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Investigation of Booroola fecundity gene polymorphism and its correlation with litter size in Sirohi and Barbari goat breeds

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Abstract

The current investigation aimed to analyze the polymorphic variants of the Booroola fecundity gene and their correlation with litter size in Sirohi and Barbari goat breeds. For this purpose, PCR-RFLP analysis was employed, wherein amplified PCR products from both breeds were subjected to digestion using the *AvaII* restriction enzyme. Upon electrophoresis on a 2% agarose gel, a single, distinct band was observed for the gene in question, suggesting a lack of polymorphism. Only the A allele was identified in both breeds, with allele frequencies of 1.00 for A and 0.00 for B. As a result, the genotype distribution was exclusively AA, with no presence of AB or BB genotypes, showing genotype frequencies of 1.00, 0.00, and 0.00, respectively. Chi-square test results indicated that both goat populations conformed to the Hardy-Weinberg equilibrium. Additionally, a comparative assessment of litter size between the breeds showed a significant difference ($p < 0.05$). The Barbari goats exhibited a notably higher litter size compared to Sirohi goats.

Keywords: Booroola gene, Barbari, Sirohi, genotype, fecundity, investigation

Introduction

Successful reproduction results from the complex interplay between genes and environmental factors, ensuring the transfer of genetic traits across generations. Identifying key mutations in candidate genes, understanding their impact on reproductive traits, and enhancing fertility such as increasing litter size can provide an efficient and cost-effective approach to accelerating breeding programs. Genetic polymorphism studies on candidate genes linked to productive and reproductive traits can facilitate the selection and propagation of elite goat populations.

A lot of research has been done on the genes that affect litter size in various species, and this has led to the discovery of important genes that play a role in this trait across different sheep breeds, such as the Booroola gene (Pardeshi *et al.*, 2009) [9]. The Booroola fecundity gene (*FecB*) has a specific mutation, Q249R (CAG → CGG). This particular mutation is associated with the hyper-prolific trait seen in Booroola ewes (Mulsant *et al.*, 2001) [8].

Materials and Methods

The research consisted of Sirohi and Barbari goat breeds. A total of 40 blood samples and related performance data including milk yield, litter size, birth weight, weaning weight, and six-month body weight were collected from 20 goats of each breed.

Genomic DNA isolation

We collected about 5 mL of blood from each animal's jugular vein, using EDTA-coated vacutainers for the samples. These were then transported to the lab while keeping them cool, stored at 4 °C until we were ready to process them. To extract genomic DNA from the whole blood, we followed the classic phenol-chloroform method outlined by John *et al.* (1991) [6], purity and concentration of the DNA checked at 260 and 280 nm.

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To ensure the genomic DNA was intact, we ran it through 0.8% agarose gel electrophoresis at 80 V for 60 minutes. After that, diluted DNA samples used for PCR amplification.

Polymerase chain reaction (PCR)

Table 1: Primer details used for PCR

Primers	Sequences (5'-3')	Product size(bp)	Reference
Booroola			
Primer F1	5'GCTGGTTCCGAGAGACAGAAATATATCA 3'	1110	Polley <i>et al.</i> (2009) ^[10]
Primer R1	3' CCCCCTCCCTTTGATATCTGCAGCAATG 5'		
Primer F2	5'GTCGCTATGGGGAAGTTTGGATGGGAA A 3'		
Primer R2	3' ATGTTTTCATGCCTCATCAACACCGTCC 5'		

To perform PCR reaction 2.0 µL of diluted genomic DNA, 1.0 µL of primers (inner and outer), 8.5 µL of nuclease-free water (NFW) along with 12.5 µL of 2X master mix was used making a total volume 25 µL. the temperature on the thermocycler was set as initial denaturation of 4 min at 94 °C, followed by 30 cycles of denaturation of 30 seconds at 94 °C, annealing at 63 °C for 40 seconds and extension at 72 °C for 1 minute. A final extension performed for 6 minutes at 72 °C. To check the PCR products 1.8% agarose gel was used along with ethidium bromide (500 ng/µL) in 1X TBE buffer. 5 µL of amplified DNA in the specific well with 100bp DNA ladder on first well was put and electrophoresis was carried out at 90 volts for 60 minutes at. DNA bands were examined under gel documentation system.

