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RESEARCH ARTICLE

Diversity analysis of DRB3 gene locus in indicus cattle- identification of novel PCR-RFLP allelic patterns

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Abstract: In the current study, genetic diversity analysis of MHC class II (DRB3) locus has been documented among indicus cattle breeds. Genotyping of 192 zebu cattle belonging to eight different breeds, was carried out to identify different allelic patterns by PCR-RFLP of BoLA DRB3 exon 2, revealing 43 different restriction patterns. Highest genetic diversity of BoLA-DRB3 gene was observed in Malnad Gidda and Gir and least in Mewati, Tharparkar and Sahiwal cattle. Allelic pattern, BM was the most frequently observed heterozygous pattern and with highest frequency among Mewati and Ladakhi cattle breeds. Few breed specific alleles also identified, which were found specifically in Tharparkar, Gir and Ongole breeds. This genetic information may be important for exploring the correlation between *BoLA*-DRB3 genetic diversity and disease susceptibility and overall allelic diversity status of the Indian cattle populations.

Keywords: Cattle, *Bos indicus*, BoLA DRB3, Allelic diversity, PCR-RFL

Introduction

The indigenous cows of India, scientifically known as *Bos indicus* or Zebu cattle, have different genetic attributes due to which they react differently to environmental stimuli. These responses are closely associated with physio-anatomical characteristics, which the animals have developed as the result of natural selection over the centuries. India is among very few nations having most of the variable natural agro-climatic conditions available within the same region. Zebu cattle are well adapted in diverse geo-climatic conditions of the country as well as under field conditions being quite refractory to several infectious diseases. In order to comprehend the distinctive disease resistance characteristics of these Zebu cattle it is important to understand the immune system of these animals. Immunity is the resistance of body against external etiological factors of disease, provided by the interaction of chemical, humoral and cellular reactions in the body. The major histocompatibility complex is one such important component of immune system responsible for modulation of innate as well as adaptive immune response.

The major histocompatibility complex (MHC) of cattle is known as bovine leukocyte antigen (BoLA), located on the short arm of bovine chromosome 23 and consisting of class I, IIa, IIb and III regions (Lewin et al. 1999). Major histocompatibility complex (MHC) class I and class II are cell surface molecules that play an important role in the intercellular recognition and self/non-self discrimination and trigger of humoral as well as cell-mediated immune responses. The *BoLA* Class IIa region comprises: *DRA*, *DRB*, *DQA* and *DQB* genes which encode the classical peptide presenting class II molecules (DR and DQ) in cattle (Lewin et al. 1999; Andersson and Davies, 1994). Three *DRB* genes, DRBP1, DRB2 and DRB3, have been identified in cattle. DRBP1 is evidently a pseudogene and functional expression of DRB2 has not been found, whereas DRB3 is functionally expressed and investigation of *BoLA-DRB3* gene of cattle is of special interest due to remarkable functional importance of the gene controlling the immune response to the viral and bacterial infections and a high level of polymorphism reported (Da Mota et al. 2002).

Genetic diversity of BoLA DRB3 gene has been studied by various researchers employing different methods. Among these, PCR-RFLP 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 (Van Eijk et al. 1992). It has been found to be a powerful tool for detecting the variation in DNA sequences of bovine lymphocyte antigens (Russell et al. 1997). Various molecular and immunological markers have been identified which are being associated with disease

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resistance and production traits, important for selection of animals with high immuno-competence or disease resistance and high performance status. It is a well-known fact that indigenous cattle breeds harbor some invaluable characteristics such as disease resistance, adaptation to heat stress, good libido and fertility, better feed conversion efficiency, compared to exotic and crossbred. Maintenance of genetic diversity at MHC loci is an important factor to ensure that population remains fit to fight against disease outbreaks and is capable of survival under continuous disease threats. This study therefore was envisaged with the objectives to explore the immune system of the Zebu cattle through investigating the allelic diversity of Major Histocompatibility Complex (MHC) DRB3 gene using simple PCR-RFLP technique among different cattle breeds adapted to varied agro-climate zones of India.

Materials and Methods

Blood samples were collected from 192 animals, 24 each of eight different cattle breeds (Konkani, Tharparker, Mewati, Gir, Ongole, Malnad-Gidda, Ladhakhi and Sahiwal), belonging to different geographical regions of India. A standard protocol, employing phenol/chloroform extraction and precipitation with ethanol, was used to isolate genomic DNA from the blood samples (Sambrook and Russell, 2001). The DNA quantification and quality was checked by using NanoDrop ND1000 (Thermo Scientific, Wilmington, DE). Agarose gel electrophoresis was also used to check the integrity of the DNA used. All samples were brought to the concentration of 50-100 ng/ul.

