

DNA polymorphism of major histocompatibility complex class II DQA and DQB genes in crossbred cattle (*Bos taurus* × *Bos indicus*)

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Received: 6 March 2011; Accepted: 30 March 2011

Key words: Crossbred cattle (*Bos taurus* × *Bos indicus*), DQA and DQB genes, Major histocompatibility complex (MHC), PCR-RFLP

Polymorphism of major histocompatibility complex (MHC) genes affects the range of antigenic peptides that are bound and presented to T-cells that causes variability in T-cell receptor (TCR) interaction. Extensive nucleotide polymorphism within these genes is implicated in variations in immune responses within a species (Racioppi *et al.* 1991). Therefore, most of the genetic analyses and typing techniques have been concentrated on this region. The MHC of cattle (*Bos taurus* and *Bos indicus*), known as bovine lymphocyte antigen (BoLA), is located on chromosome number 23 in cattle (Fries *et al.* 1986). The DQA and DQB genes are highly polymorphic in exotic cattle having approximately 40 alleles (Davies *et al.* 1994, Russel *et al.* 1997). PCR-RFLP developed by Van Eijk *et al.* (1992) has many advantages over the other methods for the genetic analysis of populations, as it requires small amount of genomic DNA and being adaptable to crude DNA preparations. Owing to the practical importance of the MHC and the extensive polymorphism shown by the class II genes the present study was undertaken to study the polymorphism of DQA and DQB genes in crossbred cattle.

Crossbred cattle (72) selected randomly from farm of the institute were used in the present investigation. These crossbreds (Holstein Friesian × Hariana) were maintained under uniform regime of feeding, management and health care. Blood (5 ml) was collected from jugular vein of each animal in a 15 ml of sterile polypropylene tube containing 0.3 ml (0.5 M) of EDTA (ethylene diamine tetra acetate) as an anticoagulant. The blood samples were mixed gently with anticoagulant and kept on ice until transportation to the laboratory. Blood samples were stored at 4°C until the isolation of genomic DNA. Genomic DNA was isolated by

phenol-chloroform extraction method (Sambrook and Russell 2001) with some modifications. The genomic DNA was checked for its quality, purity and concentration by submarine agarose gel electrophoresis and spectrophotometer. DNA samples of good quality, purity and concentration were used for further PCR-RFLP analysis.

The corresponding primers (forward and reverse) used by Davies *et al.* (2004) for the amplification of *Bison bison* DQA and DQB genes were used in the present investigation to amplify the MHC class II genes DQA exon 2-exon 3 and DQB exon 2 regions in crossbred cattle (Table 1).

PCR-RFLP analysis: PCR products of DQA gene were digested with *Hae*III and *Xba*I restriction enzymes (RE's). 12 µl of PCR product was digested with 5 units of each of the restriction enzymes separately and 5 µl buffers to a final volume of 20 µl and sample were incubated at 37°C overnight. DQB Amplicons were digested with *Pst*I and *Taq*I restriction enzymes. For PCR-RFLP study of DQB gene, 10 µl of amplified PCR product was digested with 5 units of each of *Taq*I and *Pst*I and 5 µl of buffer separately to a final volume of 20 µl and incubated at 65°C and 37 °C, respectively for overnight. Digested restriction products were resolved

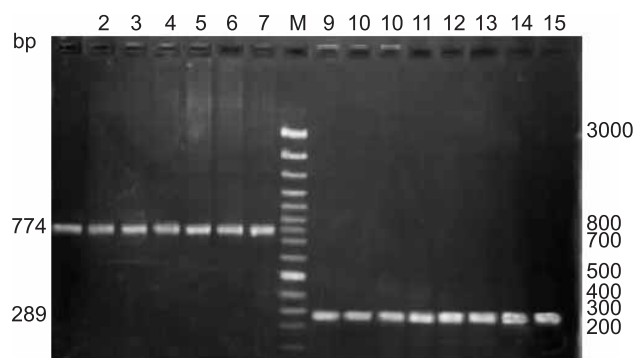


Fig. 1. PCR amplification of BoLA-DQA exon 2–3 (774 bp) and DQB exon 2 of crossbred cattle. Lane M, 100 bp DNA ladder; Lane 1–7, 774 bp DQA amplicon (exon 2–3); Lane 8–15, 289 bp DQB amplicon (exon 2).

