RESEARCH ARTICLE

Genetic Polymorphism of β-Casein Gene in Gir and Mulki Cattle of Narmadapuram Division of Madhya Pradesh State

Rohit Barwar[1], Mohan Singh Thakur[2], Shrikant Joshi[2], Asad Khan[3]*

Abstract

This study was aimed to investigate the genetic polymorphism of β-casein gene in 75 cattle (Gir=35 and Mulki=40) of Narmadapuram division of Madhya Pradesh State. Genomic DNA was extracted from white blood cells of each cattle as per the standard laboratory techniques. The concentration of DNA in isolate of each sample was found within the range of 174.90 ng/µL to 3678.80 ng/ µL. PCR product of 251 bp size was obtained by amplification of DNA samples for exon-7 of β-casein gene. The PCR-RFLP analysis of amplicons of β-casein gene revealed the distribution of only one genotype (A2A2) in both Gir and Mulki cattle. Frequency of A2 allele was found to be maximum in the population. The results of these studies have shown that the A2 allele is more abundant in populations of Gir and Mulki cattle in Narmadapuram division.

Key words: Beta casein, Cattle, Genetic, Gir, Mulki, Polymorphism

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Introduction

Casein is one of the most commonly found protein in the lacteal secretion of mammals, comprises about 80% of the total milk proteins in cattle. Casein family of milk protein consists of phosphoproteins which codify in response to various stimuli, in mammary tissue (Ramesha *et al.,* 2016). Four subtypes of casein are known i.e., αs1-casein, αs2-casein, β-casein, and κ-casein (Dmour and Taha, 2018). β-casein gene encodes for β-casein subtype and is located at sixth chromosome in bovines (Farrell *et al.,* 2004). In bovine milk, there are two major variants of the β-casein gene: A1 and A2. The A1 variant is the most common variant in the world, while the A2 variant is more common in Indian cattle (Massella *et al.,* 2017; Adoligbe *et al*., 2022). The difference between the A1 and A2 variants of β-casein is a single amino acid at position 67. In the A1 variant, the amino acid at this position is histidine, while in the A2 variant, the amino acid is proline. Bovine β-casein gene (CSN2) consists of eight introns and nine exons, the base pair change which causes the variation in amino acid chain is spotted in exon-7 (Ramesha *et al.,* 2016).

This difference in amino acids leads to a difference in the way that the two proteins are digested in the body. The A1 variant of β-casein is more likely to be broken down into a peptide called beta-casomorphin-7 (BCM-7). BCM-7 is a small opioid peptide that has been linked to a number of health problems, including diabetes, heart disease and autism (Daniloski *et al*., 2021). The A2 variant of β-casein is less likely to be broken down into BCM-7. This is because the proline at position 67 makes the protein more resistant to digestion. As a result of these differences, milk from A2 cows is generally considered to be healthier than milk from A1 cows.

[1]Division of Animal Genetics and Breeding, ICAR – Indian Veterinary Research Institute,Izatnagar, Bareilly -243122, Uttar Pradesh, India

[2]Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry Nanaji Deshmukh Veterinary Science University (NDVSU),Jabalpur – 482001, Madhya Pradesh, India

[3]Division of Animal Genetics and Breeding, ICAR – National Dairy Research Institute, Karnal – 132001, Haryana, India

Corresponding Author: Asad Khan; Division of Animal Genetics and Breeding, ICAR – National Dairy Research Institute, Karnal-132001, Haryana, India, e-mail: asadjouravi@gmail.com

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The A2 allele is present in almost all Indian cattle breeds, with a frequency of around or over 95%, whereas A1 allele is very rare in Indian cattle, with a frequency of around or less than 5% (Kumar *et al*., 2018; Sodhi *et al*., 2018; Pandey *et al.,* 2019; Saran *et al*., 2019; Thakur *et al.,* 2020; Pandey *et al.,* 2021). The A2 variant of β-casein is thought to be more digestible than the A1 variant, and it may also have other health benefits. There is a growing demand for A2 milk in India, as consumers become more aware of the potential health benefits of this type of milk (Ramesha *et al.,* 2016).

