RESEARCH ARTICLE Indian Journal of Animal Research, Volume 59 Special Issue: 30-35 (November 2025)

Insulin-like Growth Factor 1 Gene Sequence Variations in Bapedi Sheep and Their Association with Morphometric Traits

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10.18805/IJAR.BF-1890

ABSTRACT

Background: The study was conducted to identify the *Insulin-like growth factor 1* (*IGF1)* gene polymorphism and its association with morphometric traits in Bapedi sheep breed in the Limpopo Province of South Africa.

Methods: Blood samples were collected from hundred and twenty-seven (n = 127) South African Bapedi sheep aged between 1 to 5 years. The Deoxyribonucleic acid (DNA) was extracted, amplified, and sequenced for the *IGF1* gene. DNA sequencing was used to identify the sequence variations of the *IGF1* gene in South African Bapedi sheep.

Result: The results showed that two nonsynonymous single nucleotide polymorphisms (SNPs) of the *IGF1* gene in 5' flanking region positions 855G>C and 857>A were identified. Two genotypes (GG and GC) and (AA and AB) were discovered from each of the identified SNP. The results also indicated that the used population was not in the Hady-Weinberg Equilibrium. Marker trait association findings for SNPs 855G>C and 857>A showed that there was statistical relationship between the genotypes with body weight, heart girth, withers height, sternum height, rump height and body length. Dominant genotype GG and AA had the highest impact on all the traits. *IGF1* gene at 5' flanking region of Bapedi sheep had SNPs (855G>C and 857>A) at positions 855 and 857 bp with two genotypes each (GG and GC) and (AA and AB), respectively. Therefore, genotype GG of SNP 855G>C and genotype AA of SNP 857>A of *IGF1* gene might be used as a genetic marker when improving morphometric traits.

Key words: DNA sequencing, Genotypes, Indigenous sheep, Polymorphisms, Withers height.

INTRODUCTION

Sheep rearing contributes greatly to the economy, especially in areas where crop and dairy farming are not economical. It plays an important role in the livelihood of a large proportion of small farmers and landless labourers. Hasircioglu *et al.* (2024) reported that sheep can use low-order forages as source of nutrition and they are particularly useful for farmers with limited land areas.

When the immigrants scattered to inhabit different localities, giving way to segregation of the herd into currently recognised three indigenous sheep groups *i.e.* the Bapedi, Swazi and Zulu sheep in Limpopo, Swaziland and KwaZulu-Natal, respectively (Mavule, 2012). Bapedi sheep is an Indigenous breed originating from the semi-arid regions of Limpopo province in South Africa and is reared to produce lean meat and it offers a source of income to resource-limited farmers (Maqhashu, 2019; Ngcobo *et al*., 2022). Bapedi sheep is a tough, disease-resistant and a fat-tailed breed of sheep (Maqhashu *et al*., 2020). With the reports of the breed being at risk of extinction there are conservation programmes in place to make sure their genes do not disappear (Ngcobo *et al*., 2022). The purpose of improvement schemes is to increase production, product quality and cost efficiency, maintaining genetic diversity and support specific breed conservation efforts (Kunene, 2010). It is necessary to understand the genetic composition underlying these phenotypes to develop such breeds (Darissa and Irekat, 2020).

Morphometric traits and body conformation traits are the sheep industry's economically important traits (Kumar *et al*., 2023). Morphometric traits have always attracted much interest in the production of meat producing animals (Lastari *et al*., 2020). Sankhyan *et al.* (2019) reported that the physiological regulation of muscle growth in animals is under the control of numerous genes. Therefore, the candidate gene approach was introduced as a powerful tool in which the researchers recognize, map and analyse single nucleotide polymorphism (SNPs) of genes

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How to cite this article: Sako, T., Tyasi, T.L., Ng'ambi, J., Raphulu, T., Sebei, J. and Madia, K. (2025). *Insulin-like Growth Factor 1* Gene Sequence Variations in Bapedi Sheep and Their Association with Morphometric Traits. *Indian Journal of Animal Research.* **59 (Special issue):** 30-35. doi: 10.18805/IJAR.BF-1890.

