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Akhilesh Pandey
Assistant Prof. Dept. of A.G.B, Veterinary College, Jabalpur, Madhya Pradesh, India

MS Thakur
Assistant Prof. Dept. of A.G.B Veterinary College, Jabalpur, Madhya Pradesh, India

Correspondence
Akhilesh Pandey
Assistant Prof. Dept. of A.G.B, Veterinary College, Jabalpur, Madhya Pradesh, India

Nucleotide base variations in aligned sequences of AS1, AS2, B and K-casein gene along with regression study of different milk composition traits in Malvi, Nimari, Sahiwal and HF crossbred cows

Akhilesh Pandey and MS Thakur

Abstract
The aim of the present study was to evaluate the nucleotide variation by sequencing of PCR product of αs1, αs2, β and κ-casein gene of Malvi, Nimari, Sahiwal and HF crossbred cattle. In alignment of nucleotide sequences of αs1, αs2 and κ-casein gene in Malvi, Nimari, Sahiwal and HF crossbred cows revealed the nucleotide variation. The align sequence of αs1 casein gene of HF crossbred, Nimari and Malvi cattle showed similar nucleotide base sequence, whereas in Sahiwal the Guanine (G) at 44 bp site was replaced by Adenine (A). For αs2 casein gene, the HF crossbred, Sahiwal and Malvi showed similar nucleotide base sequence whereas in Nimari the Adenine (A) at 243 bp site was substituted by Thymine (T) and at 582 bp site in HF crossbred Adenine was replaced by Guanine. In same αs2 gene Thymine (T) at 713 and 715 bp site was replaced by Cytosine. There was no change observed in 121 bp of β casein gene in HF crossbred, Sahiwal, Nimari and Malvi. For K- casein gene, the Nimari, Sahiwal and Malvi showed similar nucleotide base sequence, whereas in HF crossbred, the Cytosine (C) at 262 bp site was substituted by the Thymine (T) and at 308 bp site Adenine (A) was replaced by Guanine in Nimari. The phylogenic tree represented that the four breeds were divided in to four clusters. The clusters at αS1, αS2 and K gene locus in Sahiwal and Nimari showed more genetic divergence with HF crossbred as compared to the Malvi, whereas at β gene locus only Nimari showed more genetic divergence with HF crossbred as compared to Malvi and Sahiwal.

Keywords: Phylogenetic, RFLP, Malvi, Nimari, Sahiwal and HF crossbred cattle

Introduction
Indian cattle's are well known for their adaptability to extreme climatic condition, draught efficiency, disease resistance and poor nutritional environment. Selection of appropriate populations for conservation of such genetic resources is needed in view of preventing rapid erosion of animal genetic resources [9]. Estimation of genetic diversity is essential to decide the priority of the population to be conserved. DNA- based molecular markers with high level of polymorphism have been used to evaluate genetic variation of breeds in breeding programmes and conservation. In spite of variation in coat color, size, and production traits among indigenous cattle populations, genetic differences among most of the populations have not been investigated or exploited by single nucleotide polymorphism (SNP). SNPs represent a location within a DNA sequence for which more than one nucleotide type is present within a given population [11]. RFLP and sequence analysis is efficient method to study genetic variation, gene flow, genetic distance and genetic identity of indigenous cattle breeds. Malvi, Nimari both are very efficient cattle breeds of the Madhya Pradesh with majestic-looking, medium in size, extremely docile, hardiness, faster growth rate, adoptability and suitable for steady, heavy draught under harsh tropical conditions. This might constitute a relevant threat to livestock improvement. Therefore, this study was carried out to assess the current genetic diversity and the phylogenetic relationships of the Malvi, Nimari, Sahiwal and HF crossbred cattle breeds.

Materials and Methods
The present study was carried out in the department of Animal Genetics and Breeding of College of Veterinary Science & A.H Jabalpur in year 2016-17.

