Full Length Articles

# CHARACTERISATION OF BOVINE LYMPHOCYTE ANTIGEN *BOLA-DRB3.2* ALLELES IN INDIAN ONGOLE (*Bos indicus*) CATTLE BY PCR-RFLP AND PCR-SBT

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## ABSTRACT

*Bovine Lymphocyte Antigen DRB3 (BoLA-DRB3), a gene of the major histocompatibility complex (MHC) has received attention because this is considered to be a potential genetic marker associated with disease resistance traits in cattle. Hence, a study was taken up to characterize BoLA-DRB3.2 alleles in the 60 Ongole animals. The most common alleles observed in Ongole cattle was *15 and *6 with frequency of 0.225 and 0.200, respectively. Of the 22 identified alleles detected, the seven alleles (BoLA-DRB3.2*15, *6, *12, *13, *23, *31 and *47) represented 77.5 percent of allelic frequencies. Result indicated that the BoLA-DRB3 exon 2 was highly polymorphic in Ongole cattle. Direct sequencing BoLA-DRB3.2 allele reveled that at nucleotide at position 91 and 133 of the sequence had both A and G nucleotides in contrast to T nucleotide of Bos taurus breed and the variations leads to different band pattern in gels.*

**Key words:** Onogle, MHC, BoLA-DRB3.2, Disease resistance, India

## INTRODUCTION

There are about 50 recognized native breeds of cattle in India, having special qualities of hardy nature, resistance to many diseases and adopted to adverse climatic condition. Production of high yielding crossbred dairy cows without compromising on disease resistance is a concept that is very enticing to dairy researchers and conservationist in India. Bovine Lymphocyte Antigen *DRB3* (*BoLA-DRB3*), a gene of the major histocompatibility complex (MHC) has received attention because this is considered to be a potential genetic marker associated with disease resistance traits in cattle. At present, more than 100 different alleles were investigated by PCR-RFLP (Van Eijk *et al*., 1992; Gelhaus *et al*., 1995; Maillard *et al*., 1999;

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do Nascimento *et al*., 2006). Several studies have shown that decreased MHC variability might be caused by population bottlenecks (Mason *et al*., 2011; Zhang *et al*., 2016). Bovine Leukocyte Antigens (BoLA) have been used as disease resistance markers and immunological traits in cattle due to their primary role in pathogen recognition by the immune system (David *et al*., 2020). Hence, a study was taken up to characterize *BoLA-DRB3.2* alleles in the Ongole cattle breed.

## MATERIAL AND METHODS

In the present study, blood samples from 60 Ongole animals were used for isolation of genomic DNA. DNA was isolated from above blood samples using a modified high salt method (Miller *et al*., 1988). Hemi-nested PCR was used for the amplification of the exon 2 (284 bp) of the *BoLA-DRB3* gene, as described by Van Eijk *et al*., (1992). Primers of HL030 5′- ATC CTC TCT CTG CAG CAC ATT TCC-3′, HL031 5′- TTT AAT TCG CGC TCA CCT CGC CGC T-3′ and HL032 5′- TCG CCG CTG CAC AGT GAA ACT CTC-3′ were used. In first cycle, PCR was performed with HLO30 and HLO31 primers. The amplification were carried out with a 50 ng of DNA in a 25 µl total volume containing; 5 pmols each of HLO30 and HLO31 primers, 2x PCR mastermix and 1.0 unit of *Taq* DNA Polymerase. The thermal cycling profile for the first round of amplification was an initial denaturation step of 5 minutes at 94° C followed by 10 cycles of 1minute at 94° C, 2 min ute at 60° C, 1minute at 72° C and final extension of 5 minutes at 72° C. After the first round, a semi nested second round PCR reaction was carried out with 1 µl of the first round PCR product as DNA template, containing the same volume and concentrations as described above expect with primers, HLO30 and HLO32. The thermal cycling profile for the second round was as follows, initial denaturation of 2 minutes at 94° C, followed by 30 cycles of 1minute at 94° C, 30seconds at 65.5° C, 1minute at 72° C and a final extension of 5 minutes at 72° C.