Submitted: 08-09-2024 **Accepted:** 01-04-2025 **Online:** 17-05-2025

associated with the traits of interest (Madikadike *et al*., 2024).

Insulin-like growth factor 1 gene (IGF1) which is among the genes identified for improvement of growth traits is a component of the somatotrophic axis which plays a key role in the growth and metabolism of animals (Lastari *et al*., 2020; Mokoena *et al*., 2023). It facilitates the synthesis of proteins by absorbing amino acids and lowering protein degradation, decomposition of glycogen and lastly, the absorption of glucose (Al Qasimi *et al*., 2019). The SNPs of this gene have been studied in different sheep breeds, including Santa Ines sheep breeds (Meira *et al*., 2019) and Awassi sheep (Al Qasimi *et al*., 2019). However, the *IGF1* gene polymorphism and association with growth traits in Bapedi sheep is not yet known. In this regard, the study is conducted to identify the *IGF1* gene polymorphism and to determine its association with morphometric traits in Bapedi sheep breed in the Limpopo Province of South Africa.

MATERIALS AND METHODS

The study was conducted in Limpopo province of South Africa with farmers registered in Bapedi Sheep Breeder Society. Limpopo Province (-23.4012946 S and 29.4179324 E) is semi-arid savanna biome dominant, with mean maximum temperatures reaching 28°C in summer and 18°C in winter, and annual rainfalls ranging from less than 350mm in lowlands to over 1000 mm in mountainous areas (Maluleke *et al*., 2023).

The research used 127 Bapedi sheep aged 1-5 years. The research employed a population study where all the animals within the population were used. This was due to farmers having fewer numbers of the Bapedi sheep. Data was collected from the farmers registered with the Bapedi Sheep Breeder Society. A cross-sectional study design was employed. The animals were managed under an extensive farming system in which they were allowed to go out in the morning to graze and return to their kraals in the afternoon. Bapedi sheep were monitored twice a day, in the morning before they left for grazing and when they returned to their kraals in the afternoon.

The body weight (BW) and morphometric traits such as body length (BL), heart girth (HG), withers height (WH), sternum height (SH) and rump height (RH) were measured according to the suggestions of Birteeb *et al*. (2012), Tyasi *et al*. (2020) and Dinesh *et al*. (2024). One person was nominated and trained to perform all the measurements to avoid individual errors. The morphometric traits measurements were recorded in centimetres and determined using a flexible tape. In contrast, BW was described in kilograms using an electronic weighing scale (Micro T7E Sheep/Pig Scale, South Africa) that does not impart pain to the sheep. Blood samples (5 ml) were taken aseptically from the jugular vein of each sheep using a vacutainer needle which draws blood from the animal straight to the sterilized vacutainer tube containing EDTA as anticoagulant. The blood samples were then transported to the University of Limpopo laboratory in an ice box with gel cool packs and kept there at -20°C until they were sent to Inqaba Biotechnology company (525 Justice Mahommed St Muckleneuk, 200, Pretoria, South Africa).

