1%)

Nitric acid	-	5 ml
Distilled water	-	upto 500 ml

Silver-staining solution (0.2%)

Silver nitrate	-	0.5 g
Distilled water	-	upt 500 ml

Developer solution

Sodium carbonate	-	15 g
Formaldehyde	-	750 µl
Distilled water	-	500 ml

The following are the steps involved in the silver-staining:

- The gel along with the plate was placed in a suitable size tray with the gel layer facing up. The gel was soaked in 250 ml of fixative solution for about 30 min.
- Rinsing was carried out for two to three times using double distilled water.
- About 200 ml of 1% nitric acid was poured and shake gently for 3 min. Rinsed the gel twice with double distilled water.
- About 200 ml of 0.2% silver nitrate was poured on the gel and kept in a dark room for 30 min. Rinse the gel for 30 sec with distilled water.
- About 200 ml of pre-chilled developer was poured on the gel and shaken gently for few min till the bands become brownish and distinct.
- The reaction is stopped by adding stop solution for 2 minutes.
- The gel was washed with double distilled water.

3.7.2 Visualization of gel and documentation

The gel was taken out from the tray. The bands of the gel were visualized under UV light of gel documentation system. The product sizes were estimated with the help of 50 bp ladder as a standard marker. The types of bands and their genotypes are documented and used for further analysis.

3.8 STATISTICAL ANALYSIS

The population structure, genetic variability and genetic distance were estimated and tested using suitable statistical methods. The allele data were subjected to the Excel Microsatellite Tool kit (Park, 2001) and GenAlex 6.1 (Peakall and Smouse, 2006) for estimating various parameters.

3.8.1 Allele frequency (Af)

It is the proportion of a particular allele carried by individuals in a population and was calculated by using the formula

$$Allele\ frequency = \frac{Number\ of\ alleles\ at\ a\ locus\ in\ population}{Total\ number\ of\ alleles\ at\ that\ locus\ in\ population}$$

3.8.2 Mean number of alleles per locus (MNA)

It is the average number of alleles observed at a locus and estimated by formula

$$Mean\ No.of\ alleles\ per\ locus = \frac{Total\ Number\ of\ alleles\ in\ the\ population}{Total\ number\ of\ loci\ studied}$$

3.8.3 Percentage of polymorphic loci (P)

It is the proportion of polymorphic loci observed out of the total loci studied

$$P = \frac{Number\ of\ Polymorphic\ loci\ observed}{Total\ number\ of\ loci\ studied}\ X\ 100$$

3.8.4 Shannon's Information Index (I)

It is the measure of species diversity in a community obtained by the formula

$$H = -\sum_{i=1}^{s} p_j \ln p_j$$

Where, P_j is the frequency of the i^{th} allele for the population

3.8.5 Effective number of alleles per locus (N_e)

The effective number of alleles, Ne was computed using the formula

$$N_e = {}^{1}/_{\sum p_i^2}$$

Where, p_i is the frequency of the i^{th} allele

3.8.6 Genetic diversity or Heterozygosity (observed and expected)

The heterozygosity (H) can be defined as the state of an individual having two dissimilar alleles of a gene. Genetic diversity or expected heterozygosity was measured as the amount of potential or actual heterozygosity using the formula (Nei, 1973)

$$H = 1 - \sum P_i^2$$

Where, P_i = frequency of i^{th} allele

The observed fraction of individuals, which are heterozygous for a marker in a population is known as observed heterozygosity and it was calculated by the formula,

$$Observed\ heterozygosity = \frac{Number\ of\ Heterozygotes}{Total\ number\ of\ individuals\ studied}\ X\ 100$$

$$Expected\ heterozygosity = 1 - \sum P_j^2$$

$$Unbiased\ expected\ heterozygosity = \frac{2N}{2N-1}\ X\ Expected\ heterozygosity$$

3.8.7 Polymorphic Information Content (PIC)

The Polymorphic Information Content (PIC) were calculated by using the following formula suggested by Nei (1978) as

$$PIC = 1 - (\sum_{i=1}^{n} p_i^2) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

Where,

P_i = frequency of i^{th} allele

P_j = frequency of j^{th} allele

i and j = number of alleles

3.8.8 Genetic distance

The Nei's genetic distance and genetic identity (Nei, 1972) were also estimated in GenAlex 6.1 software. Phylogenetic consensus tree was constructed using the Unweighted Pair Group Method of Analysis (UPGMA) of clustering by MEGA 3.1 software (Kumar *et al*., 2004).

3.8.9 Fixation Indices (F-statistic)

Fixation indices measure the population differentiation or variation within and between populations. The F statistic is fractional reduction in heterozygosity relative to a random mating population with the same allele frequency (Wright, 1978). The fixation indices, F_{IS}, F_{IT} and F_{ST} were estimated using FSTAT program (Goudet, 1995). These indices will guide the breeder in determining the status of inbreeding in the population before commencing a breeding programme.

3.8.9.1 F_{IS} (Within population variability)

F_{IS} measures the reduction of heterozygosity in an individual due to non-random mating within a breed/strain/population. It is the deviation of individual heterozygosity from the expected, based on allele frequencies in a random mating population. It varies from -1 to +1, but in nature it is always nearer to zero. Higher F_{IS} values indicate closer relationship among the individuals. It is similar to inbreeding coefficient or sometimes even referred as inbreeding coefficient.

3.8.9.2 F_{ST} (Measure of population differentiation)

F_{ST} is the reduction in heterozygosity due to subdivision in the population. It measures the deviation of expected heterozygosity in sub populations from the expected, based on allele frequencies in random mating total population that includes all sub-populations. The F_{ST} ranges from 0 to 1 and is classified into low (<0.15), moderate (0.15 to 0.25) and high (>0.25) genetic differentiation (Wright, 1978). The genetic diversity was measured as coefficient of gene differentiation among the breeds. The lower F_{ST} values indicate higher relationship between the populations. It is also referred as the coefficient of co-ancestry.

3.8.9.3 F_{IT} (Variation among individuals in a population)

F_{IT} is the measure of reduction in heterozygosity of an individual in relation to the total population. F_{IT} is considered as the most comprehensive measure of inbreeding as it takes into account both the effects of non-random mating (F_{IS}) and diversity among the populations/strains (F_{ST}). F_{IT} values near zero indicate no inbreeding in the population.

However, the three measures of F statistics are related in the following way

$$(1 - F_{IS})X\ (1 - F_{ST}) = (1 - F_{IT})$$

In most of the natural populations F_{IS}, the within population variability is zero, resulting in $F_{ST} = F_{IT}$.

3.8.9.4 Out crossing rate

The Out crossing rate was calculated as

$$Out\ crossing\ rate = \frac{1 - F_{IS}}{1 + F_{IS}}$$

Where, F_{IS} is the coefficient of inbreeding

3.8.10 Hardy – Weinberg Equilibrium

The deviation of observed allele frequencies from expected frequencies was tested using Chi-Square test. The breeder can assess the equilibrium status of the population with regard to the gene frequencies, which finally reflects the genotype frequencies.

RESULTS

CHAPTER IV

RESULTS

4.1 GENOMIC DNA

Fifty sheep from each of Deccani and Nellore breeds with equal representation from both the sexes were utilized in the present study. Quality of the DNA isolated was tested by electrophoresis on 0.8% agarose gel and quantification was done by UV spectrophotometer. The optical absorbance ratio (A260/A280) and the average concentration of DNA in the two breeds are presented in Table 4. The mean ratio of absorbance in both Deccani and Nellore sheep was 1.95. The concentration of the DNA varied from 0.928 µg/µl in Deccani to 0.673 µg/µl in Nellore sheep breed.

4.2 MICROSATELLITE MARKER ANALYSIS

The PCR products were checked for amplification on 2% agarose. Then the microsatellites were analyzed by running on 8% PAGE to identify DNA polymorphism. Gel images for the primers OarVH72 and SRCRSP1 are presented in Figs 8 & 9.

Genotyping of the individual animal at various loci was done based on the presence or absence of a particular allele and the genotypes of each animal at 30 different loci are detailed in Table 5. Among the 30 loci studied few alleles did not show any amplification. The presence of two alleles of similar length (bp) at a locus was considered homozygous, while that with dissimilar length was considered heterozygous.

Table 4. Genomic DNA quantification using UV spectrophotometer

S.No.	A_{260}	A_{280}	A_{260}/A_{280}	Concentration of DNA (µg/µl)
Deccani				
1	1.022	0.605	1.689	1.533
2	0.739	0.442	1.672	1.108
3	0.685	0.409	1.675	1.027
4	0.399	0.235	1.698	0.598
5	0.808	0.471	1.715	1.212
6	0.725	0.410	1.768	1.087
7	1.000	0.581	1.721	1.500
8	0.285	0.172	1.657	0.427
9	1.065	0.605	1.760	1.597
10	1.059	0.594	1.783	1.588
11	1.429	0.809	1.766	2.143
12	0.767	0.440	1.743	1.150
13	1.003	0.560	1.791	1.504
14	0.890	0.504	1.766	1.335
15	0.778	0.445	1.748	1.167
16	0.848	0.483	1.756	1.272
17	0.742	0.422	1.758	1.113
18	0.772	0.432	1.787	1.158
19	0.975	0.548	1.779	1.462
20	0.897	0.508	1.766	1.345
21	0.320	0.226	1.415	0.480
22	0.591	0.363	1.628	0.886
23	0.183	0.107	1.710	0.274
24	0.458	0.238	1.924	0.687
25	0.395	0.198	1.990	0.592
26	0.470	0.226	2.079	0.705
27	0.631	0.301	2.096	0.946
28	0.220	0.117	1.880	0.330
29	0.312	0.148	2.108	0.468
30	0.338	0.133	2.541	0.507
31	0.728	0.349	2.085	1.092
32	0.401	0.194	2.067	0.601
33	0.421	0.215	1.958	0.631
34	0.543	0.301	1.803	0.814
35	0.602	0.278	2.165	0.903

S.No.	A_{260}	A_{280}	A_{260}/A_{280}	Concentration of DNA (µg/µl)
36	0.490	0.191	2.565	0.735
37	0.204	0.074	2.756	0.306
38	0.800	0.377	2.122	1.200
39	0.199	0.088	2.261	0.298
40	0.663	0.321	2.065	0.994
41	0.608	0.308	1.974	0.912
42	0.271	0.115	2.356	0.406
43	0.496	0.232	2.137	0.744
44	0.323	0.136	2.375	0.484
45	0.535	0.233	2.296	0.802
46	0.354	0.156	2.269	0.531
47	0.384	0.175	2.194	0.576
48	0.484	0.254	1.905	0.726
49	0.548	0.280	1.957	0.822
50	1.079	0.566	1.906	1.618
Nellore				
1	0.391	0.226	1.730	0.587
2	0.802	0.449	1.786	1.203
3	0.388	0.225	1.724	0.582
4	0.616	0.350	1.760	0.924
5	0.652	0.355	1.836	0.978
6	0.344	0.196	1.755	0.516
7	0.346	0.186	1.860	0.519
8	0.549	0.305	1.800	0.823
9	0.412	0.223	1.847	0.618
10	0.574	0.314	1.828	0.861
11	0.811	0.452	1.794	1.217
12	0.498	0.271	1.837	0.747
13	0.396	0.220	1.800	0.594
14	0.659	0.350	1.882	0.989
15	0.519	0.275	1.887	0.779
16	0.540	0.291	1.855	0.810
17	0.720	0.389	1.850	1.080
18	0.494	0.265	1.864	0.741
19	0.601	0.322	1.866	0.902
20	0.472	0.256	1.843	0.708
21	0.457	0.246	1.857	0.686
22	0.548	0.292	1.876	0.822

S.No.	A_{260}	A_{280}	A_{260}/A_{280}	Concentration of DNA (µg/µl)
23	0.663	0.360	1.841	0.995
24	0.370	0.191	1.937	0.555
25	0.401	0.207	1.937	0.602
26	0.281	0.135	2.081	0.422
27	0.206	0.083	2.210	0.309
28	0.359	0.179	2.000	0.539
29	0.446	0.226	1.973	0.669
30	0.518	0.231	2.240	0.777
31	0.340	0.170	2.000	0.510
32	0.424	0.224	1.892	0.636
33	0.371	0.163	2.270	0.557
34	0.185	0.089	2.078	0.278
35	0.319	0.162	1.969	0.479
36	0.368	0.189	1.947	0.552
37	0.230	0.117	1.965	0.345
38	0.395	0.198	1.990	0.593
39	0.300	0.147	2.040	0.450
40	0.412	0.187	2.203	0.618
41	0.253	0.126	2.007	0.380
42	0.858	0.308	2.780	1.287
43	0.333	0.169	1.970	0.500
44	0.272	0.132	2.060	0.408
45	0.372	0.181	2.055	0.558
46	0.458	0.219	2.091	0.687
47	0.466	0.225	2.071	0.699
48	0.412	0.208	1.980	0.618
49	0.383	0.196	1.954	0.575
50	0.261	0.134	1.940	0.392
Mean (Deccani)	0.618	0.331	1.947	0.928
Mean (Nellore)	0.448	0.232	1.952	0.673

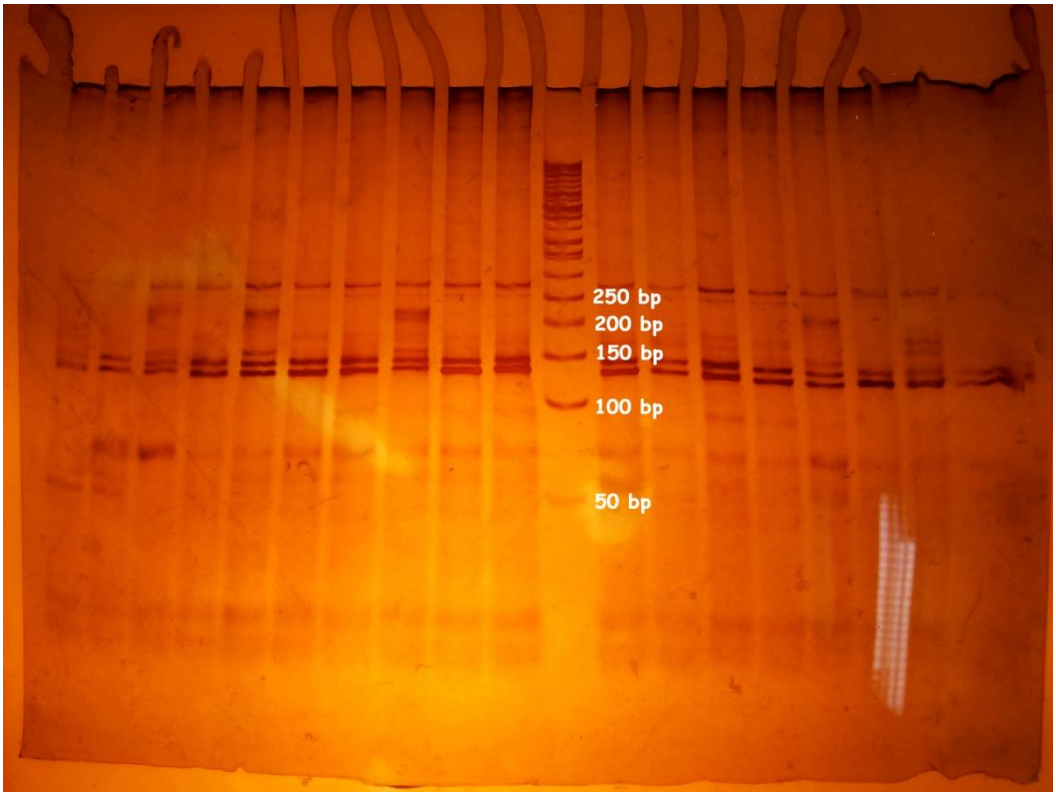

Fig 6a. Polymorphism of microsatellite marker OarVH72 resolved on Polyacrylamide gel (8%) for Deccani genome from 1 – 19.

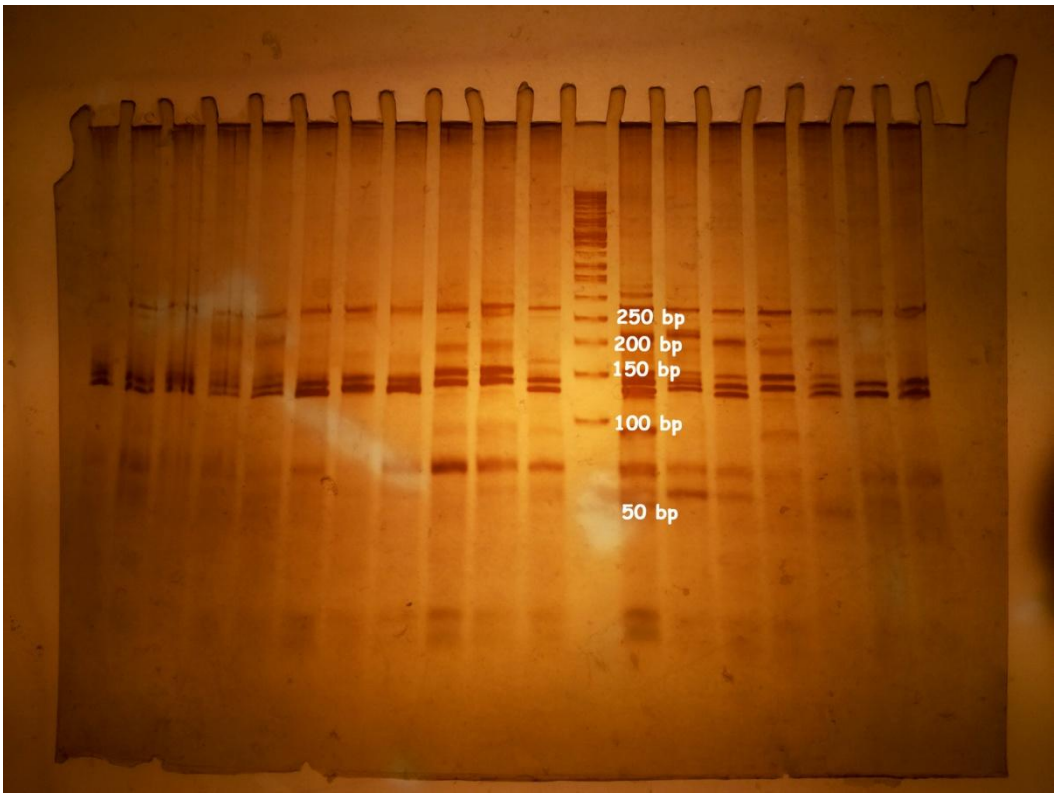

Fig 6b. Polymorphism of microsatellite marker OarVH72 resolved on Polyacrylamide gel (8%) for Deccani genome from 20 – 38.

