

A STUDY OF PROTEASE INHIBITOR GENE POLYMORPHISM  
IN MEHSANA BUFFALO (*Bubalus bubalis*)

Manisha Deshpande, D.N. Rank, P.H. Vataliya and C.G. Joshi

### ABSTRACT

Protease inhibitor (PI) genes belong to the superfamily of serine protease inhibitors that includes C1 esterase, antithrombin, and  $\alpha$ 1-antichymotrypsin, among others. The primary role of PI is to protect tissues against proteolytic digestion by neutrophil elastase. PI is also found to be associated with increased milk and fat yields, increased productive life and decreased somatic cell score. The present study was carried out to reveal PCR-RFLP polymorphism of protease inhibitor exon 2 fragment in Mehsana buffaloes. The buffaloes were registered under the progeny testing programme of the Dudhsagar Research and Development Association, Mehsana, Gujarat state (India). The fragment of 448 bp of PI gene (exon 2) locus was amplified by PCR and subsequently digested with restriction enzyme *Sfa*NI. It revealed monomorphic patterns. Further representative samples were cloned and sequenced. On comparison with published cattle sequences, the nucleotide sequence variation between cattle and buffalo was present at ten nucleotide positions, i.e., 110, 141, 146, 170, 236, 257, 277, 306, 350, and 396.

**Keywords:** Mehsana buffaloes, *Bubalus bubalis*, PCR-RFLP, PI gene, protease inhibitors, gene polymorphism

### INTRODUCTION

The present study aimed at exploring polymorphism in the PI gene by PCR-RFLP sequencing and its association with production traits in the Mehsana buffalo. Very scanty information is available on PI gene polymorphism in Indian buffaloes (*Bubalus bubalis*).

It has been reported that the PI gene is a candidate gene for production traits in dairy cattle (Khatib *et al.*, 2005). The candidate gene approach consists of the study of different genes potentially involved in physiological processes (e.g., milk protein synthesis and milk fat synthesis) and the identification for each gene of the allele responsible for the desired phenotype.

The protease inhibitor (PI) genes belong to the superfamily of serine protease inhibitors that includes C1 esterase, antithrombin and  $\alpha$ 1-antichymotrypsin among others (Barra *et al.*, 1987; Anderson and Kingston, 1983; Blank and Brantly, 1994). Khatib *et al.* (2005) reported five SNP in coding regions of the protease inhibitor (PI) gene by direct sequencing of reverse transcription-polymerase chain reaction products from a wide range of cattle tissues. A total of six different intragenic haplotypes were identified in the North American Holstein population, and these were examined for associations with milk production traits in 24 half-sib families comprising 1007 sons

---

Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat 388001, India

utilizing a granddaughter design. One common haplotype was associated with increased milk and fat yields, increased productive life and decreased somatic cell score. Another common haplotype was associated with decreased productive life and increased somatic cell score. One rare haplotype was associated with decreased milk, fat, and protein yields and increased milk protein percentage; another rare haplotype was associated with decreased milk yield, increased protein percentage, and decreased productive life.

## MATERIALS AND METHODS

### Buffalo population, sampling and DNA extraction

To analyze PI/*Sfa*NI polymorphism, blood samples were collected randomly from 60 unrelated Mehsana buffaloes registered under the progeny testing programme of the Dudhsagar Research and Development Association, Mehsana, Gujarat state. DNA was extracted using a standard protocol by phenol: chloroform extraction procedure (Sambrook *et al.*, 1989).

### Molecular genotyping

A primer pair reported by Khatib *et al.* (2005) **F:** ATG GCA CTC TCC ATC ACG CG, **R:** CCA CTA GCT TTG CAC TCT CA was used to amplify the 448 bp region of PI exon 2.

PCR was carried out in a final reaction volume of 25 µl. Amplification cycling conditions for PI involved initial denaturation at 94°C for 10 minutes, followed by 35 cycles at 94°C for 1 minute, 56°C for 45 seconds and 72°C for 1 minute with a final extension at 72°C for 10 minutes (Figure 1).

For the PCR-RFLP analysis, 10 µl of each PCR amplified product was digested with 5 units of the *Sfa*NI 5'-G C A T C (N)<sub>5</sub><sup>-</sup>3' in a 30 µl

total reaction and incubated in a water bath at 37°C for 16 h. The digestion products were separated by electrophoresis on a 2% agarose gel in 0.5 X TBE buffer.

### Cloning and sequencing

PCR products from representative sample (448 bp) were purified and cloned in pTZ57R/T vector. Ligated recombinant vector was transformed in competent *E. coli* (DH5-α) cells. Recombinant plasmids were extracted and used for cycle sequencing and were subjected to automated DNA sequencing.

Sequence data obtained were analyzed *in silico* by employing software tools, viz. NCBI BLAST, SeqScape and ClustalW, to access the genetic variation.