Blood samples were sent to Inqaba Biotechnology company for DNA extraction and amplification by polymerase chain reaction (PCR). Amplification of the *IGF1* gene covering intron 3, exon 4 and intron 5 was done using a pair of forward (5′TGA GGG GAG CCA ATT ACA AAG C3′) and reverse (5′CCG GGC ATG AAG ACA CAC ACA T3′) primers. The primers were designed using primer 3.04 software and the published nucleotide sequence of the Ovine *IGF1* gene (GenBank Accession No X69472). The PCR reaction was carried out in 20 µl reaction mixtures consisting of 10 µl of NEB OneTaq, 2X Master mix with a standard buffer, 1 µl of genomic DNA (10-30 ng/µl), 1 µl of each primer (10 µM), and up to 7 µl of nuclease free water. Amplification was done under the following conditions: denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 59°C for 30 seconds, and 72°C for 1 minute, with a 5-minutes extension at 72°C as the last step and hold at 4°C. The integrity and molecular weight of the PCR amplicons were determined using 1% agarose gel (Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye. PCR products were cleaned using ExoSAP protocol as follows: 1. Exo/SAP master mix was prepared by adding Exonuclease I 20 U/µl 50 µl and Shrimp Alkaline Phosphatase 1 U/µl 200 µl to a 0.6 ml micro-centrifuge tube. 2. Amplified PCR product 10 µl and ExoSAP Mix (step 1) 2.5 µl mixtures were prepared. 3. Mixture was mixed well and incubated at 37°C for 15 min. 4. The reaction was stopped by heating the mixture at 80°C for 15 minutes.

PCR products of a region of 5′ flanking region of the IGF1 gene were sequenced at Inqaba Biotechnology to identify the single nucleotide polymorphisms (SNPs). Fragments were sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, BRD3- 100/ 1000 according to manufacturer's instructions (NimaGen, Netherlands). Sequence alignment was performed using the NCBI/ BLAST/ blastn suit. The labelled products were then cleaned with the ZR-96 DNA Sequencing Clean-up Kit (Zymo Research, USA). The cleaned products were injected on an Applied Biosystems ABI 3500XL Genetic Analyzer or Applied Biosystems ABI 3730XL Genetic Analyzer with a 50 cm array, using POP_7. Sequence chromatogram analysis was performed using FinchTV analysis software. NCBI/BLAST site was used to perform sequence alignment.

The statistical analysis system (SAS, 2022) version 9.4 was used for data analysis. Procedure of means (PROC MEANS) was used for descriptive statistics of the morphometric traits. Procedure of analysis of variance (PROC ANOVA) was used to observe the significant difference on the genotypes. Hardy-Weinberg Equilibrium test software for Population Genetic Analysis (POPGENE)

was used to calculate genotypic and allelic frequencies. The Chi-square test was used to measure the genetic equilibrium. Student T-test was performed for marker-trait association analysis. The following model was used:

$$Yij = \mu + Gi + eij$$

Where:
Yij = Morphometric traits,
μ = Population mean,
Gi = Fixed effect of genotype,
eij = Random residual error.

RESULTS AND DISCUSSION

Amplified nucleotide sequence analysis

The amplification of a segment of the *IGF1* gene's 5' flanking region yielded a 294 bp PCR amplicon size as shown in Fig 1.

Sequence analysis and alignment on 855G>C and 857>A of 5' flanking region

Nucleotide sequence analysis of the amplicons revealed two single nucleotide polymorphisms at position 855G>C and 857>A (Fig 2). A polymorphism was detected with nucleotide transversion from guanine (G) to cytosine (C) at position 855 and insertion of adenine (A) at position 857 of 5' flanking region when compared with the *IGF1* gene (accession number: X69472) (Fig 2A). Blast was used to find the pairwise alignments of DNA as highlighted in Fig 2B. The sequence alignment results showed 855G>C and 857>A as the SNP position (red box). In addition, post-nucleotide sequence analysis results of four genotypes (GG, GC, AA and AB) were assigned. Blast was used to determine the protein sequence alignment as indicated in Fig 2C. The results indicated nonsynonymous SNPs as highlighted with a red box. Amino acid change from glutamine (Q) to histidine (H) at position 125 and arginine (R) to glutamine (Q) at position 126 were found by comparing it with the *IGF1* gene (acc. no. CAA49230).