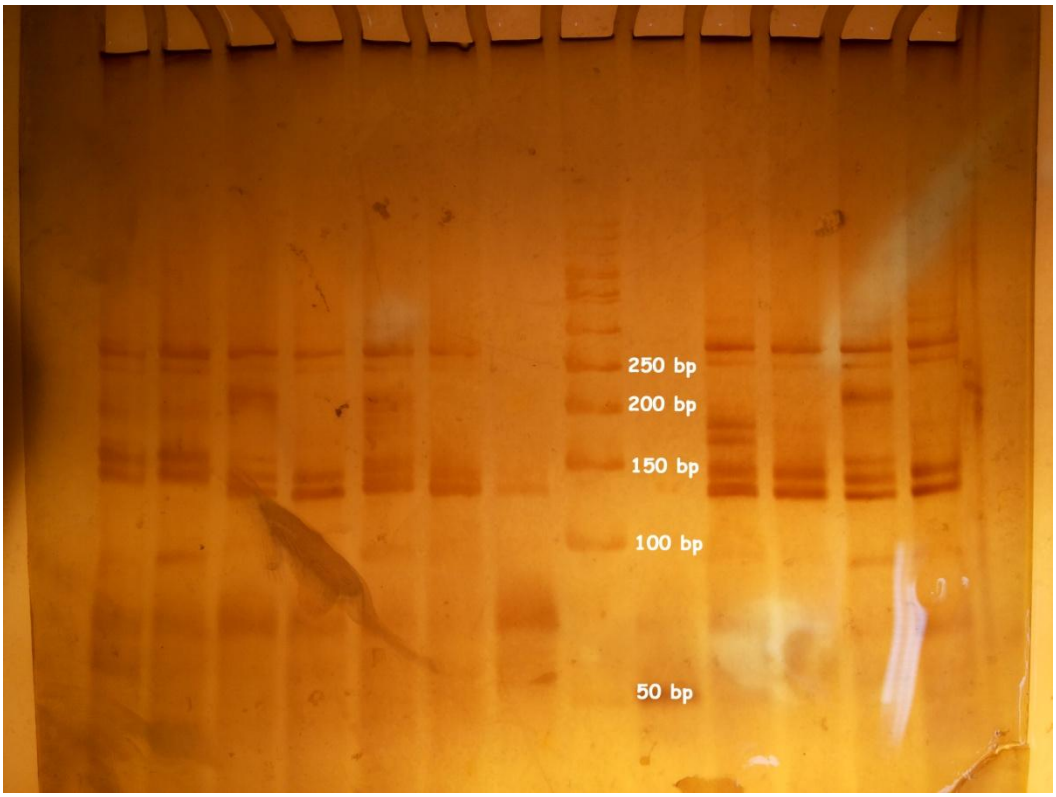

Fig 6c. Polymorphism of microsatellite marker OarVH72 resolved on Polyacrylamide gel (8%) for Deccani genome from 39 – 50.

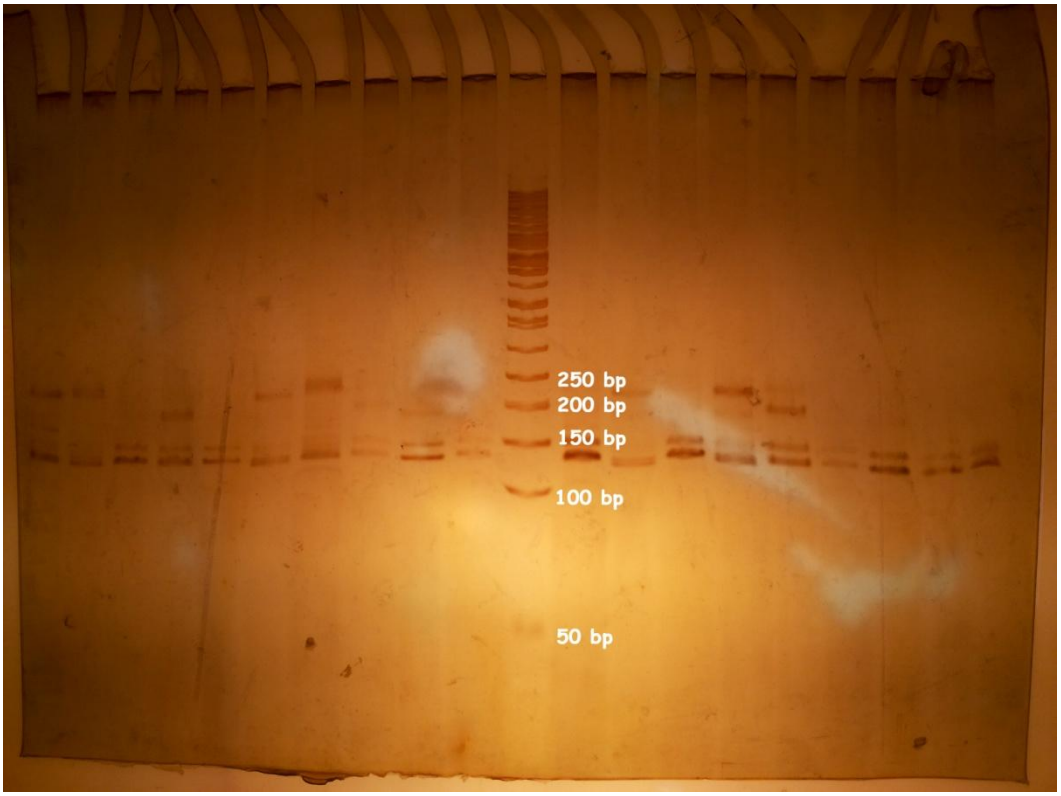

Fig 7a. Polymorphism of microsatellite marker SRCRSP1 resolved on Polyacrylamide gel (8%) for Nellore genome from 1 – 19.

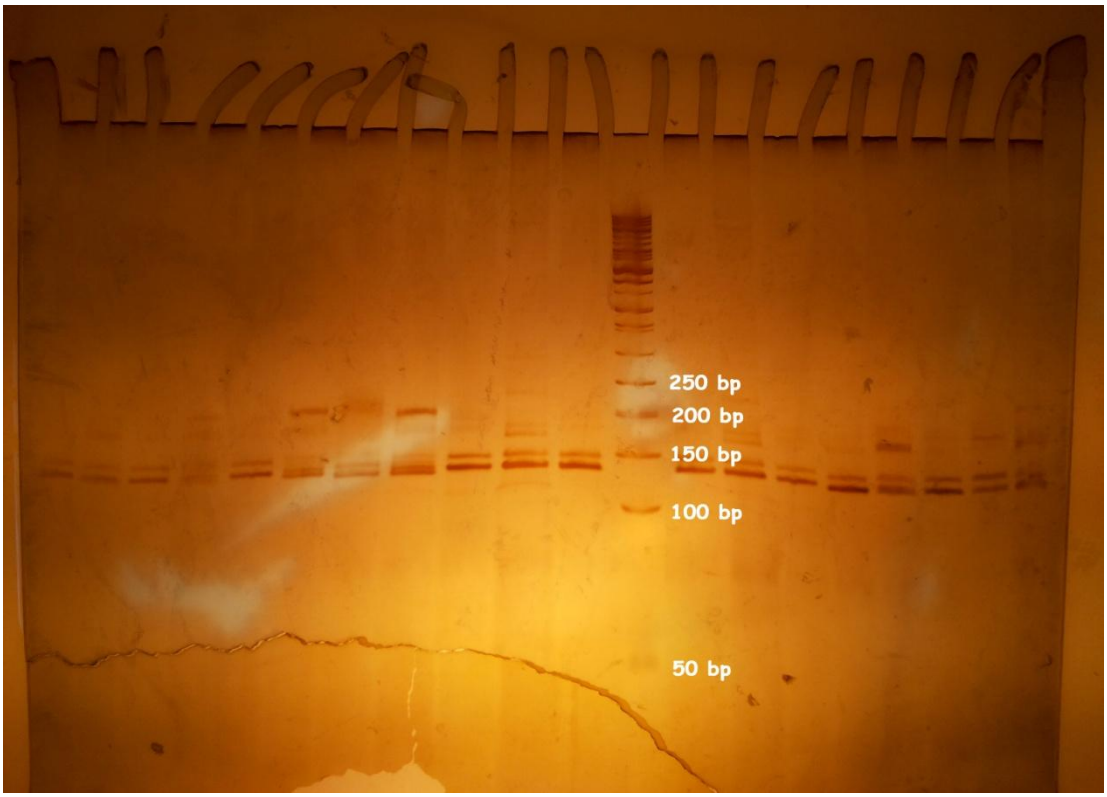

Fig 7b. Polymorphism of microsatellite marker SRCRSP1 resolved on Polyacrylamide gel (8%) for Nellore genome from 20 – 38.

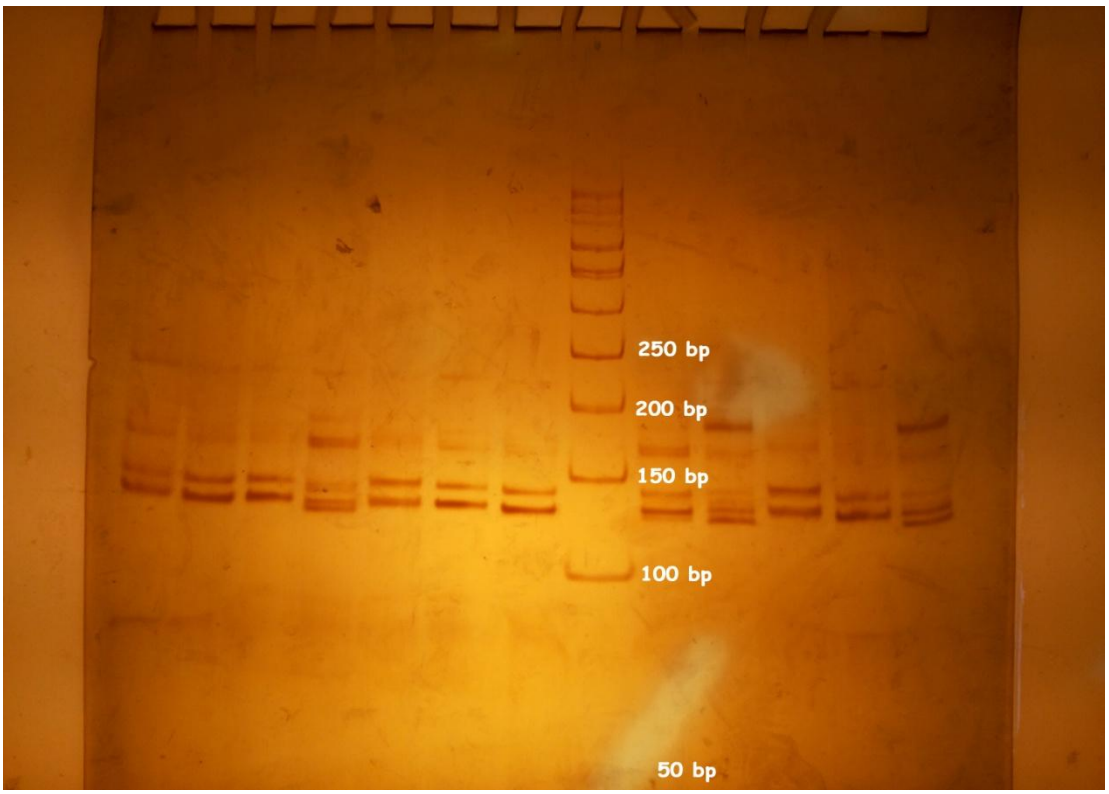

Fig 7c. Polymorphism of microsatellite marker SRCRSP1 resolved on Polyacrylamide gel (8%) for Nellore genome from 39 – 50.

Table 5. Genotypes of Deccani and Nellore sheep at various microsatellite loci studied.

Locus	BM 1314				BM6506				BM6526			
S.No.	Deccani		Nellore		Deccani		Nellore		Deccani		Nellore	
1	176	186	164	180	182	188	182	188	151	172	151	172
2	164	178	176	186	182	188	186	186	151	172	151	172
3	164	178	176	186	182	182	186	186	0	0	151	172
4	0	0	164	178	182	188	188	206	151	151	148	159
5	0	0	164	178	182	182	182	188	151	172	148	159
6	164	164	148	168	182	188	182	188	138	148	151	172
7	152	152	148	168	182	182	188	206	151	172	151	172
8	164	164	164	180	184	202	188	206	148	159	151	151
9	164	178	176	186	184	184	182	182	126	148	151	172
10	164	164	176	176	184	184	188	188	151	172	151	172
11	164	164	176	186	184	202	188	206	126	148	151	172
12	164	164	176	186	184	184	188	206	126	148	151	172
13	164	164	152	172	184	184	188	206	126	148	148	159
14	152	152	176	186	182	182	188	206	138	148	148	159
15	164	178	152	172	186	186	188	206	151	172	148	159
16	176	170	152	172	186	206	188	206	126	148	148	159
17	164	178	148	168	182	188	188	188	126	148	151	172
18	176	176	148	168	182	188	186	186	151	172	151	172
19	152	152	168	168	182	188	184	202	151	172	151	172
20	0	0	0	0	186	206	184	202	151	172	118	125
21	176	176	148	148	186	206	184	184	151	172	0	0
22	176	176	148	168	186	186	188	206	151	172	118	125
23	164	180	0	0	186	206	182	188	148	159	118	125
24	160	178	148	148	184	202	182	188	151	151	118	125
25	164	180	148	148	184	184	188	206	151	172	118	125
26	164	178	148	168	188	188	184	202	151	172	124	135
27	164	178	148	168	182	188	184	202	151	172	118	125
28	176	186	148	148	186	206	188	188	148	159	118	125
29	176	186	148	168	0	0	184	206	138	148	124	135
30	176	186	148	148	182	188	184	184	138	148	126	148
31	176	186	148	168	188	188	188	206	138	138	126	148
32	164	178	148	148	182	182	188	206	138	148	126	148
33	164	178	0	0	182	182	206	206	151	172	126	148
34	152	172	148	148	186	186	188	206	138	148	124	135
35	152	172	148	148	182	188	188	188	151	172	118	125
36	148	168	148	168	182	182	188	188	151	172	124	135
37	148	168	0	0	182	182	0	0	151	172	118	125
38	148	148	148	148	182	182	182	188	151	172	126	148
39	176	186	176	186	182	188	186	206	151	172	0	0
40	176	176	176	176	182	188	186	186	151	172	138	148
41	148	168	164	180	182	188	186	206	151	151	138	148
42	152	172	164	180	182	188	184	202	148	159	126	148
43	152	172	176	186	186	206	184	202	148	159	126	148
44	152	172	176	186	186	206	186	206	151	172	138	148
45	152	172	164	180	182	188	186	206	151	172	126	148
46	148	168	176	186	184	202	186	186	151	172	126	126
47	176	186	152	172	182	188	186	206	151	172	126	126
48	176	186	164	180	188	206	186	206	151	172	126	148
49	148	168	164	180	182	188	186	186	148	159	126	126
50	152	172	176	186	178	187	186	186	148	159	138	148

Contd.,

Table 5. continued

Locus	BM757				BM8125				CSSM31			
S. No.	Deccani		Nellore		Deccani		Nellore		Deccani		Nellore	
1	118	136	107	141	142	154	142	154	158	170	126	140
2	107	141	107	141	142	154	142	154	150	170	0	0
3	118	136	118	136	132	144	132	144	150	170	126	126
4	124	136	118	136	132	144	132	144	150	170	126	126
5	118	136	124	136	132	132	132	132	150	170	126	140
6	140	152	124	136	128	136	128	136	150	170	138	152
7	118	136	144	160	128	136	128	136	126	140	138	152
8	140	152	140	152	132	144	132	144	138	152	138	152
9	140	152	0	0	128	136	128	136	138	152	152	160
10	140	152	140	152	128	128	128	128	152	160	158	170
11	144	160	140	152	128	128	128	128	158	170	138	152
12	124	136	140	152	128	128	128	128	144	156	144	156
13	140	140	144	160	128	128	128	128	144	156	138	152
14	124	136	144	160	128	136	128	136	144	156	144	156
15	124	136	144	144	128	136	128	136	126	140	144	156
16	124	136	144	160	128	136	128	136	144	156	152	160
17	118	136	144	144	128	128	128	128	138	152	152	160
18	144	160	144	144	128	136	128	136	126	140	152	160
19	118	136	144	160	132	144	132	144	126	140	144	156
20	124	136	144	160	136	128	136	128	126	126	152	160
21	140	152	144	160	132	144	132	144	126	126	144	156
22	140	152	140	152	128	136	128	136	126	140	0	0
23	140	152	144	144	128	136	128	136	126	126	152	160
24	144	160	144	144	128	128	128	128	126	126	158	170
25	124	136	144	160	128	136	128	136	126	140	158	170
26	144	160	140	152	128	136	128	136	138	138	144	156
27	144	160	144	152	0	0	0	0	138	152	152	160
28	144	160	144	152	128	128	128	128	126	140	152	160
29	144	160	144	152	128	128	128	128	138	152	158	170
30	144	160	140	140	128	129	128	129	138	152	158	170
31	140	152	144	152	128	128	128	128	138	152	158	170
32	0	0	144	144	128	128	128	128	138	152	158	158
33	140	152	144	160	128	128	128	128	138	152	158	170
34	124	136	140	152	128	136	128	136	144	156	158	170
35	124	136	144	144	128	136	128	136	126	140	158	170
36	140	152	124	136	128	136	128	136	126	140	158	170
37	140	152	140	152	136	136	136	136	126	140	158	170
38	144	160	124	136	132	132	132	132	138	152	158	170
39	0	0	144	160	136	148	136	148	152	160	158	170
40	140	152	140	152	142	154	142	154	144	156	152	160
41	140	152	140	152	136	136	136	136	126	140	158	170
42	144	160	144	160	136	136	136	136	144	144	138	152
43	140	140	140	152	136	136	136	136	126	126	152	160
44	144	160	140	152	128	136	128	136	126	140	144	156
45	144	160	144	144	128	136	128	136	138	138	158	170
46	144	160	144	144	128	136	128	136	158	170	152	160
47	144	160	144	144	128	136	128	136	138	152	144	156
48	140	140	144	144	128	136	128	136	138	152	126	140
49	140	140	144	144	128	144	128	144	144	156	158	170
50	140	140	144	160	128	128	128	128	152	160	138	152