The state of Madhya Pradesh is a large repository of valuable cattle genetic resources possessing important cattle breeds including Malvi, Nimari, Kenkatha and Gaolao. The state also contains sizable populations of non-descript cattle (i.e., Mulki) in Betul district apart from other important indigenous milch breeds like Gir, Sahiwal and crossbreds. Keeping in view the significant role of A2 allele on human health, genetic polymorphism of the β-casein gene and its association with milk composition traits was explored for selection of suitable genotypes in Gir and Mulki cattle.

Materials and Methods

Sample Collection and Genomic DNA Extraction

The present research work comprised of total 75 local (Mulki =40 and Gir=35) cows of Betul district and Timarni tehsil of Harda district of M.P. under "Madhya Pradesh Biodiversity Board Project" entitled "Survey, documentation of ITK and Phenotypic Characterization of Non-Descript Breed of Cattle in Narmadapuram Division of Madhya Pradesh". About 3 mL blood was collected in EDTA coated vacutainer aseptically from jugular vein of each experimental cattle, after approval of technical programme by Institutional Ethical Committee. Blood samples were kept in icebox and brought to laboratory and stored at 4°C till further processing. The laboratory techniques and procedures for the DNA extraction and PCR-RFLP analysis were carried out at the Department of Animal Genetics and Breeding, CVSc & AH, NDVSU, Jabalpur. Genomic DNA was extracted from blood samples following the method of John *et al.* (1991) with slight modifications and dissolved in Tris-EDTA (TE) buffer. The concentration, purity and quality of DNA were checked by Nanodrop spectrophotometer (ND-1000, USA) and 0.8% agarose gel electrophoresis (0.8%). DNA samples with an OD 260/280 ratio of 1.6 to 1.9 were considered pure and used for further analysis. The DNA samples were diluted for obtaining a final concentration of 30 ng/ µL in miliQ water for further use.

PCR Amplification of β-Casein Gene

Bovine β-casein gene specific primers (Lien *et al.,* 1992; Dar *et al.*, 2018) were custom synthesized and used for amplification of DNA using standard PCR protocol (Table 1).

PCR amplification of β-casein was carried out in a final reaction volume of 25.0 µL. Each PCR tube contained 12.5 µL of 2X PCR Master mix (Gotaq® Green Master mix), 1.0 µL of forward and reverse primers each, 3.0 µL of genomic DNA (30 ng/µl) and 7.5 µL of nuclease free water to make the final volume of 25.0 µL, The reaction mixture was subjected to an initial 5 min of denaturation at 95^0C, followed by 35 cycles of denaturation at 95^0C for 30 sec, annealing temperature at 63^0C for 45 sec, extension for 1 min at 72^0C and final extension for 10 min at 72^0C in PCR Thermal Cycler (Applied Biosystems™). The amplified PCR products were visualized by electrophoresis in 2.0 % agarose gel, containing 500 ng/ µL of ethidium bromide in 1XTBE buffer. 5 µL of amplified PCR product was loaded along with 100 bp DNA ladder as a molecular size marker in a separate lane. The amplicons in the agarose gel were visualized by UV transilluminator and photographed using Gel documentation system (Bio-Rad).

Restriction Fragment Length Polymorphism

The restriction enzyme (RE) *TaqI* which identifies the restriction site 5'…CTGCA↓G…3' was procured from Promega Corporation and used to digest the amplified PCR products of beta casein gene. Restriction digestion was done in a separate tube in a 30.0 µL of total reaction mixture, consisting 10.0 µL of PCR product, 2.0 µL of Buffer H, 1.0 µL of RE and 17.0 µL of nuclease free water. The reaction mixture was spun for few seconds for uniform mixing and then incubated at 37°C for 1 h and inactivated at 80°C for 10 min. After enzymatic digestion with RE, the digested products were electrophoresed on 3.0 % agarose gel at constant voltage of 90 V for 1 h. The PCR-RFLP bands were visualized under UV light transilluminator and photographed using Gel documentation system (Bio-Rad). Genotyping of respective gene locus (*viz.,* β-Cn gene) was carried out according to the band pattern of respective genotypes. Gene and genotype frequencies at β-casein loci were estimated using Popgene 32 software for population genetic analysis (Yeh *et al.*, 1999).