Genotypic and allelic frequencies

Four alleles (T, C, A, and B) along with four genotypes (GG, GC, AA and AB) were identified and are detailed in Table 1. The genotypic frequencies of the heterozygous GC and AB were greater than those of the homozygous GG and AA genotypes. The chi-square (U«2) test indicated that both allelic and genotypic frequencies of both SNPs (855G>C

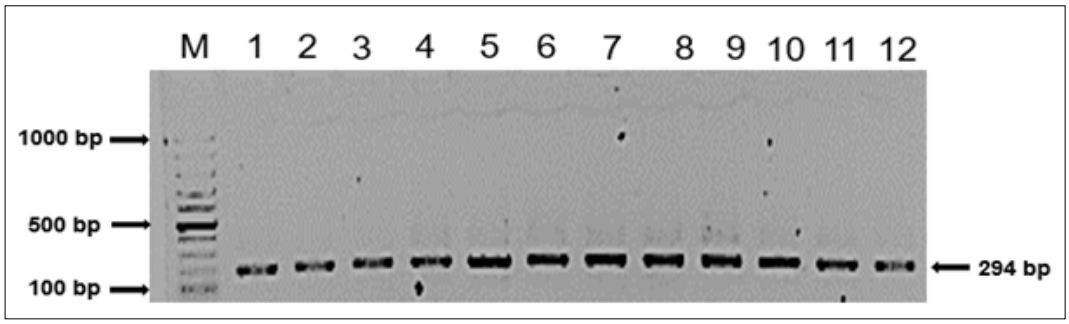
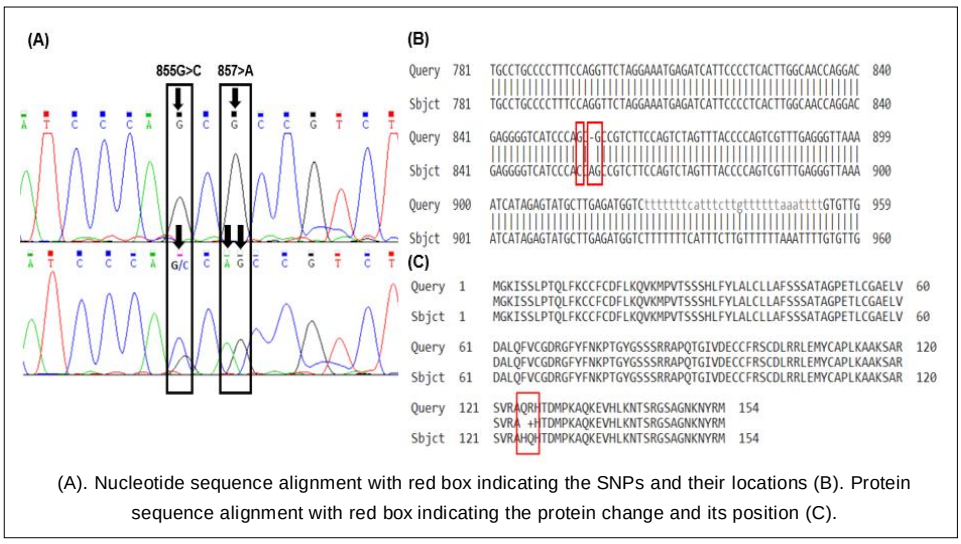


Fig 1: PCR products from the amplification of the *IGF1* gene's 5' flanking region in the Bapedi sheep.



(A). Nucleotide sequence alignment with red box indicating the SNPs and their locations (B). Protein sequence alignment with red box indicating the protein change and its position (C).

Fig 2: Nucleotide sequence analysis showing the 855G>C and 857>A gene's sites on 5' flanking region of the Bapedi sheep *IGF1* gene through chromatograms.

and 857>A) exhibited a statistical difference with the expectation of Hardy-Weinberg (χ^2 = 35.69). The chi-square results demonstrated that the frequencies (allelic and genotypic) of the *IGF1* SNPs are not characteristically under Hardy-Weinberg equilibrium, proposing that the population's frequencies change from one generation to the other.