PCR-RFLP assay

For the PCR-RFLP assay, amplified PCR products were subjected to enzymatic digestion using the *AvaII* restriction endonuclease, selected based on the presence of its recognition site within the target DNA sequence. Post-digestion, the enzyme was thermally inactivated, and the resulting restriction fragments were resolved through agarose gel electrophoresis. The banding patterns were visualized and recorded using a gel documentation system, facilitating subsequent genotypic analysis. The restriction digestion reaction was carried out in a 30 µL reaction volume, comprising 15 µL of PCR amplicon, 2 µL of 10X Buffer H (manufacturer's recommendation), 1 µL of *AvaII* enzyme (procured from Takara Bio), and 12 µL of NFW. The reaction mixture was briefly centrifuged to ensure homogeneity and incubated at 37 °C for 3 hours in a water bath to complete digestion.

Table 2: The restriction enzyme employed for the digestion of the PCR product, along with its specific recognition site

Genes	RE	Recognition site
Booroola /BMPR1B	<i>AvaII</i>	5'---G↓GTCC---3' 3'---CCAG↑G---5'

The amplified PCR products were electrophoresed on a 2.5% agarose gel with 1% ethidium bromide (5 µL per 100 mL). Gel run was performed using 0.5X TBE buffer at a constant voltage of 80-90 V for 60-90 minutes. To monitor the PCR-RFLP product, 2.0 µL of bromophenol blue was added per 10 µL of the product. A molecular weight standard was loaded using a Mass Ruler Low Range DNA Ladder (100 bp). A UV transilluminator was used to see the DNA bands, which were then recorded using a gel documentation system. Fragment sizes were assessed by comparing them to the molecular marker, and the Booroola gene was genotyped using the particular banding patterns for each genotype.

Samples with greater DNA concentrations were diluted to 30 ng/µL with nuclease-free water (Sigma). The PCR process used 3 µL of DNA as its template. To amplify the Booroola gene, custom-synthesized tetra primers unique to the gene (1110 bp) were used, as described by Polley *et al.*, 2009 ^[10].

Estimation of genotypic and allelic frequency

Gene and genotype frequencies of the fecundity genes under investigation were calculated using Popgene 32 software (version 1.32) as described by Yeh *et al.* (1999) ^[12]. To determine whether the populations of the different breeds conformed to Hardy-Weinberg equilibrium at the target locus, a Chi-square (χ^2) test was performed.

Results and Discussion

Genomic DNA extraction and amplification of Booroola gene

All genomic DNA samples were found to have sufficient concentrations and were checked for quality, using 0.8% agarose gel, all samples showed bright distinct bands under a UV transilluminator indicating that the extracted DNA was intact (Plate 1). The OD260/OD280 ratio from the Nano Drop ranged from 1.7 to 1.9 indicating high purity with little protein or contaminant contamination. All PCR products targeting the Booroola gene of Sirohi and Barbari goat breeds generated single clear bands about 1110 bp in size, as seen on a 1.8% agarose gel and 100 bp DNA ladder for reference. The system (gel) was run at 90 V for 60 minutes. The amplified PCR products were visualized under a UV light gel documentation system (Plate 02).

Restriction digestion and RFLP analysis

The PCR amplicons of the Booroola gene from each sample were subjected to digestion using the *AvaII* restriction enzyme. The analysis indicated the absence of the *AvaII* recognition site, as all samples exhibited a single undigested band of 1110 bp, with no evidence of restriction (Plate 03). Bhalavi *et al.* (2021) ^[2] also reported comparable results in their PCR-RFLP analysis of the Booroola fecundity gene, where digestion with the *AvaII* restriction enzyme showed a monomorphic banding pattern across the Barbari, Sirohi, and Black Bengal goat breeds. Their study detected only the AA genotype in all samples, with no presence of AB or BB genotypes in any of the examined individuals.

The findings of the present study are in agreement, as only the AA genotype was identified in both Sirohi and Barbari goats, and no genetic variation (i.e., AB or BB genotypes) was observed in the tested population. Similarly, a study conducted by Singh *et al.* (2013) using T-ARMS PCR on 53 Barbari goats also revealed a monomorphic trend, where all individuals displayed a heterozygous genotype, indicating limited polymorphism in the Booroola gene among the sampled animals.

Comparable findings were reported in a study involving 100 Markhoz goats from Iran, where researchers investigated polymorphisms in the Booroola (BMPR1B) gene. The study employed PCR-RFLP to screen for the previously identified

FecB mutation. Subsequently, seven PCR amplicons were selected for sequencing. The results indicated that none of the analyzed individuals carried the known FecB mutant allele (Islam *et al.*, 2019) [5].

