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Genetic variability at exon 2 of *inhibin alpha* locus in malabari goats of Kerala

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Abstract

The present study included 207 Malabari goats from six centers viz., University Goat and Sheep Farm, Mannuthy and 5 field centres of the ICAR-All India Coordinated Research Project on Goat Improvement (Malabari). The study was planned to detect single nucleotide variations in inhibin alpha gene of Malabari goats. Genomic DNA was isolated from 207 animals and PCR was done to amplify 220 bp fragment of exon 2 region of inhibin alpha (INHA) gene. Single strand conformation polymorphism (SSCP) technique was used to detect single nucleotide polymorphisms (SNPs). The SSCP showed a similar pattern of two bands (CC) for all samples and the population was found to be monomorphic for the locus.

Key words*:Malabari goat, inhibin alpha, exon, SSCP*

The goat is an important livestock species and a major source of livelihood for rural people in many developing countries. Goats are distributed worldwide due to their adaptive nature to varied climatic conditions and ability to thrive on limited resources. The Malabari breed originated in Tellicherry (Thalassery on the Malabar Coast of Kannur district in North Kerala (delete comma) and is also popularly known as the Tellicherry goat. It is a dual purpose breed famous for low fat meat and high prolificacy. These animals are well adapted to the hot and humid conditions of the state. *Inhibin* gene, a member of transforming growth factor (TGF)-β super family, codes for a glycoprotein secreted mainly from the gonads. Inhibin protein is a disulfide-linked heterodimer composed of an α-subunit and either a βA-subunit (inhibin A) or a βB-subunit (inhibin B). It functions by inhibiting follicle-stimulating hormone (FSH) secretion from the anterior pituitary, thus regulating gonadal development and function. Suppression of *inhibin* increased the ovulation rate in laboratory and domestic animals. The increased level of *inhibin alpha* (*INHA*) is also associated with poor

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embryo quality. Thus *inhibin* plays a vital role in the regulation of fertility by their twin restrictive action on the process of folliculogenesis in the ovary and FSH secretion by pituitary (Makanji *et al*., 2014). Therefore, present study was carried out to identify the variations at nucleotide level in exon 2 of *INHA* gene in Malabari goats.

Materials and Methods

The study included 207 Malabari goats from six centers *viz*., University Goat and Sheep Farm, Mannuthy and 5 field centres of the ICAR-All India Coordinated Research Project on Goat Improvement (Malabari) *viz*., Kottakkal, Thalassery, Perambra, Thaliparamba and Palakkad. Blood (5ml) was collected from each animal in tubes containing EDTA as anticoagulant and genomic DNA was extracted using phenol chloroform method. The concentration, purity and quality of DNA were checked by NanoDrop spectrophotometer (Thermo Scientific, USA). The purity of DNA was verified by measuring absorbance at 260 nm and 280 nm. A 260/280 ratio of approximately 1.8 is generally accepted as "pure" for DNA (Sambrook and Russell, 2001). DNA samples that had good quality, purity and integrity were taken for PCR-SSCP analysis. The 220 bp fragment of exon 2 of *INHA* was screened using polymerase chain reaction - single strand confirmation polymorphism analysis and sequencing. PCR amplification (25 µl, final volume) was performed in thermal cycler (BIORAD, USA) using 50ng of caprine genomic DNA, 1xPCR buffer, 1.8mM $MgCl_2$, 0.2mM each dNTP, 10pM of each primer and 1U of *Taq DNA polymerase.* Primers (forward 5'-CTT CCC TCT GCC CTC TGC-3' and reverse 5'-CCT GGA TGT CAG TAC CAG CA-3') were designed based on caprine *INHA* gene sequence (GenBAnk Accession No: NC_030809.1-whole genome shotgun sequence) using Primer 3 (V.0.4.0) software. Conditions were one cycle at 95ºC for 3 min, followed by 35 cycles (30 sec at 94ºC, 45 sec at 58ºC and 45 sec at 72ºC), followed by 1 cycle at 72ºC for 5 min, stopped at 4ºC. Amplified PCR products were loaded into the wells of 2 per cent agarose gel with a standard 50 bp DNA ladder (GenerRuler, MBI Fermentas, Germany) as a marker to check the size of the fragment. Electrophoresis was carried out at the rate 6 V/cm in 1X TBE buffer. Gels were stained with ethidium bromide and visualised under UV light and documented in a gel documentation system (Bio-Rad, USA).

Genotyping of the samples was done by Single Stand Confirmation Polymorphism (SSCP). The amplified fragments were mixed with SSCP loading buffer in the ratio of 1:2 (6 µl sample with 12 µl dye), denatured at 95°C for 10 min and immediately snap chilled in ice. The products were run in 12% poly-acrylamide gel at 4°C for 3 h at 120 V. The composition of poly-acrylamide gel was 30% acrylamide/bis-acrylamide (29:1)–6 ml, 10% ammonium per sulphate–75 µl, TEMED–15 µl, 10X TBE–1.5 ml and nuclease free water-7.5 ml. Gels were stained with silver nitrate as per the procedure described by Byun *et al*. (2009) and SSCP fragments were visualised directly. Representative samples were sequenced to confirm the nucleotide sequence. The obtained sequence was subjected to BLAST analysis to retrieve similar sequences of other breeds of goats.