Polymorphism information analysis

The polymorphism information analysis along with the genetic diversity of the population were computed and are indicated in Table 2. In both SNPs (855G>C and 857>A), the gene heterozygosity *(He)* was lower than the gene homozygosity *(H0)* with an effective number (*Ne*) of 1.83 and a polymorphism information content *(PIC)* which indicated the existence of medium ranged polymorphisms within the Bapedi sheep population.

Association analysis between identified genotypes of 855G>C and 857>A and morphometric traits

The marker-trait association between the recognised genetic markers to the traits of interest are indicated in Table 3. The results indicated that there were significant differences (P<0.05) between the genotypes GG and GC of SNP 855G>C regarding all the recorded traits with genotype GG performing better than GC in all the recorded traits. The results (Table 3) further indicated that there were significant differences (P<0.05) between the genotypes AA and AB of SNP 857>A regarding all the recorded traits with genotype AA performing better than AB in all the recorded traits.

Insulin-like growth factor 1 (IGF1) gene plays a key role in various physiological processes, such as reproduction, development of the foetus and animal growth (He *et al*., 2012). Hence, the objective of this study was to identify the single nucleotide polymorphisms of *IGF1* gene and its association with morphometric traits of South African Bapedi sheep. Findings of the current study revealed 2 non-synonymous SNPs (G/C) and (A) at positions 855 and 857 of the 5′ flanking region of IGF1 gene which were named 855G>C and 857>A, respectively. Two single nucleotide polymorphisms (g.855G>C and g.857G>A) in the 5′ flanking region in IGF-1 gene were discovered by Kumar *et al*. (2023) who conducted a study on the influence of single nucleotide polymorphisms in the *IGF-1* gene on performance and conformation traits In Munjal sheep in India. Scatà *et al*. (2010) investigated *IGF1* gene polymorphisms in three dairy sheep namely, Sarda, Altamurana and Gentile di Puglia sheep breeds and reported the detection of the SNPs g.855G>C and g.857G>A positioned at the 5' flanking region. Sebastino *et al*. (2020) also reported two *IGF1*

Table 1: Genotypic and allelic frequencies at the site of single nucleotide polymorphisms locus of the *IGF1* gene in Bapedi sheep.

SNPs	Genotype	Number of animals	Genotypic frequency	Allele	Allele frequency	χ^2
855G>C	GG	39	0.31	G	0.65	35.69*
	GC	88	0.69	C	0.35	
857>A	AA	39	0.31	A	0.65	35.69*
	AB	88	0.69	B	0.35	

SNPs: Single Nucleotide Polymorphisms, χ^2: Chi-square * p<0.05: Statistically significant when the data were analysed using a Pearson's goodness-of-fit chi-square test (degree of freedom = 1), ns: non-significant (p<0.05).

Table 2: Polymorphism information analysis of the *IGF1* gene's 5' regulatory region of Bapedi sheep.

SNPs	Gene homozygosity (*Ho*)	Gene heterozygosity (*He*)	Effective allele number (*Ne*)	Polymorphism information content (*PIC*)
855G>C	0.55	0.45	1.83	0.35
857>A	0.55	0.45	1.83	0.35

SNPs: Single nucleotide polymorphisms.

Table 3: Association of the 855G>C and 857>A polymorphism with morphometric traits (Mean±SE).

Traits	855G>C		857>A	
	Genotype GG	Genotype GC	Genotype AA	Genotype AB
Body weight (kg)	44.03±1.11^a	32.59±0.61^b	44.03±1.11^a	32.59±0.61^b
Heart girth (cm)	79.00±0.75^a	70.83±0.62^b	79.00±0.75^a	70.83±0.62^b
Withers height (cm)	67.87±0.61^a	62.78±0.46^b	67.87±0.61^a	62.78±0.46^b
Sternum height (cm)	47.85±0.47^a	45.08±0.39^b	47.85±0.47^a	45.08±0.39^b
Rump height (cm)	70.21±0.64^a	65.74±0.43^b	70.21±0.64^a	65.74±0.43^b
Body length (cm)	66.82±0.72^a	60.77± 0.69^b	66.82±0.72^a	60.77±0.69^b