Results and Discussion

Genomic DNA Extraction

Optical density ratio (OD260/280) of most of the extracted Genomic DNA samples were falling between 1.70-1.92 indicating that DNA samples were highly pure. Samples with OD ratio beyond this range were reprocessed by two to three repeated washes with equal volume of phenol: chloroform: iso-amyl alcohol, chloroform: iso-amyl alcohol, then reprecipitated by isopropyl alcohol and sodium acetate, washed with ethanol and redissolved in TE buffer. The concentration of DNA in isolate of each sample was found within the range of 174.90 ng/ µL to 3678.80 ng/ µL The extracted genomic DNA samples were found to be of good quality in 0.8% agarose gel electrophoresis (Fig. 1).

PCR Amplification of β-Casein Gene

Exon-7 of β-casein gene was successfully amplified in all the DNA isolates. The amplified fragments of β-casein showed a size of 251 bp, indicating the primer specific amplification

Table 1: Primer sequence and restriction enzymes

Name of primer	Nucleotide sequence
Beta casein (ß-CN, 251 bp) gene	(F): 5´-GAGTCGACTGCAGATTTTCAAATCAGTGAGAGTCAG -3´ (R): 5´ CCTGCAGAATTCTAGTCTATCCCTTCCCTGGGCCCATC- 3´

(Fig. 2). The PCR product of similar size using same set of primers was also reported in earlier investigations for β-casein gene (Jawane *et al*., 2018; Thakur *et al*., 2020 and Shivashanker *et al*., 2022).

Restriction Fragment Length Polymorphism

The digested PCR product of β-casein gene (CSN2) (251 bp) by *TaqI* (RE) resulted single uncut compact band of 251 bp because of absence of restriction site for *TaqI* restriction endonuclease in amplicons β-casein gene of Gir and Mulki cattle (Fig. 3) . All the screened animals were found to be monomorphic for β-casein/*TaqI* gene. In accordance to present findings, a single compact band of 251bp for β-Cn gene was reported by Ramesha *et al*. (2016) in Deoni and Khillar; Jawane *et al*. (2018) in Dangi cattle; Thakur *et al*. (2020) in Gir, Sahiwal and Tharparkar and Pandey *et al*. (2021) in Malvi and Nimari cattle.

Gene and Genotypic Frequencies at β- Casein/*TaqI* Gene Locus

Gene and genotypic frequencies at β- casein/*TaqI* gene locus in Gir and Mulki cattle has been presented in figure 4. The frequencies of A1A1, A1A2 and A2A2 genotypes were found to be 0.00, 0.00 and 1.00 in both Gir and Mulki breed of cattle. The respective gene frequency for A1 and A2 alleles were found to be 0.00 and 1.00 in both breeds. High frequency of

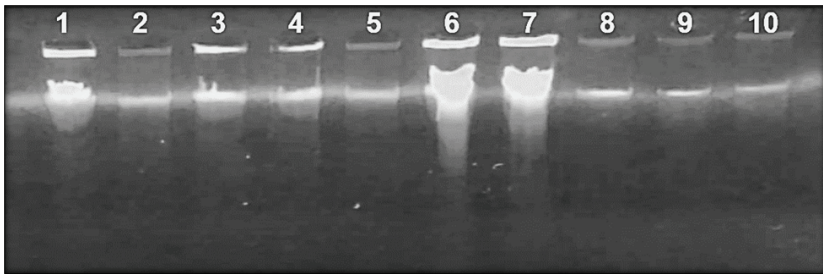

Fig. 1: Genomic DNA electrophoresed on 0.8% agarose gel; Lanes – 1-5 (Gir) and 6-10 (Mulki)