Gene and Genotype frequency at Booroola gene locus

In the current study, all individuals from the Sirohi (N=20) and Barbari (N=20) goat populations were found to be monomorphic at the Booroola gene locus. The allele frequencies for the A and B alleles were determined to be 1.00 and 0.00, respectively, in both breeds. Similarly, the observed genotype frequencies for AA, AB, and BB were 1.00, 0.00, and 0.00, respectively, in both Sirohi and Barbari goats (refer to Table 3).

A single restriction pattern corresponding to the AA genotype (1110 bp) of the Booroola gene was detected in all samples. No individuals with AB or BB genotypes were identified in the studied populations of both Sirohi and Barbari goats. The genotypic frequency of AA was calculated as 1.00. Results of the Chi-square analysis for deviation from HWE revealed to be non-significant between the observed and expected genotype frequencies ($p>0.05$), indicating that both Sirohi and Barbari populations were in HWE at the Booroola gene locus. Ahlawat *et al.* (2014) [1] observed similar findings related to the when they observed BMP1B gene in eight native Indian goat breeds. They also reported uniform absence of polymorphism in all goat breeds. The results of this study are in accordance with previous findings, of Kumar *et al.* (2008) [7] observing HWE in Malpura and Kendrapada sheep, He *et al.* (2010) [4] reporting in Chinese goat populations and Godara *et al.* (2012) [3] also in Marwari goats which lack genetic variation at the Booroola gene locus in goats.

Table 3: Gene and genotypic frequency of Booroola gene variants of Sirohi and Barbari goats

Breeds	Genotype Frequencies			Chi-square (X^2) Value	Gene Frequencies	
	AA	AB	BB		A	B
Sirohi	1.00	0.00	0.00	0.00 ^{NS}	1.00	0.00
Barbari	1.00	0.00	0.00	0.00 ^{NS}	1.00	0.00

NS-Non significant, ($p>0.05$)

Association study of genotypes of Booroola gene with Litter size in Sirohi and Barbari goat breeds

All screened samples from both Sirohi and Barbari goats was found to be monomorphic at the Booroola gene locus. As a result, it was not possible to conduct an association analysis between this gene and litter size variation. Nonetheless, a comparative analysis of mean litter size between the two breeds is presented below.

Litter Size (LS)

A statistically significant influence of genotype ($p<0.05$) was observed on the litter size trait. The average litter size at first kidding was analyzed and compared between Sirohi and Barbari goat breeds. Comparable results were documented by Bhalavi *et al.* (2021) [2], who reported a markedly greater litter size in Black Bengal goats when compared to Barbari and Sirohi breeds. In the current investigation, only the AA genotype was identified across all individuals from both Sirohi and Barbari breeds. The average litter size corresponding to the AA genotype was 1.70 ± 0.16 in Barbari goats, while in Sirohi goats it was recorded at 1.25 ± 0.09 . The effect of breed on litter size was statistically noteworthy ($p<0.05$), with Barbari goats showing a significantly higher litter size than their Sirohi counterparts (see Table 4).

Table 4: Least square means for litter size of Sirohi and Barbari breeds of goat

Genotype	Breeds	
	Sirohi	Barbari
AA	$1.25^a\pm0.09$	$1.70^b\pm0.16$
AB	0.00 ± 0.00	0.00 ± 0.00
BB	0.00 ± 0.00	0.00 ± 0.00
Overall	$1.25^a\pm0.09$ (20)	$1.70^b\pm0.16$ (20)

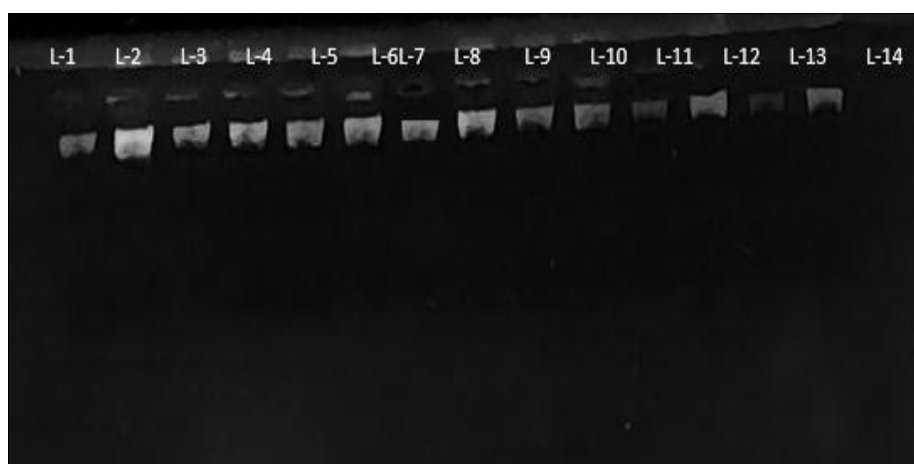


Plate 1: Genomic DNA of goats electrophoresed on 0.8% agarose [lanes 1-14 are genomic DNA samples of Sirohi and Barbari goats]