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Table 1. Primer sequences used in the present investigation

Locus	Primer name	Location	Primer sequence (5'-3')	Product size (bp)
DQA	BoDQA1FP-E2A	Intron 1/ Exon 2	CTC CGA CTC AGC TGA CCA CAT TGG	774
	BoDQA2FP-E2B	Intron 1/ Exon 2	CCT CAA TTA TCA GCT GAC CAC GTT GG	
	BoDQA-RP-E3A	Exon 3 5' end	GAC AGA TGA GGG TGT TGG GCT G	
DQB	BoDQA2FP-E2B	Intron 1/ Exon 2	TCC CCC GCA GAG GAT TTC GTG	289
	BoDQA-RP-E3A	Intron 1/ Exon 2	CTC CCC GCA GAG GAT TTC GTG	
	BoDQB-RP-E2C	Exon 2/ Intron 2	CGC ACT CAC CTC GCC GCT GC	

Table 2. BoLA-DQA exon 2-3 type specific restriction patterns and their frequencies in crossbred cattle

Genotype	Fragment size (bp)	Genotypic frequency
<i>HaeIII</i> -A	78, 289, 410	0.25
<i>HaeIII</i> -B	77, 220, 476	0.073
<i>HaeIII</i> -C	55, 77, 255, 400	0.097
<i>HaeIII</i> -D	57, 76, 200, 440	0.208
<i>HaeIII</i> -E	75, 200, 500	0.139
<i>HaeIII</i> -F	270, 425	0.111
<i>HaeIII</i> -G	75, 270, 425	0.122
<i>XbaI</i> -A	60, 225, 485	0.361
<i>XbaI</i> -B	75, 200, 500	0.253
<i>XbaI</i> -C	60, 715	0.386

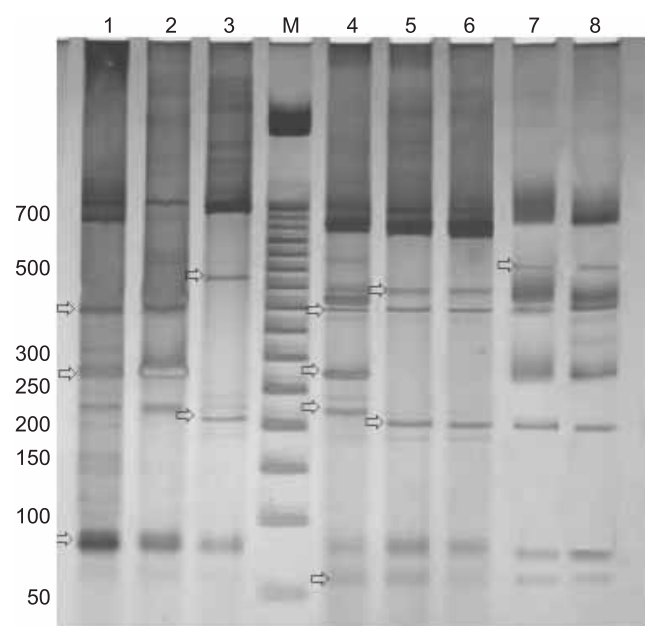


Fig. 2. *HaeIII* digestion pattern of BoLA-DQA exon 2-3. Lane M, 50 bp DNA ladder; Lane 1-2, *HaeIII* A; Lane 3, *HaeIII* B; Lane 4, *HaeIII* C; Lane 5-6, *HaeIII* D; Lane 7-8, *HaeIII* E.