The aim of study was to know the nucleotide variation by sequencing of PCR product of αs1, αs2, β and κ-casein gene of Malvi, Nimari, Sahiwal and HF crossbred cattle. The research work was conducted on 200 lactating cows comprising 50 each of Malvi Nimari, Sahiwal and HF Crossbred cattle.

About 5 ml blood sample was collected from the cow with Identification number in EDTA coated test tube. Collected samples are maintained in cold chain during transportation in laboratory. In next phase of research work blood samples are processed and genomic DNA was extracted by modified John *et al.* [2] method. The Quality of DNA was assessed through 0.80% horizontal submarine agarose gel electrophoresis. The concentration, purity of DNA was checked by Nanodrop Spectrophotometer. The casein gene primers were used for the amplification of PCR product as described in Table 01.

Table 1: List of primers specific to four standard haplotypes of αs1, αs2, β and κ-casein genes

S. No.	Name of primer	Nucleotide sequence	bp size	References
1	αs1- casein	(F): 5-TGCATGTTCTCATAATAACC-3 (R): 5-GAAGAAGCAGCAAGCTGG-3	310 bp	Mir *et al.* [5]
2	αs2-casein	(F): 5'-TATGACATGTCGAGAAATGAG-3' (R): 5'-TTGGAACAATGCTATTAGGT T-3'	1267 bp	Szymanowska *et al.* [8]
3	β-casein	(F): 5´- CCT TCT TTC CAG GAT GAA CTCCAG G-3´ (R): 5´ - GAG TAA GAG GAG GGA TGT TTTGTG GGAGGC TCT- 3´	121 bp	Miluchova *et al.* [4]
4	κ-casein	(F): 5' -GCTGAGCAG GTATCCTAGTTAT- 3' (R): 5' - CTTCTTTGATGTCTCCTTAGAG - 3'	443 bp	Schlieben *et al.* [7]

A master mix for desired number of samples was prepared and liquated 22 µl in each PCR tube. 3 µl genomic DNA (30 ng/pl) was added in each tube to make the final volume 25 µl. The PCR tubes were kept in a preprogrammed thermo cycler (Mastercycler gradient, Eppendorf) and set at the standardized reaction programme.

Digested PCR products were analyzed on 2.50 % agarose gel (5 µl of PCR product mixed with 1 µl of gel loading dye). The electrophoresis at constant voltage of 90 volt for 50 minutes at 37˚C using 0.5X TBE buffer was conducted. The mass ruler DNA ladder (100 bp- 1000 bp) as a molecular size marker was used for sizing of the DNA bands.

Statistical Analysis

Gene and genotype frequencies for different casein genes under study were estimated using Popgene 32 (version1.32), microsoft Windows-based freeware for population genetic analysis [10]. Association study of various polymorphic variants of milk protein genes for Milk yield (MY), Daily milk yield (DMY), Protein (%), Fat (%), Lactose (%), SNF (%) and Milk density (Kg/L) data were subjected to least squares analysis of variance employing linear model.

Results and Discussion

The alignment of sequences of αs1, αs2, β and κ-casein gene in Malvi, Nimari, Sahiwal and HF crossbred cows revealed that the nucleotide variation.The align sequence of αs1 casein gene of HF crossbred, Nimari and Malvi cattle showed similar nucleotide base sequence, whereas in Sahiwal the Guanine (G) at 44 bp site was replaced by Adenine (A) (Fig. 1). For αs2 casein gene, the HF crossbred, Sahiwal and Malvi showed similar nucleotide base sequence whereas in Nimari the Adenine (A) at 243 bp site was substituted by Thymine (T) and at 582 bp site in HF crossbred Adenine was replaced by Guanine (Fig. 4). In same αs2 gene Thymine (T) at 713 and 715 bp site was replaced by Cytosine. There was no change observed in 121 bp of β casein gene in HF crossbred, Sahiwal, Nimari and Malvi (Fig. 2). For K- casein gene, the Nimari, Sahiwal and Malvi showed similar nucleotide base sequence, whereas in HF crossbred, the Cytosine (C) at 262 bp site was substituted by the Thymine (T) and at 308 bp site Adenine (A) was replaced by Guanine in Nimari. Similar results are also reported by Ndiaye *et al.* [6] broad genetic diversity in Senegalese cattle breeds will allow for greater opportunities for improvement of productivity and adaptation relative to global changes. For the development of sustainable breeding and crossbreeding programs of Senegalese local breeds, effective management is needed towards genetic selection and transhumance to ensure their long-term survival.

