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ABSENCE OF C>T POLYMORPHISM IN THE INTRON 1 (5′-UTR) REGION OF THE *MTHFR* GENE IN INDIAN BARBARI GOATS

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SUMMARY

The methylenetetrahydrofolate reductase (*MTHFR*) gene plays a crucial role in one-carbon metabolism and has been investigated for its association with production traits in livestock. This study aimed to examine a previously reported polymorphism in the *MTHFR* gene (rs641815079) in 272 adult female Barbari goats maintained at the University Goat Farm, Department of Physiology, DUVASU, Mathura, using PCR-RFLP with the *BsaH*I restriction enzyme.

The results revealed a monomorphic banding pattern, where all individuals exhibited the CC genotype, confirming the absence of C>T polymorphism at this site. Sanger sequencing further validated the presence of the allele, leading to the formation of the *BsaH*I restriction site. Due to the monomorphic nature of the population, no association study with milk production traits was conducted. These findings suggest that this region of the *MTHFR* gene does not contribute to genetic variation in Barbari goats, emphasising the need for alternative genetic markers for selection programs.

INTRODUCTION

Genetic selection plays a significant role in improving milk yield, composition, and metabolic efficiency in dairy animals. The *MTHFR* gene, a key regulator of one-carbon metabolism and DNA methylation, has been linked to various productive traits in cattle and goats. Several studies have demonstrated that polymorphisms within this gene can impact milk yield, protein percentage, and metabolic efficiency in ruminants. Previous research in Xinong Saanen and Guanzhong dairy goats (An *et al*. 2015; Hou *et al*. 2015) identified three genotypes (CC, CT, and TT) at the g.1951C>T locus (rs641815079) of the *MTHFR* gene. The rs641815079 SNP, located at NC_030823.1:g.40362342C>T, has been previously associated with milk composition traits in other dairy goat breeds (An *et al*. 2015). However, there is a lack of studies investigating *MTHFR* polymorphisms in Indian goat breeds, particularly Barbari goats, which are known for their high adaptability and moderate milk production. The objectives of this study were to genotype the *MTHFR* rs641815079 variant using PCR-RFLP in Barbari goats and determine allele and genotype frequencies in the studied population.

MATERIALS AND METHODS

Animal selection and sample collection. A total of 272 adult female Barbari goats from the University Goat Farm, DUVASU, Mathura were included in the study. Blood samples (5 mL) were collected via jugular venipuncture using EDTA-coated vacutainers and stored at −20°C for further analysis. Ethical approval for this study was obtained from the Institutional Animal Ethics Committee (IAEC), DUVASU, Mathura, under the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India (Approval No. V-11011(13)/6/2022-CPCSEA-DADF, dated 09 June 2022).

DNA extraction and PCR amplification. Genomic DNA was extracted using the phenol-chloroform method and assessed for quality using 1% agarose gel electrophoresis and NanoDrop spectrophotometry.

The targeted SNP, rs641815079, is a known variant in goats and corresponds to the T→C substitution at genomic coordinate NC_030823.1:g.40362342C>T based on the ARS1.2 goat reference genome. The ARS1.2 reference genome lists the C allele at this position. A 546 bp fragment within non-coding region of the *MTHFR* gene was amplified using PCR as per the PCR condition mentioned in Table 1, with the following previously reported primer set (An *et al*. 2015)

F 5'-CCGAACATCTGTTGACCTC-3'

R 5'-AGGAAGAAGGCTGGTGAG-3'

PCR amplification was performed in a 25 µl reaction volume containing 12.5 µl of 2X PCR Master Mix (EmeraldAMP GT PCR master mix, Takara Bio, RR310A), 0.5 µl each of 10 pmole/µl forward and reverse primers, 1.0 µl of genomic DNA (~100 ng), and 10.5 µl of nuclease-free water.

Table 1. PCR conditions for the amplification of the *MTHFR* gene

Steps		**1**	**2**			**3**	**4**
		Initial denaturation	35 Cycles of			Final extension	Storage
MTHFR	Temp.	94°C	94°C	58°C	72°C	72°C	12°C
	Time	5 min	30 sec	30 sec	30 sec	8 min	∞

Amplified fragments were visualized using 1.0% agarose gel electrophoresis, and the results were documented using a gel documentation system (Figure 1).

***MTHFR/BsaH*I PCR-RFLP Assay.** The PCR products were digested using the *BsaH*I restriction enzyme following standard protocols. The digested fragments were separated by 2.0% agarose gel electrophoresis, stained with ethidium bromide, and visualized under a UV transilluminator.

Sanger sequencing. To confirm the presence of the restriction site for the *BsaH*I enzyme and to validate the monomorphic nature of the rs641815079 locus, PCR products from three randomly selected Barbari goats were purified and subjected to Sanger sequencing. The obtained sequences were aligned using standard bioinformatics tools to detect the presence of the T→C substitution, which forms the recognition site for *BsaH*I.