Contd.,

Table 5. continued

Locus	ETH121				HUJ616				ILSTS030			
S. No.	Deccani		Nellore		Deccani		Nellore		Deccani		Nellore	
1	191	191	0	0	118	136	118	118	148	160	158	168
2	182	182	0	0	120	120	118	118	158	168	160	178
3	182	192	0	0	120	136	118	118	160	182	166	182
4	182	192	182	182	126	142	118	136	166	190	160	178
5	182	195	182	182	0	0	118	142	158	168	160	168
6	182	195	0	0	130	146	0	0	166	182	166	190
7	182	195	186	186	134	146	120	136	166	166	166	190
8	0	0	188	188	134	146	126	142	166	190	166	182
9	186	197	188	202	134	152	130	136	166	182	166	182
10	186	197	188	188	134	152	130	146	158	168	166	182
11	182	195	188	188	134	146	142	158	158	168	166	182
12	182	182	188	188	0	0	134	152	158	168	166	190
13	182	195	182	182	134	146	130	142	166	182	160	178
14	182	195	182	182	134	146	142	158	166	182	160	178
15	182	195	182	182	130	142	142	158	166	190	166	182
16	0	0	182	182	130	142	142	142	166	182	166	182
17	182	182	182	182	126	142	142	158	158	168	160	178
18	188	202	182	182	0	0	142	158	158	168	148	160
19	188	202	182	182	126	142	142	158	158	158	148	160
20	182	182	186	186	142	142	134	152	158	168	148	148
21	182	182	186	186	142	142	142	158	158	160	148	160
22	182	195	188	188	142	158	136	152	148	160	148	160
23	182	182	188	188	142	142	136	146	148	160	148	148
24	182	182	182	182	142	142	136	146	140	160	148	148
25	182	182	188	188	134	134	136	142	140	140	148	148
26	182	182	188	188	142	142	142	158	158	158	148	160
27	186	186	188	188	142	142	142	158	158	168	148	160
28	186	186	188	188	134	134	142	158	158	168	148	160
29	182	182	188	188	130	130	142	158	158	178	158	168
30	182	182	188	188	142	142	142	158	158	178	166	190
31	186	186	188	188	142	142	130	142	158	178	166	166
32	182	182	186	186	134	134	142	152	0	0	158	168
33	182	182	182	182	130	130	120	136	158	178	158	168
34	182	182	182	182	134	134	120	136	158	178	148	160
35	182	182	182	182	142	142	118	136	148	168	158	168
36	0	0	182	182	142	142	120	136	148	178	158	168
37	182	182	182	182	142	142	120	136	158	182	158	168
38	182	195	182	182	134	134	120	136	0	0	148	160
39	188	188	186	186	130	130	142	158	166	190	160	182
40	188	188	186	186	134	146	142	158	166	190	160	182
41	0	0	0	0	134	142	142	158	166	190	158	168
42	188	188	0	0	134	142	142	158	166	182	158	168
43	188	188	182	182	134	134	142	158	166	182	160	182
44	0	0	182	182	130	142	142	158	166	166	158	168
45	188	188	182	182	142	142	130	146	166	190	158	168
46	188	188	182	182	126	142	126	142	158	182	166	166
47	188	188	186	186	126	142	120	136	166	166	166	166
48	0	0	188	188	126	136	120	136	158	178	160	178
49	188	188	188	188	134	146	118	136	158	178	158	168
50	188	188	188	188	142	158	118	136	166	182	160	178

Contd.,

Table 5. continued

Locus	ILSTS11				ILSTS28				MAF209			
S. No.	Deccani		Nellore		Deccani		Nellore		Deccani		Nellore	
1	236	248	236	248	150	164	150	164	112	124	132	144
2	226	248	238	274	136	150	150	164	122	122	122	138
3	226	246	226	246	136	150	136	150	132	144	122	138
4	226	246	240	278	136	150	136	136	112	124	118	132
5	226	246	238	238	136	150	136	136	112	124	122	138
6	226	246	238	274	126	146	136	150	118	132	122	138
7	226	246	236	247	150	164	136	136	132	144	132	144
8	236	248	236	269	150	164	150	164	132	144	118	132
9	240	274	236	274	136	150	126	144	0	0	132	144
10	236	246	240	274	126	126	126	144	0	0	122	138
11	240	274	252	288	136	150	136	150	122	138	122	138
12	236	278	252	288	126	146	136	150	122	138	132	144
13	240	278	258	288	136	136	136	150	118	132	127	138
14	252	288	236	246	126	146	150	164	122	138	132	144
15	226	246	236	246	126	146	126	146	0	0	132	144
16	226	248	236	248	116	132	126	146	118	132	122	138
17	226	246	240	278	116	116	126	146	112	124	122	138
18	226	246	0	0	126	146	116	116	112	112	112	124
19	0	0	236	248	116	146	136	150	112	134	112	124
20	236	248	236	248	136	150	116	116	0	0	132	144
21	236	248	238	246	136	150	116	116	112	134	132	144
22	226	246	226	246	150	164	116	116	118	132	122	138
23	236	246	226	246	136	150	112	130	118	132	122	138
24	223	246	236	248	136	150	136	150	0	0	122	138
25	252	274	240	288	136	150	112	130	122	138	132	144
26	240	278	236	246	126	146	136	136	132	144	122	138
27	226	246	252	288	136	150	136	136	132	144	122	138
28	236	248	252	288	136	136	116	132	0	0	132	144
29	236	248	252	288	136	150	136	150	122	138	132	144
30	252	278	252	288	136	150	136	150	132	132	132	132
31	240	288	262	288	150	164	136	150	0	0	132	132
32	238	274	262	288	136	150	150	164	132	144	122	122
33	240	278	240	274	126	146	150	164	0	0	122	138
34	226	246	252	278	0	0	150	164	132	144	122	138
35	226	246	238	248	116	116	150	164	118	132	122	122
36	0	0	226	246	116	116	136	150	118	132	122	122
37	252	288	226	246	116	132	136	150	122	138	122	122
38	252	288	236	248	116	132	150	164	122	138	122	138
39	262	288	252	278	150	164	112	130	132	144	132	132
40	252	288	240	278	150	164	136	136	132	144	0	0
41	240	240	226	226	136	150	136	150	0	0	122	138
42	240	240	226	226	136	150	136	136	132	144	122	122
43	252	288	240	240	136	150	136	136	112	124	118	132
44	240	274	252	288	136	136	136	136	0	0	118	132
45	240	278	252	288	136	136	150	164	112	124	122	138
46	252	288	262	288	150	164	150	164	112	124	122	138
47	240	274	262	288	150	150	150	164	112	112	122	138
48	240	274	262	288	150	150	136	150	0	0	122	138
49	252	288	262	288	150	164	150	164	112	124	122	138
50	262	288	0	0	150	150	112	130	112	124	118	132

Contd.,

Table 5. continued

Locus	MAF214				MAF33				MAF70			
S. No.	Deccani		Nellore		Deccani		Nellore		Deccani		Nellore	
1	180	192	192	210	144	158	114	126	144	156	150	154
2	180	192	198	206	144	158	114	126	138	144	158	164
3	180	192	192	206	144	158	122	134	138	144	158	172
4	180	192	180	192	142	158	126	142	138	144	150	150
5	180	192	180	192	142	158	130	146	136	144	150	164
6	180	192	192	210	130	146	144	148	144	144	158	158
7	180	192	192	210	130	142	144	158	138	154	158	164
8	180	180	192	214	144	158	144	158	140	154	150	164
9	180	196	192	214	130	142	144	158	140	154	156	156
10	180	196	192	214	130	146	144	158	150	164	164	164
11	180	206	192	206	144	144	144	148	150	156	164	164
12	198	214	192	206	144	158	144	144	150	156	164	164
13	192	206	198	206	144	158	144	144	156	156	150	164
14	180	192	180	192	130	146	144	158	150	150	158	172
15	198	206	180	192	130	146	126	142	138	154	158	164
16	192	206	180	192	142	148	126	134	138	144	158	164
17	192	206	180	192	144	158	126	142	138	144	158	164
18	180	206	180	180	144	158	122	134	138	144	158	158
19	192	206	180	192	144	158	122	134	140	154	158	158
20	180	196	180	192	144	158	142	142	158	172	158	172
21	180	198	180	192	144	144	142	148	150	164	172	172
22	180	198	180	192	144	144	130	142	144	156	172	172
23	180	192	182	196	130	146	130	142	136	144	0	0
24	180	196	180	192	130	142	142	142	144	156	158	172
25	180	196	184	198	144	144	144	158	144	156	158	172
26	180	214	192	198	144	146	130	142	150	164	144	156
27	198	214	192	206	144	148	130	142	150	164	144	164
28	198	214	192	206	144	158	142	148	158	172	150	164
29	196	196	200	214	144	158	130	146	144	156	150	164
30	198	198	200	214	144	168	142	146	158	172	144	154
31	198	214	192	210	144	158	130	142	144	156	144	154
32	200	214	198	200	0	0	126	134	144	156	158	164
33	200	214	192	198	130	142	122	134	144	150	144	154
34	200	214	180	198	142	148	122	134	138	154	144	144
35	200	214	192	214	144	158	122	122	140	154	144	144
36	200	214	0	0	144	158	126	142	136	144	144	144
37	200	200	198	214	144	158	130	146	136	144	138	144
38	200	214	200	214	144	148	130	146	136	136	138	144
39	180	206	200	214	122	134	144	158	158	158	140	140
40	180	200	198	214	122	134	144	148	172	172	150	164
41	180	198	198	200	122	134	144	158	158	172	140	154
42	180	196	192	206	122	134	144	158	158	172	136	144
43	180	206	192	214	122	134	142	158	150	164	136	154
44	180	206	192	210	130	130	142	146	158	164	140	154
45	180	198	192	210	0	0	136	148	158	164	140	154
46	200	214	200	214	122	122	136	148	0	0	144	156
47	180	196	192	206	122	122	142	158	158	158	140	144
48	196	214	200	200	122	122	130	146	158	158	150	164
49	200	214	200	200	122	134	142	158	158	164	144	156
50	214	214	200	214	122	122	144	158	158	158	0	0

Contd.,

Table 5. continued

Locus	MCM140				OarAE129				OarCB226			
S. No.	Deccani		Nellore		Deccani		Nellore		Deccani		Nellore	
1	170	190	152	170	174	186	174	186	146	158	128	144
2	162	190	148	148	166	180	174	186	146	158	0	0
3	162	180	148	148	166	180	156	172	134	148	128	128
4	162	180	148	166	164	176	164	176	142	152	128	128
5	170	190	148	148	174	186	174	186	128	128	128	128
6	170	190	148	166	174	186	174	186	146	158	128	128
7	170	190	152	170	164	176	174	180	134	148	154	166
8	170	190	158	158	164	176	174	186	160	172	128	144
9	170	170	158	158	156	172	166	180	134	148	128	144
10	170	190	148	148	164	176	0	0	0	0	128	128
11	170	170	148	148	164	166	156	172	128	128	0	0
12	171	170	148	148	140	160	174	186	134	148	146	158
13	170	170	148	148	140	160	174	186	142	152	134	148
14	158	176	152	152	140	160	166	180	142	152	134	148
15	148	166	152	170	140	160	174	186	128	144	128	128
16	148	166	0	0	140	160	166	186	154	166	134	148
17	148	166	162	180	156	172	174	186	134	148	128	144
18	148	148	162	180	156	172	174	180	134	148	128	144
19	148	166	158	176	156	172	174	174	0	0	142	152
20	148	166	152	176	164	164	156	172	128	144	146	150
21	152	170	148	148	166	180	156	172	128	144	146	153
22	148	166	148	148	166	166	156	180	128	128	134	158
23	152	152	0	0	174	186	166	180	128	144	128	144
24	158	176	148	148	174	186	156	180	128	144	134	134
25	170	190	148	166	174	174	174	186	128	144	134	134
26	162	180	148	166	174	180	164	180	134	148	128	128
27	162	180	0	0	174	180	164	186	134	134	134	134
28	162	180	0	0	174	186	174	186	128	144	134	148
29	0	0	148	148	174	174	174	186	146	152	134	148
30	162	180	152	170	174	174	166	186	142	152	128	144
31	162	180	152	170	166	166	156	172	146	152	134	134
32	162	180	0	0	174	180	166	186	142	142	134	148
33	170	190	152	170	174	174	174	186	134	144	134	144
34	170	190	158	176	164	176	156	172	142	152	134	134
35	170	190	158	170	174	176	174	186	146	146	128	144
36	170	190	158	176	174	186	174	186	146	158	128	144
37	170	170	162	180	174	186	174	186	142	152	134	134
38	0	0	162	180	174	186	164	172	154	166	0	0
39	170	170	162	180	174	180	166	186	142	166	154	166
40	170	170	162	162	0	0	166	180	154	166	0	0
41	0	0	148	166	174	186	156	172	142	152	146	158
42	190	190	148	148	174	180	164	180	134	152	142	152
43	190	190	152	152	174	180	156	180	154	166	128	148
44	0	0	148	148	174	180	156	172	128	144	134	134
45	190	190	148	148	156	180	156	172	134	148	128	128
46	0	0	148	148	0	0	164	176	134	134	128	128
47	0	0	152	152	0	0	164	176	160	172	128	128
48	0	0	152	152	174	186	156	172	146	158	128	128
49	190	190	158	158	174	186	174	180	134	158	134	152
50	190	190	158	176	174	186	174	180	146	166	142	158

Contd.,

Table 5. continued

Locus	OarCP20				OarCP34				OarFCB128			
S. No.	Deccani		Nellore		Deccani		Nellore		Deccani		Nellore	
1	71	71	79	92	126	126	148	164	132	134	110	120
2	71	71	79	79	126	140	148	164	132	134	116	122
3	71	71	76	76	126	140	138	150	134	134	118	118
4	71	74	71	74	126	140	126	140	110	120	116	116
5	71	74	71	74	132	150	0	0	120	132	110	110
6	71	71	71	74	138	150	132	142	128	132	116	120
7	74	76	71	74	148	164	138	150	128	134	118	126
8	74	76	71	74	138	150	138	142	128	134	120	132
9	74	76	74	74	138	150	0	0	132	134	116	122
10	0	0	74	76	0	0	148	164	134	134	110	120
11	74	74	74	76	148	164	0	0	134	134	0	0
12	76	81	71	74	0	0	138	164	122	122	132	134
13	76	76	74	76	148	164	0	0	128	134	132	134
14	76	81	71	74	148	164	0	0	120	132	120	132
15	76	81	71	74	148	164	138	164	132	132	128	134
16	74	76	71	74	156	168	132	142	132	132	128	128
17	0	0	71	74	138	150	0	0	134	134	132	134
18	74	76	71	74	156	168	156	168	134	134	134	134
19	76	81	74	76	148	164	156	168	116	126	132	134
20	76	81	74	76	132	142	126	140	128	128	132	132
21	79	92	76	81	138	150	126	140	128	134	132	134
22	71	74	76	81	126	140	126	126	128	134	128	128
23	71	74	74	76	132	142	126	140	128	132	120	134
24	71	74	76	81	132	142	126	126	128	128	120	120
25	74	74	79	92	126	142	126	140	128	132	120	126
26	71	71	76	81	126	142	138	150	128	132	120	126
27	71	71	76	91	0	0	126	140	132	134	116	122
28	71	71	74	76	126	142	132	142	128	134	116	116
29	71	71	76	81	126	140	132	142	134	134	116	126
30	71	71	79	92	126	140	0	0	134	134	118	126
31	71	74	79	92	126	126	126	126	134	134	110	120
32	71	71	79	79	0	0	132	142	132	134	116	126
33	71	71	71	71	126	140	132	142	128	134	116	126
34	71	71	71	74	126	140	138	150	116	122	110	126
35	71	74	71	74	126	140	138	150	122	122	120	134
36	79	92	74	76	0	0	138	150	134	134	0	0
37	79	79	71	74	132	142	138	150	110	120	132	134
38	76	81	71	74	132	142	132	150	120	120	132	132
39	79	79	74	76	138	144	138	150	128	134	132	132
40	79	92	71	74	132	142	132	142	118	118	128	134
41	79	79	71	74	126	140	132	142	118	118	120	132
42	79	92	71	71	126	140	138	138	132	132	128	134
43	79	92	71	74	126	140	132	142	132	134	134	134
44	76	81	71	74	0	0	126	140	134	134	0	0
45	0	0	79	92	126	140	126	140	134	134	128	134
46	76	81	79	92	138	142	126	126	134	134	120	132
47	76	92	79	92	156	168	126	140	132	134	128	134
48	74	76	71	74	148	164	126	126	132	134	116	122
49	74	76	71	71	138	150	126	140	128	132	116	122
50	74	76	71	74	148	164	126	126	128	134	134	134

Contd.,

Table 5. continued

Locus	OarFCB20				OarFCB48				OarHH35			
S. No.	Deccani		Nellore		Deccani		Nellore		Deccani		Nellore	
1	128	142	128	140	152	162	148	158	86	102	86	102
2	128	142	126	126	152	162	152	162	98	112	0	0
3	126	146	126	140	148	158	152	162	98	112	98	98
4	130	144	124	142	144	154	152	162	86	102	86	102
5	128	146	126	142	144	154	152	152	86	102	98	112
6	126	126	128	128	148	158	152	152	86	86	98	112
7	130	142	124	142	152	162	152	162	86	102	98	112
8	128	142	124	142	152	162	148	158	86	86	114	122
9	132	142	124	144	152	152	148	148	98	119	114	122
10	126	142	126	142	152	152	148	148	98	112	114	122
11	128	142	126	142	152	152	148	158	86	102	120	128
12	128	142	126	126	152	162	152	152	0	0	120	128
13	130	142	126	126	148	158	152	162	86	102	114	122
14	126	146	128	140	148	158	152	162	86	86	114	122
15	126	126	128	128	144	154	152	162	86	86	104	119
16	132	132	126	140	142	142	148	158	86	102	104	119
17	126	142	126	142	142	150	152	162	86	86	104	119
18	126	142	126	126	142	142	144	154	86	86	104	119
19	126	142	126	140	144	154	152	162	86	86	104	119
20	128	142	128	146	148	158	142	150	86	86	104	119
21	126	142	128	146	148	158	142	142	86	102	104	119
22	128	142	126	142	158	158	142	150	86	86	104	119
23	128	128	126	140	158	158	142	150	86	102	114	122
24	130	142	126	126	148	158	142	150	86	86	104	119
25	128	142	126	140	152	162	142	150	86	86	120	128
26	128	142	126	140	152	162	142	150	86	86	120	128
27	128	146	126	140	152	162	142	142	98	112	120	128
28	132	146	126	140	152	162	142	142	86	96	120	128
29	128	142	126	142	152	162	152	162	98	112	114	122
30	128	142	126	144	152	162	152	162	86	102	114	122
31	128	128	128	128	152	162	152	162	86	102	114	122
32	126	142	128	128	152	162	152	162	86	86	120	128
33	126	144	126	140	148	158	148	158	86	86	114	122
34	128	142	128	144	148	158	148	158	98	112	114	122
35	130	142	128	142	148	158	152	162	98	98	114	122
36	128	140	126	144	144	154	148	162	86	86	114	114
37	134	146	126	126	144	154	148	162	86	86	114	114
38	0	0	128	144	144	154	152	162	98	112	114	114
39	128	142	126	142	148	168	152	162	86	86	114	122
40	128	142	126	126	148	168	148	158	86	86	120	128
41	132	144	128	142	152	162	144	154	86	86	114	122
42	126	146	128	146	152	162	144	154	86	102	114	122
43	128	140	126	126	152	162	144	154	86	86	104	119
44	126	142	128	142	152	162	144	154	86	102	104	119
45	128	146	126	144	142	150	144	154	86	102	104	119
46	128	146	126	144	142	150	148	158	86	86	114	114
47	128	128	126	144	142	142	152	162	0	0	114	114
48	126	126	126	142	152	162	152	162	86	86	114	122
49	128	142	128	128	152	162	148	158	86	86	104	119
50	126	126	128	144	152	152	152	162	86	86	104	119