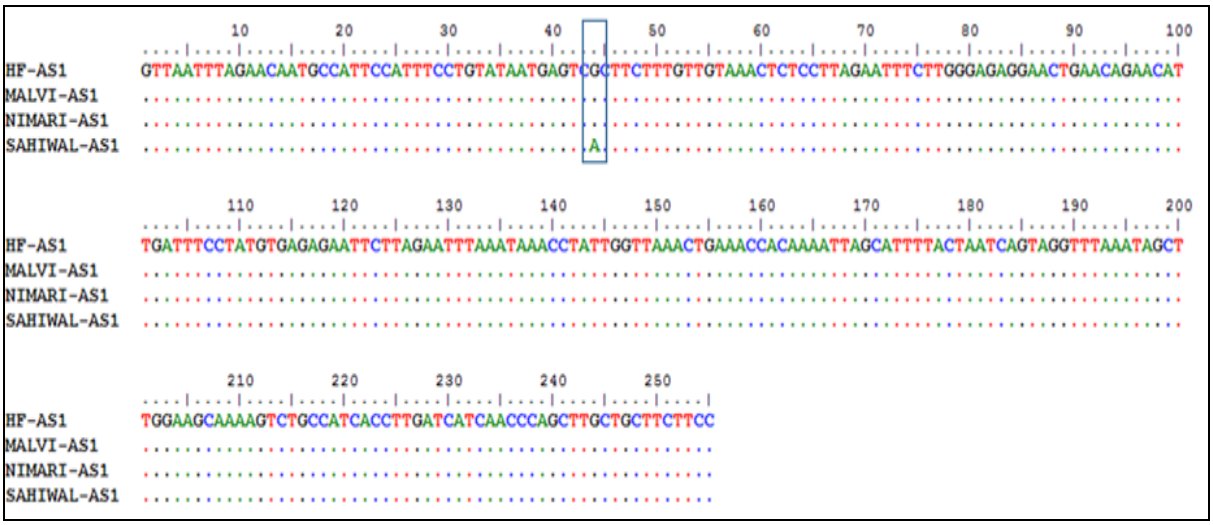


Fig 1: Multiple sequence alignment of αS1 (AS1) gene among different breed Rectangular box shows the SNP (G>A)

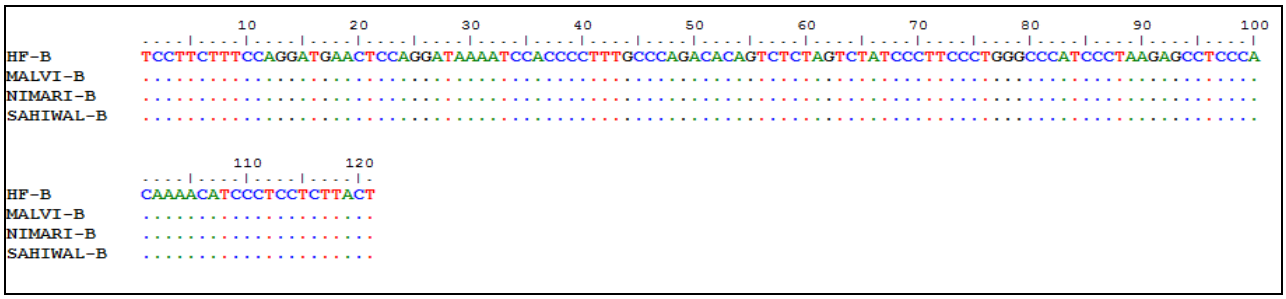


Fig 2: Multiple sequence alignment of β casein gene among different breed.

