GENETIC POLYMORPHISM STUDY OF IGF-I GENE IN BUFFALOES OF GUJARAT

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ABSTRACT

The IGF plays an important role in lactation. Polymorphs of IGF gene are reported to be significantly associated with milk production and constituent traits. This study was undertaken to detect polymorphism at the genetic level and to explore allelic variability at this locus. A total of 150 animals, belonging to three breeds of riverine buffalo, viz. Mehsani, Surti and Jaffarabadi, were scanned. A 265 bp segment of gene was amplified by polymerase chain reaction and subsequently, subjected to single strand conformation polymorphism (SSCP) to identify different allelic patterns. All the three breeds exhibited three different SSCP patterns, but the sequence analysis could not confirm polymorphism. However, the sequence showed variation at three positions (at 89C/T, 98G/T and 167T/C) when compared with reference sequence. The study will augment the information and will be useful in further studies to determine the role of IGF in the regulation of milk synthesis and improvement of quality and quantity of milk in riverine buffaloes.

Keywords: IGF-I gene, buffalo, SSCP, sequencing

INTRODUCTION

India is the world leader in milk production, producing 86.96 MMT per year. The country has a buffalo population of 96.9 million which is about 56% of the world buffalo population and contributes more than 50% of total milk production of the country. Some of the best-known breeds are Murrah, Nili-Ravi, Jaffarabadi, Surti, Mehsani and Nagpuri. In the dairy sector, the buffalo could emerge as a promising alternative to crossbred cattle due to its adaptability to varied ecological conditions, higher milk yield and higher fat content, realizing a premium price. Hence, analyzing candidate genes and identifying polymorphism associated with high milk production can be a tool for improving milk production in buffaloes.

Insulin-like Growth Factor (IGF) is an important locus governing lactation traits. It plays an important role in lactation and is involved in a variety of physiological processes including reproduction, fetal development and growth (Adam *et al*., 2000, Shen *et al*., 2003). IGF1 gene is also considered to be a factor that regulates growth, differentiation, and the maintenance of differentiated function in numerous tissues and in specific cell types of mammals through binding to a family of specific membrane-associated glycoprotein receptors (Werner *et al*., 1994). For several centuries, genetic variation in animals (economic traits) have been used by breeders to select the best animal in a breed. Among various mutation detection methods single-strand conformation polymorphism (SSCP) is a powerful method for identifying sequence variation in amplified DNA. SSCP analysis of DNA has been used for detection of genetic mutations in humans (Orita *et al*., 1989), rats (Pravenec *et al*., 1992), cattle (Kirkpatrick, 1992) and in various bacteriological (Morohoshi *et al*., 1991) and viral (Fujita *et al*., 1992) systems. The most significant studies using the SSCP approach were accomplished on bovines in linkage analysis (Neibergs *et al*., 1993) and to define intragenic haplotypes at the growth hormone (Lagziel *et al*., 1996). The search for SSCP polymorphism could lead to the finding of genetic markers useful for improved selection of populations,

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namely, when applied to candidate genes associated with quantitative genetic variation in traits of economic importance. The objective of this study was to search for the IGF polymorphism in buffaloes, using SSCP analysis.

MATERIALS AND METHODS

Genomic DNA was extracted from blood samples collected from 50 animals each of the Surti, Mehsani and Jaffarabadi breeds.

PCR amplification: The IGF1 gene was amplified by PCR using the primer pairs (Ge *et al*., 1997) shown in Table 1. PCR reactions consisted of 25 to 50 ng of genomic DNA; 1X Mastermix (MBI Fermentas), 10 picomoles of each primer in a final volume of 25 µl. Cycling conditions were denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension for 30 seconds at 72°C for 30 cycles. The product of each amplification was analyzed by agarose gel electrophoresis. The amplified PCR products were subjected to SSCP analysis.