Contd.,

Table 5. continued

Locus	OarHH41				OarHH47				OarJMP29			
S. No.	Deccani		Nellore		Deccani		Nellore		Deccani		Nellore	
1	112	118	124	132	156	162	140	140	146	152	146	172
2	114	122	124	132	162	170	140	152	146	152	146	172
3	112	118	124	132	156	162	140	152	146	146	138	150
4	112	118	124	132	156	162	146	158	146	152	150	150
5	112	118	124	132	162	170	140	158	146	152	146	152
6	112	112	124	132	156	156	146	162	152	152	146	152
7	122	128	124	132	146	162	146	158	138	150	146	152
8	128	128	124	132	146	162	162	170	146	152	132	140
9	122	128	124	132	146	158	170	170	140	140	128	140
10	124	132	124	124	0	0	152	162	146	152	146	152
11	122	128	122	128	140	152	146	162	138	150	146	152
12	122	128	124	124	146	158	156	162	138	150	138	152
13	128	128	122	122	162	170	0	0	138	150	150	150
14	124	132	114	122	140	152	162	170	128	140	0	0
15	122	128	114	122	140	152	146	162	128	140	146	152
16	112	118	0	0	140	152	146	162	138	150	146	152
17	114	122	114	120	140	146	140	140	146	152	0	0
18	124	132	114	120	152	158	140	152	138	150	152	152
19	124	132	122	128	152	162	140	152	138	150	152	152
20	112	118	124	132	156	170	162	170	132	140	124	132
21	112	118	124	132	156	162	170	170	124	132	128	140
22	112	118	124	124	146	158	170	170	128	150	128	140
23	122	128	124	132	146	162	0	0	138	150	132	140
24	122	128	124	132	156	162	170	170	128	140	132	140
25	124	132	124	132	0	0	156	170	128	140	132	140
26	124	124	124	132	146	162	170	170	138	150	128	140
27	124	124	124	124	156	162	170	170	138	150	132	140
28	124	124	0	0	156	162	170	170	146	152	124	132
29	124	132	124	132	152	162	170	170	138	150	138	150
30	124	132	124	124	156	162	156	170	152	152	138	150
31	132	132	124	132	146	152	156	162	146	152	152	152
32	124	124	124	132	146	162	152	162	132	140	146	152
33	124	132	124	132	146	162	156	170	132	140	146	152
34	124	132	124	132	140	152	152	158	132	140	146	162
35	124	132	124	132	0	0	162	170	128	140	146	152
36	124	124	124	132	140	140	140	162	128	140	138	150
37	124	124	124	124	140	140	140	162	128	140	152	152
38	122	128	114	122	140	152	152	162	128	140	146	152
39	124	132	124	124	146	152	140	152	146	152	146	152
40	124	132	124	124	0	0	140	140	146	152	132	140
41	124	124	124	132	158	158	140	152	152	152	146	146
42	124	132	124	132	156	162	140	152	146	152	146	146
43	124	124	124	124	152	162	140	140	146	152	0	0
44	124	124	0	0	140	146	140	140	146	152	132	140
45	124	132	124	132	140	152	140	152	152	152	146	146
46	124	132	124	132	140	152	0	0	138	150	152	152
47	124	132	0	0	152	158	140	152	146	152	152	152
48	124	132	124	132	152	162	140	158	152	152	146	152
49	124	132	0	0	152	162	140	140	152	152	152	152
50	124	132	124	132	162	170	140	152	146	152	146	152

Contd.,

Table 5. continued

Locus	OarVH72				SRCRSP1				SRCRSP5			
S. No.	Deccani		Nellore		Deccani		Nellore		Deccani		Nellore	
1	132	140	132	140	140	146	132	146	148	158	148	158
2	132	140	132	140	126	138	126	138	148	158	134	146
3	132	140	132	140	118	128	126	138	148	158	140	148
4	126	136	132	140	118	128	126	140	158	158	134	146
5	126	136	126	140	118	128	126	140	148	158	134	146
6	126	132	132	140	118	118	126	140	158	158	134	146
7	126	132	132	140	132	140	126	138	140	148	134	146
8	132	140	132	140	138	146	132	144	128	136	134	146
9	132	140	132	140	138	144	132	144	134	146	140	148
10	132	140	0	0	140	146	132	144	148	158	140	148
11	126	136	132	140	140	146	140	146	148	158	148	158
12	126	140	132	132	140	146	126	138	148	158	148	158
13	126	136	132	132	140	146	138	146	148	158	140	148
14	132	140	126	126	140	146	132	144	148	158	148	158
15	132	140	132	140	138	146	126	138	148	158	140	148
16	132	140	132	140	132	132	126	138	148	148	134	146
17	132	140	126	126	132	132	126	138	148	148	140	148
18	132	140	126	136	126	138	126	138	148	148	140	148
19	126	132	126	136	126	138	132	140	140	148	148	158
20	132	140	132	140	140	146	126	138	140	148	134	146
21	132	140	132	140	132	140	132	144	134	146	128	136
22	132	140	132	140	138	146	126	138	140	148	124	136
23	126	140	132	140	140	146	126	138	140	148	124	136
24	126	136	119	128	146	146	126	140	140	148	124	136
25	132	140	126	132	140	146	126	140	140	148	128	136
26	132	140	132	140	146	146	126	138	148	158	134	146
27	132	140	126	136	140	146	132	140	140	148	140	148
28	132	140	126	136	140	146	132	140	140	148	134	146
29	132	140	132	140	140	146	138	146	148	158	134	146
30	132	140	132	140	138	144	138	144	148	158	140	148
31	132	140	132	140	146	146	132	140	148	158	140	148
32	132	140	132	140	146	146	132	140	140	148	140	148
33	132	140	132	140	140	146	126	138	140	148	140	148
34	132	140	126	136	140	146	118	128	134	146	134	146
35	126	132	132	140	140	146	118	128	128	136	134	146
36	132	140	132	140	140	146	118	128	140	148	124	136
37	132	140	132	140	140	146	118	128	134	146	134	146
38	0	0	132	140	140	146	118	118	134	146	134	146
39	132	132	132	140	138	146	132	140	134	134	134	146
40	132	140	126	136	126	138	132	140	134	134	134	134
41	132	140	132	140	126	138	132	140	134	134	134	146
42	132	140	126	136	126	140	126	138	134	146	134	146
43	132	140	132	140	126	140	132	140	134	146	148	158
44	132	132	132	140	126	140	132	140	134	146	140	148
45	132	132	126	140	132	140	132	132	140	140	158	158
46	0	0	126	140	132	146	132	140	140	148	140	148
47	132	132	132	140	132	144	126	126	134	146	140	148
48	126	136	132	140	140	146	126	138	134	146	148	158
49	126	140	132	140	146	146	132	144	134	146	140	148
50	132	140	132	140	140	146	132	144	134	146	140	148

4.3 POLYMORPHISM

Polymorphism is the occurrence of two alleles at a locus. All the microsatellite loci utilized in the present study were found to be polymorphic and informative. The sample size, mean number of alleles, effective number of alleles, Shannon's information index, observed, expected and unbiased expected heterozygosity, Polymorphism information content, fixation index and out crossing rates obtained in the present study are presented in Table 6 & 7.

4.3.1 Alleles and Allelic patterns

4.3.1.1 Number of alleles

A total of 254 alleles in Deccani and 260 alleles in Nellore were amplified in the present study across the 30 microsatellite loci studied. The loci BM1314 (11), ILSTS11 (12), OarCB226 (12) and OarCP34 (11) in Deccani and ILSTS (14), MAF (33) and OarCB226 (12) in Nellore have amplified a total of more than 10 alleles whereas the rest of the loci had less than 10 alleles. Among all the loci OarVH72 (4) amplified lowest number of alleles in Deccani and ETH121 (4) in Nellore sheep.

The allelic patterns across the 30 microsatellite loci studied are presented in Table 8. The product size (bp) varied from 71 for OARCP20 to 288 for ILSTS11 in both the breeds. The size of the most frequent alleles ranged from 71 to 246 bp in Deccani and 74 to 288 bp in Nellore sheep.

Table 6. Sample size (N), Mean number of alleles (N_a), Effective number of alleles (N_e), Shannon's Information Index (I), Observed (H_o) and Expected heterozygosity (H_e) in Sheep

Locus	N		N_a		N_e		I		H_o		H_e	
	Deccani	Nellore	Deccani	Nellore	Deccani	Nellore	Deccani	Nellore	Deccani	Nellore	Deccani	Nellore
BM1314	47	46	11	9	6.903	5.878	2.097	1.957	0.702	0.739	0.855	0.830
BM6506	49	49	8	6	4.455	4.685	1.678	1.648	0.612	0.673	0.776	0.787
BM6526	49	48	6	10	4.346	7.863	1.600	2.166	0.918	0.917	0.770	0.873
BM757	48	49	9	9	6.427	4.250	1.955	1.737	0.896	0.735	0.844	0.765
BM8125	49	49	7	8	3.294	3.104	1.466	1.413	0.673	0.633	0.696	0.678
CSSM31	50	48	10	9	7.429	7.314	2.130	2.079	0.840	0.938	0.865	0.863
ETH121	44	44	8	4	3.213	2.683	1.482	1.070	0.341	0.023	0.689	0.627
HUJ616	47	49	10	10	4.306	6.033	1.768	1.992	0.532	0.918	0.768	0.834
ILSTS030	48	50	9	8	6.391	6.868	2.009	1.989	0.875	0.860	0.844	0.854
ILSTS11	48	48	12	14	8.913	10.240	2.278	2.433	0.958	0.917	0.888	0.902
ILSTS28	49	50	7	10	4.573	4.822	1.701	1.837	0.776	0.740	0.781	0.793
MAF209	39	49	8	8	6.404	4.381	1.949	1.644	0.897	0.837	0.844	0.772
MAF214	50	49	7	10	5.855	5.683	1.864	1.931	0.900	0.939	0.829	0.824
MAF33	48	50	9	11	5.840	8.157	1.936	2.222	0.813	0.900	0.829	0.877
MAF70	49	48	10	10	8.195	7.133	2.201	2.093	0.816	0.708	0.878	0.860
MCM140	43	45	10	8	5.391	4.644	1.916	1.802	0.698	0.489	0.814	0.785
OarAE129	47	49	10	8	6.375	6.377	2.085	1.947	0.851	0.980	0.843	0.843
OarCB226	48	46	12	12	9.580	4.804	2.347	1.912	0.854	0.609	0.896	0.792
OarCP20	47	50	6	7	4.641	4.468	1.649	1.644	0.617	0.860	0.785	0.776
OarCP34	44	43	11	10	7.918	6.874	2.204	2.079	0.955	0.837	0.874	0.855
OarFCB128	50	47	9	9	3.949	7.339	1.652	2.082	0.560	0.723	0.747	0.864
OarFCB20	49	50	9	7	4.890	4.333	1.784	1.664	0.837	0.740	0.796	0.769
OarFCB48	50	50	9	8	6.165	6.158	1.971	1.936	0.820	0.840	0.838	0.838
OarHH35	48	49	6	10	2.135	6.428	1.079	2.030	0.479	0.878	0.532	0.844
OarHH41	50	45	7	6	4.500	2.716	1.691	1.235	0.740	0.778	0.778	0.632
OarHH47	46	47	7	7	5.845	5.161	1.846	1.764	0.913	0.702	0.829	0.806
OarJMP29	50	47	8	10	5.800	5.241	1.873	1.895	0.840	0.745	0.828	0.809
OarVH72	48	49	4	6	2.871	3.184	1.171	1.305	0.917	0.918	0.652	0.686
SRCRSP1	50	50	8	8	4.525	5.988	1.739	1.899	0.840	0.940	0.779	0.833
SRCRSP5	50	50	7	8	4.730	5.794	1.679	1.867	0.820	0.960	0.789	0.827
Mean	47.80	48.10	8.46	8.66	5.52	5.62	1.82	1.84	0.77	0.78	0.79	0.80

Table 7. Unbiased Expected heterozygosity (uH_e), Polymophism Information Content (PIC), Fixation Indices (F_{IS}) and Out crossing rates at various microsatellite loci studied.

Locus	uH_e		PIC		F_{IS}		Outcrossing rate	
	Deccani	Nellore	Deccani	Nellore	Deccani	Nellore	Deccani	Nellore
BM1314	0.864	0.839	0.877	0.894	0.179	0.109	0.70	0.80
BM6506	0.784	0.795	0.752	0.766	0.211	0.144	0.65	0.75
BM6526	0.778	0.882	0.825	0.737	-0.193	-0.050	1.48	1.11
BM757	0.853	0.773	0.809	0.804	-0.061	0.039	1.13	0.92
BM8125	0.704	0.685	0.809	0.865	0.033	0.067	0.94	0.87
CSSM31	0.874	0.872	0.866	0.844	0.029	-0.086	0.94	1.19
ETH121	0.697	0.635	0.792	0.762	0.505	0.964	0.33	0.02
HUJ616	0.776	0.843	0.828	0.824	0.307	-0.101	0.53	1.22
ILSTS030	0.852	0.863	0.886	0.767	-0.037	-0.007	1.08	1.01
ILSTS11	0.897	0.912	0.754	0.743	-0.079	-0.016	1.17	1.03
ILSTS28	0.789	0.801	0.861	0.839	0.007	0.066	0.99	0.88
MAF209	0.855	0.780	0.714	0.849	-0.063	-0.084	1.13	1.18
MAF214	0.838	0.833	0.767	0.739	-0.085	-0.139	1.19	1.32
MAF33	0.838	0.886	0.819	0.818	0.020	-0.026	0.96	1.05
MAF70	0.887	0.869	0.498	0.827	0.070	0.176	0.87	0.70
MCM140	0.824	0.794	0.750	0.574	0.143	0.377	0.75	0.45
OarAE129	0.852	0.852	0.807	0.779	-0.009	-0.162	1.02	1.39
OarCB226	0.905	0.801	0.806	0.786	0.046	0.231	0.91	0.62
OarCP20	0.793	0.784	0.587	0.629	0.214	-0.108	0.65	1.24
OarCP34	0.884	0.865	0.750	0.811	-0.093	0.020	1.21	0.96
OarFCB128	0.754	0.873	0.759	0.805	0.250	0.162	0.60	0.72
OarFCB20	0.804	0.777	0.877	0.894	-0.052	0.038	1.11	0.93
OarFCB48	0.846	0.846	0.752	0.766	0.021	-0.003	0.96	1.01
OarHH35	0.537	0.853	0.825	0.737	0.099	-0.039	0.82	1.08
OarHH41	0.786	0.639	0.809	0.804	0.049	-0.231	0.91	1.60
OarHH47	0.838	0.815	0.809	0.865	-0.101	0.129	1.22	0.77
OarJMP29	0.836	0.818	0.866	0.844	-0.015	0.080	1.03	0.85
OarVH72	0.659	0.693	0.792	0.762	-0.407	-0.339	2.37	2.03
SRCRSP1	0.787	0.841	0.828	0.824	-0.078	-0.128	1.17	1.29
SRCRSP5	0.797	0.836	0.886	0.767	-0.040	-0.160	1.08	1.38
Mean	0.806	0.812	0.773	0.777	0.029	0.031	0.94	0.94

Table 8. Allelic patterns across the microsatellite loci

Locus	Deccani			Nellore		
	Product size (bp)	No. of alleles	Most frequent allele	Product size (bp)	No. of alleles	Most frequent allele
BM1314	148-186	11	164	148-186	9	148
BM6506	178-206	8	182	182-206	6	188
BM6526	126-172	6	151	118-172	10	148
BM757	107-160	9	140	107-160	9	144
BM8125	128-154	7	128	128-154	8	128
CSSM31	126-170	10	126	126-170	9	158
ETH121	182-202	8	182	182-202	4	182
HUJ616	118-158	10	142	118-158	10	142
ILSTS030	140-190	9	166	148-190	8	160
ILSTS11	223-288	12	246	226-288	14	288
ILSTS28	116-164	7	150	112-164	10	136
MAF209	112-144	8	132	112-144	8	122
MAF214	180-214	7	180	180-214	10	192
MAF33	114-168	9	144	114-158	11	142
MAF70	136-172	10	144	136-172	10	164
MCM140	148-190	10	170	148-180	8	148
OarAE129	140-186	10	174	156-186	8	174
OarCB226	128-172	12	134	128-166	12	128
OarCP20	71-92	6	71	71-92	7	74
OarCP34	126-168	11	126	126-168	10	126
OarFCB128	110-134	9	134	110-134	9	134
OarFCB20	126-146	9	128	124-146	7	126
OarFCB48	142-168	9	152	142-162	8	152
OarHH35	86-119	6	86	86-128	10	114
OarHH41	112-132	7	124	114-132	6	124
OarHH47	140-170	7	162	140-170	7	140
OarJMP29	124-172	8	152	124-172	10	152
OarVH72	126-140	4	132	126-140	6	132
SRCRSP1	118-146	8	140	118-146	8	126
SRCRSP5	124-158	7	148	124-158	8	148

4.3.1.2 Mean number of alleles

The mean number of alleles was 8.46 and 8.66 for Deccani and Nellore respectively. The effective number of alleles ranged from 2.135 to 9.580 in Deccani and 2.683 to 10.240 in Nellore whereas the overall mean effective number of alleles varied between 5.52 for Deccani and 5.62 for Nellore sheep.

