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Polymorphism of *FecB* Gene in Black Bengal Goat by PCR-SSCP method

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Abstract

The objective of present study was to search for *FecB* gene polymorphism in Black Bengal goats. Blood samples were collected from 96 animals. Genomic DNA was isolated using phenol-chloroform extraction method. The quantity and quality of isolated DNA was checked by agarose gel electrophoresis and spectrophotometery. W ith the primers having forward and reverse base sequence as 5'-CCAGAGGACAATAGCAAAGCAAA-3' and 5'-CAAGATGTTTTCATGCCTCATCAACAGGTC-3' respectively, PCR products of 190 bp were obtained. For genotyping, amplified products were subjected to polyacrylamide gel electrophoresis for the detection of Single Strand Conformational Polymorphism (SSCP). As a result, three different SSCP variants were found which were designated as AA, AB and BB. The highest genotype frequency was observed for AB (0.38), which was followed by BB (0.33) and AA (0.29). Nucleotide sequences of the allelic variants were also analyzed. The DNA sequences showing polymorphism observed were used to identify SNPs. Principal SNP was found at 78 position of gene sequence, which shows transition of Adenine (AA genotype) to Guanine (BB genotype).

Key Words : FecB, Black Bengal Goat, PCR-SSCP

Introduction

In India, goat is the major meat source. Therefore, not only growth traits but also an increase in proliferation is important for improvement of the goat's performance. Unfortunately, the genetic mechanism and genetic markers for caprine proliferation gene has yet to be explored. A study of *FecB* gene as a genetic marker could lead to breeding goats with more fecundity.

The *FecB* (Booroola fecundity) gene is the first major gene to be described that affects ovulation rate and proliferation in sheep. It was first identified in 1980s. In addition, this gene has been shown to be involved in *FecB*, *FecX[1]*, *FecX[H]*, *FecX[B]* and *FecX[G]* (Davis, 2004). The Booroola fecundity gene (*FecB*) is a single autosomal gene, which increases ovulation rate and litter size in sheep [co-dominant for ovulation rate & partially dominant for litter size (Piper *et al.*, 1985; Montgomery *et al.*, 1992)]. Piper *et al.* (1985) and Piper and Bindon (1996) found that the effect of *FecB* mutation is additive for ovulation rate and each copy increases ovulation rate by about 1.6 and approximately one to two extra lambs in Booroola Merinos.

High prolificacy in Booroola sheep is due to a non-conservative mutation (q249r) in a highly conserved intracellular kinase signaling domain of the bone morphogenetic protein receptor-1B (BMPR-1B) expressed in the ovary and granulosa cells (Mulsant *et al.,* 2001; Wilson *et al.*, 2001). Thus, considering the above mentioned points, the present research has been designed to study the polymorphism of *FecB* gene in Black

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Bengal Goats.

Materials and Methods

For present investigation, blood samples (5 ml each) were collected from the jugular vein from a total of 96 Black Bengal Goats. These samples were collected in vaccutainer tubes. An anticoagulant (EDTA) was mixed in blood. Genomic DNA was isolated and purified from white blood cells using proteinase-K digestion and standard phenol-chloroform extraction as per the standard protocol described by Sambrook *et al.* (1989). 20 ml of stock solution of DNA was diluted serially to a concentration of 50-100ng/µl as working stock for further analysis. Rest of the stock solution of DNA was stored at -20°C. Quality and quantity check of isolated genomic DNA sample was done by agarose gel electrophoresis. On completion of electrophoresis, the gel was visualized under UV trans-illuminator (Bio-Rad) and quality and quantity was judged. A pair of synthetic oligonucleotide (primers) was required to prime DNA synthesis. Forward and reverse base sequence of primers used were as 5'-CCAGAGGACAATAGCAAAGCAAA-3' and 5'-CAAGATGTTTTCATGCCTCAT CAACAGGTC-3' (Supakorn *et al.,* 2010) respectively. The product size of *FecB* gene was 190 bp. (W ilson *et al*., 2001). Primers were synthesized by Xcelris Lab, Ahmedabad.

After optimization of PCR for proper temperature range, final PCR was run which was followed by SSCP through polyacrylamide gel electrophoresis performed at 4^0 C for 4 hours at 200 V. After running was over, gel was kept for silver staining according to Bassam *et al.* (1991). The gel was visualized for banding pattern under light and documented. Selected samples were sent for sequencing (Xcelris Lab, Ahmedabad) to confirm polymorphism. Sequencing results were analyzed with MegAlign software included in DNASTAR (Lasergene 10).

Results and Discussion

In the present investigation, PCR-SSCP studies were carried out on BMPR1B gene for detection of the point mutation of *FecB* gene in Black Bengal Goats. Gupta *et al.* (2005) reported that the PCR-SSCP method is one of the best method employed in detection of SNPs and nucleotide base change.