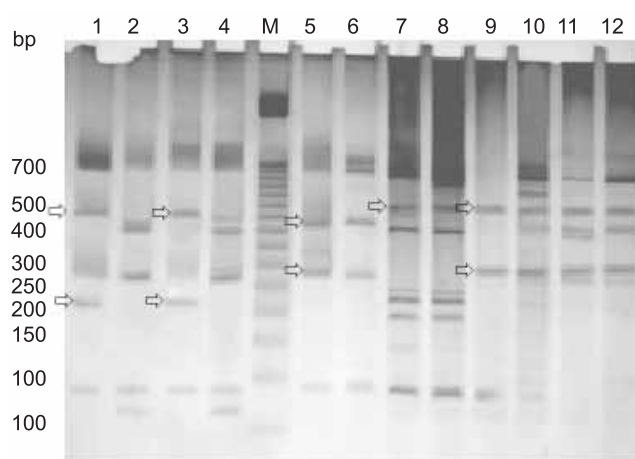


Fig. 3. *HaeIII* digestion pattern of BoLA-DQA exon 2-3.

Lane M, 50 bp DNA ladder; Lane 1-2, *HaeIII* B; Lane 3-4, *HaeIII* C; Lane 5-6, *HaeIII* G; Lane 7-8, *HaeIII* E; Lane 9-12, *HaeIII* F.

on 8% polyacrylamide gel and visualized under gel documentation system after silver staining. The amplification of DQA (774 bp) and DQB (289 bp) genes crossbred cattle is shown in Fig.1.

PCR-RFLP of BoLA-DQA gene with *HaeIII* and *XbaI*: Digestion of 774bp fragment of DQA gene with *HaeIII* restriction enzyme produced 7 RFLP patterns in crossbred cattle. The sizes of these patterns were 78, 289, 410 bp; 77, 220, 476 bp; 55, 77, 255, 400 bp; 57, 76, 200, 440 bp; 75, 200, 500 bp; 270, 425 bp and 75, 270, 425 bp for *HaeIII*-A, *HaeIII*-B, *HaeIII*-C, *HaeIII*-D, *HaeIII*-E, *HaeIII*-F and *HaeIII*-G, respectively (Figs 2 and 3). The genotypic frequencies of these patterns were 0.250, 0.073, 0.097, 0.208, 0.139, 0.111 and 0.122, respectively (Table 2).

Patterns *XbaI*-A, *XbaI*-B and *XbaI*-C were observed, when the 774bp amplicon of MHC class II DQA gene was digested with *XbaI* restriction enzyme (Fig 4). The fragment sizes of these patterns were 60, 225, 485 bp; 75, 200, 500 bp and 60, 715 bp, respectively. The frequencies of *XbaI*-A, *XbaI*-B and

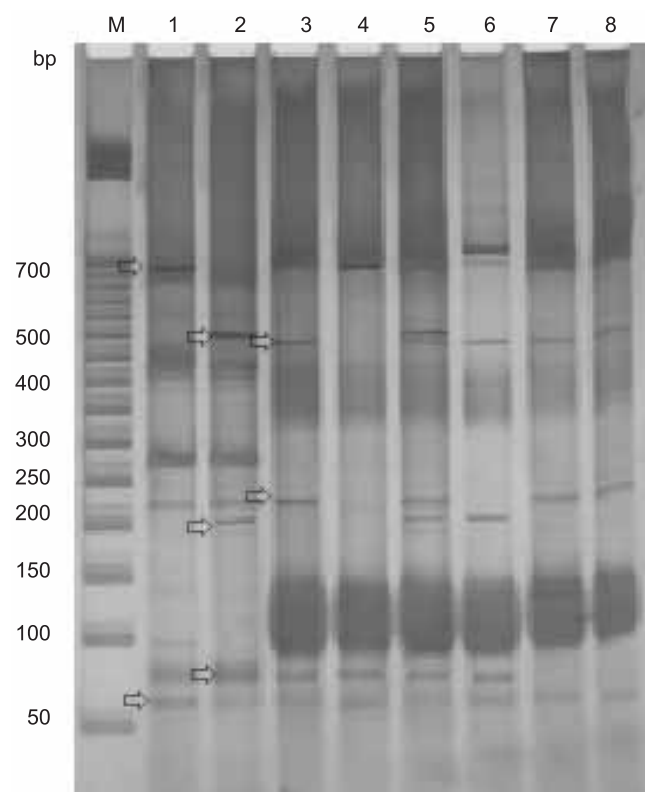


Fig. 4. *XbaI* digestion pattern of BoLA-DQA exon 2-3.