PCR-RFLP analysis of the *BoLA-DRB3.2* alleles was carried out to determine different allelic patterns in Ongole animals. In this study, the PCR-RFLP was carried out with three different restriction enzymes *Rsa*I, *BstY*I and *Hae*III. Restriction fragments were revealed by gel electrophoresis on 10 per cent polyacrylamide using Vertical electrophoretic system (Consort, Belgium) with low molecular weight marker (M20).The PCR RFLP patterns were determined according to the BoLA nomenclature (Russell, 1997)as reported in the BoLA nomenclature homepage (*http://www.projects.roslin.ac.uk/bola/drb3pcr.htmltable*). Results obtained for PCR-RFLP analysis are presented in Table 1. Allele frequencies were determined by $H_i = \square n_i/N$, where $H_i$ is the frequency for allele *i*, $n_i$ is the number of alleles *i* in a population, and *N* is the total number of alleles in the population.

Further, direct sequencing of *BoLA-DRB3.2* alleles was carried out in a 17 selected PCR samples using the automated DNA sequencer to get the complete picture of polymorphism at nucleotide level. However, a duplicate sample of each was also sequenced but with different animal number. The nucleotide sequences were analysed using DNASTAR (Lasegene, Inc. USA).

## RESULTS AND DISCUSSION

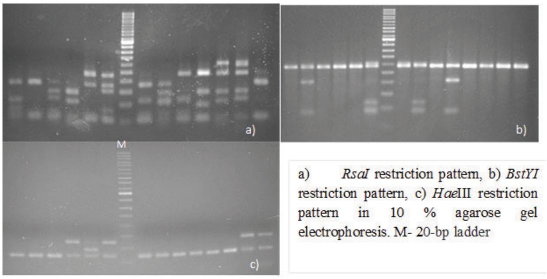
A 284 bp fragment of *BoLA-DRB3* gene, composed of 10 bp of the 5′ intron, 267 bp of exon 2 and 7 bp of the 3′ intron, was analysed in 60 animals. This 284 bp PCR amplified fragment of the *BoLA-DRB3.2* gene was digested with *Rsa*I, *Bst*YI, and *Hae*III (Fig. 1). The detection of PCR RFLP alleles were attempted combining the data for all restriction enzymes by comparing the patterns as per *BoLA* nomenclature (Russell *et al*., 1997) and the frequency distributions of BoLA alleles described in Table 1. The results of the present study indicated that the *BoLA-DRB3* exon 2 was highly polymorphic in Ongole cattle. The distribution of the allele frequencies shown in Fig. 2 should that the total number of alleles identified in Ongole cattle was twenty two with frequencies ranging from 0.008 to 0.225 and all the 22 alleles were similar to those reported in earlier studies (Van Eijk *et al*., 1992; Gelhaus *et al*., 1995; *http://www.projects.roslin.ac.uk/bola/drb3pcr.htmltable*).

**Table 1 Comparative allele frequencies of *BoLA –DRB3.2* alleles for cattle breeds studied**

| ***DRB3.2* alleles** | **Ongole (N=60)** |
|---|---|
| 3 | 0.025(3) |
| 6 | 0.200(24)* |
| 8 | 0.008(1) |
| 9 | 0.008(1) |
| 11 | 0.008(1) |
| 12 | 0.092(11)* |
| 13 | 0.108(13)* |
| 14 | 0.025 (3) |
| 15 | 0.225 (27)* |
| 16 | 0.025 (3) |
| 20 | 0.008 (1) |
| 23 | 0.075 (9) |
| 27 | 0.017 (2) |
| 28 | 0.017 (2) |
| 31 | 0.042 (5) |
| 32 | 0.008 (2) |
| 34 | 0.014 (3) |
| 36 | 0.008 (1) |
| 37 | 0.017 (2) |
| 42 | 0.017 (2) |
| 47 | 0.033 (4) |
| 51 | 0.008 (1) |
| **Total** | **22** |

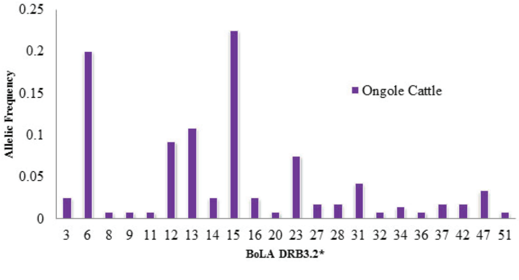
* Significantly ($P<0.05$) higher gene frequency based on Chi-square test

**Fig. 1. PCR-RFLP patterns of BoLA-DRB3 gene digested by different restriction enzymes**



a) *RsaI* restriction pattern, b) *BstYI* restriction pattern, c) *Hae*III restriction pattern in 10 % agarose gel electrophoresis. M- 20-bp ladder

