Jurnal Biologi Tropis

Original Research Paper

Genetic Diversity Analysis of Growth Differentiation Factor 9 (GDF-9) Exon 1 in Boerka Goats via PCR-RFLP

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Article History

Received : February 14th, 2026

Revised : February 25th, 2026

Accepted : March 04th, 2026

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Abstract: This study aims to identify polymorphisms in the Growth Differentiation Factor-9 (GDF-9) gene in Boerka goats using the PCR-RFLP method. A total of 60 blood samples were used for DNA isolation. Amplification of the GDF-9 gene Exon 1 produced a 462 bp fragment. Genetic diversity analysis using the *HhaI* restriction enzyme revealed three genotypes: GG (462 bp), GA (462, 410, and 52 bp), and AA (410 and 52 bp). The frequency of the G allele (0.925) was found to be higher than the A allele (0.075), with a dominant GG genotype frequency of 0.867. The Polymorphic Information Content (PIC) value was 0.129, indicating a low level of genetic diversity in the studied population. Descriptive analysis showed that the heterozygous GA genotype tended to have a higher average litter size than the homozygous genotypes. These results indicate that the GDF-9 gene is polymorphic in Boerka goats and has the potential to be used as a molecular marker in breeding programs.

Keywords: Boerka goat, GDF-9, genetic diversity, polymorphism, PCR-RFLP.

Introducting

Enhancing the productivity of Boerka goats as a premium meat commodity in Indonesia is highly dependent on reproductive efficiency. A key parameter of reproductive success is prolificacy, or the ability to produce a high litter size. At the molecular level, this trait is regulated by specific candidate genes, notably Growth Differentiation Factor-9 (GDF-9). This gene plays a pivotal role in ovarian follicular development and the ovulation rate of ruminants (Noshahr & Rafat, 2014).

The mechanism of action of the Growth Differentiation Factor 9 (GDF-9) gene involves the regulated processing and production of the GDF-9 protein. Synthesized by the oocyte within the ovarian follicle, GDF-9 is secreted into the follicular microenvironment (Song et al., 2023). Initially synthesized as a precursor protein, GDF-9 undergoes proteolytic cleavage to transition into its biologically active mature form. Once activated, GDF-9 functions either as a homodimer or as a heterodimer in conjunction with BMP-15 (Fillingim, 2017). The signaling cascade is initiated when GDF-9 binds to specific Bone Morphogenetic Protein (BMP) receptors on granulosa or cumulus cells. This process involves the recruitment of Type I and Type II transmembrane receptors, which subsequently propagate intracellular signals via the SMAD2/3 pathway (Chen et al., 23023). By utilizing this canonical TGF-β signaling pathway, GDF-9 modulates the expression of target genes that critically regulate the proliferation and differentiation of granulosa cells (Amandykova et al., 2023).

Despite the genetic potential derived from the crossbreeding of Boer and Kacang goats, information regarding the polymorphism of the GDF-9 gene in this population remains limited. Identifying polymorphisms in this gene is a

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critical initial step in Marker-Assisted Selection (MAS) programs (Ciptayasa et al., 2016). Therefore, this study was conducted to identify GDF-9 genotypic variations in Boerka goats using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method (Agus Hidayat & Maskur, 2015).

Materials and Methods

Research Sample

The study utilised 60 blood samples from Boerka goats, collected via the jugular vein using Venoject tubes containing EDTA as an anticoagulant.

Procedure

Genomic DNA was isolated using the Phenol-Chloroform method. Amplification of the GDF-9 gene (Exon-1) was performed via the PCR technique, yielding a 462 bp product. Mutation detection was carried out using the RFLP method with the *HhaI* restriction enzyme. The digested products were then incubated at 36°C for 16-18 hours and visualised using 2.5% agarose gel electrophoresis.

Data Analysis

Genotype and allele frequency data were analysed using Popgene software version 1.32.

Results and Discussion

PCR Amplification

PCR amplification successfully produced specific products with a length of 462 bp across all 60 samples, as visualised on the agarose gel electrophoresis in Figure 1. No evidence of non-specific amplification was observed.

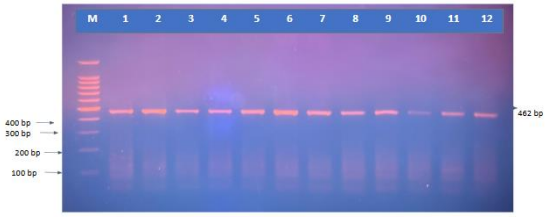

Figure 1. Agarose gel electrophoresis of GDF-9 gene fragments amplified via PCR from Boerka goat DNA samples

Identification of Gene Mutation

Mutations occurring within the GDF-9 gene resulted in genotypic polymorphism in Boerka goats, as shown in Figure 2. The genetic diversity analysis of the GDF-9 gene revealed two types of alleles, namely G and A, with three identified genotypes: GG, GA, and AA.