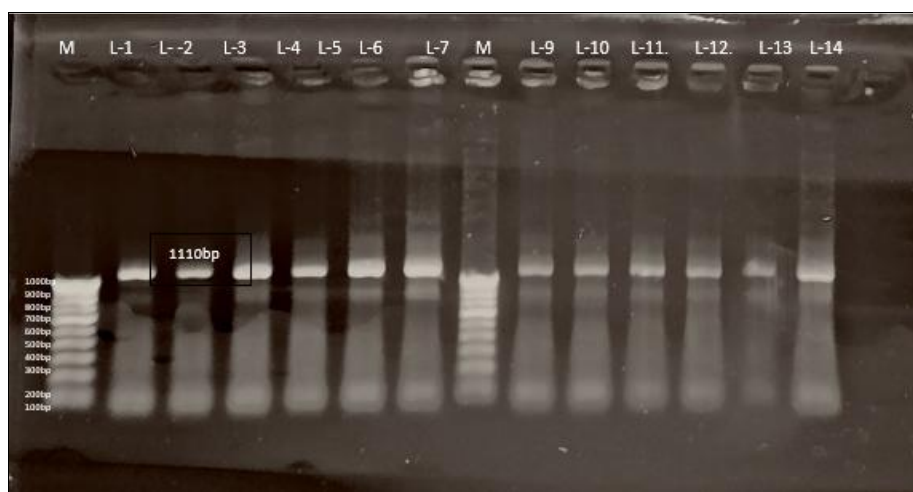


Plate 2: Amplified PCR product of Booroola gene of goats on 1.8% agarose M: 100bp DNA Ladder, Lanes amplicons (1110bp), 2-7 Sirohi and -14 Barbari]

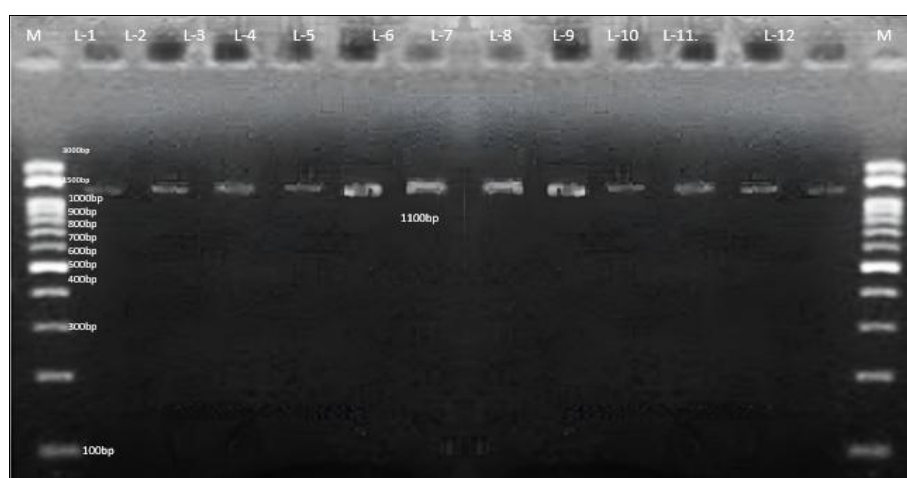


Plate 3: PCR-RFLP pattern of Booroola digested with *AvaII* restriction enzyme [lanes M: 3000bp DNA Ladder; AA genotype (1100bp), lanes 1-7 Sirohi and 8-15 Brabari]

Conclusion

In the present study, the Booroola fecundity gene (1110 bp) exhibited a monomorphic pattern in both Sirohi and Barbari goat breeds. The A allele was found to have a gene frequency of 1.00, while the B allele was absent (frequency = 0.00). Correspondingly, the genotypic frequencies of AA, AB, and BB genotypes were 1.00, 0.00, and 0.00, respectively, in both breeds. The comparative analysis of litter size between the two breeds revealed a significantly higher average litter size in Barbari goats compared to Sirohi goats.

Conflict of Interest

Not available

Financial Support

Not available

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