4.3.1.3 Allele frequency

The frequency of various alleles at different loci in the two populations are detailed in the Table 9.

The allele frequency ranged from 1.0 percent (178 and 187 bp at BM6506, 107 and 141 bp at BM757, 223 and 238 bp at ILSTS11, 168 bp at MAF33, 126 bp at OarFCB128, 134 bp at OarFCB20, 96 bp and 119 bp at OarHH35 and 124 bp at OarJMP29) to 65.6 percent (86 bp at OarHH35) in Deccani and 1.0 percent (129 and 148 bp at BM8125, 247, 258 and 269 bp at ILSTS11, 132 bp at ILSTS128, 127 bp at MAF209, 182, 184 and 196 bp at MAF214 and 91 bp at OarCP20) to 51.1 percent (124 bp at OarHH41) in Nellore sheep.

4.3.1.4 Population specific alleles

The list of locus wise population specific alleles, the allele size (bp) and frequencies are presented in Table 10 & 11. A total of 23 out of 254 and 37 out of 260 alleles amplified were found to be population specific for Deccani and Nellore sheep respectively. The locus OarFCB20 recorded highest number of specific alleles (3) in Deccani and the locus OarHH35 recorded the highest number of specific alleles (5) in Nellore sheep. However the frequency of most of these population specific alleles was very low and only 2 (9%) and 4 (11 %) alleles had the frequency

Table 9. Allele size (bp) and Allele frequency at various microsatellite loci studied

S.No.	Locus	Allele size (bp)	Allele frequency		S.No.	Locus	Allele size (bp)	Allele frequency	
			Deccani	Nellore				Deccani	Nellore
1	BM1314	148	0.074	0.304			144	0.082	0.061
		152	0.138	0.043			148	0.041	0.010
		160	0.011	0.000			154	0.031	0.031
		164	0.245	0.098	6	CSSM31	126	0.220	0.073
		168	0.053	0.130			138	0.160	0.073
		170	0.011	0.000			140	0.120	0.031
		172	0.074	0.043			144	0.090	0.083
		176	0.181	0.163			150	0.050	0.000
		178	0.106	0.022			152	0.150	0.188
		180	0.021	0.076			156	0.070	0.083
		186	0.085	0.120			158	0.030	0.188
2	BM6506	178	0.010	0.000			160	0.030	0.115
		182	0.357	0.082			170	0.080	0.167
		184	0.143	0.112	7	ETH121	182	0.489	0.455
		186	0.133	0.204			186	0.091	0.159
		187	0.010	0.000			188	0.227	0.375
		188	0.224	0.306			191	0.023	0.000
		202	0.041	0.061			192	0.023	0.000
		206	0.082	0.235			195	0.102	0.000
3	BM6526	118	0.000	0.094			197	0.023	0.000
		124	0.000	0.042			202	0.023	0.011
		125	0.000	0.094	8	HUJ616	118	0.011	0.112
		126	0.061	0.156			120	0.032	0.082
		135	0.000	0.042			126	0.064	0.020
		138	0.082	0.042			130	0.106	0.051
		148	0.194	0.198			134	0.245	0.020
		151	0.327	0.146			136	0.032	0.173
		159	0.071	0.063			142	0.383	0.276
		172	0.265	0.125			146	0.085	0.041
4	BM757	107	0.010	0.020			152	0.021	0.041
		118	0.063	0.020			158	0.021	0.184
		124	0.094	0.041	9	ISTS030	140	0.031	0.000
		136	0.156	0.061			148	0.052	0.170
		140	0.240	0.143			158	0.250	0.120
		141	0.010	0.020			160	0.063	0.200
		144	0.146	0.408			166	0.219	0.170
		152	0.135	0.163			168	0.115	0.130
		160	0.146	0.122			178	0.083	0.070
5	BM8125	128	0.459	0.469			182	0.115	0.100
		129	0.000	0.010			190	0.073	0.040
		132	0.082	0.092	10	ILSTS11	223	0.010	0.000
		136	0.276	0.296			226	0.146	0.094
		142	0.031	0.031			236	0.094	0.125

Contd.,

Table 9. Continued

S.No.	Locus	Allele size (bp)	Allele frequency		S.No.	Locus	Allele size (bp)	Allele frequency	
			Deccani	Nellore				Deccani	Nellore
		238	0.010	0.063			126	0.000	0.080
		240	0.146	0.083			130	0.115	0.100
		246	0.156	0.094			134	0.063	0.070
		247	0.000	0.010			136	0.000	0.020
		248	0.083	0.073			142	0.083	0.200
		252	0.094	0.104			144	0.292	0.170
		258	0.000	0.010			146	0.063	0.070
		262	0.021	0.063			148	0.042	0.070
		269	0.000	0.010			158	0.188	0.130
		274	0.073	0.052			168	0.010	0.000
		278	0.063	0.052	15	MAF70	136	0.061	0.021
		288	0.104	0.167			138	0.092	0.021
11	ILSTS28	112	0.000	0.040			140	0.041	0.063
		116	0.102	0.090			144	0.204	0.177
		126	0.092	0.050			150	0.102	0.104
		130	0.000	0.040			154	0.071	0.083
		132	0.031	0.010			156	0.112	0.052
		136	0.265	0.320			158	0.163	0.177
		144	0.000	0.020			164	0.082	0.208
		146	0.082	0.030			172	0.071	0.094
		150	0.337	0.270	16	MCM140	148	0.093	0.389
		164	0.092	0.130			152	0.035	0.167
12	MAF209	112	0.192	0.020			158	0.023	0.122
		118	0.090	0.051			162	0.105	0.078
		122	0.115	0.327			166	0.070	0.056
		124	0.115	0.020			170	0.291	0.078
		127	0.000	0.010			171	0.012	0.000
		132	0.244	0.224			176	0.023	0.056
		134	0.026	0.000			180	0.093	0.056
		138	0.090	0.235			190	0.256	0.000
		144	0.128	0.112	17	OarAE129	140	0.053	0.000
13	MAF214	180	0.290	0.143			156	0.053	0.133
		182	0.000	0.010			160	0.053	0.000
		184	0.000	0.010			164	0.085	0.071
		192	0.130	0.316			166	0.085	0.082
		196	0.100	0.010			172	0.043	0.112
		198	0.110	0.102			174	0.309	0.224
		200	0.110	0.122			176	0.064	0.031
		206	0.100	0.092			180	0.117	0.133
		210	0.000	0.061			186	0.138	0.214
		214	0.160	0.133	18	OarCB226	128	0.146	0.348
14	MAF33	114	0.000	0.020			134	0.156	0.250
		122	0.146	0.070			142	0.104	0.033

Contd.,

Table 9. Continued

S.No.	Locus	Allele size (bp)	Allele frequency		S.No.	Locus	Allele size (bp)	Allele frequency	
			Deccani	Nellore				Deccani	Nellore
		144	0.094	0.109			134	0.010	0.000
		146	0.104	0.043			140	0.020	0.110
		148	0.083	0.076			142	0.276	0.140
		150	0.000	0.011			144	0.031	0.090
		152	0.104	0.033			146	0.092	0.030
		153	0.000	0.011	23	OarFCB48	142	0.090	0.120
		154	0.042	0.022			144	0.070	0.060
		158	0.063	0.043			148	0.120	0.150
		160	0.021	0.000			150	0.030	0.060
		166	0.063	0.022			152	0.270	0.250
		172	0.021	0.000			154	0.070	0.060
19	OarCP20	71	0.330	0.270			158	0.140	0.090
		74	0.202	0.320			162	0.190	0.210
		76	0.202	0.170			168	0.020	0.000
		79	0.117	0.110	24	OarHH35	86	0.656	0.020
		81	0.085	0.050			96	0.010	0.000
		91	0.000	0.010			98	0.104	0.051
		92	0.064	0.070			102	0.146	0.020
20	OarCP34	126	0.227	0.256			104	0.000	0.143
		132	0.080	0.116			112	0.073	0.031
		138	0.091	0.151			114	0.000	0.265
		140	0.148	0.116			119	0.010	0.143
		142	0.114	0.116			120	0.000	0.082
		144	0.011	0.000			122	0.000	0.163
		148	0.091	0.035			128	0.000	0.082
		150	0.080	0.105	25	OarHH41	112	0.100	0.000
		156	0.034	0.023			114	0.020	0.056
		164	0.091	0.058			118	0.080	0.000
		168	0.034	0.023			120	0.000	0.022
21	OarFCB128	110	0.020	0.064			122	0.100	0.078
		116	0.020	0.138			124	0.370	0.511
		118	0.040	0.043			128	0.120	0.022
		120	0.060	0.149			132	0.210	0.311
		122	0.050	0.053	26	OarHH47	140	0.152	0.277
		126	0.010	0.085			146	0.141	0.064
		128	0.180	0.096			152	0.185	0.149
		132	0.210	0.170			156	0.130	0.053
		134	0.410	0.202			158	0.076	0.053
22	OarFCB20	124	0.000	0.040			162	0.261	0.160
		126	0.194	0.380			170	0.054	0.245
		128	0.276	0.210	27	OarJMP29	124	0.010	0.021
		130	0.051	0.000			128	0.090	0.043
		132	0.051	0.000			132	0.050	0.096

Contd.,

Table 9. Continued

S.No.	Locus	Allele size (bp)	Allele frequency	
			Deccani	Nellore
		138	0.120	0.053
		140	0.140	0.117
		146	0.180	0.245
		150	0.130	0.085
		152	0.280	0.309
		162	0.000	0.011
		172	0.000	0.021
28	OarVH72	119	0.000	0.010
		126	0.135	0.153
		128	0.000	0.010
		132	0.448	0.388
		136	0.063	0.071
		140	0.354	0.367
29	SRCRSP1	118	0.050	0.060
		126	0.080	0.220
		128	0.030	0.040
		132	0.090	0.210
		138	0.110	0.180
		140	0.260	0.170
		144	0.030	0.080
		146	0.350	0.040
30	SRCRSP5	124	0.000	0.040
		128	0.020	0.020
		134	0.180	0.200
		136	0.020	0.060
		140	0.150	0.170
		146	0.120	0.180
		148	0.330	0.240
		158	0.180	0.090

Table 10. Population specific alleles in Deccani sheep

Locus	Deccani	
	Allele size (bp)	Allele frequency
BM1314	160	0.011
	170	0.011
BM6506	178	0.010
	187	0.010
CSSM31	150	0.050
ILSTS030	140	0.031
ILSTS11	223	0.010
MAF209	134	0.026
MAF33	168	0.010
MCM140	171	0.012
	190	0.256
OarAE129	140	0.053
	160	0.053
OarCB226	160	0.021
	172	0.021
OarCP34	144	0.011
OarFCB20	130	0.051
	132	0.051
	134	0.010
OarFCB48	168	0.020
OarHH35	96	0.010
OarHH41	112	0.100
	118	0.080

Table 11. Population specific alleles in Nellore sheep

Locus	Nellore	
	Allele size (bp)	Allele frequency
BM6526	118	0.094
	124	0.042
	125	0.094
	135	0.042
BM8125	129	0.010
ETH121	191	0.023
	192	0.023
	195	0.102
	197	0.023
ILSTS11	247	0.010
	258	0.010
	269	0.010
ILSTS28	112	0.040
	130	0.040
	144	0.020
MAF209	127	0.010
MAF214	182	0.010
	184	0.010
	210	0.061
MAF33	114	0.020
	126	0.080
	136	0.020
OarCB226	150	0.011
	153	0.011
OarCP20	91	0.010
OarFCB20	124	0.040
OarHH35	104	0.143
	114	0.265
	120	0.082
	122	0.163
	128	0.082
OarHH41	120	0.022
OarJMP29	162	0.011
	172	0.021
OarVH72	119	0.010
	128	0.010
SRCRSP5	124	0.040

of more than 10 per cent. The alleles with frequency above 10 per cent may be more reliable and can be used to identify the population.

4.3.2 Polymorphic Information Content (PIC)

The overall mean PIC values ranged from 0.773 in Deccani to 0.777 in Nellore breed. And across the loci the values ranged from 0.587 (OarVH72) to 0.886 (OarCB226) in Deccani and 0.552 (ETH121) to 0.894 (ILSTS11) in Nellore. The PIC values for all the loci were above 0.50 except for the loci OarHH35 (0.49) in Deccani.

4.4 GENETIC DIVERSITY

Genetic diversity measures the genetic variation among the populations in terms of estimates of heterozygosity.

4.4.1 Heterozygosity

The mean expected heterozygosity (H_e) among the breeds ranged from 0.79 (Deccani) to 0.80 (Nellore). The expected heterozygosity was the highest in Nellore for locus ILSTS11 (0.90) and the least in Deccani for the locus OarHH35 (0.53).

The mean observed heterozygosity levels ranged from 0.77 in Deccani to 0.78 in Nellore sheep. Observed heterozygosity in Deccani sheep ranged from 0.34 (ETH121) to 0.96 (ILSTS11) and in Nellore sheep the values ranged from 0.02 (ETH121) to 0.96 (SRCRSP5).

The unbiased expected heterozygosity in Deccani sheep ranged from 0.54 (OarHH35) to 0.91 (OarCB226) and in Nellore sheep, it ranged from 0.64 (ETH121) to 0.91 (ILSTS11).

4.4.2 Fixation Indices (F – statistic)

The coefficient of inbreeding (F_{IS}) across the loci in both the breeds is presented in Table 12. Fixation indices give an idea about the population structure in terms of inbreeding coefficient and population differentiation. The overall mean inbreeding coefficient was 0.029 in Deccani and 0.031 in Nellore sheep.

Of the 30 loci studied, 14 loci in Deccani and 16 loci in Nellore sheep showed negative inbreeding coefficient indicating the presence of outbreeding. Eight loci (BM1314, BM6506, BM8125, ILSTS28, MAF70, MCM140, OarCB226 and OarFCB128) showed positive, mild to moderate inbreeding ranging from 0.007 to 0.25 in both the breeds studied.

The mean values of F_{IS}, F_{IT} and F_{ST} over all the loci were 0.03, 0.05 and 0.02, respectively (Table 12). The F_{IS} values ranged from -0.372 (OarVH72) to 0.74 (ETH121), F_{ST} values ranged from 0.001 for the locus BM8125 to 0.172 for the locus OarHH35 and F_{IT} values ranged from -0.370 for the locus OarVH72 to 0.728 for the locus ETH121.

4.5 GENETIC DISTANCE

The Nei's genetic distance matrix is presented in Table 13. The value of genetic distance was 0.225 between the two populations studied.

4.6 GENETIC IDENTITY

The Nei's genetic similarity or identity matrix is just reciprocal of the genetic distance matrix (Table 14). The value of genetic identity between Deccani and Nellore sheep was 0.799.

Table 12. Estimation of Fixation indices at different loci

Locus	Fixation indices		
	F_{IS}	F_{ST}	F_{IT}
BM1314	0.145	0.029	0.170
BM6506	0.177	0.035	0.206
BM6526	-0.117	0.025	-0.089
BM757	-0.013	0.028	0.015
BM8125	0.050	0.001	0.050
CSSM31	-0.028	0.023	-0.005
ETH121	0.724	0.015	0.728
HUJ616	0.095	0.039	0.130
ILSTS030	-0.022	0.016	-0.006
ILSTS11	-0.047	0.006	-0.041
ILSTS28	0.037	0.006	0.042
MAF209	-0.073	0.032	-0.039
MAF214	-0.112	0.021	-0.089
MAF33	-0.004	0.013	0.010
MAF70	0.123	0.008	0.130
MCM140	0.258	0.067	0.308
OarAE129	-0.086	0.009	-0.076
OarCB226	0.133	0.020	0.150
OarCP20	0.054	0.006	0.060
OarCP34	-0.037	0.003	-0.033
OarFCB128	0.203	0.025	0.223
OarFCB20	-0.008	0.025	0.017
OarFCB48	0.009	0.002	0.011
OarHH35	0.014	0.172	0.184
OarHH41	-0.077	0.020	-0.055
OarHH47	0.012	0.023	0.035
OarJMP29	0.032	0.005	0.037
OarVH72	-0.372	0.002	-0.370
SRCRSP1	-0.104	0.043	-0.056
SRCRSP5	-0.101	0.007	-0.093
Mean	0.029	0.024	0.052

Table 13. Nei's genetic distance matrix between the populations

	Deccani	**Nellore**
Deccani	0.000	
Nellore	0.225	0.000

Table 14. Nei's genetic identity matrix between the populations

	Deccani	**Nellore**
Deccani	1.000	
Nellore	0.799	1.000

4.7 PHYLOGENY

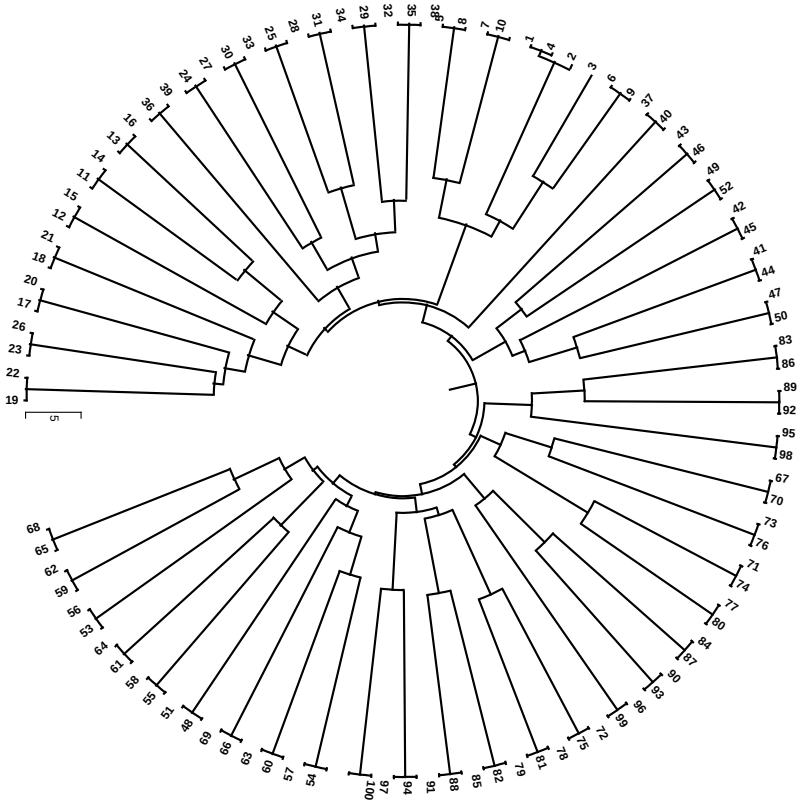

Fig. 8 Dendrogram showing the phylogenetic relationships among the two sheep breeds

The dendrogram showing the Phylogenetic relationship among the two sheep breeds included in the present study is presented in Fig. 8. The two breeds were separated from each other as two clusters. There is blending of germplasm between the two breeds regarding two animals, 48th animal from Deccani and 52nd animal from Nellore sheep.