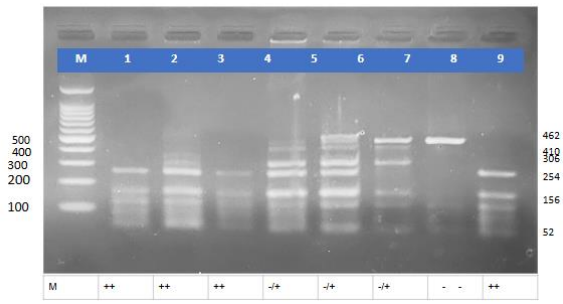

Figure 2. PCR-RFLP amplification results of the GDF-9 gene using the *HhaI* restriction enzyme.

The obtained PCR-RFLP results were then calculated to determine the genotype and allele frequencies. The results of these frequency calculations are presented in Table 1.

Table 1. Genotype and allele frequencies of the GDF-9 gene in Boerka goats

Gen	Frekuence						
	Genotipe			Alel		X^2 (HWE)	X^2 Table (0,05)
	GG	GA	AA	G	A		
GDF-9	0.867 (52)	0.117 (7)	0.017 (1)	0.925	0.075	121,8	3.841

Notes: $X^2_{count} < X^2_{table(0,05)(1)}$

The population measurements for the local goats, including observed heterozygosity (Ho), expected heterozygosity (He), and Polymorphic Information Content (PIC), are presented in Table 2.

Table 2. Observed heterozygosity (Ho), expected heterozygosity (He), and Polymorphic Information Content (PIC) of the GDF-9 gene in Boerka goats

Gen	N	Ho	He±Se
GDF-9	**60**	**0.117**	**0.139±0.0000**

PCR Result Amplification

Amplification of the candidate gene controlling prolificacy in the Boerka goat genome was conducted using forward and reverse primers previously designed and utilised across various goat and sheep breeds, including both local and non-local livestock. The GDF-9 gene primers were specifically designed to amplify a 462 bp target DNA fragment. According to Hidayat and Maskur (2015), the size of the resulting PCR products can be verified using a 100 bp DNA Ladder (HindIII), confirming that the amplification products are indeed the target gene. The target gene in all samples was successfully amplified with clear resolution, visualised via PCR product electrophoresis on a 2.5% agarose gel at a temperature of 59°C.

The success of GDF-9 gene amplification is influenced by the annealing temperature applied to the DNA template (target gene), which ensures that other reagents function optimally. Furthermore, sample preparation techniques—including both the collection of materials and the mixing process—can significantly impact amplification success; inadequate preparation may result in suboptimal PCR performance and the failure to detect the target gene (Hidayat & Maskur, 2015).

GDF-9 Gene Amplification

The PCR-RFLP amplification of the GDF-9 gene in Boerka goats revealed genetic polymorphism, characterised by variations in DNA banding patterns on the agarose gel electrophoresis (Figure 2. These findings are consistent with the study by Noshahr and Rafat (2014) conducted on Iranian Moghani sheep, which reported the same fragment length of 462 bp. The PCR process yielded a 462 bp DNA fragment, which was subsequently digested using the *HhaI* restriction enzyme. The digestion process produced distinct banding patterns, specifically at 410 bp and 52 bp, indicating a mutation in the A allele. The GA heterozygous genotype exhibited a combination of bands from both alleles, specifically at 462 bp, 410 bp, and 52 bp.

Table 1 shows the obtained genotype frequencies of GG = 0.867 (52 heads), GA = 0.117 (7 heads), and AA = 0.017 (1 head). These data indicate that the GG genotype is highly dominant within the observed population. This dominance resulted in a significantly higher frequency of the G allele (0.925) compared to the A allele (0.075). Such conditions identify the G allele as the major allele, while the A allele is the minor allele in the Boerka goat population. This pattern is consistent with the findings of Elieser et al. (2019) and Chairunnisa et al. (2022), who reported that in several Indonesian goat breeds, the frequency of the dominant (wild-type) GDF-9 allele tends to be higher, whereas the mutant allele frequency is relatively lower. The Hardy-Weinberg Equilibrium (HWE) test showed a calculated X^2 count value of 121.8, compared to theX^2 table value of 3.841 (α = 0.05$). Statistically, this value indicates a deviation from the Hardy-Weinberg equilibrium, suggesting that the Boerka goat population is not in a state of genetic equilibrium.

Table 2 shows that the observed heterozygosity (Ho) of the GDF-9 gene in Boerka goats is 0.117, while the expected heterozygosity (He) is 0.139 ± 0.0000. The obtained Polymorphic Information Content (PIC) value is 0.129, based on an analysis of 60 individuals. These results indicate a population imbalance. This condition may arise due to several factors, including non-random mating, artificial selection, and the repeated use of superior sires in breeding programs (Chairunnisa et al., 2022). Nonetheless, the GDF-9 gene maintains its crucial role in livestock production and reproduction.

Conclusion

The GDF-9 gene in Boerka goats is polymorphic, exhibiting three genotypic variations (GG, GA, and AA). Although the genetic diversity is categorised as low, the GA genotype shows potential superiority for prolificacy traits, suggesting its utility as a basis for future livestock selection programs.

Acknowledgments

Thank you to all parties involved in this research.

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