## RESULTS AND DISCUSSION

On screening the PI/*Sfa*NI in the 60 Mehsana buffalo individuals, all the samples showed an identical restriction pattern with RE site at 242 bp and 366 bp on both chromosomes resulting in the appearance of three bands of 242 bp, 124 bp, 82 bp with BB genotype (Figure 2). This result indicates that there was no *Sfa*NI polymorphism at the PI locus in Mehsana buffalo. Allele B for PI is fixed in Mehsana buffaloes. As there is no polymorphism association, studies for production traits were not undertaken.

The RFLP results are not in accordance with Khatib *et al.* (2005) as there was no *Sfa*NI polymorphism at PI locus in Mehsana buffalo. Khatib *et al.* (2005) reported five SNP in coding regions of the protease inhibitor (PI) gene. Published reports for PI/*Sfa*NI polymorphism in buffaloes were not available to compare with till preparation of this manuscript.

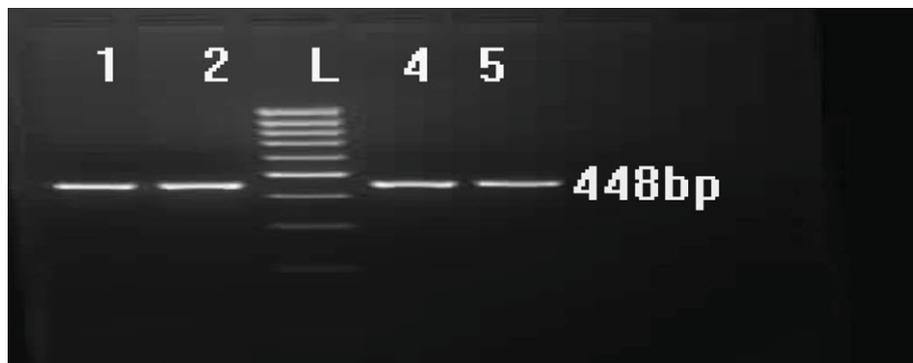


Figure 1. PCR product of protease inhibitor exon 2 locus.

Lane 1, 2, 4, 5: 448 bp PI gene product.

Lane 3: 100 bp ladder.

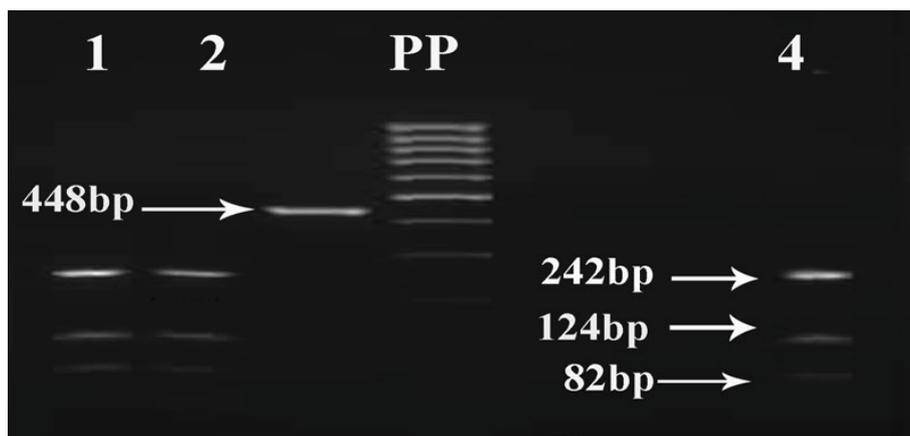


Figure 2. PI exon 2 -SfaNI RFLP: 448 bp PCR fragment in Mehsani buffalo digested by SfaNI and electrophoresed on 2% agarose in 0.5 X TBE at 80 V.

Lanes 1,2: PI /SfaNI digest with three fragments of 242 bp, 124 bp, 82 bp.

Lane 3: PCR product of 448 bp.