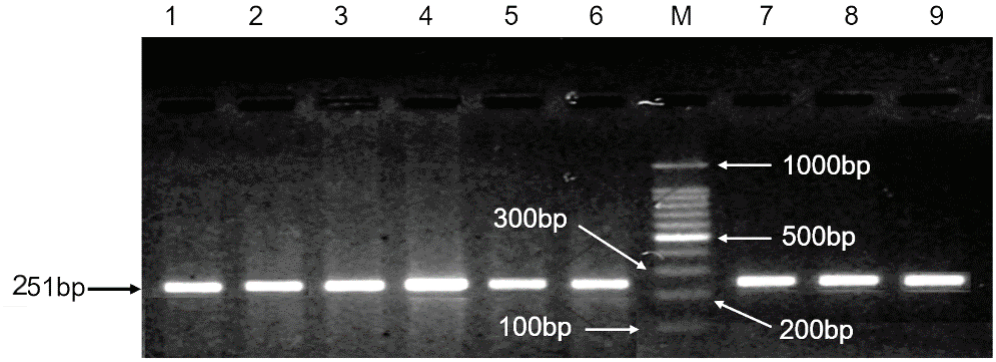

Fig. 2: Amplicons of exon-7 of β-casein Gene electrophoresed on 2.0% agarose gel; M – 100 bp DNA Ladder; Lanes – 1-4 (Gir) and 5-9 (Mulki)

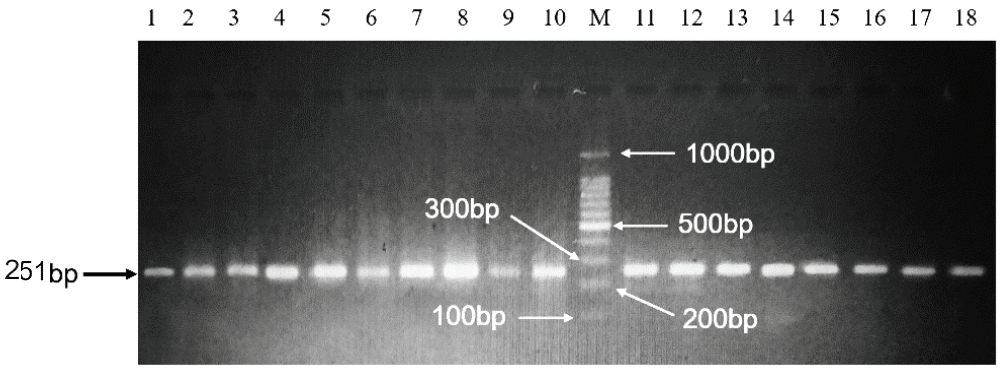

Fig. 3: RFLP products of amplicons of exon-7 of β-casein Gene electrophoresed on 3.0% agarose gel; M – 100 bp DNA Ladder; Lanes – 1-8 (Gir) and 9-18 (Mulki)