PCR-SSCP analysis of FecB Gene : Three different SSCP variants were found which were designated as AA, AB and BB (figure 1). Similar findings were reported by Polley *et al.* (2009) who worked upon BMPR1B gene (*FecB*) polymorphism in Black Bengal Goat and reported three types of genotype in *FecB* region. Chu *et al.* (2010) also found polymorphism of BMPR1B gene and obtained three types of genotypes in Jining Grey goats which support our current finding.

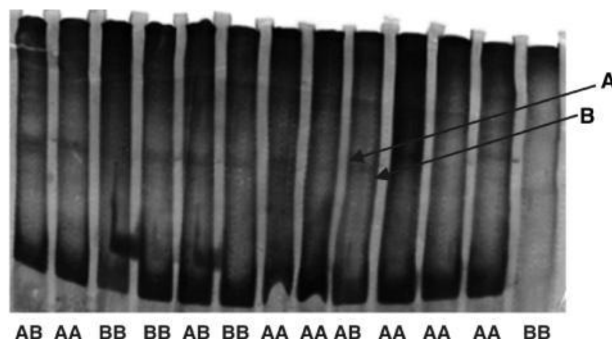

Figure 1: Different genotypic pattern of *FecB* gene

Genotype Frequency: In Black Bengal goat population, the highest genotype frequency was observed for AB (0.38), which was followed by BB (0.33) and AA (0.29) (Table 1).

Table 1: Allelic and genotype frequencies of Black Bengal goat for *FecB* gene

Gene	No. of Animal	Allele frequency		Genotype frequency		
		A	B	AA	AB	BB
FecB	96	0.48	0.52	0.29	0.38	0.33

Sequence analysis of FecB gene: The PCR products representing different SSCP (namely for genotype AA and genotype BB) patterns in Black Bengal Goat resource population of present study were directly sequenced using DNA sequencing service (Xcelaris lab, Hyderabad).

From the reverse and forward sequence a complete sequence of genotype AA (33_PF_S6986_F11_085.ab1 and genotype BB (61_6PF_S6292) was formed. The nucleotide sequence alignments were carried out using alignment tools, ClustalW in MegAlign embedded in DNASTAR software.

Nucleotide sequences of the allelic variants were analyzed. The DNA sequence polymorphism was observed at position 78 of the nucleotide base, which showed the transition of Adenine to Guanine (Figure 2). The *FecB* locus is situated in the region of ovine chromosome 6 corresponding to the human chromosome 4q22-23 (Montgomery *et al.,* 1993) that contains the bone morphogenetic protein receptor-1B (BMPR1B) gene. The Adenine to Guanine transition at nucleotide position 746

