

Original Research Article

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Investigation of *FecB* and *POUIF1* Gene Polymorphism in Assam Hill Goat

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ABSTRACT

The present study was carried out for investigation of polymorphism of *FecB* and *POUIF1* gene in Assam Hill goat. Blood samples pertaining to 80 randomly selected Assam Hill goats having kidding history of single as well as multiple births maintained at three field units viz., Batabari, Nahira and Tetelia under “AICRP on Goat Improvement” were utilized. DNA was extracted using modified phenol chloroform extraction procedure. The quantity and quality of extracted DNA were assessed using spectrophotometry and agarose gel electrophoresis. A 190 bp fragment of *FecB* gene and a 450 bp fragment of *POUIF1* gene were amplified using Polymerase Chain Reaction (PCR). RFLP analysis of *FecB* using *AvaII* enzyme revealed undigested 190 bp product for all the samples. Digestion of *POUIF1* with *DdeI* produced three fragments of 102 bp, 118 bp and 200 bp in agarose gel electrophoresis for all the samples revealing monomorphism. The *POUIF1* PCR-RFLP products were also visualized by loading on 12% PAGE in 0.5X TBE buffer which resulted in similar banding patterns. RFLP analysis of *POUIF1* gene was also performed by using *PstI*, resulting in undigested product of 450 bp. In sequence analysis, no restriction site was found for *AvaII* in *FecB* gene and four restriction sites were found for *DdeI* in *POUIF1* gene. Sequence analysis of the samples revealed 99-100% homology for both the genes among all the samples irrespective of litter size.

Keywords

Assam Hill goat,
FecB, *POUIF1*,
Polymorphism,
PCR-RFLP

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Introduction

India possesses the second largest goat population in the world with 135.17 million goats (26.40% of the country's total livestock) which corresponds to 15.68% of total goat population in the world (Basic Animal Husbandry and Fisheries Statistics, 2014). The goat population of Assam is 6.169

million contributing 4.56% of total goat population of India (Basic Animal Husbandry and Fisheries Statistics, 2014). The Assam Hill goat is one of the important meat type animals distributed throughout the state of Assam, which is characterized by small body size, shorter generation interval and high prolificacy with a higher percentage of multiple births. Almost the entire population

of North-Eastern region of India is non-vegetarian and chevon is the meat of choice. The chevon production in Assam in the year 2012-13 was 11,000 tonnes which was 20% of the total meat produced in the state (Basic Animal Husbandry and Fisheries Statistics, 2014). The gap between demand and production of meat could be bridged by augmenting the reproductive efficiency and growth performance of the animals. This can conventionally be achieved with the help of established methods of selection and breeding as well as with the modern molecular genetics techniques.

Candidate gene approach provide a good breeding tool that can enhance the frequency of multiple births early in life, which has been proposed as a direct search for Quantitative Trait Loci (QTL) to improve quantitative traits (Tambasco *et al.*, 2003). The information utility from candidate genes in breeding programs has potential to substantially enhance the accuracy of selection and increasing selection differential (Saleha *et al.*, 2012). Detection of genetic markers, along with mutants of the genes associated with economically important traits, could assist the breeders in designing practical animal breeding plans (Davis *et al.*, 2006). Studies have revealed that the ovulation rate and litter size of domesticated sheep could genetically be regulated by a set of different genes, collectively named as *Fec* genes. *FecB* and Pituitary Transcription Factor 1 (*POUIF1*) are important candidate genes affecting growth and reproduction in small ruminants (Supakorn, 2009).

