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Molecular Characterization of exon 2 of Leptin gene in Bos- indicus

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Abstract

Milk production traits are quantitative traits controlled by numerous genes and environmental factors. The 167 amino acid protein product of the ob gene was named leptin and consists of three exons of which the first exon is not transcribed into the leptin protein of 16-Kilo Dalton. The bovine leptin gene is located at BTA4q32 and is involved in the growth and metabolism of animals, and plays an important role in the regulation of feed intake, energy metabolism, growth, production and reproduction of cattle. This study aimed to find out the polymorphism in the leptin gene of Nimari (n=70) and Gaolao (n=75) bovine breeds. PCR-SSCP (Single Strand conformational polymorphism) of 317 bp amplicon of leptin gene revealed SSCP patterns I, II and III in both the breeds. The chi-square test revealed that difference between observed frequencies of different patterns in Nimari and Gaolao were non-significant at 5% level of significance.
Keywords: Leptin gene, Gaolao, Nimari, SSCP

Introduction

Milk production traits are quantitative traits controlled by numerous genes and environmental factors. The leptin gene is a potential candidate gene for quantitative trait loci (QTL) studies. The obesity (ob) gene was discovered by the positional cloning technique (Zhang *et al*., 1994). Leptin is 167 amino acid proteins which is product of the ob gene and constituted by three exons of which the first exon is not transcribed into the leptin protein of 16-Kilo Dalton. The bovine leptin gene is located at BTA4q32 (Pfister-Genskow *et al.*, 1996) and is involved in the growth and metabolism of animals .Its plays an important role in the regulation of feed intake, energy metabolism, growth, production and reproduction of cattle (Liefers *et al*., 2003).

In India there are 30 documented breeds of zebu cattle besides numerous populations found in various states of India are yet to be characterized and defined (Nivsarkar *et al*., 2000). Nimari and Gaolao are the documented breeds of zebu cattle found in Madhya Pradesh (M.P) and adjoining areas of Maharastra and Uttar Pradesh. Nimari breed is known as "Biological Engine of Nimar" Nimari breed originated from cross of Gir and Khillari breeds and the home tract is concentrated in central part of Khargone and Barwani districts. The Gaolao is dual purpose breed for draught and milk production. This breed is found in Balaghat, Chindwara, Durg, Rajnandgaon and Seoni districts of M.P and Wardha district of Maharastra. In the present study, genetic polymorphism in the exon 2 of leptin gene in Gaolao and Nimari breeds of cattle were observed using a PCR-SSCP technique.

Materials and methods

Total of 145 blood samples (approximately 10 mL) of genetically unrelated from Nimari (n=70) (Ujjain and Shajapur districts of MP) and Gaolao (n=75) (Chindwara region and Betul districts of MP and Wardha district of Maharastra) were collected and stored in EDTA-coated vacutainer tubes. The DNA was isolated by the method as described by John *et al.* (1991). Quality check and quantification was done by gel electrophoresis (0.8% agarose) and NanodropTM spectrophotometer at optical density (OD) 260nm/280nm respectively. The DNA concentration was determined and

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samples were diluted 10-30 times (approx. 30 ng/µl) with MiliQ water.

DNA amplification of the leptin gene was achieved by PCR. Oligonucleotide primers F-AGGGAAGGGCAGAAAGATAG and R- ATG-GCAGACTGTTGAGGATC were used for the PCR amplification of the leptin gene. Reaction was carried out in a final volume of 25 µl. Containing of 90 ng of genomic DNA, 1 X master mix (MBI Fermentas), 10 picomole of each forward and reverse primer and remaining volume was adjusted with nuclease free water. The PCR was carried out in a Biorad thermal cycler. The thermal cycling profile was as follows: initial denaturation for 10 min. at 94°C; followed by 30 cycles of denaturation at 94°C for 1min, annealing at 52°C for 1 min and extension at 72°C for 1 min .The final extension was carried out for 10 min at 72°C.

Each PCR product was diluted in a denaturing solution (95% formamide, 10 mM NaOH, 0.05% xylene cynol and 0.05% bromophenol blue, 20 mM EDTA), denatured at 94°C for 5 min, immediately chilled on ice and resolved on 6% polyacrylamide gel. The electrophoresis was carried out in a Sequi-Gen GT nucleic acid sequencing cell (BIO-RAD) vertical electrophoresis unit using 1X TBE buffer at constant 5 W for SSCP analysis of all the fragments. Gel was silver-stained as per Sambrook & Russell (2001) and dried on cellophane gel was scanned by GS-800 calibrated densitometer (Bio-rad).

Results

A 317 bp fragment of leptin gene was amplified. PCR-SSCP of the amplified Leptin fragments was performed to detect any mutation that might be present. Three types of SSCP band patterns were observed in the two breeds of cattle as shown in Fig.1. Three SSCP patterns observing in these breeds were numbered SSCP pattern I, SSCP pattern II and SSCP pattern III. The frequencies of patterns in these breeds for leptin gene loci are showed in table.1. SSCP pattern III is most common is both the breeds with genotypic frequency 60.71 % and 72.73 % in Nimari and Gaolao respectively. Pattern II is less common in both the breeds with percentage 12.50 and 9.09% in Nimari and Gaolao breed respectively. The chi-square test revealed that difference between observed frequencies of different patterns in Nimari and Gaolao were non-significant at 5% level of significance.

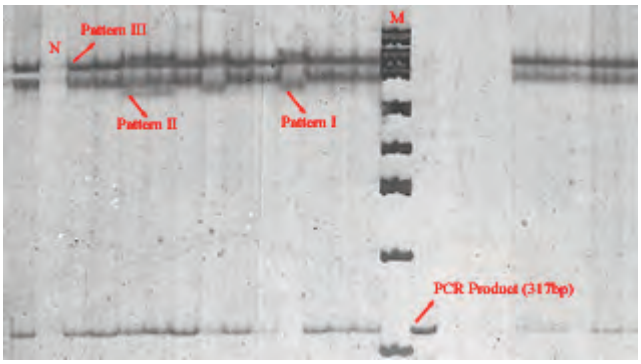

Fig. 1. Types of SSCP band patterns

Table 1. SSCP patterns and there allelic frequency in two Nimari and Galoao

SSCP Patterns	Nimari	Gaolao
I	26.67	18.18
II	12.50	9.09
III	60.71	72.73

Discussion

SSCP offers a simple, inexpensive and sensitive method for detecting sequence variation even at single base pair level, and so can greatly reduce the amount of sequencing necessary. The exon -2 of leptin polymorphism founded in this study was lower than that of Dubey *et al*. 2008, who analyzed the same exon using the same methodology and same region but reported four patterns in Sahiwal cattle. PCR-RFLP analysis of almost same region of exon 2 of leptin gene by Tahmoorespur *et al*. 2007 reported 3 genotype viz AA, AB & BB in Sistani cattle (Bos-indicus).

These variants can further be sequenced to develop SNP markers. These SNP markers would be helpful to breeders for future association studies, selecting superior germplasm and conservation strategies. Our results provided the first evidence of genetic variability of the leptin gene within the Indian cattle breed viz Nimari, Gaolao. The data generated by current studies may be useful for establishing possible associations between productive parameters and genetic variants and help in the process of decision making at the farmer's level for improvement and sustainable management of this cattle breed.

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