BoLA-DRB3 exon 2 region was amplified using primers (HL030; 5'- ATCCTCTCTCTGCAGCACATTTCC-3' and HL031; 5'-TTTAAATTCGCGCTCACCTCGCCGCT-3') as reported by earlier workers (Van Eijk et al. 1992). The primers designed were meant for complete DRB3 exon 2 amplification. PCR amplification was carried out in 20 µl of reaction volume, containing 50-100 ng of genomic DNA, 0.5 µl of 10 pmol of each primer, 0.5 µl of 10mM dNTPs mix, 10X PCR buffer containing 15 mM $MgCl_2$ and 1 unit of Taq DNA polymerase (New England Bio Labs, USA). The thermal cycling conditions were set for an initial denaturation at 95°C for 2.5 min followed by 32 cycles at 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min with final extension at 72°C for 5 min. Amplified PCR products, were confirmed on agarose gel. All PCRs were performed in a 96-well C1000 thermal cycler (Bio-Rad Laboratories, Inc. USA).

The amplified products were subjected to PCR-RFLP using 5 µl of PCR products, digested for approximately 6 h at 37°C with 2 units of RsaI (New England Bio Labs, USA) in a total volume of 20 µl. RsaI restriction enzyme was selected for PCR-RFLP on the basis of earlier reports using it for the cattle and bison sequences to identify allelic patterns. For the analysis of different allelic patterns at *BoLA*-DRB3 region through differential restriction patterns the digested products were resolved and differentiated by 3% Metaphor high resolution agarose gel electrophoresis (Sigma, USA) with 50 bp ladder (GenRuler, Fermentas) in 1X TAE buffer at 80V for 90 min. After staining with ethidium bromide, the fragments were visualized on a UV trans-illuminator and analyzed for genotyping on the basis of different restriction patterns recorded manually. Nomenclature of these allelic patterns were done simply by alphabetic order to avoid any confusion because of the complexity generated through high allelic diversity and high heterozygosity at DRB3 locus.

Results and Discussion

Most of the polymorphism of the BoLA-DRB3 gene is located in exon 2, which encodes the peptide binding cleft, and its sequence variation plays an important role in the variability of immune response and disease resistance (Baxter et al. 2008). In the present study 310 bp fragment of exon 2 region of BoLA-DRB3 was successfully amplified in 192 animals (Figure 1). The BoLA-DRB3 exon 2 alleles in cattle have been found to be associated with resistance or susceptibility to various diseases as well as different milk protein traits (Starkenburg et al. 1997). Genotyping of BoLA-DRB3 was carried out to identify different alleles by PCR-RFLP, it was first done by Van Eijk and co-workers (1992).

The amplified BoLA-DRB3 exon 2 PCR products were digested with RsaI restriction enzyme, revealing a large number of restriction patterns (Figure 2) as combinations of different DRB3 alleles, present in either homozygous or heterozygous at single locus. Total 43 different restriction patterns or alleles were identified across 192 zebu cattle, out of this 22 (51%) were homozygous and 21 (49%) were heterozygous. Highest genetic diversity of BoLA-DRB3 gene was observed in Malnad Gidda and Gir and least in Mewati, Tharparkar and Sahiwal (Table 1). Results obtained also suggest that within breed genetic variation across breeds is higher than between breeds. This genetic information will be important for investigating the relationship between BoLA DRB3 and disease incidences in various cattle breeds. It has an implication on designing breeding programs that will aim at monitoring overall genetic diversity and herd health status and planning breeding programs to keep allelic diversity at this locus sufficient to combat the diseases.

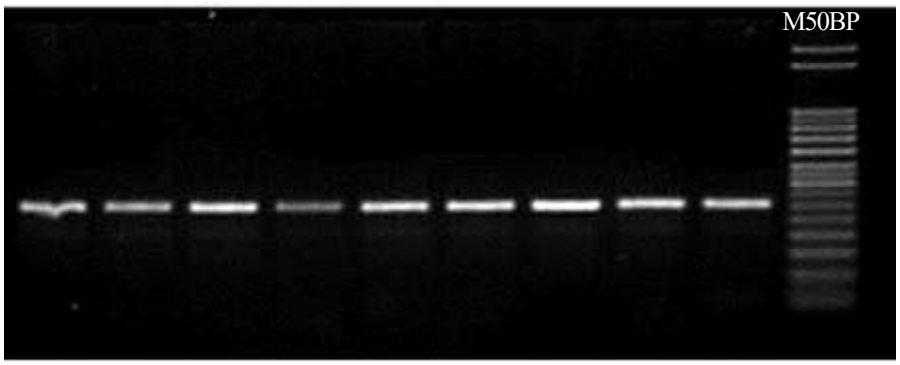
High variation for standard diversity indices were observed among the cattle breeds studied. These results corroborated with the previous findings (Takeshima et al. 2015) reporting, a total of 46 alleles of BoLA-DRB3.2 in the cattle breeds in this study. Previous workers (Wang et al. 2012) have reported that DRB3 gene is the most widely studied class II gene as it is extremely polymorphic. Among various alleles identified in this study, BM allelic pattern was the most frequently observed heterozygous pattern in indicus population and it was highest among Mewati and Ladakhi. Some breed specific alleles, AU, AG and AL were also identified, which were only found in Tharparkar, whereas KG allelic pattern was observed only in Gir and Ongole breeds.