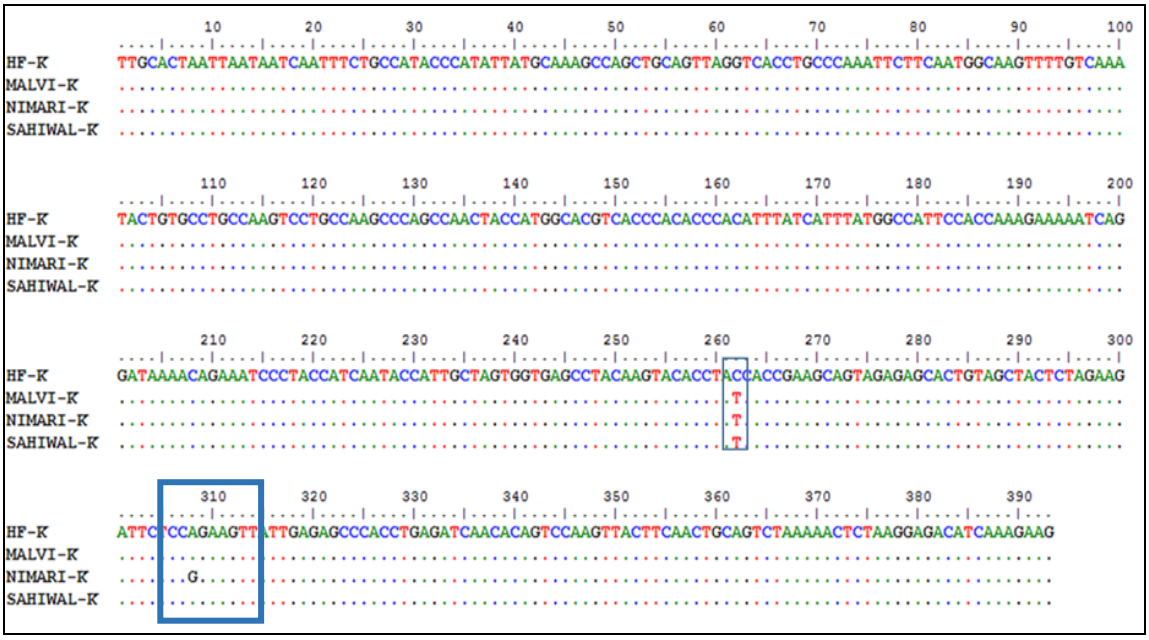


Fig 3: Multiple sequence alignment of K gene among different breed. Rectangular box shows the SNP (C>T)