FecB gene was first identified in Booroola ewes by Piper and Bindon (1982). It is a single autosomal gene in chromosome number 6 in sheep which is the main reason for higher prolificacy of certain breeds (Montgomery *et al.*, 1994). Original source of Booroola Merino sheep *FecB* gene is the

Garole sheep from Sunderban (West Bengal) area of India. *FecB* gene has effects on granulosa cell maturation, oocyte development and its function (Abraham and Thomas, 2012). Each mutant *FecB* gene results in an increase to the ovulation rate of 1.6 times (Montgomery *et al.*, 2001). The *FecB* locus contain a conservative substitution mutation Q249R (CAG®CGG), in a highly conserved intracellular kinase signaling domain of the bone morphogenetic protein receptor -1B (*BMPR-1B*), has been associated with the hyper-prolific phenotype of Booroola ewes (Mulsant *et al.*, 2001).

POU domain, class 1, Transcription factor 1 (*POUIF1*) is otherwise known as *PIT 1* and GH factor 1 (Supakorn, 2009). Wollard *et al.*, (2000) stated that *POUIF1* gene is located on chromosomes 1q21-22 and comprises 6 exons containing the POU domain. *POUIF1* gene is a positive regulator of growth hormone, prolactin and thyroid stimulating hormone in mammals (Cohen *et al.*, 1997). The published reviews have reported that genetic polymorphisms of *POUIF1* gene were significantly associated with growth, development and lactation in swine, bovine and caprine (Li *et al.*, 1990).

Since *FecB* and *POUIF1* gene have been found to be responsible for prolificacy and, the tendency to twinning and triplet is inherited in both sheep and goats, the present study was carried out to identify the polymorphism of these genes in Assam Hill goats for its possible association with prolificacy and growth performance.

Materials and Methods

Collection of blood and extraction of DNA

A total of 80 blood samples from randomly selected Assam Hill goats having kidding history of single as well as multiple births,

maintained at three field units *viz.*, Batabari, Nahira and Tetelia under 'AICRP on Goat Improvement', Goat Research Station, Burnihat were utilized in the present study. Out of these, 10 samples from animals with history of single birth were taken as control. Five ml of blood was collected aseptically from the jugular vein in a vacutainer tube containing 2.7% EDTA as anticoagulant. The samples were brought to the laboratory in double walled ice-boxes containing ice packs and stored at -20°C until the genomic DNA was extracted. Genomic DNA was extracted using phenol chloroform extraction procedure (Sambrook and Russell, 2001) with slight modifications by using DNAzol reagent instead of SDS and Proteinase K. The purity of genomic DNA was assessed by UV spectrophotometer (Nanodrop Spectrophotometer, Model: UV/VIS 916) and Optical Density (OD) values were measured at 260 and 280 nm with TE buffer as blank. The concentration of genomic DNA was estimated spectrophotometrically by taking OD value at 260 nm. Quality of isolated genomic DNA samples was checked by using agarose gel electrophoresis which was visualized under gel documentation system (Kodak 100).

PCR amplification

The primer pairs F: 5'-CCAGAGGAGAA TAGCAAAGCAA3' and R: 5'CAAGATG TTTTCATGCCTCAT CAACACGGTC3' (Jamshidi *et al.*, 2013) and, F: 5'-CCATCAT CTCCCTTCTT-3' and R: 5'-AATGTACA ATGTGCCTTCTGAG-3' (Lan *et al.*, 2007) were used to amplify *FecB* and *POUIF1* gene, respectively. PCR was carried out in 50 µl volume containing 1 µl of 10pmol/µl each primer, 2 µl DNA template, 1 µl MgCl₂, 25 µl Master Mix and 20 µl Nuclease Free Water. Amplification conditions for the two genes were as follows;

The obtained PCR products were separated and confirmed by horizontal submarine agarose gel electrophoresis (1.5%) in 1X TAE buffer at 110V using 100 bp DNA ladder.