SSCP analysis: For SSCP analysis, to 5 µl of each amplification product was added 6x SSCP loading dye. The samples were heat-denatured at 95°C for 5 minutes, chilled at 0°C, and resolved on 6% polyacrylamide gels. Gels were run at 5 W (4°C cold room) constant power in 1 X Tris-borate EDTA. After electrophoresis, DNA was detected by silver staining and gels were transferred to Whatman 3 M paper and vacuum dried.

Sequence analysis: DNA fragments that displayed a modified electrophoretic pattern were selected for sequencing and were purified in low melting point agarose following the method described by Sambrook and Russel (2001). The concentration of the purified PCR product was determined and ligated using the InsT/Aclone™ PCR product cloning kit (MBI Fermentas) following the manufacturer's instructions. Ligated plasmids were transformed in DH5α and recombinant clones were selected by blue white screening. Recombinant plasmids were extracted and purified as per the method described by Sambrook and Russel (2001). The purified recombinant plasmids were used as templates for cycle sequencing. Cycle sequencing was performed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Electrophoresis and data analysis was carried out on the ABI PRISM®310 Genetic Analyzer using appropriate modules, basecallers, dyesets/primers and matrix files.

RESULTS AND DISCUSSION

A 265 bp fragment of IGF1 gene was amplified. PCR-SSCP of the amplified IGF fragments was performed to detect any mutation that might be present. Three types of SSCP band patterns were observed in the three buffalo breeds. The patterns were simple and consisted of only three bands. Pattern 1 consisted of band 2; pattern 2 of bands 1, 2 and 3 and pattern 3 of bands of bands 2 and 3 (Figure 2). The pattern frequency observed in the three breeds is given in Table 1. The samples showing differential band patterns were subjected to cloning and sequencing. The sequences obtained for segment of IGF1 in Mehsani, Surti and Jaffarabadi buffaloes have been submitted to the NCBI database (EU159114, EU159115 and EU159116). The sequence analysis of the IGF1 region in the three breeds of buffalo revealed no sequence variation. However, it revealed changes in nucleotide positions compared to reference sequence (Figure 3). It indicated that polymorphism revealed by SSCP could be independent of sequence variation.

Candidate genes have known biological functions related to the development or physiology of an important trait. Such genes can encode structural proteins or a member in a regulatory or biochemical pathway affecting the expression of the trait (Bryne and McMullen 1996) and can be tested as putative QTLs (Yao *et al*., 1996).

The study by Ge *et al.* (2001) characterizes a G → A transition polymorphism within an Eco130I site of intron 3 of the IGF1 gene in swamp buffaloes (*Bubalus b. bubalis kerebau*). Polymorphisms in the bovine IGF-I gene are associated with circulating IGF-I concentrations and growth traits. Additional polymorphisms in growth hormone axis genes that

are associated with production traits in ruminants have been reported by Grochowska *et al*. (2001). Similarly Lien *et al*. (2000) revealed three polymorphisms in noncoding regions of the IGF gene in the Norwegian cattle population. Li *et al*. (2006) studied allele frequencies of IGF1 which exhibited significant ($P<0.05$) deviation from neutral expectation and therefore, might be associated with divergence in North Eurasian cattle because of genetic selection.

Growth in animals is controlled by a complex system, in which the somatotropic axis plays a key role. Genes that operate in the somatotropic axis are responsible for the postnatal growth, mainly GH that acts on the growth of bones and muscles mediated by IGF-1 (Sellier 2000). The IGF-1 gene is a candidate for growth in the bovine, since it plays a key role in growth regulation and development (Breier, 1999; Hossner *et al*., 1997; Tuggle and Trenkle, 1996).

If specific haplotypes can be defined at this candidate gene that can be associated with milk production, protein and fat content, it would be rendered available as a valuable genetic resource for improvement of these buffalo breeds.

Table 1. SSCP pattern frequency in the three breeds.