**Fig. 2. Comparative allele frequencies of BoLA –DRB3.2 alleles in Ongole cattle breed**



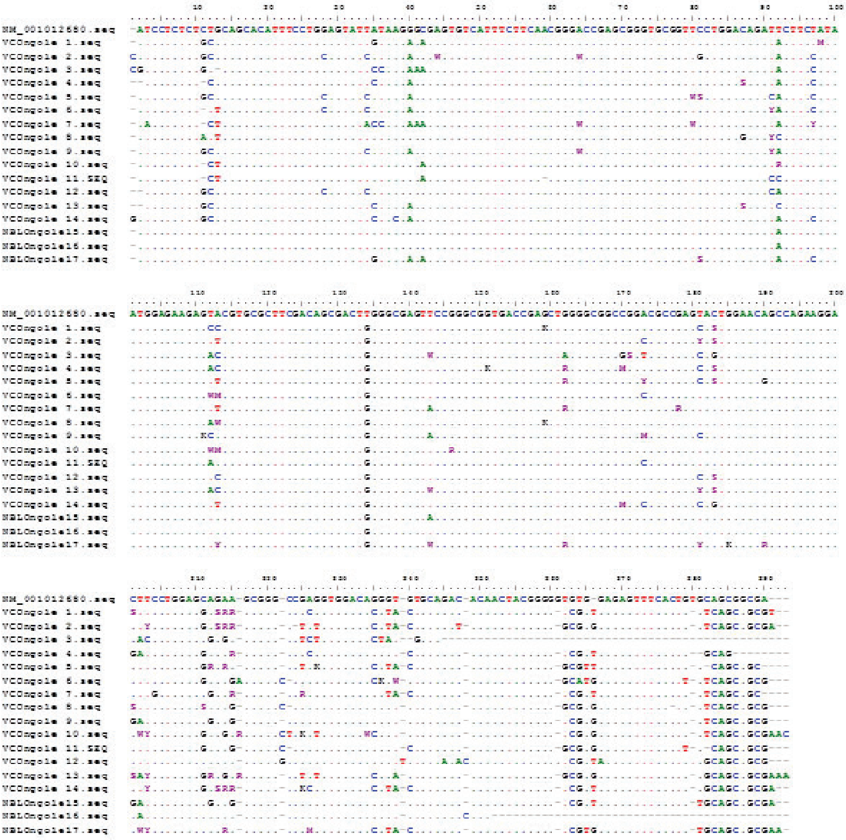
Most common allele of *BolA -DRB3.2*15* were present at the rate of 22.5 per cent. High degree of polymorphism in exon 2 of *BoLA-DRB3* by the PCR-RFLP technique was also revealed by Van Eijk *et al*., (1992). The second highest number of allele *BoLA* allele **6* was present at a frequency of 0.200. The seven alleles of Ongole (*BoLA-DRB3.2*15, *6, *12, *13, *23, *31* and **47*) was represented 77.5 per cent of allelic frequencies. Alleles **3, *8, *12, *13, *16, *32* and **42* in Ongole were unique to this breed. Duangjinda *et al*., (2009) revealed that the most frequently detected alleles of Holstein x Zebu were *DRB3*16, *51, *23, *11, *8* and **1* accounting for 61.12 per cent of the alleles.

A high degree of polymorphism in the *BoLA-DRB3.2* has also been reported in various studies carried out in other breeds. For example, in a study carried out on 66 Jersey cows by Sharif *et al*. (1998), the most frequently detected *BoLA* alleles reported were *BoLA-DRB3.2 *7, *10, *17, *21, *28* and **32*. In a later study on Jersey cattle by Gilliespie *et al*., (1999), it was observed that the most frequently isolated alleles were *BoLA-DRB3.2 *8, *10, *15, *21, *36* and **ibe*. The allele *DRB3.2 *7*, which was the most common allele type detected in the Jersey cows in the study carried out by Sharif *et al*., (1998), was not observed to be present in the Jersey herd studied by Gilliespie *et al*., (1999). This is comparable to observation of our findings studied in two cattle breeds.