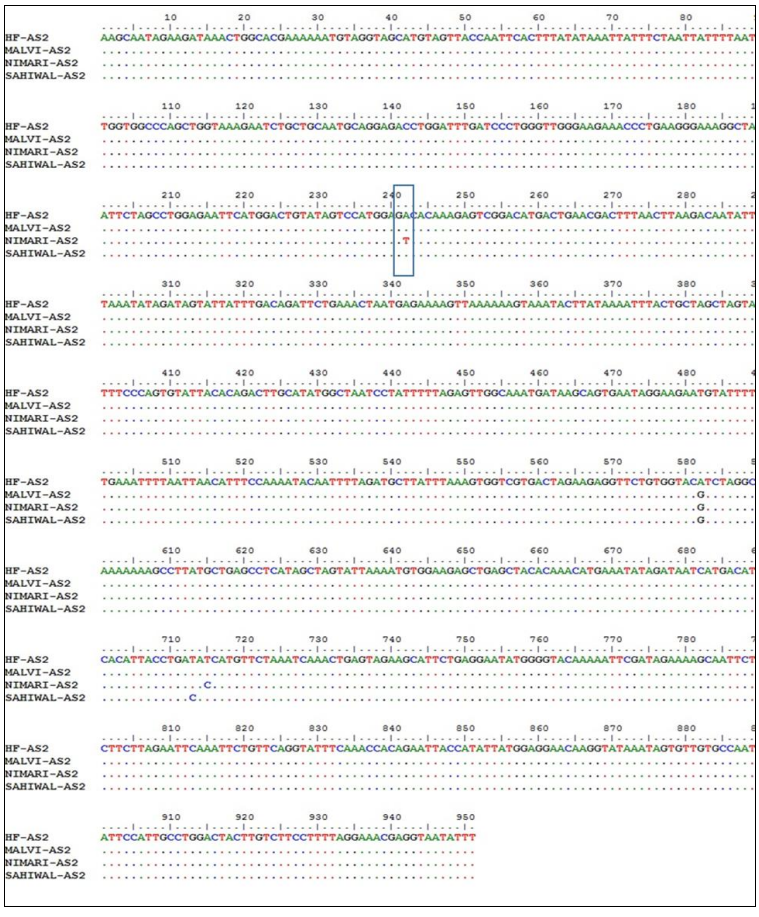
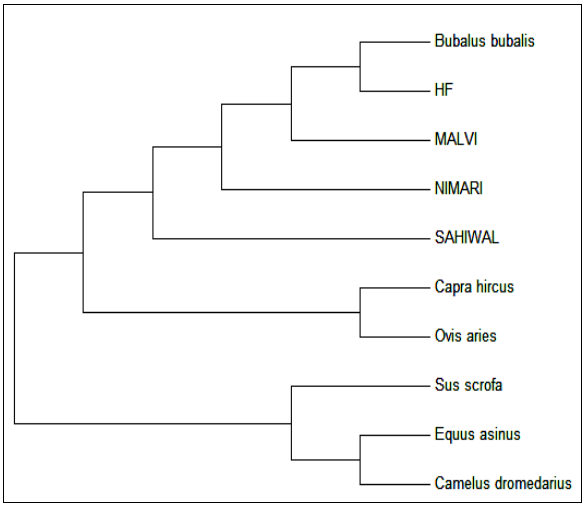
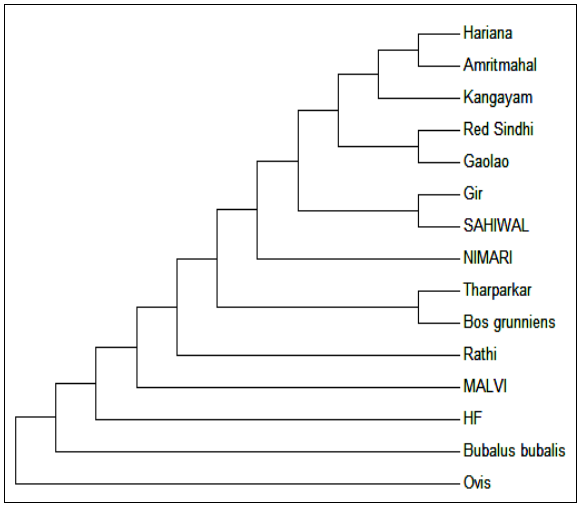


Fig 4: Multiple sequence alignment of αS2 (AS2) gene among different breed. Rectangular box shows the SNP (A>T)