4.8 HARDY WEINBERG EQUILIBRIUM

The populations were tested for departure from Hardy-Weinberg equilibrium frequencies for all the loci. The Chi-square values with their respective degree of freedom for different loci utilized for genotyping in the present study are given in the Table 15. All the loci departed from the equilibrium frequency in the two populations studied.

Table 15. χ^2 values for testing HWE at various loci

Locus	Deccani		Nellore	
	df	χ^2	***df***	χ^2
BM1314	55	208.25***	36	256.40***
BM6506	28	210.52***	15	94.41***
BM6526	15	125.67***	45	312.75***
BM757	36	303.33***	36	260.49***
BM8125	21	165.99***	28	156.36***
CSSM31	45	278.24***	36	286.74***
ETH121	28	138.06***	6	88.04***
HUJ616	45	124.77***	45	174.25***
ILSTS030	36	139.90***	28	175.67***
ILSTS11	66	216.14***	91	203.08***
ILSTS28	21	138.37***	45	287.27***
MAF209	28	153.78***	28	197.40***
MAF214	21	68.16***	45	170.04***
MAF33	36	138.99***	55	190.78***
MAF70	45	164.70***	45	99.12***
MCM140	45	248.47***	28	154.47***
OarAE129	45	259.33***	28	157.59***
OarCB226	66	324.52***	66	249.34***
OarCP20	15	98.25***	21	124.83***
OarCP34	55	310.64***	45	271.11***
OarFCB128	36	182.77***	36	100.90***
OarFCB20	36	68.61***	21	51.61***
OarFCB48	36	260.58***	28	248.96***
OarHH35	15	81.44***	45	379.19***
OarHH41	21	169.63***	15	105.20***
OarHH47	21	59.33***	21	66.78***
OarJMP29	28	198.42***	45	158.62***
OarVH72	6	65.77***	15	178.57***
SRCRSP1	28	132.79***	28	158.56***
SRCRSP5	21	194.37***	28	236.48***

*** Significant ($P \leq 0.001$)

DISCUSSION

CHAPTER V

DISCUSSION

5.1 GENOMIC DNA

The overall mean absorbance ratio of genomic DNA was 1.947 in Deccani sheep while it was 1.952 in Nellore sheep (Table 4) which is similar to the ratios recommended for pure DNA preparations free from protein (Sambrook and Russell, 2001). DNA with an optical absorbance ratio of 1.60 to 2.00 is considered to be of good quality without any protein contamination. The ratio obtained in the present study is little more than that reported by Vani (2012) in Nellore Jodepi sheep (1.789).

The average DNA concentration recorded in the present study was 0.928 µg/µl in Deccani and 0.673 µg/µl in Nellore sheep. Quantity of DNA depends on the number of alcohol washings at the time of isolation, which plays a major role in PCR amplification. Pictures of agarose gel electrophoresis of DNA samples isolated (Fig. 6 & 7) revealed intact single band for each of the DNA samples, which indicated that there was no shearing of genomic DNA (De *et. al.*, 2000).

5.2 MICROSATELLITE MARKER ANALYSIS

A total of 100 sheep belonging to Deccani and Nellore breeds were genotyped using thirty microsatellite markers. The primers were amplified by PCR and the bands resolved in polyacrylamide gel. The allele size range was estimated by using a 50 bp sequencing ladder as the standard molecular weight marker. From the allelic pattern, the homozygotes (presence of single band) could be distinguished from heterozygotes (presence of double band) with respect to each microsatellite locus.

5.3 POLYMORPHISM

A total of 254 alleles in Deccani and 260 alleles in Nellore were amplified in the present study across the 30 microsatellite loci studied. The loci BM1314 (11), ILSTS11 (12), OarCB226 (12) and OarCP34 (11) in Deccani and ILSTS (14), MAF (33) and OarCB226 (12) in Nellore have amplified a total of more than 10 alleles whereas the rest of the loci had less than 10 alleles. Among all the loci OarVH72 (4) amplified lowest number of alleles in Deccani and ETH121 (4) in Nellore sheep. The allelic patterns across the 30 microsatellite loci studied are presented in Table 8. The product size (bp) varied from 71 for OARCP20 to 288 for ILSTS11 in both the breeds.

Published literature revealed a wide range for the number of alleles along with the size of alleles depending upon the genetic group studied and the number of microsatellites used. Thus, many authors (Farid *et. al.,* 2000; Arora and Bhatia, 2004; Arora and Bhatia, 2006; Girish *et. al.,* 2007; Arora and Bhatia, 2008; Prema *et. al.,* 2008a, 2008b and Nanekarani *et. al.,* 2010) reported a lower mean number of alleles while some authors reported higher number of alleles (Diez *et. al.,* 2000; Ozerov *et. al.,* 2008; Arora *et. al.,* 2010; Jyotsana *et. al*., 2010; Rodrigo *et. al.,* 2010 and Radha *et. al.,* 2011) than the present findings. The differences in allele number and size may be attributable to the study of unrelated local populations spread over distant geographical area, which harboured high degree of genetic variation. The loci genotyped in the present study were found to be polymorphic in both the breeds with moderate to high informativeness.

5.3.1 Allele and Allele Patterns

5.3.1.1 Number of alleles

The total number of alleles observed in the present study was 254 and 260 with an average of 8.46 and 8.66 alleles per locus in Deccani and Nellore breeds respectively (Table 9).

5.3.1.2 Mean number of alleles

The mean number of alleles in the present study ranged from 4 to 12 in both the sheep breeds studied (Table 6). Effective number of alleles (N_e) is the non linear function of the expected heterozygosity in the population. It gives an idea of how wider the allele frequencies are distributed in the population. The effective number of alleles in the present study varied between 2.135 to 9.580 in Deccani and 2.683 to 10.240 in Nellore sheep. The mean effective number of alleles observed in the present study was 5.52 and 5.62 in Deccani and Nellore sheep.

The allele distribution and characteristics of different microsatellite loci are discussed for each locus separately in terms of product size, mean number of alleles, effective number of alleles and frequent allele(s).

BM1314

The allele BM1314, a di-nucleotide repeat amplified a total of 11 alleles in Deccani and 9 alleles in Nellore sheep with a product size of 148 – 186 bp in both the breeds. The size of the most frequent allele was 164 bp (Table 8). The total number of alleles in the present study is higher than that reported by Arora and Bhatia (2006) in Magra sheep (9), Girish *et.*

al. (2007) in Nilagiri (8), Arora and Bhatia (2008) in Magra (9), Marwari (9) and Sonadi (7), Prema *et. al.* (2008a) in Mecheri Sheep (6) and (2008b) in Madras Red sheep (6), Pramod *et. al.* (2009) in Vembur sheep (8), Hepshiba *et. al.* (2014) in Coimbatore sheep (6) and Ahmed *et. al.* (2014) in Kail sheep (5). Few studies recorded 14 alleles at this locus in Kazak sheep breeds (Ozerov *et. al.,* 2008) and in sheep breeds from Rajasthan (Arora *et. al.,* 2011a).

The effective number of alleles at the locus in the present study was 6.903 and 5.878 for Deccani and Nellore sheep, respectively which were lower than those reported by Arora and Bhatia (2006) in Magra sheep (7.3) and higher than those reported by Prema *et. al.* (2008a) in Mecheri sheep (3.68) and (2008b) in Madras Red sheep (4.11) and Hepshiba *et. al.* (2014) in Coimbatore sheep (1.95).

BM6506

A total of 8 alleles in Deccani and 6 alleles in Nellore were amplified at BM6506 locus which is a $(CA)_{23}$ di-nucleotide repeat with the size of the alleles ranging from 178-206 bp. Diez-Tascon *et. al.* (2000) studied the same locus in Merino sheep and reported 9 alleles from the study. Ozerov *et. al.* (2008) reported 8 alleles from the same locus in Kazakh sheep breeds. A perusal of other published reports revealed a range of 1.9 to 6 alleles with 3 alleles in Magra (Arora and Bhatia, 2006); 2 alleles in Bellary (Kumar *et. al.*, 2007); 5.97 alleles in Jalauni (Arora *et. al.*, 2008) and 5 alleles in Mecheri ((Prema *et. al.*, 2008a); 6 alleles in Vembur sheep (Pramod *et. al.*, 2009) and 4 alleles in Coimbatore sheep (Hepshiba *et. al.*, 2014).

The effective number of alleles in the present study ranged from 4.455 in Deccani and 4.685 in Nellore sheep which was higher than the values reported by Kumar *et. al.*, 2007 in Bellary sheep (1.704), Arora *et. al.*, 2008 in Jalauni sheep (2.52), Prema *et. al.*, 2008a in

Mecheri sheep (2.85) and Hepshiba *et. al.,* 2014 in Coimbatore sheep (2.83). The most frequent allele was 182 bp in Deccani where as it is 188 bp for Nellore sheep which is lower than the allele 195 bp reported by Hepshiba *et. al.*, 2014 in Coimbatore sheep.

BM6526

The BM6526 locus amplified a total of 6 alleles in Deccani and 10 alleles in Nellore sheep. The allele size ranged from 118-172 bp in both the sheep breeds. Earlier reports on this locus indicated the presence of 11 alleles in Kazakh sheep (Ozerov *et. al.*, 2008), 12 alleles in Egypt breeds (El-Nahas *et. al.*, 2008), 4 alleles in Muzzafarnagri sheep (Arora and Bhatia, 2004), 6 alleles in Jalauni sheep (Arora *et. al.*,2008), 3 alleles in Madras Red sheep (Prema *et. al.,* 2008a), 6 alleles in Vembur sheep (Pramod *et. al.*,(2009), 19 alleles in Rajasthan sheep (Arora *et. al.,* 2010), 9 alleles in Kilakarsal sheep (Radha *et. al.,* 2011) and 7 alleles in Coimbatore sheep (Hepshiba *et. al.*,2014).

The effective number of alleles was 4.346 for Deccani and 7.863 for Nellore which were quite more than the values reported by Arora and Bhatia (2004) as 2.571 in Muzzafarnagri, Arora *et. al.* (2008) as 4.42 in Jalauni, Prema *et. al.* (2008a) as 2.97 in Madras Red, Pramod *et. al.* (2009) as 4.351 in Vembur, Radha *et. al.* (2011) as 5.06 in Kilakarsal and Hepshiba *et. al.* (2014) as 4.42 in Coimbatore sheep. The most frequent alleles were 151 bp and 148 bp for Deccani and Nellore sheep breeds respectively which were nearer to the values 155 & 167 reported by Hepshiba *et. al.* (2014) in Coimbatore sheep.

BM757

The BM757 a $(GT)_{14}$ di-nucleotide repeat has amplified a total of 9 alleles in both the sheep breeds studied with the allele size ranging between 107-160 bp. The allele numbers

obtained in the present study are in line with reports of Tascon *et. al.* (2000), Ozerov *et. al.* (2008), Pramod *et. al.* (2009) and Jyotsana *et. al.* (2010) who reported from 7 to 9 alleles. However, lesser number of alleles (4-6) for the same locus was reported by Arora and Bhatia (2006) Arora and Bhatia (2008), Girish *et. al.* (2007), Kumar *et. al.* (2007) and Prema *et. al.* (2008a) in Magra, Marwari, Sonadi, Bellary and Mecheri sheep. Whereas, Arora *et. al.* (2011a) reported a higher number of alleles (14) at this locus in six sheep breeds of Rajasthan.

The allele size in the present study is lower than that obtained by Kumar *et. al.* (2007) in Bellary (225-241 bp), Prema *et. al.*(2008a) in Mecheri (176-206 bp), and Arora and Bhatia (2009) in Jalauni, Marwari and Sonadi (178-200 bp).

The effective number of alleles ranged from 4.250 in Nellore sheep to 6.427 in Deccani sheep. Published literature revealed a range of 2.6 to 5.0 alleles at this locus (Arora and Bhatia, 2006; Girish *et. al.*, 2007; Kumar *et. al.*, 2007; Prema *et. al.*, 2008a; Pramod *et. al.*, 2009). The most frequent alleles at this locus were 140 bp and 144 bp alleles for Deccani and Nellore sheep respectively. These are smaller than the allele of 180 bp length reported by Hepshiba *et. al.*, (2014) in Coimbatore sheep.

BM8125

The allele size range for the locus BM8125 was 128-154 bp with amplification of 7 alleles in Deccani and 8 alleles in Nellore sheep. The number of alleles recorded in the present study is similar to that reported by Ozerov *et. al.* (2008) in Kazakh sheep (8 alleles), Hoda *et. al.* (2012) in Albanian sheep (7 alleles), Kumar *et. al.* (2007) in Bellary sheep (7 alleles) and Jyotsana *et. al.*(2010) in three Gujarat sheep breeds (7 alleles). However, more number of alleles (10 to 11) were reported by Rodrigo *et. al.* (2010) in Chilean breeds and

Arora *et. al.* (2011) in Rajasthan sheep breeds. Lesser number of alleles are also reported at the same locus by Arora and Bhatia (2004) in Muzaffarnagari sheep (5), Girish *et. al.* (2007) in Nilagiri sheep (5), Arora *et. al.* (2008) in Jalauni sheep (6)Pramod *et. al.* (2009) in Vembur sheep (6 alleles).

The most repeated allele was 128 bp in both the sheep breeds and the effective number of alleles ranged from 3.104 in Nellore to 3.294 in Deccani sheep. Published reports on effective number of alleles at the same locus depict a range of 1.85 to 3.37 (Hoda *et. al.*, 2012, Girish *et. al.*, 2007, Kumar *et. al.*, 2007, Arora *et. al.*, 2008 and Pramod *et. al.*, 2009) in Albanian, Nilagiri, Bellary, Jalauni and Vembur sheep breeds.

CSSM31

This $(AC)_{35}$ di-nucleotide microsatellite repeat produced 10 and 9 alleles in Deccani and Nellore sheep respectively with the allele size ranging between 126 - 170 bp. The number of alleles in the present study was lower when compared to the reports of 17 alleles in Kazakh sheep by Ozerov *et. al.* (2008), 25 alleles in Chilean sheep by Rodrigo *et. al.* (2000) and 24 alleles in sheep breeds from Rajasthan by Arora *et. al.*, (2010). Arora and Bhatia (2006), Arora *et. al.* (2008), Radha *et. al.* (2011) also reported around 10 alleles based on studies on Magra, Jalauni, and Kilakarsal sheep. Whereas only 5 alleles were reported in Nilagiri and Vembur sheep by Girish *et. al.* (2007) and Pramod *et. al.* (2009) and only 2 alleles were reported in Coimbatore sheep by Hepshiba *et. al.* (2014).

The mean effective number of alleles was 7.429 in Deccani and 7.314 in Nellore sheep and the most frequent alleles were 126 bp in Deccani and 158 bp in Nellore sheep. Mean effective number of alleles as observed from published literature ranged from 2 to 4.9

(4.9 in Magra sheep by Arora and Bhatia, 2006; 2.94 in Nilagiri sheep by Girish *et. al.,* 2007; 3.90 in Jalauni sheep by Arora *et. al.*, 2008; 4.158 in Vembur sheep by Pramod *et. al.,* 2009; and 2.00 in Coimbatore sheep by Hepshiba *et. al.*, 2014). The most frequent alleles at this locus in Coimbatore sheep were 128 & 146 as reported by Hepshiba *et. al.* (2014).

ETH121

This is a $(GT)_{22}$ di-nucleotide bovine specific microsatellite and amplified a total of 8 alleles in Deccani and 4 alleles in Nellore sheep in the present study.

The allele size ranged between 182-202 bp and the effective number of alleles was 3.213 and 2.693 in Deccani and Nellore sheep respectively. The most frequent allele was 182 bp in both the sheep breeds studied.

HUJ616

In the present study, the HUJ 616 allele amplified a total of 10 alleles in each of the breeds with the allele size ranging between 118-158 bp. Published reports indicate a range of 2 to 15 alleles. Hoda *et. al.*(2012) reported 15 alleles in Albaninan sheep; Arora and Bhatia (2004) reported 2 alleles in Muzzafarnagri and Arora and Bhatia, 2006 reported 5 alleles in Magra sheep, Girish *et. al.*, 2007 reported 4 alleles in Nilagiri sheep, Kumar *et. al.*, 2007 reported 8 alleles in Bellary sheep, Prema *et. al.*, 2008a reported 5 alleles in Macheri sheep, Pramod *et. al.*, 2009 reported 9 alleles in Vembur sheep and Jyotsana *et. al.*, 2010 reported 12 alleles in the three Gujarat sheep breeds.

The effective mean number of alleles was 4.306 in Deccani and 6.033 in Nellore with the most frequent allele to be 142 bp in the both the sheep. The effective number of alleles

reported in Albanian sheep by Hoda *et. al.*, 2012 was 5.03, 1.50 in Muzzafarnagri sheep by Arora and Bhatia (2004), 3.96 in Nilagiri sheep by Girish *et. al.* (2007), 4.618 in Bellary sheep by Kumar *et. al.* (2007) and 6.836 in Vembur sheep by Pramod *et. al.* (2009).

ILSTS030

This marker which is a di-nucleotide produced about 9 alleles in Deccani and 8 alleles in Nellore sheep and the most frequent alleles were 166 bp and 160 bp in Deccani and Nellore sheep respectively.

The allele size range for this marker was 140 – 190 bp and the effective number of alleles was 6.391 in Deccani and 6.868 in Nellore sheep.

ILSTS11

The locus ILSTS11 is found to be highly polymorphic in the present study and produced a total of 12 alleles in Deccani and 14 alleles in Nellore sheep. The allele size range was between 223 – 288 bp in both the breeds. Less number of alleles were recorded in the present study than that by Rodrigo *et. al.* (2000) who reported a total of 23 alleles in Chilean sheep. But, the values by Hoda *et. al.*, 2012 as 9 alleles in Albanian sheep and 5 alleles in Kail sheep as reported by Ahmed *et. al.* in 2014 were lower than those observed in the present study.