Table 1 Genetic diversity of BoLA-DRB3 locus in different *Bos Indicus* breeds

S. No.	Breed	Total number of alleles	Number of homozygous alleles	Number of heterozygous alleles	Percentage of alleles	Heterozygous alleles with highest frequency*
1.	Mewati	9	7	2	19%	BM (21%)
2.	Ladakhi	12	8	4	26%	BM (21%)
3.	Malnad Gidda	14	10	4	30%	BL (8%)
4.	Gir	14	5	9	30%	KG (21%)
5.	Ongole	13	7	6	28%	KG (12%)
6.	Konkani	11	6	5	24%	BJ (12%)
7.	Tharparkar	9	2	7	19%	AU (25%)
8.	Sahiwal	9	4	5	19%	RG (17%)

*Alphabetical nomenclature given to each unique allelic patterns is as shown in representative agarose gel pictures (Figure 2).

Fig.1 PCR amplified products (310bp) of BoLA-DRB3 gene exon 2, resolved on agarose gel. M50BP- 50bp ladder marker



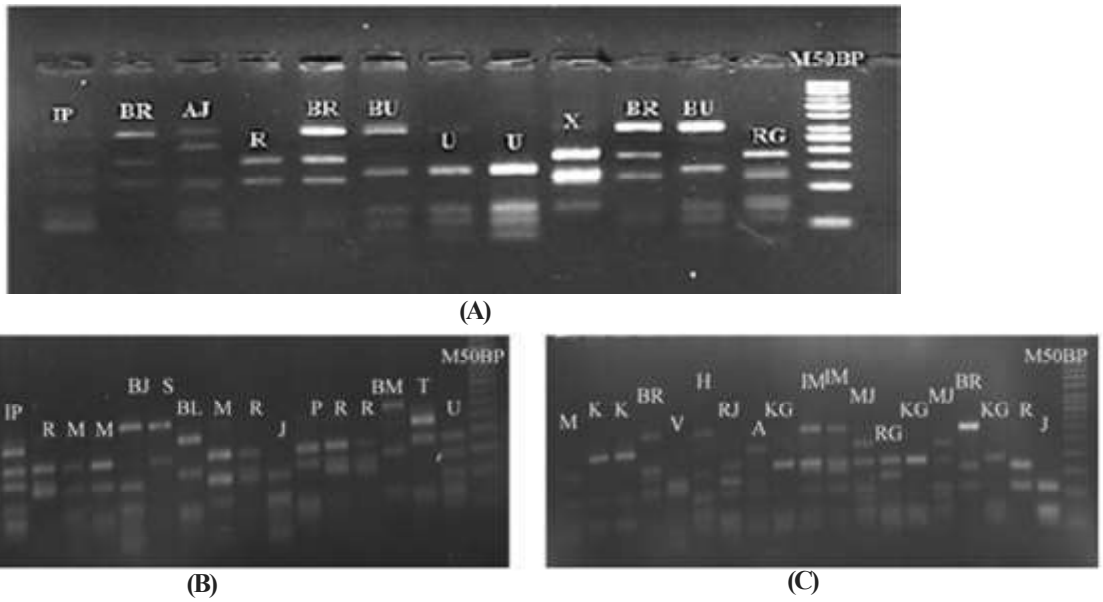


Fig. 2 PCR-RFLP allelic patterns of DRB3 gene 310 bp exon 2 using RsaI restriction enzyme on different cattle breeds. (A) Holstein Friesian, (B) Malnad Gidda and (C) Ongole, are the representative allelic patterns generated on specific breeds. Nomenclature of the patterns given is arbitrary alphabetically. M50BP- 50bp ladder marker

Earlier workers (Das et al. 2012), also identified breed specific alleles in three different breeds of cattle indicated their breeding structure or equilibrium and possible reservoir of rare alleles. In another study, Giovambattista et al. (2020), reported 71 distinct alleles, including three new variants of BoLA-DRB3 in local Myanmar cattle populations, while exotic Holstein-Friesian population, as a result of the different degrees of native admixture demonstrated a high degree of dispersion.

Conclusion

The higher heterozygosity values and greater genetic diversity of BoLA-DRB3 alleles observed in the present study among indicus cattle populations may be visualized as a positive genetic adaptations, which could be largely influenced by greater exposures to natural environmental conditions, pathogenic organisms and hot humid tropical climatic conditions, faced by them during free grazing in their native breeding tracts.

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