Restriction Fragment Length Polymorphism (RFLP) analysis

The PCR products (20 µl) of *FecB* gene were digested with restriction enzyme *AvaII* (New England Biolab, UK). For *POUIF1* gene, two restriction enzymes *DdeI* and *PstI* (New England Biolab, UK) were used. The reaction mixture was vortexed for few seconds for uniform mixing and then incubated at 37° C for overnight. The enzyme digested products were loaded @ 10 µl on 2.5% agarose gel. Electrophoresis was carried out at 110 V for 1 hour and 15 minutes and the bands were visualized and documented using gel documentation system. The bands were analyzed by comparing with 50 bp DNA ladder. In case of *POUIF1* gene digested by *DdeI*, the digested products were also visualized by loading on 12 % PAGE in 0.5X TBE buffer. Electrophoresis was carried out at 100 V for 3 hours.

Sequence analysis

The PCR products were sent for sequencing to first base DNA sequencing division, Malaysia. The sequences were analyzed by using Clustal W method of DNASTAR Software (Lasergene, USA) to generate sequence alignment reports and residue substitution.

Results and Discussion

A single band on agarose gel confirmed the extraction of DNA. The yield of DNA extracted from 2 ml of whole blood ranged from 106 ng/µl to 247 ng/µl with a mean of 181 ng/µl. The OD ratio was in the range of 1.7-1.9 indicating purity of extracted DNA.

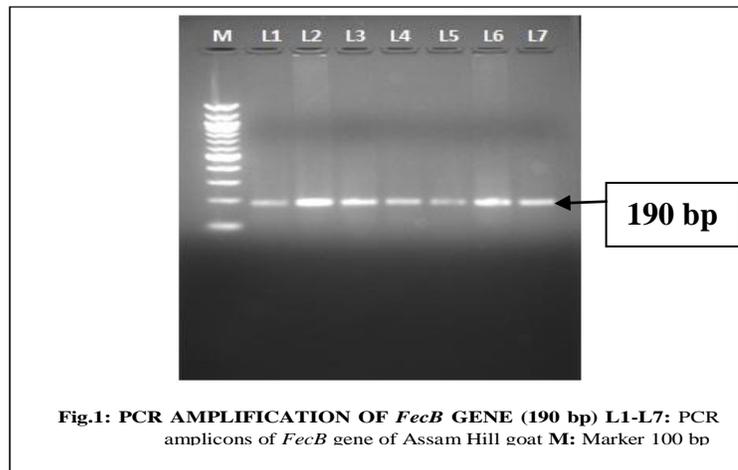
Amplification of *FecB* gene resulted in generation of 190 bp DNA fragment (Fig 1) which is consistent with the expected size as reported by Jamshidi *et al.*, (2013) in Sangsari sheep of Iran and Davis *et al.*, (2002) in prolific sheep breeds from eight countries. Amplification of *POUIF1* gene resulted in generation of 450 bp product (Fig 2) of the exon 6 and partial intron 5, 3' UTR region which is in agreement with the results reported by Lan *et al.*, (2007) in Inner Mongolia White Cashmere goats.