The most repeated allele was 246 bp allele in Deccani and 288 bp allele in Nellore sheep and the effective number of allele was 8.913 in Deccani sheep and the highest 10.240 in Nellore sheep.

ILSTS28

The allele size range for this allele was 112-164 bp and a total of 7 and 10 alleles were amplified in Deccani and Nellore sheep respectively. Hoda *et. al.* (2012) found 13 alleles in three Albanian sheep breeds with the allele size being 127 – 169 bp.

The effective number of alleles was 4.573 in Deccani and 4.822 in Nellore sheep in the present study while Hoda *et. al.*(2012) reported a higher number of 6.33 in Albanian sheep. The most frequent allele for this locus was 150 bp in Deccani and 136 bp in Nellore sheep.

MAF209

This di-nucleotide repeat marker produced 8 alleles each in Deccani and Nellore sheep with a size range of 112-144 bp in the present study. The number of alleles for the locus in the present study was lower than the reports by other authors as 13 alleles in Merino sheep as reported by Diez-Tascon *et. al.* (2000), 12 alleles by Hoda *et. al.,* 2012 in three Albanian sheep where the allele size was 110 – 140 bp.

The effective number of alleles was 6.404 for Deccani and 4.381 in Nellore which was nearer to the value of 5.11 reported by Hoda *et. al.,* 2012 in three Albanian sheep and the most frequent allele was 132 bp and 122 bp in Deccani and Nellore sheep respectively

MAF214

This $(GT)_{56}$ di-nucleotide repeat amplified 7 and 10 alleles in Deccani and Nellore sheep respectively. The allele number reported by earlier researchers varied with 12 alleles reported in Romanian sheep by Kevorkian *et. al*. (2010), 15 alleles in Albanian sheep by

Hoda *et. al.* (2012), 12 alleles in Kilakarsal sheep by Radha *et. al.* (2011) and 13 alleles in Coimbatore sheep by Hepshiba *et. al.* (2014).

The allele size range was 180 – 214 bp in the present study where as earlier workers reported 184 – 228 bp by Radha *et. al.* (2011) and Hepshiba *et. al.* (2014) based on studies on Kilakarsal and Coimbatore sheep, respectively.

The effective number of alleles was 5.855 in Deccani and 5.683 in Nellore sheep while lower number of alleles was reported by Radha *et. al.* (2011) in Kilakarsal sheep (3.64) and by Hepshiba *et. al.* (2014) in Coimbatore sheep. Most frequent allele was of 180 and 192 bp in Deccani and Nellore sheep respectively and the most frequent allele reported by Hepshiba *et. al.*(2014) in Coimbatore sheep also has 192 bp.

MAF33

The locus showed 9 alleles in Deccani and 11 in Nellore with size range of 114 – 168 bp in both the breeds. Earlier reports revealed a wide range of 8 to 21 alleles at this locus, 16 by Hoda *et. al.* (2012) in Albanian sheep and 8 by Ahmed *et. al.* (2014) in Kail sheep).

The effective number of alleles was 5.840 in Deccani and 8.157 in Nellore sheep. Hoda *et. al.* (2012) reported 4.27 alleles in Albanian sheep at the same locus. The most frequent allele for this locus in the present study was 144 bp in Deccani and 142 bp in Nellore sheep.

MAF70

The marker MAF70, which is a $(CA)_{19}$ repeat di-nucleotide microsatellite amplified 10 alleles each in Deccani and Nellore sheep. The allele size range was 136 – 172 bp in the

present study. Ahmed *et. al.*(2014) observed 8 alleles in Kail sheep. However, rather higher number of alleles were reported by Kevorkian *et. al.* (2010) in Romanian sheep (30 alleles) and by Hoda *et. al.* (2012) in Albanian sheep (16 alleles).

The effective number of alleles was 8.195 and 7.133 in Deccani and Nellore sheep respectively. Hoda *et. al.*(2012) and Ahmed *et. al.* (2014) reported lesser number of alleles (5.65 and 4.84) in Albanian Kail sheep, respectively. The most frequent allele in this study is found to be of 144 bp length in Deccani and 164 bp length in Nellore sheep.

MCM140

In the present study, MCM140 locus produced 10 alleles in Deccani and 8 in Nellore sheep with allele size ranging between 148 – 190 bp. Hoda *et. al.* (2012) found 13 alleles at this locus in Albanian sheep with a size range of 161-192 bp.

The effective number of alleles ranged from 4.644 in Nellore sheep to 5.391 in Deccani sheep. The present finding is in agreement with that of Hoda *et. al.* (2012) who reported 5.97 alleles at the same locus in Albanian sheep. The most frequent allele was 170 and 148 bp in Deccani and Nellore sheep respectively.

OarAE129

The marker OarAE129 was primed to amplify 10 alleles in Deccani and 8 alleles in Nellore sheep. The allele size range for this locus was identified as 140 – 186 bp in the present study. A wide range (3-18) of alleles were reported by earlier workers working on different breeds. Farid *et. al.* (2000) and Pramod *et. al.* (2009) reported 8 alleles in Tan and Vembur breeds respectively while 7 alleles were reported by Nanekarani *et. al.* (2010) and Hoda *et. al.* (2012) in Iranian pelt sheep and Albanian sheep respectively.

However, Arora *et. al.* (2010) reported a whopping 18 alleles in six Rajasthan sheep breeds while smaller number of alleles ranging from 3 to 4 was reported by Nahas *et. al.*(2008), Arora and Bhatia(2004), Arora and Bhatia(2006) Mukesh *et. al.*(2006)Girish *et. al.*(2007)Kumar *et. al.*(2007) Prema *et. al.*(2008a) Arora *et. al.*(2010) Hepshiba *et. al.*(2014).

The allele size reported by earlier authors for this locus ranged from 138 -164 bp (Arora and Bhatia, 2004; Mukesh *et. al.,* 2006; Girish *et. al.,* 2007; Kumar *et. al.,* 2007; Pramod *et. al.,* 2009 and Radha *et. al.,* 2011).

The effective number of alleles was 6.375 and 6.377 in Deccani and Nellore sheep respectively. A perusal of published literature on this locus revealed the effective number of alleles to range from 1.88 to 5.66 (Hoda *et. al*., 2012; Ahmed *et. al*., 2014; Arora and Bhatia, 2004; Mukesh *et. al.,* 2006; Girish *et. al.,* 2007; Kumar *et. al.,* 2007; Pramod *et. al*., 2009 and Hepshiba *et. al.,* 2014).

The most frequent allele was 174 bp in both the sheep breeds studied while Hepshiba *et. al.* (2014) reported 142 bp as allele size in Coimbatore sheep.

OarCB226

The number of alleles amplified at the locus OarFCB226 which is a $(CA)_{14}$ di-nucleotide microsatellite repeat was 12 in both Deccani and Nellore sheep breeds. The size of the allele ranged between 128 – 172 bp in the present study.

The effective number of alleles for this locus was 9.580 in Deccani which was the highest number for that population for all the loci studied and 4.804 in Nellore sheep and the most frequent allele was 134 bp in Deccani and 128 bp in Nellore sheep.

OarCP20

The marker which is $(GT)_{14}$ microsatellite repeat amplified a total of 6 alleles in Deccani and 7 alleles in Nellore sheep with allele size between 71 – 92 bp. The results for allele number were in accordance with the studies reported as 12 alleles by Diez-Tascon *et. al.,* 2000 in Merino sheep, 10 alleles in by Ozerov *et. al.,* 2008 in Kazakh sheep breeds, 11 alleles by Kevorkian *et. al.,* 2010 in Romanian sheep and 13 alleles by Arora *et. al.,* 2010 in Rajasthan sheep. However, the observed values were higher than the findings reported by Nahas *et. al.,* 2008 in three Egypt breeds (5), Arora and Bhatia, 2004 in Muzzafarnagri (5), Arora and Bhatia, 2006 in Magra (3), Girish *et. al.,* 2007 in Nilagiri (6), Arora *et. al.,* 2008 in Jalauni sheep (4), Prema *et. al.,* 2008a in Mecheri (6), Prema *et. al.,* 2008b in Madras Red (4), Pramod *et. al,* 2009 in Vembur (5) and Radha *et. al.,* 2011 in Kilkarsal sheep (6). The allele size was similar to the present findings in almost all the earlier studies.

The most frequent allele was 71 bp allele in Deccani and 74 bp allele in Nellore sheep and the effective number of allele was 4.641 and 4.648 in Deccani and Nellore sheep, respectively. The effective number reported in earlier studies was 2.63 by Arora and Bhatia (2004), 4.11 by Girish *et. al.* (2007), 3.17 by Prema *et. al.* (2008a), 3.45 by Pramod *et. al.* (2009) and 3.15 by Radha *et. al.* (2011).

OarCP34

The number of alleles amplified at the locus was 11 in Deccani and 10 in Nellore sheep. The reports which showed lower values than the present study were 7 alleles by Diez-Tascon *et. al.* (2000), 6 alleles by Ozerov *et. al.* (2008), 7 alleles by Hoda *et. al.* (2012), 7 alleles by Arora and Bhatia (2004), 4 alleles by Mukesh *et. al.* (2006), 6 alleles by Girish *et. al.* (2007), 5 alleles by Pramod *et. al.* (2009), 9 alleles by Jyotsana *et. al.* (2010) and 4 alleles

by Hepshiba *et. al.* (2014). Whereas the report which showed higher values was 14 alleles by Kevorkian *et. al.* (2010).

The effective number of alleles obtained at the locus was 9.580 and 4.804 for Deccani and Nellore sheep, respectively. While other reported values for the same locus are 5.02 by Hoda *et. al.* (2012), 5.18 by Arora and Bhatia (2004), 3.67 by Mukesh *et. al.* (2006), 4.22 by Pramod *et. al.* (2009), 3.78 by Radha *et. al.* (2011) and 4.39 by Hepshiba *et. al.* (2014). The size of the allele for the two sheep breeds ranged between 126 – 168 bp, which is little higher than that reported by Arora and Bhatia, 2006 (110-128), Mukesh *et. al.*, 2006 (114-128), Jyotsana *et. al.*, 2010 (107-123) and Radha *et. al.*, 2011 (111-129). The most repeated alleles was 126 bp in both the sheep breeds studied where as it was 113 bp allele in Coimbatore sheep as studied by Hepshiba *et. al.* (2014).

OarFCB128

The OarFCB128, a di-nucleotide repeat motif $(GT)_{15,}$ showed a total of 9 alleles each in both the sheep breeds. The allele size ranged between 110 – 134 bp and the most frequent allele was 134 bp in both Deccani and Nellore sheep. The observation on the allele number was in accordance with the reports made by few authors as 9 alleles by Tascon *et. al.*, 1999, 11 alleles by Ozerov *et. al.*, 2001, 9 alleles by Hoda *et. al.*, 2012, 9 alleles by Arora and Bhatia, 2006, 11 alleles by Jyotsana *et. al.*, 2010, 18 alleles by Arora *et. al.*, 2010 and 13 alleles by Radha *et. al.*, 2011. However, the observed value was higher than the findings reported by Arora and Bhatia, 2004 (6), Girish *et. al.*, 2007 (4), Kumar *et. al.*, 2007 (2), Pramod *et. al.*, 2009 (5) and Hepshiba *et. al.*, 2014 (5).

The effective number of alleles was 3.949 and 7.339 in Deccani and Nellore sheep, respectively. The values were in accordance with the earlier studies made by Arora *et. al.*,

2004 (5.62), Girish *et. al.*, 2007 (3.11), Pramod *et. al.*, 2009 (4.27), Jyotsana *et. al.*, 2010 (4.08) and Hepshiba *et. al.*, 2014 (4.39).

OarFCB20

OarFCB20, which was a di-nucleotide $(GT)_{15}$ repeat motif showed amplification of 9 and 7alleles in Deccani and Nellore sheep, repectively, with size range of 124 – 146 bp. The number of alleles was higher than the estimates of 13 alleles by Diez-Tascon *et. al.* (2000), 20 alleles by Kevorkian *et. al.* (2010), 13 alleles by Hoda *et. al.* (2012) and lower than the findings of 6 alleles each by El-Nahas *et. al.* (2008), Nanekarani *et. al.* (2010), Girish *et. al.* (2007), Prema *et. al.* (2008a and 2008b) and Pramod *et. al.* (2009). Previous literature on the same marker showed the allele of size 92-118 bp by Hoda *et. al.* (2012), 124-148 bp by Girish *et. al.* (2007), 94-110 bp by Prema *et. al.* (2008a and 2008b) and 92-116 bp by Pramod *et. al.* (2009).

The effective number of alleles was 4.890 in Deccani sheep and 4.333 in Nellore sheep which were in accordance with the reports of 4.39 by Ahmed *et. al.* (2014), 5.17 by Nanekarani *et. al.* (2011), 4.01 by Girish *et. al.* (2007), 5.14 by Prema *et. al.* (2008a) and 4.32 by Pramod *et. al.* (2009) and the most frequent alleles was 128 and 126 bp in Deccani and Nellore sheep.

OarFCB48

The number of alleles for the locus in the two sheep were 9 (Deccani) and 8 (Nellore). The allele size ranged from 142 – 168 for the locus. Similar allele number was seen by Arora and Bhatia, 2006 (9), Mukesh *et. al.*, 2006 (8) and Kumar *et. al.*, 2007 (9). However, the observations of the present study were higher in the studies made by Diez-

Tascon *et. al.,* 2000 (14), Ozerov *et. al.,* 2008 (14), Jyotsana *et. al.,* 2010 (13), and Arora *et. al.,* 2010 (13) and the lower values were reported in studies made by Arora and Bhatia, 2004 (6), Prema *et. al.,* 2008a (5) and Pramod *et. al.,* 2009 (5). The allele size in the present study was similar to the findings obtained by the previous authors.

The effective number of alleles observed was 6.165 and 6.158 for Deccani and Nellore sheep. The effective number of alleles in other studies was 5.62 by Arora and Bhatia (2004), 4.87 by Mukesh *et. al.* (2006), 4.91 by Kumar *et. al.* (2007), 4.43 by Prema *et. al.* (2008a), 4.16 by Pramod *et. al.* (2009), 6.68 by Jyotsana *et. al.* (2010) and 4.22 by Radha *et. al.* (2011). The most frequent allele was 152 for the locus where as it was 132 bp in the study made by Hepshiba *et. al.* (2014).

OarHH35

A total of 6 and 10 alleles were amplified by this locus for Deccani and Nellore sheep, respectively. The allele size ranged from 86 – 128 bp. The previous reports at the same locus showed 6 alleles by Arora and Bhatia (2004), 8 alleles each by Arora and Bhatia and Mukesh *et. al.* (2006), 7 alleles by Girish *et. al.* (2007), 10 alleles by Kumar *et. al.* (2007), 6 alleles by Pramod *et. al.* (2009), 11 alleles by Jyotsana *et. al.* (2010) and 10 alleles by Hepshiba *et. al.* (2014). The size of the allele ranged from 87-142 bp in the literature made by Arora and Bhatia (2006), Mukesh *et. al.* (2006), Girish *et. al.* (2007), Kumar *et. al.* (2007) and Hepshiba *et. al.* (2014).

The effective number of alleles for Deccani and Nellore sheep for this locus was 2.135 and 6.428 respectively which were similar to those obtained in all other studies done

for this locus. The most frequent allele is 86 bp for Deccani and 114 bp for Nellore sheep where as it was 123 bp allele as reported by Hepshiba *et. al.* (2014).

OarHH41

Seven alleles were amplified at this locus for Deccani and Six alleles for Nellore sheep. The allele size ranged between 112 – 132 bp for this locus in the present study. All the other studies for this locus varied between 4 to 9 alleles where as the size of the alleles were similar to the present findings.

The effective number of alleles was 4.500 and 2.716 for Deccani and Nellore sheep. Perusal of the previous literature showed the value as 3.30 by Arora and Bhatia (2004), 3.70 by Arora and Bhatia (2006), 3.27 by Girish *et. al.* (2007), 2.74 by Kumar *et. al.* (2007), 5.34 by Arora *et. al.* (2008), 2.76 by Prema *et. al.* (2008a), 4.59 by Prema *et. al.* (2008b), 4.834 by Pramod *et. al.* (2009), 3.21 by Arora *et. al.* (2010), 4.77 by Jyotsana *et. al.* (2010), 3.46 by Radha *et. al.* (2011) and 1.97 by Hepshiba *et. al.* (2014). The most frequent allele was 124 bp each for both the sheep breeds when compared to 118 bp allele in the study made by Hepshiba *et. al.* (2014).

OarHH47

The OarHH47 is a di-nucleotide with $(CA)_{22}$ repeat motif and produced 7 alleles each in both the sheep breeds under the present study. The size of the alleles ranged between 140 – 170 bp. The findings were in accordance to the reports made as 7 alleles by Arora and Bhatia, 2004 in Muzzafarnagri, 7 alleles by Mukesh *et. al.,* 2006 in three Indian sheep and 7 alleles by Radha *et. al.,* 2011 in Kilakarsal. The values are little higher than values of 4 alleles by Girish *et. al.,* 2007 in Nilagiri, 6 alleles by Arora *et. al.,* 2008 in Jalauni, 5 alleles by Prema et

al (2008a and 2008b) and 2 alleles by Pramod *et. al.,* 2010 in Vembur and lesser than the studies shown as 9 alleles by Kumar *et. al.,* 2007 in Bellary, 8 alleles by Arora *et. al.,* 2010 in Ganjam, 15 alleles by Jyotsana *et. al.,* 2010 in three Gujarath sheep, 15 alleles by Arora *et. al.,* 2010 in six Rajasthan sheep and 10 alleles by Hepshiba *et. al.,* 2014 in Coimbatore sheep.

The effective number of alleles for this locus was 5.845 in Deccani and 5.161 in Nellore which were similar to the reports shown as 5.43 by Hoda *et. al.* (2012) and 5.67 by Arora *et. al.* (2010) and the other reports for the same locus was 3.89 by Arora *et. al.* (2004), 3.30 by Girish *et. al.* (2007), 6.23 by Kumar *et. al.* (2007), 3.51 by Prema *et. al.* (2008b), 1.90 by Pramod *et. al.* (2009) and 4.52 by Radha *et. al.* (2011). The most frequent allele was 162 bp and 140 bp in Deccani and Nellore sheep, respectively which was similar to the finding made by Hepshiba *et. al.* (2014).