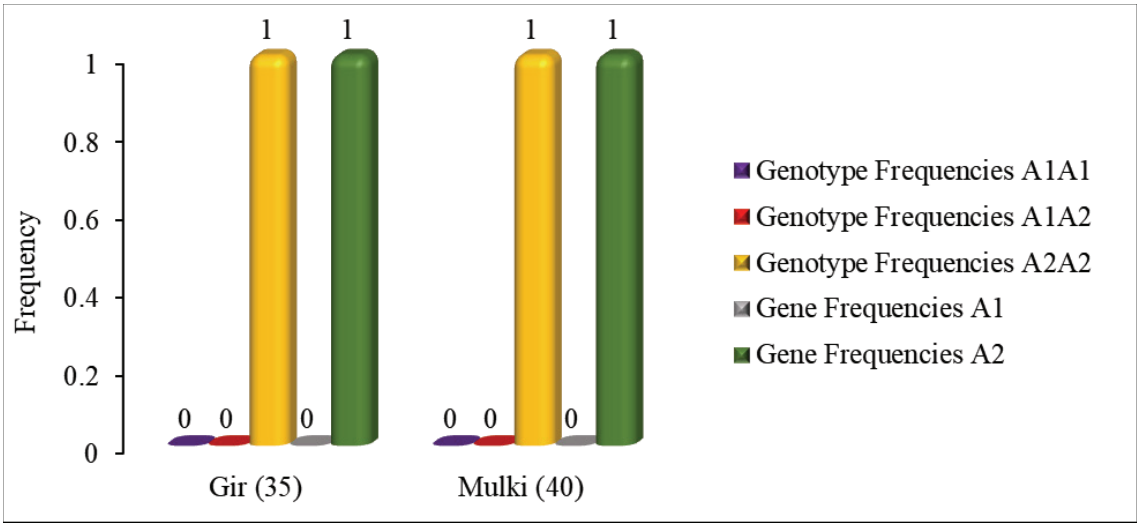

Fig. 4: Distribution of gene and genotypic frequencies at β- casein gene locus in Gir and Mulki cattle

A2 allele was observed in both breeds of cattle under study (Fig. 4).

At β-casein gene locus, only single genotype (i.e., A2A2) was detected, hence Chi-Square test for testing correspondence between observed and expected genotypic frequencies at this locus could not be applied. Similar to the present results, Malarmathi *et al*. (2014) reported A2A2 genotype in Kangeyam cattle breed, Pandey *et al*. (2019) reported A2A2 genotype in Malvi and Nimari cattle, Saran *et al*. (2019) reported monomorphic patterns for β-casein gene with only A2A2 genotype and Thakur *et al*. (2020) reported A2A2 genotype in Gir, Sahiwal and Tharparkar cattle with 100 per cent frequency of A2 allele. On the contrary to our findings, Ramesha *et al*. (2016) reported A1A2 and A2A2 genotypes in Malnad Gidda, HF, Jersey and HF crossbred cattle. They reported low frequency of A1 allele in Malnad Gidda (0.014) and higher frequency of A1 allele in HF crossbreds (0.295). Dar *et al*. (2018) reported two genotypes with genotypic frequencies of 0.76 and 0.24 for A2A2 and A1A2, respectively in Badri cattle. Similarly, Sodhi *et al*. (2018) reported A1A1 and A2A2 genotypes with maximum frequency (0.90) of A2 allele in local Ladakhi cattle, Kumar *et al*. (2018) reported two genotypes in Gir and Tharparkar cattle with higher frequency of A2 allele and Sahin and Boztepe (2022) reported all the three genotypes in Holstein Frisian, Brown Swiss and Jersey cattle at β-Cn gene locus with the highest frequency of A2 allele (0.785) in Jersey cattle.

In the present research, only one genotype was observed in the populations of Gir and Mulki. It demonstrated that the A1 allele had not been introduced into the populations of both breeds. It was hypothesised that these populations had HWE at the β-casein gene locus. In accordance to above findings, Pandey *et al*. (2019) reported that the populations of Malvi and Nimari cattle were HWE for β-Cn gene locus. Gorkhali *et al*. (2020) also reported HWE in Lulu cattle of Nepal. The HWE inferring that the present population of Gir and Mulki cattle were in the pure state, where there was no genetic introgression with the exotic breed for the sake of improvement of productivity.

Conclusions

The amplified PCR products of specific size 251 bp were obtained for β-casein gene. PCR-RFLP assay of Beta casein/ *TaqI* gene revealed monomorphic pattern in both the population of Gir and Mulki cattle. Only A2A2 genotype was found with 100 % frequency of A2 allele. This fact could be considered an indicator that existing breeding strategies adopted by farmers have not affected the equilibrium of gene frequencies in the milk production related loci in these two breeds of cattle.

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