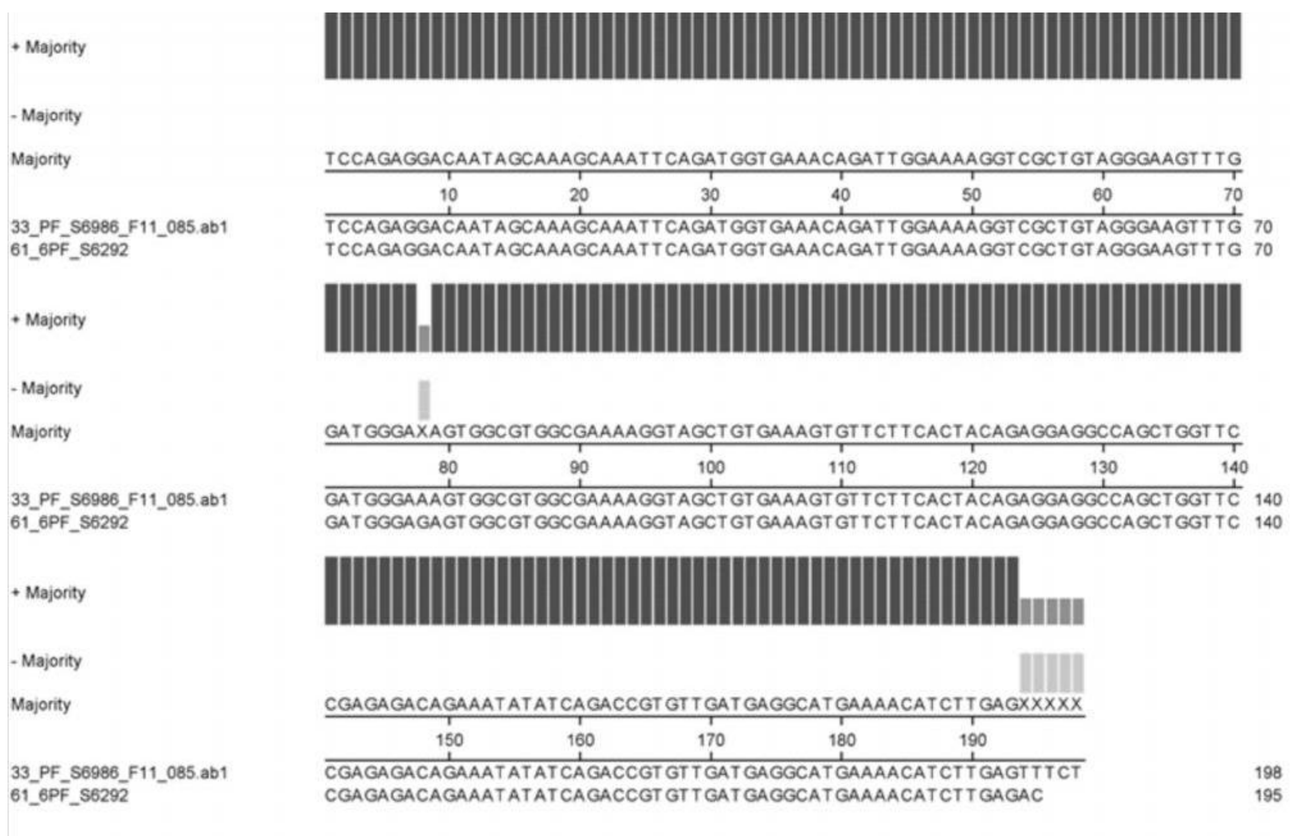

Figure 2: Sequence analysis of *FecB* gene

of the cDNA sequence induces a non-synonymous substitution of glutamine with an arginine corresponding to position 249 of the mature protein (Q249R, Souza *et al*., 2001; Wilson *et al*., 2001; Mulsant *et al*., 2001).

Summary and Conclusion

Presence of polymorphism in *FecB* gene is indicative of gene flow among different variants of Black Bengal goat. Further the allelic variants can be used for marker assisted selection (MAS) based on their superiority with respect to different economic traits. Pattern of inheritance of *FecB* gene along with nature of dominance of the gene could help strengthen the cause of MAS in Black Bengal Goats.

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References

Bassam *et al.* 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.* **196** : 80-83.

Chu *et al.* 2010. Polymorphisms of BMPR-IB gene and their relationship with litter size in goats. *Molecular Biology Reports* **37(8)** : 4033-4039

Davis G.H. 2004. Fecundity genes in sheep. *Anim. Reprod. Sci.* **82–83** : 247–253

Gupta *et al*. 2005. Single Strand Conformational Polymorphism–technique for SNP and mutation detection. Molecular Technique for Gene Characterization and genome analysis. *Indian J. Anim. Sci*. **31:**146-151.

Harvey W.R. 1990. Mixed Model Least Squares and Maximum Likelihood Computer Program. User's Guide for LSMLMW and MIXMDL.

Markoff *et al*. 1997. Optimzation of single–strand conformation polymorphism analysis in the presence of polyethylene glycol. *Clinical Chemistry* **43(1) :** 30-33.

Montgomery *et al.* 1992. Physiology and molecular genetics of mutations that increases ovulation rate in sheep. *Endo. Rev.,***13(2):**309-327

Montgomery *et al.* 1993. The ovine Booroola fecundity gene (*FecB*) is linked to markers from a region of human chromosome 4q. *Nat. Genet.*, **4** : 410–414

Mulsant *et al.* 2001. Mutation in the bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola ewes. *Proc. Natl. Acad. Sci.* **98** : 5104–5109

Piper L.R. and Bindon B.M., 1996. The Booroola Merino. *Prolific Sheep* 152–160

Piper L.R., Bindon B.M. and Davis G.H., 1985. The single gene inheritance of the high litter size of the Booroola Merino. *Genetics of Reproduction in Sheep* 115–125

Polley *et al.* 2009. Polymorphism of fecundity genes (BMPR1B, BMP15 and GDF9) in the Indian prolific Black Bengal goat. *Small Ruminant Research* **85** : 122–129

Sambrook *et al.* 1989. Molecular cloning : A Laboratory Manual 2nd ed. *Cold spring Harbour, Cold Laboratory press, NY*

Smith *et al.* 1993. Effects of the Booroola gene (*FecB*) on body weight, ovarian development and hormone concentrations during fetal life. *Journal of Reproduction and Fertility* **98** : 41–54.

Souza *et al.* 2001. The Booroola (*FecB*) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (BMPR1B) gene. *Journal of Endocrinology* **169** : R1–6.

Supakorn C. and Pralomkarn W., 2010. Sheep *FecB* Gene Polymorphism Role In Thai Meat Goat Proliferation Rate. In:*Proceedings of the 9th World Congress on Genetics Applied to Livestock Production, Leipiz, Germany,2010.*

Wilson *et al.* 2001. Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biology of Reproduction* **64:**1225-1235.