RESULTS AND DISCUSSION

***MTHFR*/*BsaH*I PCR-RFLP assay.** The *BsaH*I/PCR-RFLP assay revealed only one banding pattern (CC genotype, 426 bp & 120 bp) (Figure 2). CT and TT genotypes were absent, confirming the monomorphic nature of the Barbari goat population for this variant in the studied population. The study found that all screened animals lacked variation for the *MTHFR* rs641815079 variant, with all individuals exhibiting the CC genotype. Consequently, no association study with milk production traits was conducted.

***MTHFR/BsaH*I DNA sequencing.** Revealing that all sequenced were homozygous for the allele of the C allele of the rs641815079 variant. Sanger sequencing confirmed the PCR-RFLP results (Figure 3). The C allele, which is also present in in the ARS1.2 reference genome, the recognition sequence for *BsaH*I, explaining the uniform digestion pattern in all individuals. Due to the monomorphic nature of the population, no association study with milk production and quality traits were conducted.

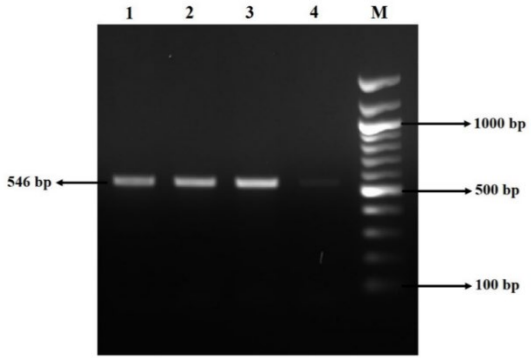

Figure 1. Agarose (1.0%) gel electrophoresis showing a 546 bp PCR products in all lanes (1-4), M= Marker (100bp ladder)

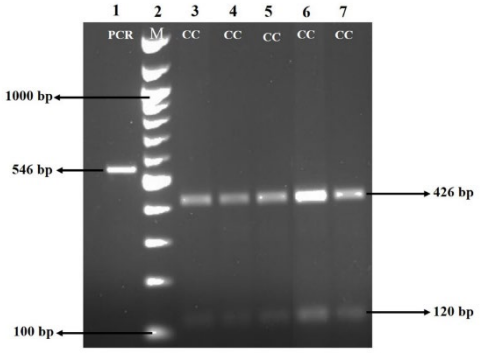

Figure 2. *MTHFR/BsaH*I PCR-RFLP assay showing genotype pattern in 2.0% agarose gel; Lane 1: Undigested PCR product, 2: Marker (100 bp ladder), 3-7: CC genotype (426 & 120 bp)

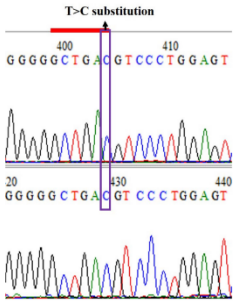

Figure 3. DNA sequencing showing the rs641815079 C allele highlighted by the purple box in the GCTGAC recognition site of *BsaH*I enzyme

Comparison with previous studies. An *et al.* (2015, 2016) and Hou *et al.* (2015) found several polymorphic variants in Xinong Saanen and Guanzhong dairy goats and reported significant associations with milk protein content for some of these variants, including the rs641815079 variant.

The frequency of the C allele for the rs641815079 variant was 0.72, whereas in our study, it was 1.00, indicating a fixed allele in Barbari goats.

Implications of monomorphism. The absence of observed genetic variation for the rs641815079 variant at the *MTHFR* locus in this study may be attributed to the relatively small population size, limiting the detection of rare alleles. To validate these findings, a larger and more diverse sample of Barbari goats should be screened. Although monomorphism was observed at the investigated locus, the original study plan included assessing the association of this polymorphism with various milk production and quality traits. These included daily milk yield, fat percentage, solids-not-fat (SNF), protein content, and lactose percentage. If polymorphism had been detected, statistical analyses would have been conducted to evaluate its association with these economically important traits in Barbari goats. It is also important to note that the rs641815079 variant is likely a marker and not necessarily a causal variant. In other goat breeds, such as Xinong Saanen and Guanzhong, other polymorphisms within the *MTHFR* gene (e.g., *c.*2494G>A, *g.2244A>G*) have shown significant associations with milk protein traits (An *et al*. 2015; *Hou et al*. 2015; An *et al*. 2016). Future studies in Barbari goats should consider exploring these additional variants or identifying novel SNPs through targeted resequencing. A genome-wide association study (GWAS) could also be more effective in identifying whether the *MTHFR* region or other genomic loci are linked to milk production and composition traits in this breed.

CONCLUSION

This study investigated the rs641815079 variant in the non-coding region of the *MTHFR* gene in Barbari goats using PCR-RFLP with the *BsaH*I restriction enzyme. The amplified region (546 bp) was monomorphic, with all individuals exhibiting the CC genotype. Sanger sequencing confirmed the results of the PCR-RFLP assay. Due to monomorphism, no association study with milk production traits was performed.

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