Breed Relationships



Alpha S1 casein gene



Alpha S2 casein gene

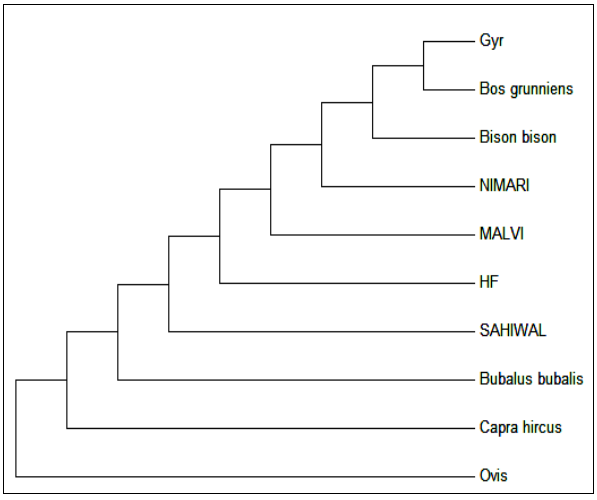


Fig 5: Beta casein gene

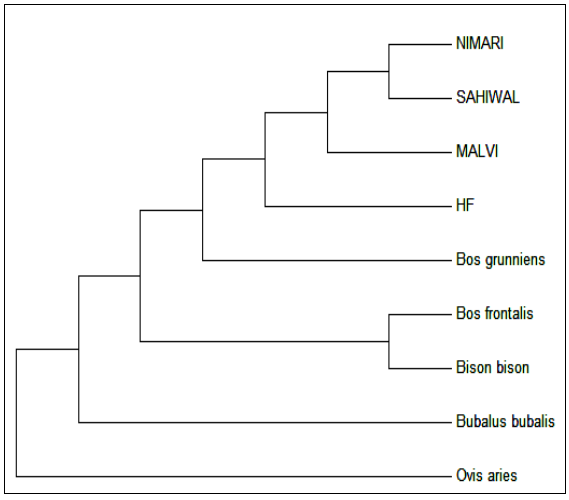


Fig 6: Kappa casein gene

Fig 5: Neighbour Joining tree for αS1, αS2, β and κ Casein gene in Sahiwal, Nimar, Malvi and H.F. cross breed cattle along with some more species.

In the study of genetic distances among four genetic groups using sequenced data, the phylogenetic analysis using the software *MEGA6: Molecular Evolutionary Genetics Analysis version 6.0* by using cluster x method was performed. The obtained cluster groups have been presented in figure 05. The phylogenic tree represented that the four breeds were divided in to four clusters. The clusters at αS1, αS2 and K gene locus in Sahiwal and Nimari showed more genetic divergence with HF crossbred as compared to the Malvi, whereas at β gene locus only Nimari showed more genetic divergence with HF crossbred as compared to Malvi and Sahiwal. Martin *et al.* [3] reported that the genetic relationship between the ten populations was determined using Nei's standard genetic distance (DS) as well as Cavalli-Sforza and Edwards, DC. The largest Nei's standard genetic distance, Ds was estimated between Punganur and Kangayam cattle while the least distance was between Hariana-Kankrej and Hariana-Mewati cattle which were in similar range that was reported in Spanish cattle. The five populations of Hariana, Kankrej, Mewati, Nagori and Tharparkar individuals with admixture, whereas, Ghumusari, Hill Cattle and Kangayam individuals showed clear distinctness and formed separate clusters whereas Binjharpuri and Punganur formed separate clusters [1].

Conclusion

The study of align sequence of αs1 casein gene of Sahiwal, the Guanine (G) at 44 bp site was replaced by Adenine (A). In αs2 casein gene, Nimari the Adenine (A) at 243 bp site was substituted by Thymine (T) and at 582 bp site in HF crossbred Adenine was replaced by Guanine. Thymine (T) at 713 and 715 bp site was replaced by Cytosine. For K- casein gene in HF crossbred, the Cytosine (C) at 262 bp site was substituted by the Thymine (T) and at 308 bp site Adenine (A) was replaced by Guanine in Nimari. The phylogenic tree showed that the four breeds were divided in to four clusters. The clusters at αS1, αS2 and K gene locus in Sahiwal and Nimari showed more genetic divergence with HF crossbred as compared to the Malvi, whereas at β gene locus only Nimari showed more genetic divergence with HF crossbred as compared to Malvi and Sahiwal.

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