Results and Discussion

The concentration and purity of DNA were checked by NanoDrop spectrophotometer (Figure 1). Those samples with a 260/280 ratio between 1.7 and 1.8 were assessed by one per cent agarose gel electrophoresis and visualized with gel documentation system. The 220 bp fragment of *INHA* gene was amplified using the specific set of primers designed with Primer 3 and procured from Sigma Aldrich. The amplified products are displayed in figure 2.

The SSCP pattern for 220 bp fragment of *INHA* gene showed a similar pattern of two bands (CC) for all samples and the screened population was found to be monomorphic with respect to this fragment (Figure 3). The PCR products were sequenced to confirm the same. The BLAST analysis discovered only one sequence pertaining to *Capra hircus* breed - San Clemente chromosome 2, ASM170441v1

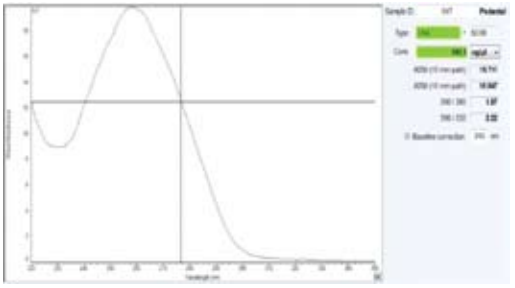

Figure 1. Concentration and optical density of DNA measured in NanoDrop spectrophotometer

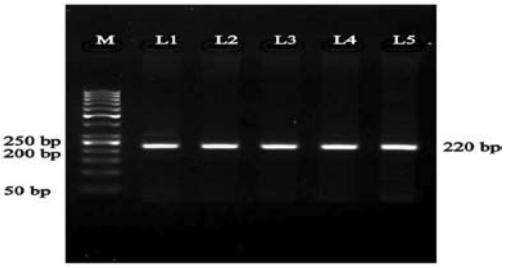

Figure 2. PCR amplification of 220 bp fragment of *INHA* gene (exon 2)
Lane 1-5: 220 bp product
Lane M: 50 bp DNA marker

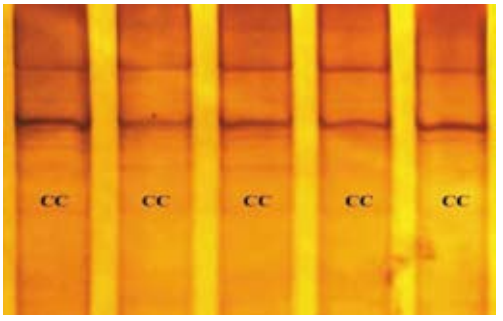

Figure 3. SSCP pattern of 220 bp fragment of *inhibin alpha* (CC genotype)

which had 100 per cent similarity. This result suggests the conserved nature of this fragment in the screened Malabari goat population of Kerala.

However Hua *et al*. (2007) reported a new mutation G284A by PCR-RFLP analysis using *Hae III* enzyme in Matou, Nubi, Boer and Haimen breeds of goat. He *et al*. (2009) observed three SNPs in exonic region of *INHA* gene in Haimen goats which were synonymous mutations. They noticed that nucleotide homology of *INHA* precursor gene ranged from 12.7% to 96.5% in goat, cattle, pig, human, chicken, horse, rat and dog. In Boer goats, Wu *et al*. (2009) reported twelve single nucleotide polymorphisms, including the non-synonymous variations at 911(*T/C)* and 946(*A/C)* position in exon 2 of *INHA* gene, resulting in corresponding amino acid change *Val/Ala* at 299th and *Thr/Pro at* 311th positions, respectively. A silent C865T mutation was also observed by He *et al*. (2010) in the exon 2 of *INHA* gene in Haimen, Boer, and Huanghuai goat breeds. They reported three genotypes BB, Bb and bb with frequency ranging from 0.00 to 0.02 for BB, 0.08 to 0.84 for Bb and 0.16 to 0.92 for bb. The allelic frequency of B was 0.04, 0.44 and 0.39 for Haimen, Boer, and Huanghuai goats, respectively. The allelic frequency of b was 0.96, 0.56 and 0.61 for Haimen, Boer, and Huanghuai goat, respectively. The bb genotype frequency was significantly higher in Haimen goats compared to other goat breeds. Studies done by Isa *et al*. (2017) revealed two variations in the second exon of *INHA* gene at 2518(G>A) and 3041(A>G) in West African Dwarf (WAD), Red Sokoto (RS) and Kalahari Red (KR) breeds of goat. Out of these g.2518G>A was detected only in Kalahari Red population. They noticed predominantly homozygous wild type GG (G>A) at 2581th position in the KR goats. Liu *et al*. (2017) detected an SNP in first exon of *INHA* gene in highly prolific Jining Grey breed and five medium prolific goats such as Boer, Liaoning Cashmere, Wendeng Dairy, Taihang and Mongolian Cashmere goats. They found G→A transiton at 759th position of the amplified region in genotype BB compared to Bb genotype.

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