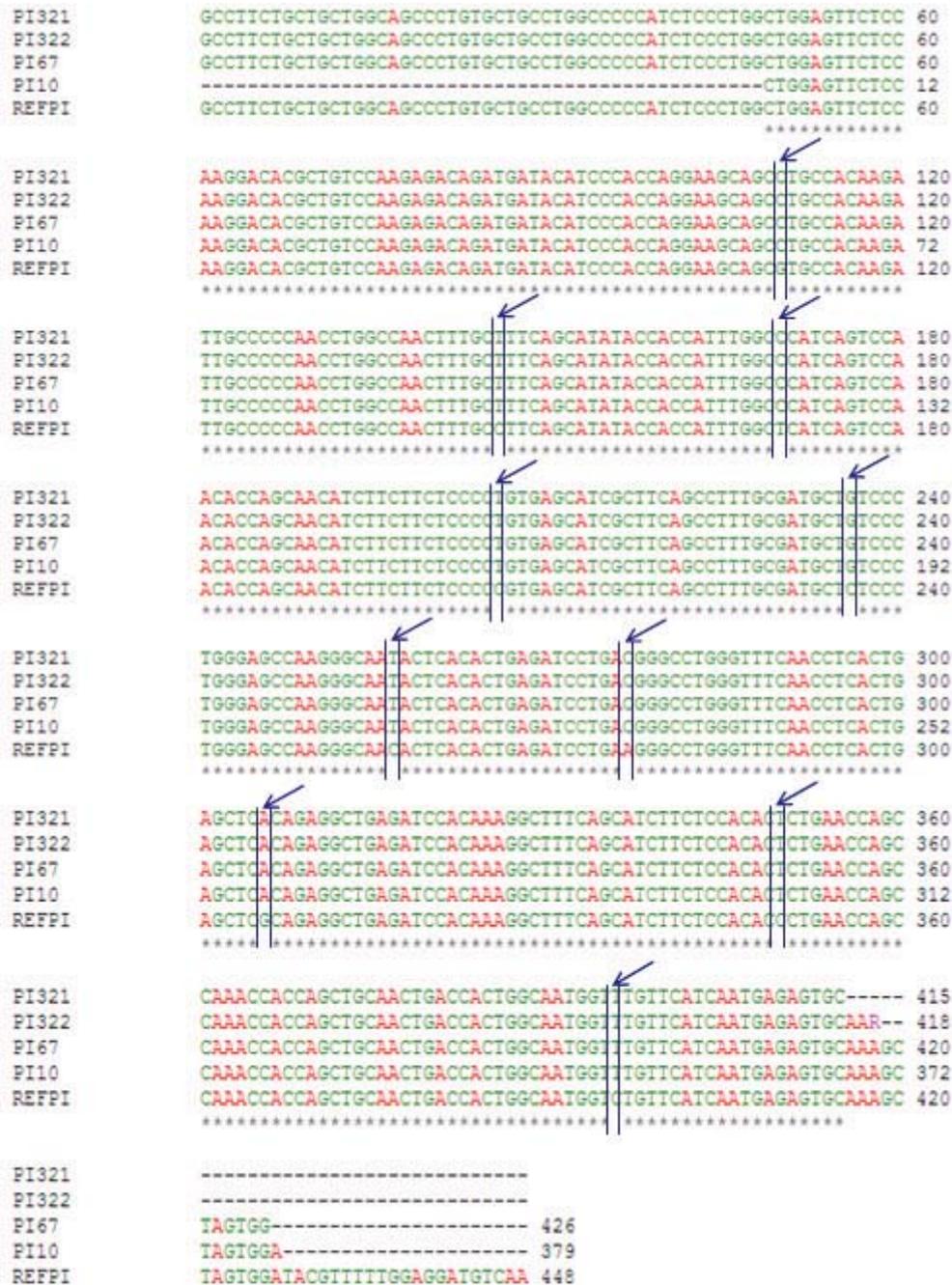


Plate 1. Clustal W results of PI exon 2.

Representative samples from Mehsana buffaloes for PI locus were sequenced and forward and reverse sequences were assembled along with reference sequences on *Seq Scape* software. A consensus sequence of 426 bp was obtained. Consensus sequence was then aligned with published in GenBank for PI gene exon-2 in cattle using NCBI BLAST, ClustalW programme revealed strong homology of 94 % to 98 % (Table 1). There was no sequence available in Genbank database for buffaloes until this manuscript was prepared to compare with. The nucleotide sequence variation between cattle and buffalo was present at ten nucleotide positions, i.e., 110,141,146,170, 236, 257, 277, 306, 350, and 396 (Plate 1 ).

PI (exon2) nucleotide sequences were submitted to NCBI Genbank database accession number GQ385225.

### REFERENCES

Anderson, S. and I.B. Kingston. 1983. Isolation

of a genomic clone for bovine pancreatic trypsin inhibitor by using a unique-sequence synthetic DNA probe. *Proc. Natl. Acad. Sci. U.S.A.*, **80**: 6838-6842.

Barra, D., S.M. Simmaco, S.F. Bossa, E.F. Angelettill and A. Franca. 1987. Primary structure of a protease isoinhibitor from bovine spleen: a possible intermediate in the processing of the primary gene product. *J. Biol. Chem.*, **262**(29): 13916-13919.

Blank, C.A. and M. Brantly. 1994. Clinical features and molecular characteristics of  $\alpha$ 1-antitrypsin deficiency. *Ann. Allergy*, **72**: 105-120.

Khatib, H., E. Heifetz and J.C.M. Dekkers. 2005. Association of the protease inhibitor gene with production traits in Holstein dairy cattle . *J. Dairy Sci.*, **88**(3): 1208-1213.

Sambrook, J. and D.W. Russell. 2001. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York, U.S.A.

Table 1. *Bubalus bubalis* Protease Inhibitor - Blastn in GenBank + EMBL DDBJ.

Sr. No.	Accession	Description	Location/ Source	Max Ident
1	BT025459.1	Bos taurus serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 (SERPINA1), mRNA, complete cds	1-426 (164-589)	97%
2	BC102730.1	Bos taurus serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1, mRNA (cDNA clone MGC:127781 IMAGE:30957372), complete cds	1-426 (72-497)	97%
3	X 63129.1	Bos taurus mRNA for alpha-1-antitrypsin	1-426 (55-480)	97 %