Low variability at Hinf I locus of Major Histocompatibility Complex (MHC) – DQB gene in Indian Mithun *(Bos frontalis)*

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ABSTRACT

Genomic region corresponding to exon 2 of DQB gene was amplified from 79 Indian mithuns. Three primers were used (two forward and one reverse) to carry out Polymorphism studies. Amplified products of DQB genes were digested with HinfI restriction enzymes, which revealed a total of 2 restriction patterns in DQB. Nearly all the mithun population was found to be fixed for AA restriction pattern. PCR-RFLP results revealed very low variability at Hinf I site in Bofr-DQB, contrary to PCR-RFLP studies of DQB gene in other bovines. Very low genetic diversity was observed at Hinf I loci at exon 2 region of mithun DQB, indicating conserved recognition site of Hinf I. Our study indicated that Hinf I enzyme may not be good enough for the typing of DQB alleles in mithun population, on other side tetracutters may a good choice for the genotyping of Bofr-DQB.

Key words: Mithun; MHC; DQ genes; duplicated haplotype; PCR-RFLP

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INTRODUCTION

Mithun (*Bos frontalis*), is a massive, semi-domesticated and rare bovine species and considered descendent from wild Indian gaur (Simoons 1984). Through contributing in terms of meat, leather and drafting power, it plays an important role in the day-to-day socioeconomic, cultural and religious life of the local tribal population of North East region of the India (Arora, 1998). Presently, India possesses 0.29 million Mithun distributed mainly in Arunachal Pradesh, Nagaland, Mizoram and Manipur states (19th Livestock Census, 2012). In view of small and scattered populations of mithun in the region, major efforts are being done to increase the population by the various government and non-government agencies in this region. Census shows the recent increase of the mithun population in region during recent time (18th Livestock Census 19th Livestock Census, 2012). However, it is essential to assess the genetic diversity of functional markers in mithun, as it reflects the fitness as well adaptation of the population to the local environment.

Functional markers like Major Histocomaptability Complex (MHC) are preferred over neutral markers like microsatellite for assessing the genetic diversity in rare wild species as these, being directly associated with host's immune response. Evidence suggests that populations with extreme monomorphism shown by neutral markers may persists sufficient genetic variation at the MHC (Aguilar et al, 2004). Apart from critical role in immune response, the genetic variations at MHC loci are thought to have a significant impact on population fitness and regarded as being exploited for increasing the diversity under conservation programme for some of the rare wild animal species (Yasukochi et al. 2012). Generally, MHC region is an organized cluster of highly polymorphic genes related with immunity. MHC class II genes, specifically DRB3, DQA and DQB are extremely polymorphic in ruminants Andersson and Rask, 1988, Sigurdardottir *et al* 1992, Marello et al 1995, Ballingall *et al.,* 1997). However, no such study has been conducted to assess the variability at MHC loci in mithun species. In view of small and scattered populations of Mithun in the region, the genetic diversity of these populations has been prime

importance to assess the fitness for their better survival and adaptation to the local environment. Therefore, we conducted a study to identify genetic variability at MHC-DQB loci in Mithun using PCR-RFLP.

MATERIAL AND METHODS

Seventy nine mithun blood samples were collected from different field areas of Nagaland and Arunachal Pradesh of India. Genomic DNA was isolated by by phenol-chloroform extraction method by standard protocol (Sambrook and Russel, 2001) The purity of the genomic DNA was assessed by spectrophotometry the ratio of optical densities value at 260 and 280 nm was used as a criteria for purity. To amplify genomic sequence of DQB encompassing exon 2, a set of three primers were used referred by Traul et al., (2005). For amplification of Bofr-DQB, two forward primers (B1G-F-5'-TCCCCCGCAGAGGATTTCGTG-3' and

B2G-F -5'-CTCCCCGCAGAGGATTTCGTG-3') with reverse primer BG-R -5'-CGCACTCACCTCGCCGCTGC-3). were multiplexed.

The optimized concentrations for 20 µl volume of reaction mixture were 1.5 mM of $MgCl_2$, 2mM of dNTPs and 10 pmol of primers each, 100 ng of genomic DNA as template, 10x PCR assay buffer and 3 units of *Taq* DNA polymerase. Amplification conditions were: initial denaturation at 95 °C for 3 minutes, 32 cycles of denaturation for DQA and 34 cycles for DQB at 94 °C for 30 seconds, annealing 65 °C f for 30 seconds and extension at 72 °C for 1 minute for DQA and 40 seconds for DQB and final extension at 72 °C for 10 minutes. Amplified PCR products were digested with *HinfI* restriction enzyme for RFLP analysis. Digested products were analyzed by gel electrophoresis by using 2.5% high resolution agarose gel in gel electrophoresis at 80V for 2 hour. The frequency of different patterns was estimated by using standard method.

RESULTS AND DISCUSSION

289 long nucleotide region encompassing respective highly variable regions (exon 2) nucleotide sequences of DQB genes, respectively were amplified in 79 Mithun. Amplified products of DQB genes were digested with HinfI restriction enzymes, which revealed a total of 2 restriction patterns in DQB (Figure 1). The frequencies of different patterns with fragment size are given in table 1. Nearly all the mithun population was found to be fixed (96.2%) for AA restriction pattern. PCR-RFLP results revealed very low variability at Hinf I site in Bofr-DQB, contrary to PCR-RFLP studies of DQB gene in other bovines (Marello et al, 1995, Niranjan et al, 2010a & b). Although, more number of restriction patterns has been identified in cattle and buffalo DQB, however by using other enzymes. Niranjan and coworkers (2010b) also adopted the Hae III enzyme for typing of DQA alleles in buffaloes and revealed six patterns.

Our results indicated that the recognition site (G/ANTC) of Hinf I in DQB alleles may be conserved. Further, Hinf I enzyme was tried in this study, however, never been reported for typing of DQB alleles in any species, to the best of our knowledge, by any worker. Our study indicated that Hinf I enzyme may not be good enough for the typing of DQB alleles in mithun population, on other side tetracutters like Hae III, Rsa I may a good choice for the genotyping of Bofr-DQB.

In conclusion, very low genetic diversity was observed at Hinf I loci at exon 2 region of mithun DQB, indicating conserved recognition site of Hinf I.

Figure 1: Electrophoretic mobility of RE fragments obtained by digestion of Bofr-DQB exon-2 region with Hinfl (in 2.5% agarose gel)
Lane 1-15: Different restriction patterns
Lane M: 100 bp DNA ladder

Table 1. Hinf I restriction patterns of Bofr-DQB

S.N.	Patterns	Animals	Frequency (%)
1	AA	76	96.20
2	AB	3	3.80

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