Lane M, 50 bp DNA ladder; Lane 1 & 4, *XbaI* C; Lane 2, 5 and 6, *XbaI* B; Lane 3, 7 and 8, *XbaI* A

XbaI-C genotypes were 0.361, 0.253 and 0.386 respectively, which were comparatively higher than those observed for *HaeIII* genotypes (Table 2). However, Ballingall *et al.* (1997) reported 4, 5 and 3 DQA1 RFLP patterns using *HinfI*, *MnII*, and *TaqI* restriction enzymes in African *Bos taurus* cattle. Besides they also observed two RFLP patterns with *MnII* and monomorphic patterns in DQA2 gene for each of the *MseI* and *BsaJI* restriction enzymes.

PCR-RFLP of BoLA-DQB gene with *PstI* and *TaqI*: RFLP patterns namely *PstI*-A, *PstI*-B, *PstI*-C, *PstI*-D and *PstI*-E

Table 3. BoLA-DQB exon 2 type specific restriction patterns and their frequencies in crossbred cattle

Genotype	Fragment size (bp)	Genotypic frequency
<i>PstI</i> -A	50,70,170	0.125
<i>PstI</i> -B	70, 220	0.18
<i>PstI</i> -C	90, 200	0.153
<i>PstI</i> -D	50,100,140	0.292
<i>PstI</i> -E	80, 210	0.25
<i>TaqI</i> -A	90, 200	0.155
<i>TaqI</i> -B	30, 40, 50, 70, 100	0.253
<i>TaqI</i> -C	50, 80, 160	0.238
<i>TaqI</i> -D	30, 80, 180	0.169
<i>TaqI</i> -E	30, 260	0.185

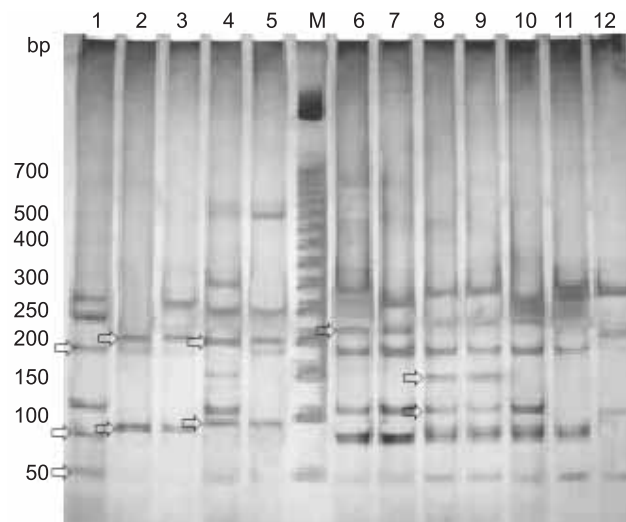


Fig. 5. *PstI* digestion pattern of BoLA-DQB exon 2.

Lane M, 50 bp DNA ladder; Lane 1, 10 & 11, *PstI* A; Lane 2 and 3, *PstI* E; Lane 4, 5 and 12, *PstI* C; Lane 6 and 7, *PstI* B; Lane 8 and 9, *PstI* D.

