GENETIC VARIABILITY OF BOLA-DRB3.2 GENE IN KENKATHA BREED OF CATTLE ASSESSED BY PCR-SSCP

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ABSTRACT

Present study was conducted to investigate Bola (Bovine leucocyte antigen) gene polymorphism in indigenous draft Kenkatha (n= 52) breed of cattle at DRB3.2 loci by PCR-SSCP (Single strand conformational polymorphism). This Bola region encodes cell surface glycoprotein, which binds to foreign antigens of T lymphocytes that play central role in the induction and regulation of adaptive immunity. A PCR-SSCP of 284bp amplicon of Bola-DRB3.2 gene revealed 13 SSCP patterns. This suggests that exon 2 region of Bola-DRB3 is highly polymorphic in nature. This may be due to natural selection on a Bola allele pool, leading to the development of resistance to local infectious diseases.

Key words: Bola, Kenkatha, PCR –SSCP.

INTRODUCTION

Bola-DRB 3.2 is one of the prime gene controlling the immune response of the organisms to viral as well bacterial infections. It has been identified that Bola-DRB 3.2 alleles are associated with resistance and susceptibility to leukaemia, mastitis, ovarian disease and milk fever in cattle. Association between cattle Bola alleles with fertility, growth and milk production traits have been also reported (Beever et al. 1990). In cattle, Bola genes are located on chromosome 23 (BTA23). This Bola region encodes cell surface glycoprotein, which binds to foreign antigens of T lymphocytes that play central role in the induction and regulation of adaptive immunity (Germain, 1994). The MHC class I and class II genes are highly polymorphic and are known to be related to immune response related auto-immune disease, infectious disease and responses to immunization (Ellis and Ballingall, 1999). The MHC Class II region of Bola, the DR region contained three DRB genes and high expression was observed in peripheral blood lymphocytes (Moon et al. 1997).

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). Kenkatha is draught cattle breed of Panna, Chattarpur and Tikamgarh districts of Madhya Pradesh and adjoining areas of Bundelkhand comprising Lalitpur, Hamirpur and Banda districts of Uttar Pradesh.

Genetic polymorphism at candidate genes affecting economic traits have stimulated substantial research interest, because of their impending utilization as an aid to genetic selection and to demarcate evolutionary relationships in different livestock breeds. Nucleotide variation in the coding region of a gene may lead to change in amino acids which alter expressed protein(s) and although intronic variation does not change the amino acid sequence of the protein it may play a significant role in marker assisted selection. In livestock such variation in DNA may also be associated with economic traits, which are governed by many genes each having a small effect. The objective of the present investigation was to identify the genetic polymorphism of Bola-DRB 3.2 gene in Kenkatha breed of cattle using a PCR-SSCP technique.

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MATERIALS AND METHODS

Genomic DNA Sample: Blood samples (approximately 8 to 10 mL) were collected from 52 genetically unrelated animal representative of the Kenkatha breed. The samples were collected from their respective home tracks viz Tikamgarh, Chattarpur and Panna districts of MP. The DNA was isolated by the method as described by John et al. (1991). Quality check and quantification was done by NanodropTM spectrophotometer and electrophoresis on 0.8% agarose gel. The DNA concentration was determined and samples were diluted 10-30 times (approx. 30 ng/u) with MiliQ water.

Amplification of Bola-DRB.3 gene: B o L A - DRB3.2 gene was amplified from genomic DNA samples by polymerase chain reaction. The HL-030 (5’ATCTCTCTTGCACGACATTTCC3’) and HL-031 (5’TCTTAATTCGGCTCAGCTGGCT 3’) primers were used for the first round of PCR and the HL-030 and HL-032 (5’TCGCGCTAGTCTGGCTGCA CAGTGAAACTCTC 3’) primers were used for the second round PCR. The reaction was carried out in 25 µl consisted of 90 ng template genomic DNA, 10 Pico mol of each primer and 1X Mastermix (MBI Fermentas, USA) and volume of reaction was adjusted with nuclease free water. The PCR condition included an initial denaturation 94°C for 4 min followed by 15 cycles of denaturation at 94°C for 1min, annealing at 60°C for 2 min and extension at 72°C for 1 min with a final extension at 72°C for 5 min using a MJ Research thermo cycler (MJ Research, USA). The second PCR reaction was carried out in a total volume of 25 µl containing 1 µl of the first PCR product, 10 Pico mol of each primer and 1X Mastermix (MBT Fermentas, USA) and volume of reaction was adjusted with nuclease free water. The second PCR condition included an initial denaturation at 94°C for 5 min followed by 40 cycles at 94°C for 1min at 65°C for 30sec and 72°C for 1 min with a final extension at 72°C for 10min using a MJ Research thermo cycler (MJ Research, USA). The PCR products were run on 2% agarose gels and visualized under UV light.