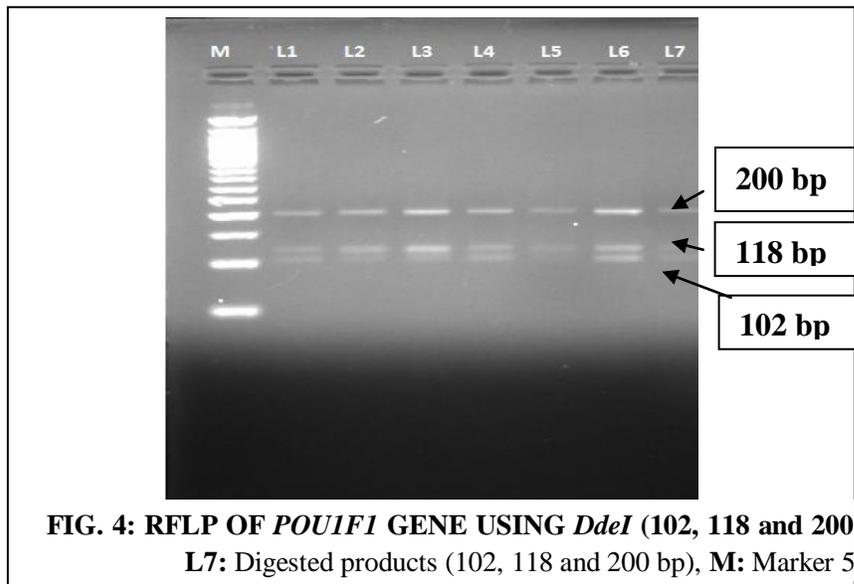
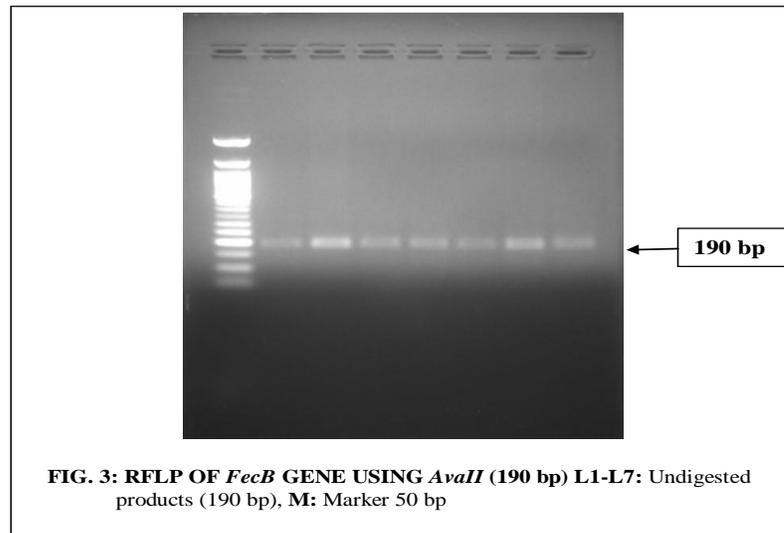
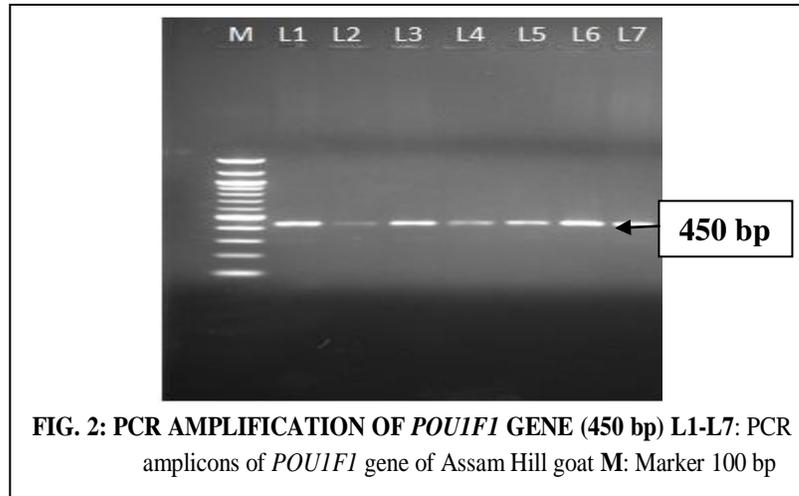
The RFLP technique was used to identify the variants in *FecB* gene based on the variants produced by digestion of 190 bp amplified product with restriction enzyme *AvaII*. The restriction enzyme was not able to fragment the amplicon of 190 bp as shown in Fig 3. The amplified 190 bp fragment of *FecB* gene