Dietz *et al*., (1997) carried out polymorphism studies on the *BoLA-DRB3.2* locus in a population of 127 Holstein cows. They observed that *BoLA-DRB3.2 *8, *11, *16, *22, *23* and **24* were the six most frequently (70.3 per cent) detected alleles. In an another study, on Holstein animals (n = 835), Sharif *et al*. (1998) observed that seven alleles of *BoLA-DRB3.2 *3, *8, *11, *16, *22, *23 and *24* represented 88.7 per cent of the total alleles. But in contrast to our observations, in Argentine Creole cattle (n = 194), 68 per cent of the gene frequencies were represented by five alleles (*DRB 3.2 *15, *18, *20, *24, and *27* (Giovambattista *et al*., 1996). Approximately 70 per cent of the alleles in the Japanese Shorthorn cattle were accounted for by six alleles (*BoLA-DRB3.2 *8, *9, *21, *27, *7, and *24* (Takeshima *et al*., 2002). The BoLA-DRB3 gene is the most polymorphic bovine MHC class II DR gene and is the only one having been shown to be fully functional, although the total amount of alleles is low compared to species such as human (Shiina *et al*., 2017). In a study carried out on 125 Saavedreno Creole dairy cattle, it was observed that the most frequently occurring alleles were *BoLA-DRB3.2 *7, *8, *11, *16, *27, *36, and *37*acounteing for 70 per cent of the total variation (Ripoli *et al*., 2004). In a study on Iranian Golpayegani cattle, five alleles (*BoLA-DRB3.2 *16, *7, *19, *28, and *11*) accounted for 50 per cent of the alleles (Mosafer and Nassiry, 2005). Therefore, it could be observed that differences in allelic frequencies existed among different breeds of cattle. The present study on native breed of Ongole cattle clearly indicates that the allelic variations in the *BoLA* region are distinctively different from other breeds.

All the sequences from ongole breed revealed that the *BoLA-DRB3* exon 2 amplified was 284 bp in length. The interesting feature noticed in Ongole breed was that at position 91 and 133 of the sequence, it had both A and G nucleotide in contrast to BoLA reference sequence (NC_007324) of *Bos taurus* breed, which had only TT nucleotides. Out of the total 60 animals studied, almost all animals are showing the same nucleotide patterns which were not present in the *Bos taurus* breed. There were three deletions noticed at positions of 49, 58 and 97 as revealed by pattern with 4 bands. Different nucleotide variations were also noticed at positions of 34(A-G), 39 (G-A), 41(C-A), 80(C-G), 96(T-C), 111(A-T,C), 142 (T-A), 200(C-G), 201(T-A), 202(T-C), 211(A-G), 213(A-G), 214(A-G), 220(A-C), 232(G-C) and 234(GTG-TAC), 256 and 257(GT-TC) and 259 and 260 (TG-GT) revealed by the different combinations of RFLP patterns. Therefore all these variations proved existence of heterozygosity at *BoLA-DRB3.2* in Ongole breeds of cattle. The Multiple Sequence Alignment of Ongole sequence with taurine sequences is shown in Fig.3. The variations in nucleotides of *BoLA-DRB3* exon2 were confirmed to similar findings of the ISAG BoLA Nomenclature Committee report (Russell *et al*., 1997).

**Fig. 3. Multiple Sequence Alignment variations of *BoLA –DRB3.2* alleles in Ongole breed of cattle**



Therefore this study showed the prevalence of nucleotides AA, GG, GG, CC and TT in exon 2 at positions 91, 133, 211, 232 and 256, respectively in Ongole and their respective position found to be highly conserved in Indian breeds. This is comparable with the report of Rupp *et al.,* (2007) that the lysine encoding allele (AA) is highly prevalent in *Bos indicus* breeds. Similar polymorphic patterns were also detected in 25 *BoLA-DRB3* alleles including two deletions in five Indian cattle breeds (De and Singh, 2006). The aligned nucleotide sequences confirmed unquiness of aligned sequences with other sequences available in NCBI gene bank data base. The similar polymorphism was also observed by Takeshima *et al*., 2002), analyzed PCR-SBT in a total of 176 Japanese Shorthorn cattle and identified 21 distinct alleles with different amino acid sequences in the translated *BoLA-DRB3* alleles. The aligned amino acid sequences were in accordance with the detailed DNA sequence as reported by Russell *et al.,* (2004).

PCR-RFLP and PCR-SBT are the powerful techniques to detect polymorphism in the exon 2 of *BoLA-DRB3* genein Ongole cattle. The number of RFLP variants and types of DNA sequences indicate the involvement of more than one alleles in PCR amplified genomic DNA. BoLA DRB3.2 alleles of **15* (0.225) and **6*(0.200) were found to be most common alleles in Ongole (*Bos indicus*) breed. Further, exploring inheritance pattern of the *BoLA-DRB3* gene in large number of Ongole cattle breed may further reveal crucial role *BoLA-DRB3* gene in providing disease resistance.

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