SE: Standard error, n: Number of animals, kg: Kilograms, cm: Centimetres. ^{ab}Different superscripts on the same row show significant differences (P<0.05).

polymorphisms at positions g184028491C>G and g184028489C>T of the 5′ flanking region in Sarda sheep. The results of this study suggest that SNPs 855G>C and 857>A causes an amino acid change from glutamine to histidine and arginine to glutamine, respectively, which influences protein structure and function.

The results of the current study reported high allelic frequency for A allele in SNP 857>A. Similar results were reported by Kumar *et al*. (2023) for SNP g.857G>A. Chi-square results of the current study demonstrated that the population used was not under Hardy-Weinberg equilibrium (HWE) for SNPs 855G>C and 857>A. Results are in agreement with Kumar *et al*. (2023) findings. The observed results imply that the genotypic and allelic frequency for SNPs 855G>C and 857>A of *IGF1* gene on Bapedi sheep changes from generation to generation. Alim *et al*. (2015) reported that when the chi-square test for SNP in the population is in Hardy-Weinberg equilibrium the selection pressure on that SNP in the population in not too powerful.

Marker trait association findings for SNP 855G>C showed that there was statistical relationship between the genotypes with body weight, heart girth, withers height, sternum height, rump height and body length. Genotype GG had the highest impact on all the traits. Marker trait association findings for SNP 857>A showed that there was statistical relationship between the genotypes with body weight, heart girth, withers height, sternum height, rump height and body length. Genotype AA had the highest impact on all the traits. The results of the current study are not in agreement with Al Qasimi *et al*. (2019) and Kumar *et al*. (2023) for number of genotypes reported (AG CC and GC) and (AA, GA and GG) in Awassi lambs and Munjal sheep, respectively. The findings of the current study disagree with Kumar *et al*. (2023) for the significant effect of the genotypes on measured traits except for body weight and body length. Furthermore, disagree with Al Qasimi that reported non-significant of genotypes on body length and heart girth in Awassi lambs at weaning. Differences could be attributed to use of different sheep breeds.

The results of the current study agree with Kumar *et al*. (2013) that reported higher body weight for AA genotype compared to other genotypes. GG genotype for SNP 855G>C of *IGF1* gene might be used as a genetic marker when improving body weight, heart girth, withers height, sternum height, rump height and body length. Genotype AA of SNP 857>A of *IGF1* gene might be used as a genetic marker when improving body weight, heart girth, withers height, sternum height, rump height and body length.

CONCLUSION

DNA analysis revealed 2 nonsynonymous SNPs (855G>C, 857>A) on 5' flanking region of *IGF1* gene of Bapedi sheep, that caused a change in amino acids glutamine to histidine and arginine to glutamine respectively. There marker-assistant selection suggests that there was an association in SNP 855G>C between genotypes and morphometric traits with genotype GG having high impact in all traits. There was an association in SNP 857>A between genotypes and morphometric traits with AA having high impact in all traits. The positive effect of the *IGF1* gene on morphometric traits as observed in this study suggests that this area of the ovine *IGF1* gene is particularly important and warrants further investigation.

ACKNOWLEDGEMENT

The authors are thankful to the Bapedi Sheep Breeder Society and Limpopo Department of Agriculture and Rural Development for allowing us to conduct our study using their animals. The authors are also thankful to National Research Foundation (PMDS230508103565) for financially supporting the study.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions.

Informed consent

All procedures were performed following the standards and protocols set by the University of Limpopo Animal Research Ethics Committee (AREC) project number: AREC/46/2023: PG. This approval from the AREC underscores the commitment to ensuring the humane treatment and welfare of the animals involved in the study.

Conflict of interest

All authors declare that they have no conflict of interest.

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