Breeds/SSCP Pattern	I	II	III
Surti	46.0	43.0	11.0
Mehsani	4.5	60.0	35.5
Jaffarabadi	51.0	36.1	12.9

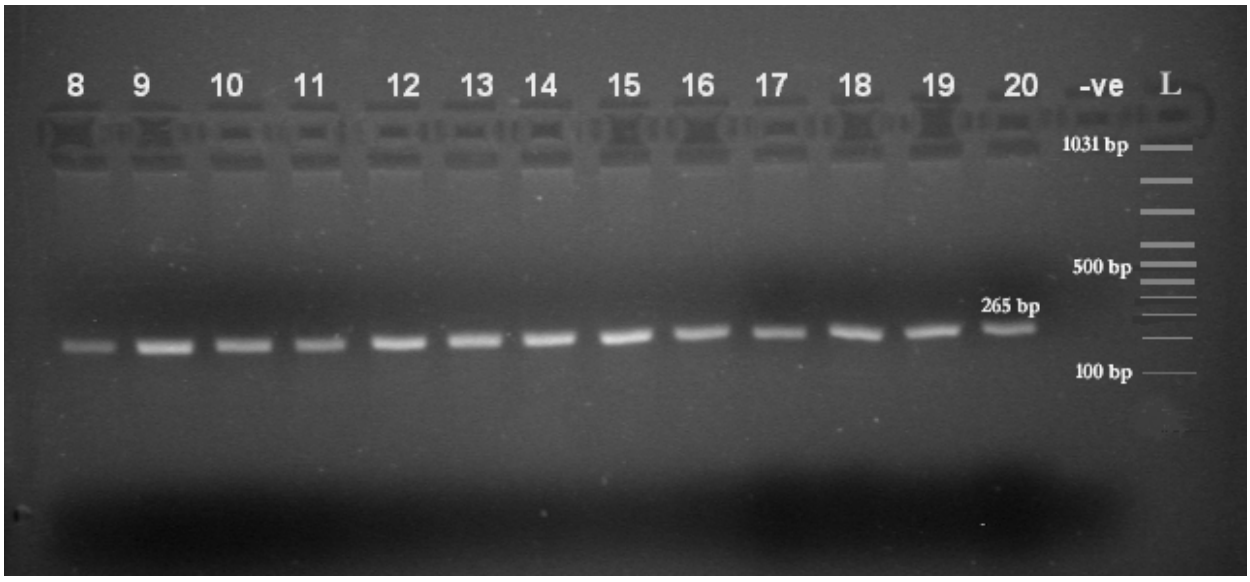

Figure 1. Amplified product of IGF-1 gene (265 bp) resolved on 2% Agarose Gel.