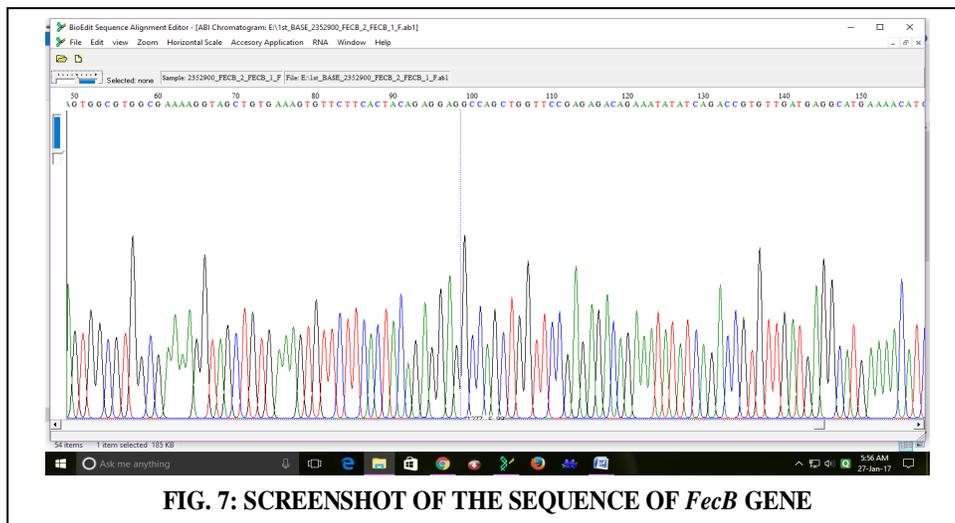
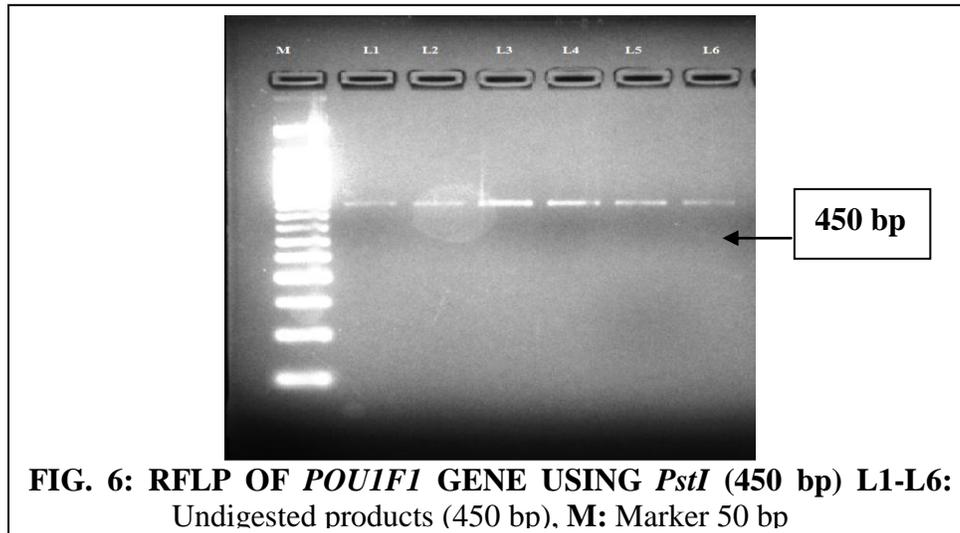
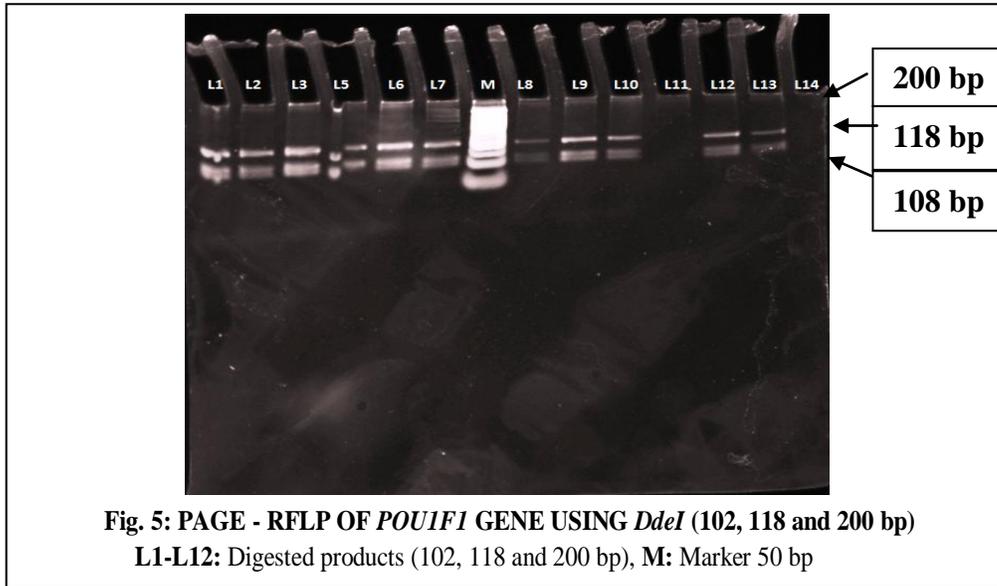
upon *AvaII* digestion produced a single band of 190 bp. All the animals under the study were found to be monomorphic. The results revealed the absence of mutant type B nucleotide, indicating that the investigated Assam Hill goats were wild homozygous type. Similar results are also reported by Hua *et al.*, (2007) in Boer, Haimen, Boer x Huanghuai goat, Huanghuai, Nubi and Matou goat, and Jamshidi *et al.*, (2013) in Sangshari sheep. Reports on Jining Grey goats, Boer goats, Wending dairy goats, Liaoning Cashmere goats, Inner Mongolia Cashmere goats, Beijing native goats (He *et al.*, 2006) and Raighar goats (Palai *et al.*, 2013) state the same view of absence of polymorphism in *FecB* gene. However, *FecB* mutation is present in Garole (Davis *et al.*, 2002) and Hu (Davis *et al.*, 2006) sheep.