SSCP analysis of Bola-DRB.3 gene: Each PCR product was diluted in a denaturing solution (95% formamide, 10 mM NaOH, 0.05% xylene cyanol 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 (Sambrook and Russell, 2001) and dried on cellophane gel was scanned by GS-800 calibrated densitometer (Biorad).

RESULTS AND DISCUSSION

SSCP patterns are generated as a result of nucleotide sequence change at one or several positions together. Different patterns, developed because of altered migration due to changed conformation in single strands of fragments. A 284 bp fragment of BoLA-DRB3.2 gene was amplified. PCR-SSCP of the amplified BoLA-DRB3.2 fragments was performed on 6% PAGE to detect any mutation that might be present. The major bands in upper region of the gel were scored. The gel containing native band (bottom part) was excluded from analysis. A total of 10 bands constituted the 13 SSCP patterns in Kenkatha breed as shown in figure1 (P1 to P13). SSCP pattern I has highest frequency 0.12. The BoLA-DRB3.2 locus in the Bos indicus Kenkatha breed of cattle is highly polymorphic. The SSCP patterns and their respective frequencies are given in Table 1.

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>I</td>
<td>0.12</td>
</tr>
<tr>
<td>II</td>
<td>0.096</td>
</tr>
<tr>
<td>III</td>
<td>0.064</td>
</tr>
<tr>
<td>IV</td>
<td>0.032</td>
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<tr>
<td>V</td>
<td>0.064</td>
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<td>VI</td>
<td>0.096</td>
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<tr>
<td>VII</td>
<td>0.096</td>
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<tr>
<td>VIII</td>
<td>0.032</td>
</tr>
<tr>
<td>IX</td>
<td>0.096</td>
</tr>
<tr>
<td>X</td>
<td>0.096</td>
</tr>
</tbody>
</table>

TABLE 1. SSCP patterns and their respective frequency of Kenkatha cattle
carried out on other breeds. PCR –SSCP analysis by Ranjan et al. (2010) reported 18 SSCP patterns in Malvi breed of cattle with maximum gene frequency 0.116. PCR -RFLP of Behl et al. (2007) reported that there are 24 different alleles in Kankrej cattle in which twenty-one alleles of the total 24 alleles were similar to those reported earlier; 3 alleles were new and had not been reported previously. Almost all the previous studies of BoLA-DRB3.2 locus in cattle were carried out by PCR-RFLP molecular marker. At present, 103 BoLA-DRB3.2 alleles have been identified by the sequencing of cloned genomic DNA, cDNA, PCR products and PCR-Sequences based typing (SBT) (Takeshima et al. 2002; http://www.projects.roslin.ac.uk/ bola/reports.html). The present study is based on PCR-SSCP; it revealed that exon 2 region of BoLA-DRB3 was a highly polymorphic in nature. This may be the due to natural selection on a BoLA allele pool, leading to the development of resistance to local infectious diseases. The observed high degree of genetic variation in the cattle could be crucial for the long-term survival of this population. Maintenance of high polymorphism, as a genetic resource could be an important issue that will demand attention in future breeding programmes in species under high selective pressures. Information on BoLA diversity in cattle is important to aid our understanding of immune responses and may contribute to maintenance of healthy cattle populations. Apart from all these understanding the mechanisms involved in generating this diversity may shed light on the complex nature of mammalian MHC evolution.

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