OarJMP29

The OarJMP29, which is a di-nucleotide repeat, $(CA)_{21}$ amplified a total of 8 alleles (Deccani) and 10 alleles (Nellore) in the present study. The allele size ranged from 124 – 172 bp. The studies made on the same locus by other authors are 15 alleles by Hoda *et. al.* (2012), 6 alleles by Arora and Bhatia (2004), 7 alleles by Mukesh *et. al.* (2006) and Girish *et. al.* (2007), 11 alleles by Jyotsana *et. al.* (2010), 8 alleles by Arora *et. al.* (2010) and 7 alleles by Radha *et. al.,* (2011). The allele size in the present study were within the range reported by other authors.

The effective number of alleles was 5.800 and 5.241 in Deccani and Nellore sheep respectively. The effective number of alleles in other findings were as shown 5.82 by Hoda *et. al.* (2012), 3.56 by Arora *et. al.* (2004), 4.02 by Mukesh *et. al.* (2006), 5.40 by Girish *et.*

al. (2007), 3.15 by Kumar *et. al.* (2007), 5.16 by Pramod *et. al.* (2009), 2.93 by Hepshiba *et. al.* (2014). The most frequent allele for this locus was 152 each in the two populations studied.

OarVH72

The number of alleles for this locus varied between 4 and 6 for Deccani and Nellore sheep, respectively. The size of the alleles ranged from 119 – 140 bp in both the sheep studied. The allele number in the present study was similar to the previous findings made as 6 alleles by Ahmed *et. al.* (2014) in Kail sheep, 5 alleles by Arora and Bhatia (2004) in Muzzafarnagri, 5 alleles by Girish *et. al.* (2007) in Nilagiri, 6 alleles by Kumar *et. al.* (2007) in Bellary and 5 alleles by Pramod *et. al.* (2009) in Vembur. However, higher values were reported Hoda *et. al.*, 2012 in Albanian sheep (9), Arora *et. al.*, 2010 in Rajasthan sheep (10) and Radha *et. al.*, 2011 in Kilakarsal (8). The allele size reported in other findings was 125-141 bp by Hoda *et. al.* (2012), 113-137 bp by Arora and Bhatia (2006), 128-140 by Girish *et. al.* (2007), 124-142 bp by Kumar *et. al.* (2007), 124-134 by Pramod *et. al.* (2009), 121-151 bp by Jyotsana *et. al.* (2010), 126-140 bp by Radha *et. al.* (2011) and 126-134 bp by Hepshiba *et. al.* (2014).

The effective number of alleles in the present study was 2.871 in Deccani and 3.184 in Nellore which were similar to the findings of the previous literature made on the same locus on different sheep breeds and the most frequent allele was 132 each for both Deccani and Nellore and it was 128 bp in Coimbatore sheep as shown by Hepshiba *et. al.* (2014).

SRCRSP1

A total of 8 alleles in Deccani and 7 alleles in Nellore were amplified by this locus in the present study which were higher than that reported by Hoda *et. al.,* 2012 as 4 alleles in three Albanian sheep and the allele size ranged between 118 – 146 bp.

The effective number of alleles ranged between 4.525 and 5.988 in Deccani and Nellore sheep breeds, respectively and it was 2.56 in Albanian sheep as reported by Hoda *et. al.* (2012). The most frequent allele was 140 bp in Deccani and 126 bp in Nellore.

SRCRSP5

The allele size for the locus SRCRSP5 ranged between 124 – 158 bp. The number of alleles varied from 7 and 8 for Deccani and Nellore sheep respectively which were in accordance with the findings reported by Hoda *et. al.* (2012). The effective number of alleles for this locus was 4.730 in Deccani and 5.794 in Nellore which were little higher than the value (2.86) reported by Hoda *et. al.* (2012) in the Albanian sheep and the most frequent allele was 148 bp each for both the sheep populations.

5.3.1.3 Allele frequency

The allele frequencies in the present study were as low as 1.00 per cent and as high as 65.60 per cent (Table 9). The distribution of allele frequency in the present study is very discrete. Similar findings were also reported by El-Nahas *et. al.* (2008) in Egyptian sheep breeds, Nanekarani *et. al.* (2010) in Iranian sheep and Kumar *et. al.* (2007) in Bellary sheep. The single base pair differences observed for some of the nucleotide repeat alleles might be because of the point mutations in the flanking regions.

5.3.1.4 Population specific alleles

This value shows the amount of genetic diversity across populations, which can only be supplied by the population having the unique alleles towards the metapopulation global diversity. A total of 60 alleles were found to be unique in both the populations in the present study, though the frequencies of most of the alleles were very low (Tables 10 and 11). Twenty three alleles in Deccani are unique with 9 percent alleles above 10 per cent frequency. Similarly thirty seven alleles in Nellore were unique with only 4 per cent above 10 per cent frequency.

5.3.2 Polymorphic Information Content (PIC)

The mean polymorphic information content (PIC) is an ideal index to measure the polymorphism of alleles. The PIC value of more than 0.5 indicates high polymorphism, 0.25 to 0.50 a moderate and less than 0.25, a low polymorphism. The overall PIC values of all the loci genotyped in both Deccani and Nellore were above 0.5 (Table 7). Almost all the loci showed the PIC values more than 0.5 except for OarHH35 in Deccani which showed the value as 0.498. The mean PIC values in the present study ranged from 0.498 (OarHH35) to 0.886 (OarCB226 in Deccani and from 0.551 (ETH121) to 0.894 (ILSTS11) in Nellore sheep.

Published literature revealed a wide range of PIC values among Indian sheep breeds. The PIC values ranged from 0.533 to 0.808 in Muzzafarnagri sheep (Arora and Bhatia, 2004); 0.210 to 0.831 in Nali and 0.346 to 0.768 in Chokla sheep (Sodhi *et. al.,* 2006); 0.458 to 0.827 in Nilagiri sheep (Girish *et. al.,* 2007); 0.240 to 0.820 in Jalauni sheep (Arora *et. al.,* 2008); 0.079 to 0.806 in Kheri sheep (Bhatia and Arora, 2008); 0.454 to 0.785 in Madras Red

sheep (Prema *et. al.*, 2008b) and from 0.371 to 0.836 in Vembur sheep (Pramod *et. al.*, 2009). Pandey *et. al.* (2007) and Radha *et. al.* (2011) reported the average PIC values of 0.60 and 0.831 in Shahabadi and Kilakarsal sheep breeds, respectively.

The mean PIC values estimated for Nellore sheep in the present study were lower than the PIC values reported in Shahabadi sheep (Pandey *et. al.*, 2007), Jalauni sheep (Arora *et. al.*, 2008), Kheri (Bhatia and Arora, 2008), Vembur (Pramod *et. al.*, 2009) while Radha *et. al.* (2011), reported higher value of PIC than the present study in Kilakarsal sheep. PIC values estimated for all the markers used in the present study were more than 0.5, indicating that these microsatellite markers can effectively be used for molecular characterization, genome mapping and genetic diversity studies in sheep.

5.4 GENETIC DIVERSITY

5.4.1 Heterozygosity

The heterozygosity is the unit of measurement for population diversity and variation. In the present study the population revealed high degree of polymorphism and genetic variation with an overall expected heterozygosity of 0.79 in Deccani and 0.80 in Nellore sheep. The average observed heterozygosity values in the present study ranged from 0.77 in Deccani to 0.78 in Nellore.

In Deccani sheep, the lowest heterozygosity was observed for locus ETH121 (0.341) and the highest for locus ILSTS11 (0.958) while in Nellore, the lowest heterozygosity is observed for locus ETH121 (0.023) and the highest for locus OarAE129 (0.980). Present findings are in agreement with the heterozygosity observed by Ozerov *et. al.* (2008) in Kazakh sheep (0.75). However, lower heterozygosity was observed by Farid *et. al.* (2000) in Ten sheep breeds of US, Canada and Denmark (0.59); Kevorkian *et. al.* (2010) in Romanian

sheep (0.61); Arora and Bhatia (2004) in Muzzafarnagri (0.65); Kumar *et. al.* (2007) in Bellary (0.512); Pramod *et. al.* (2009) in Vembur (0.520); Jyotsana *et. al.* (2010) in Gujarat breeds (0.63) and Radha *et. al.* (2011) in Kilakarsal sheep (0.618).

In the present study, Deccani showed the highest expected heterozygosity for the locus OarCB226 (0.896) and lowest for OarHH35 (0.532) where as in Nellore the highest heterozygosity was observed for the locus ILSTS11 (0.902) and lowest for the locus ETH121 (0.627). The values obtained in the present study are in accordance with the values obtained by Farid *et. al.* (2000) in Ten sheep breeds (0.74), Ozerov *et. al.* (2008) in Kazakh sheep (0.78), Kevorkian *et. al.* (2010) in Romanian sheep (0.0.73), Pramod *et. al.* (2009) in Vembur (0.733), Radha *et. al.* (2011) in Kilakarsal (0.725) and higher than the values obtained by Arora and Bhatia (2004) in Muzzafarnagri (0.69), Kumar *et. al.* (2007) in Bellary (0.684), Jyotsana *et. al.* (2010) in three Gujarat breeds (0.69).

5.4.2 Fixation Indices

The fixation indices measure the genetic divergence of sub-populations within the total population. The F – statistic describes the amount of inbreeding like effects within sub-population (F_{IS}), among the sub-populations (F_{ST}) and in the total population (F_{IT}).

The mean F_{IS} estimates among the two populations, estimated based on thirty microsatellite loci in the present study were obtained as 0.029 for Deccani and 0.031 for Nellore. In Deccani sheep, the F_{IS} varied from -0.407 (OarVH72) to 0.505 (ETH121) and in Nellore from -0.339 (OarVH72) to 0.964 (ETH121). The mean F_{IS} values obtained in the present study were little lower than the values obtained by Hoda *et. al.* (2012) in Albanian sheep (0.041), Ahmed *et. al.* (2014) in Kail sheep (0.052), Arora and Bhatia (2004) in

Muzzafarnagri (0.159), Prema *et. al.* (2008b) in Madras Red sheep (0.048), Pramod et al (2009) in Vembur (0.2954), and Radha *et. al.* (2011) in Kilakarsal (0.147).

The mean F_{ST} values obtained in the present study was 0.024 which is slightly higher than the values obtained by Hoda *et. al.* (2012) in Albanian sheep (0.011)

5.5 GENETIC DISTANCE

The genetic distance between populations provides a relative estimate of the time that have elapsed since the population existed as a unified and cohesive unit and helps in characterizing the breeds in the populations. The genetic distance obtained in the present study was very least and shown as 0.225 (Table 13).

5.6 GENETIC IDENTITY

The genetic identity is the reciprocal of the genetic distance, the higher the genetic distance the lower the genetic similarity and *vice versa*. In the present investigation the genetic identity between the two populations was obtained as 0.799 (Table 14).

5.7 PHYLOGENY

The relationship among the groups or breeds can be assessed using the genetic distances/genetic identities, which are estimated from allele frequencies. The dendrogram obtained in the present study revealed that the two breeds were identified as two separate clusters and departed from each other showing considerable genetic identity among themselves. But there is slight intermixing of germplasm between the breeds regarding two animals. The phylogeny of 48^{th} animal from Deccani and 52^{nd} animal from Nellore sheep

were found blended. This might be due to the intermixing of the breeds which is the order of the day as farmers are excited to use Nellore breed of sheep on Deccani sheep. There is an empirical advantage as the crossbreds are showing enhanced performance. However, this needs to be confirmed by a scientific study. In the present investigation, the animals were selected on the phenotypic appearance as Deccani and Nellore breeds and probably the phenotypic selection might have gone wrong.

5.8 HARDY WEINBERG EQUILIBRIUM

The Hardy-Weinberg equilibrium status of the populations was tested for all the loci. All the loci deviated significantly from equilibrium (Table 15).

The presence of null alleles might have amplified in the PCR leading to overestimation of particular homozygote or heterozygote and hence the deviation in the frequencies is seen causing the departure from equilibrium.

SUMMARY

CHAPTER VI

SUMMARY

The present study was undertaken to assess the genetic variability within and between the Deccani and Nellore sheep breeds. A total of 100 sheep, 50 each from Deccani and Nellore breeds were utilized for genotyping using 30 dinucleotide microsatellite marker primers. The genomic DNA was isolated using standard phenol-chloroform method. The mean optical absorbance ratio in Deccani sheep was 1.95 and in Nellore sheep the ratio of absorbance is 1.95 indicating good quality of genomic DNA.

The PCR products of the microsatellites were analyzed by running on 8% PAGE to identify DNA polymorphism. All the thirty microsatellite loci used in the study were polymorphic with a reasonable informativeness ranging from moderate to high. A total of 254 alleles in Deccani and 260 alleles in Nellore were obtained with the allele size range varied from 71 for loci OarCP20 to 288 for ILSTS11 in both the breeds. The number of alleles ranged from 4 (OarVH72) to 12 (ILSTS11) in Deccani with an average of 8.46 and 4 (ETH121) to 14 (ILSTS11) with an average of 8.66.

The effective number of alleles ranged from 2.135 to 9.580 in Deccani and 2.683 to 10.240 in Nellore whereas the overall mean effective number of alleles varied between 5.52 for Deccani and 5.62 for Nellore sheep. The allele frequency ranged from 1.0 per cent to 65.6 per cent in Deccani and 1.0 per cent to 51.1 per cent in Nellore sheep.

A total of 23 out of 254 and 37 out of 260 alleles amplified were found to be population specific for Deccani and Nellore sheep respectively. The locus OarFCB20 recorded highest number of specific alleles (3) in Deccani and the locus OarHH35 recorded the highest number of specific alleles (5) in Nellore sheep. However the frequency of most of

these population specific alleles was very low and only 2 (9%) and 4 (11 %) alleles had the frequency of more than 10 per cent. The alleles with frequency above 10 per cent may be more reliable and can be used to identify the population.

The overall mean PIC values ranged from 0.773 in Deccani to 0.777 in Nellore breed. And across the loci the values ranged from 0.587 (OarVH72) to 0.886 (OarCB226) in Deccani and 0.552 (ETH121) to 0.894 (ILSTS11) in Nellore. The PIC values for all the loci were above 0.50 except for the loci OarHH35 (0.49) in Deccani.

The mean expected heterozygosity among the breeds ranged from 0.79 (Deccani) to 0.80 (Nellore). The expected heterozygosity was the highest in Nellore for locus ILSTS11 (0.90) and the least in Deccani for the locus OarHH35 (0.53). The mean observed heterozygosity levels ranged from 0.77 in Deccani to 0.78 in Nellore sheep. Observed heterozygosity in Deccani sheep ranged from 0.34 (ETH121) to 0.96 (ILSTS11) and in Nellore sheep the values ranged from 0.02 (ETH121) to 0.96 (SRCRSP5).

The overall mean inbreeding coefficient was 0.029 in Deccani and 0.031 in Nellore sheep. Of the 30 loci studied, 14 loci in Deccani and 16 loci in Nellore sheep showed negative inbreeding coefficient indicating the presence of outbreeding. Eight loci (BM1314, BM6506, BM8125, ILSTS28, MAF70, MCM140, OarCB226 and OarFCB128) showed positive, mild to moderate inbreeding ranging from 0.007 to 0.25 in both the breeds studied. The F_{IS} values ranged from -0.372 (OarVH72) to 0.74 (ETH121), F_{ST} values ranged from 0.001 for the locus BM8125 to 0.172 for the locus OarHH35 and F_{IT} values ranged from -0.370 for the locus OarVH72 to 0.728 for the locus ETH121 between the Deccani and Nellore sheep breeds studied. The unbiased expected heterozygosity in Deccani sheep ranged from 0.54 (OarHH35) to 0.91 (OarCB226) and in Nellore sheep, it ranged from 0.64 (ETH121) to 0.91 (ILSTS11).

The genetic distance was 0.225 and genetic identity was 0.799 between the two sheep studied. The phylogenetic studies between the two breeds showed two separate clusters which revealed considerable genetic identity between and among the breeds. It was observed that there is slight intermixing of germplasm between the breeds regarding two animals, which puts the present identification of animals based on phenotype. This supports the understanding that there is rampant interbreeding among the two breeds in this region. All the loci departed from the equilibrium frequency significantly in both the sheep breeds. This might be due to homozygote deficiency as the animals from both the breeds were selected from different regions across the states.

The results of this study contribute to the knowledge of genetic structure of Deccani and Nellore sheep. The results reveal high genetic variation within the breeds and hence the significant characters of the two breeds *Viz.*, the dual utility of Deccani sheep and tallest character of the Nellore sheep makes it imperative to strengthen the conservation measures. Also, integrating genetic improvement programme with marker oriented production strategies will raise the economy of the farmers of this region.

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APPENDICES

APPENDIX I

LIST OF CHEMICALS

- Agarose (Himedia)
- Acrylamide (GeNei)
- Ammonium Per Sulphate (Sigma)
- Bisacylamide (GeNei)
- Boric acid (Merck)
- Bromophenol blue (Sigma)
- Chloroform (Fischer Scientific)
- Ethylene Diamine Tetra Acetic acid (Sigma)
- Ethidium Bromide (Amresco)
- Ethyl alcohol (Himedia)
- Formaldehyde (Molychem)
- Iso amyl alcohol (Merck)
- Iso propanol (Merck)
- Magnesium chloride (Merck)
- Nitric acid (Fischer Scientific)
- Proteinase K (GeNei)
- Silver nitrate (Qualigens)
- Sodium acetate (Fischer Scientific)
- Sodium carbonate (Fischer Scientific)
- Sodium Chloride (Fischer Scientific)
- Sodium Dodecyl Sulphate (Himedia)

- Sucrose (Sigma)
- TEMED (Sigma)
- Tris base (Sigma)

APPENDIX II

LIST OF EQUIPMENT

- Agarose Gel Electrophoresis system (Owl)
- Autoclave
- Electronic balance (Shimadzu)
- DNA thermal cycler (Eppendorf)
- Freezer of -20^{0}C (Blue star)
- Gel Documentation System (Syngene)
- Hot air oven (Bio technichs India)
- Laboratory centrifuge (Remi)
- Laminar air flow chamber
- Magnetic Stirrer (GeNei)
- Microwave oven (Bajaj)
- Microcentrifuge (Eppendorf)
- Pipetteman (Eppendorf)
- P^H meter (Eutech)
- Poly Acrylamide Gel Electrophoresis System (GeNei)
- Refrigerator of 4^{0}C (Godrej)
- UV – absorbance spectrophotometer (Bio-Rad)
- Vortex mixer (GeNei)
- Water bath with thermostat (30^{0}C to 100^{0}C) (GeNei)