Table.1

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension
<i>FecB</i>	94°C for 5 minutes	94°C for 15 seconds	60°C for 30 seconds	72°C for 30 seconds	72°C for 5 minutes and 99°C for 15 minutes
	35 cycles				
<i>POUIF1</i>	94°C for 5 minutes	94°C for 45 seconds	54.5°C for 45 seconds	72°C for 1 minute	72°C for 10 minutes
	35 cycles				







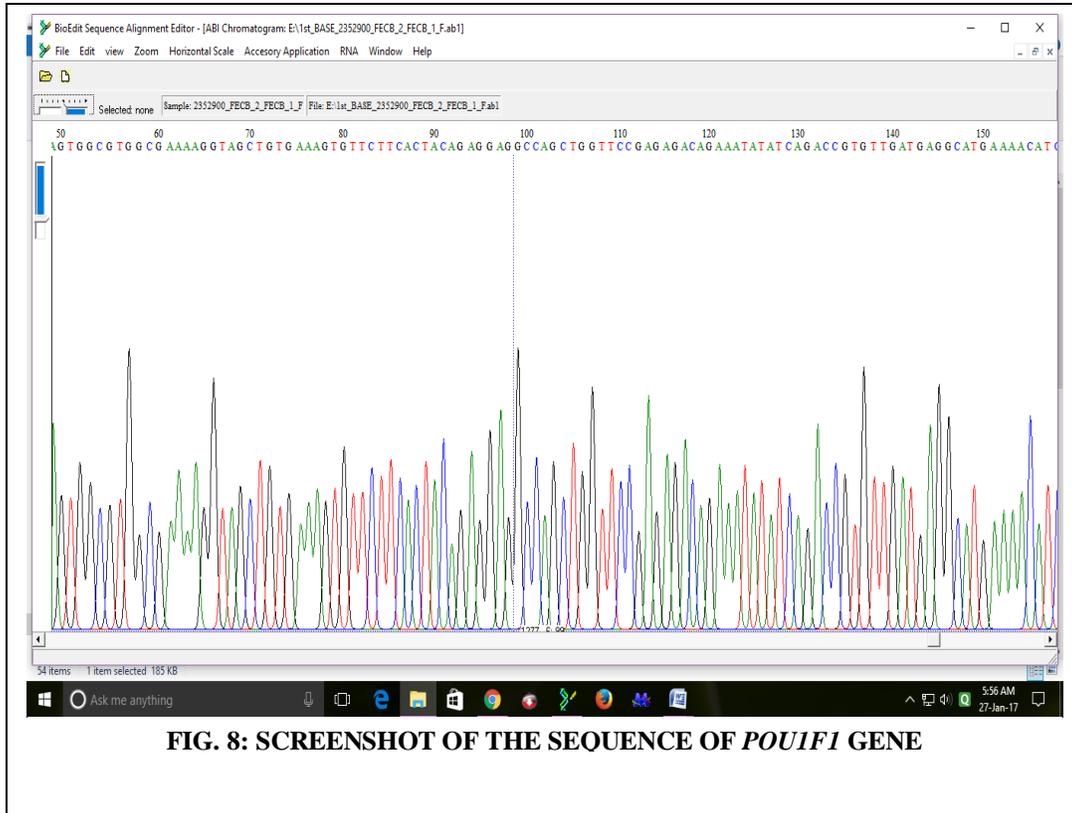


FIG. 8: SCREENSHOT OF THE SEQUENCE OF *POU1F1* GENE

The amplified 450 bp fragment of *POU1F1* upon *DdeI* digestion was expected to produce five fragments of 200, 118, 102, 20 and 11 bp. However, only three bands of 200, 118 and 102 bp were visible (Fig. 4). The remainder two bands were not visible both in agarose gel electrophoresis as well as in 12.0% PAGE (Fig. 5) owing to their small sizes. These findings are in accordance with the findings of Li *et al.*, (2016) in 709 indigenous Chinese goats. All the animals under study revealed monomorphic banding patterns. However, the reports of Lan *et al.*, (2007) state polymorphism in exon 6 and its flanking region in Chinese goats. Another restriction enzyme *PstI* treated PCR products resulted in undigested 450 bp product (Fig 6) which is similar to the findings observed by Sharma *et al.*, (2013) in Barbari goats using PCR-RFLP methods. The *PstI*/PCR-RFLP assay of the 450 bp PCR product indicates the presence of single genotype (450 bp) with a genotypic

frequency of 1.0 as no polymorphic band pattern was observed. On the contrary, Saleha *et al.*, (2012) in their study on Barki, Zaribi, Ardi and Masri breeds of goat in Egypt and Saudi observe two different banding patterns, undigested product of 450 bp and digested product with two fragments of 370 and 80 bp after digestion by *PstI*; thus, all samples of goat were typed as allele T (450 bp) and allele C (370 and 80 bp) with genotype TT and CC. So, the band pattern obtained after digestion of *POU1F1* with *PstI* in Assam Hill Goat indicates that all the animals under study revealed TT genotype.

The partial sequences of *FecB* and *POU1F1* gene were analyzed by BLAST. No restriction site was found for *AvaII* in *FecB* gene and four restriction sites were found for *DdeI* in *POU1F1* gene. All the sequences of the *FecB* and *POU1F1* genes showed 99-100% similarity among all the 24 sequenced

samples irrespective of variation in kid size and growth.

The present findings of monomorphic banding patterns indicate that the amplified fragments of *FecB* and *POUIF1* gene have no affinity for greater prolificacy in Assam Hill goats. The high prolificacy which was evident from the collected data may be due to some other genes or some other factors which are yet to be explored. This study has highlighted the importance of further investigation for the genes influencing reproductive performance in these goats. Therefore, there is a need to undertake a further research on substantially large number of individuals in Assam Hill goat.

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