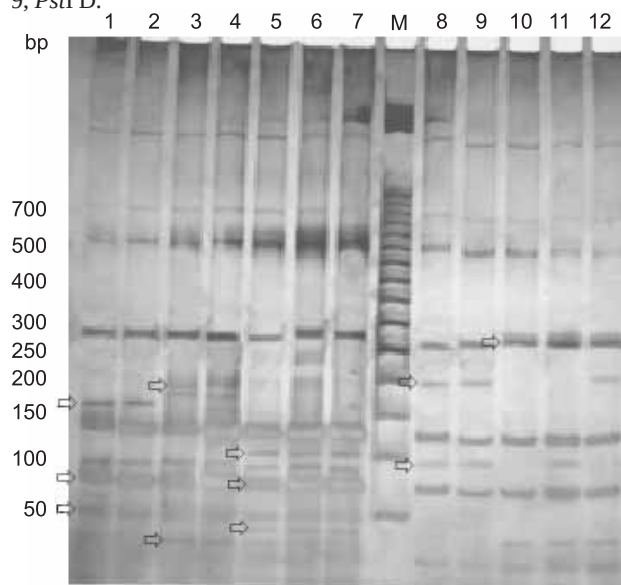


Fig. 6. *TaqI* digestion pattern of BoLA-DQB exon 2.

Lane M, 50 bp DNA ladder; Lane 1 and 2, *TaqI* C; Lane 3 and 4, *TaqI* D; Lane 5, 6 and 7, *TaqI* B; Lane 8 and 9, *TaqI* A; Lane 10, 11 and 12, *TaqI* E.

were found, when a 289 bp fragment of DQB exon 2 of crossbred cattle was digested with *PstI* restriction enzyme. The fragment sizes of these patterns were 50, 70, 170 bp; 70, 220 bp; 90, 200 bp; 50,100,140 bp and 80, 210 bp, respectively (Fig. 5). The genotype frequencies of these patterns were 0.125, 0.180, 0.153, 0.292 and 0.250, respectively (Table 3). Similarly, digestion of 289bp amplicon of DQB exon 2 with *TaqI* revealed five RFLP patterns with the fragment sizes as 90, 200 bp; 30, 40, 50, 70, 100 bp; 50, 80, 160 bp; 30, 80, 180 bp and 30, 260 bp (Fig. 6). The RFLP patterns namely *TaqI*-A, *TaqI*-B, *TaqI*-C, *TaqI*-D and *TaqI*-

E had the genotype frequencies as 0.155, 0.253, 0.238, 0.169 and 0.185, respectively (Table 3). Mareello *et al.* (1995) investigated the polymorphism of expressed bovine MHC class II DQB gene in a group of nine MHC homozygous Kenya Boran cattle (*Bos indicus*). They reported that digestion of DQB exon 2 PCR products with *RsaI* showed clear bands representing both the expressed sequences although band intensity varied. But in the present study, we could not find the site for *RsaI* in MHC class II DQB gene in crossbred cattle. Olle *et al.* (1990) also used *TaqI*-RFLP analysis for prediction of disease of siblings of affected children with 21-hydroxylase deficiency. They also reported that 7 of the 8 sibling pairs concordant for 21-hydroxylase deficiency had pairwise identical *TaqI* HLA-B-DRB-DQA-DQB haplotypes.

The results showed that the crossbred cattle used in the present study were in heterozygous condition. Therefore, it may be concluded that the MHC class II BoLA-DQA and DQB genes are highly polymorphic in crossbred cattle like other bovine species.

SUMMARY

The present study was undertaken to study the polymorphism of DQA and DQB genes in crossbred cattle. Crossbred cattle (72) selected randomly from farm of the institute were used in the present investigation. The results showed that these crossbred cattle were in heterozygous condition. Therefore, it may be concluded that the MHC class II BoLA-DQA and DQB genes are highly polymorphic in crossbred cattle like other bovine species.

ACKNOWLEDGEMENT

The authors are thankful to Director and Joint Director (Academic), Indian Veterinary Research Institute (IVRI), Izatnagar for providing the necessary facilities to conduct the study. The help received from Incharges of all the Farms for collection of the blood samples is also duly acknowledged.

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