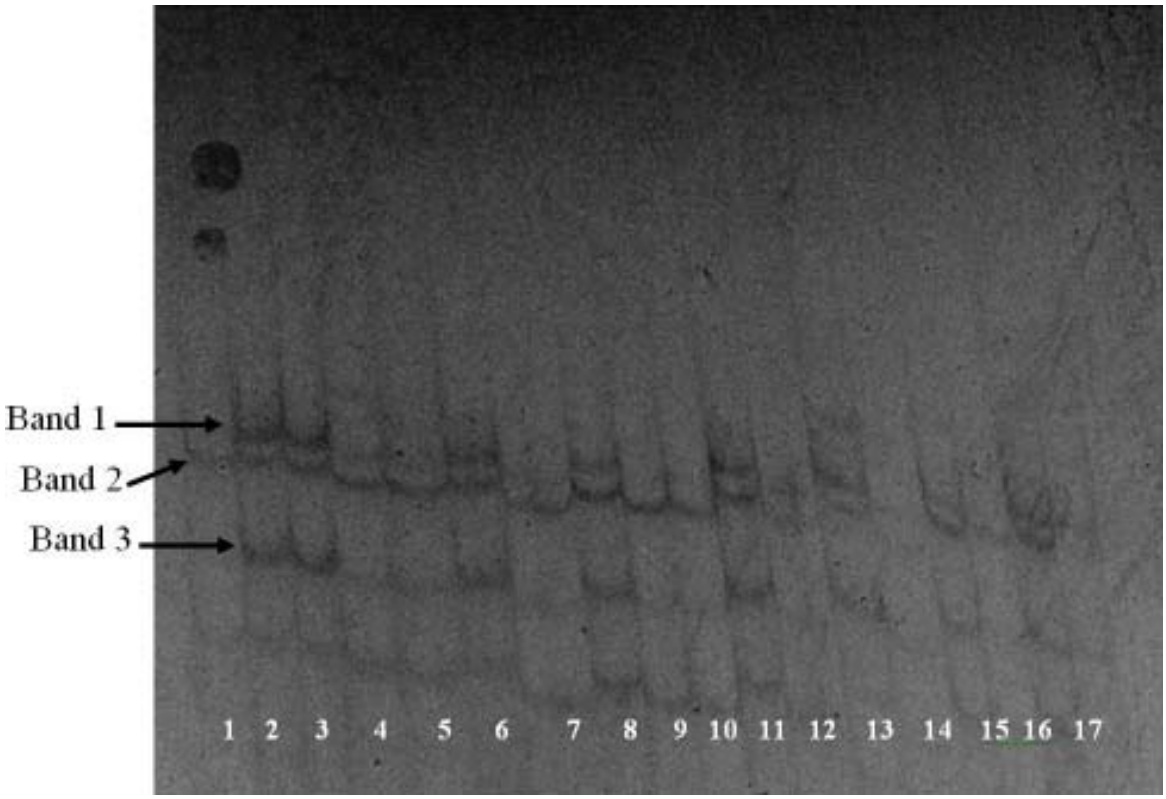

Figure 2. SSCP patterns of IGF-1 gene in representative samples on 6 % PAGE.
Pattern 1: Lane no. 1
Pattern 2: Lane no. 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 17

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Surti          GTTCTAGGAAATGAGATCATTCCCCTCACTTGGCAACCAGGACGAGGGGTCATCCCAGCG 60
Hehsani        GTTCTAGGAAATGAGATCATTCCCCTCACTTGGCAACCAGGACGAGGGGTCATCCCAGCG 60
Jafarabadi     GTTCTAGGAAATGAGATCATTCCCCTCACTTGGCAACCAGGACGAGGGGTCATCCCAGCG 60
Reference      GTTCTAGGAAATGAGATCATTCCCCTCACTTGGCAACCAGGACGAGGGGTCATCCCAGCG 60
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Surti          CTGTCTTCCATTCTAGTTTACCCCAGTCGTTTGAGGGTTAAAATCATAGAGTATGCTTGA 120
Hehsani        CTGTCTTCCATTCTAGTTTACCCCAGTCGTTTGAGGGTTAAAATCATAGAGTATGCTTGA 120
Jafarabadi     CTGTCTTCCATTCTAGTTTACCCCAGTCGTTTGAGGGTTAAAATCATAGAGTATGCTTGA 120
Reference      CCGTCTTCCAGTCTAGTTTACCCCAGTCGTTTGAGGGTTAAAATCATAGAGTATGCTTGA 120
               * ******** ************************************************

Surti          GATGGTCTTTTTTTCATTTCTTGTTTTTTAAATTTTGTGTTGGCTCTGGAATATAAAATT 180
Hehsani        GATGGTCTTTTTTTCATTTCTTGTTTTTTAAATTTTGTGTTGGCTCTGGAATATAAAATT 180
Jafarabadi     GATGGTCTTTTTTTCATTTCTTGTTTTTTAAATTTTGTGTTGGCTCTGGAATATAAAATT 180
Reference      GATGGTCTTTTTTTCATTTTTTGTTTTTTAAATTTTGTGTTGGCTCTGGAATATAAAATT 180
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Surti          GCTCGCCCATCCTCCACG 198
Hehsani        GCTCGCCCATCCTCCACG 198
Jafarabadi     GCTCGCCCATCCTCCACG 198
Reference      GCTCGCCCATCCTCCACG 198
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Figure 3. Sequence Alignment of IGF-1 gene with reference sequence (AY803777).

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