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**Research Article**

# GENETIC POLYMORPHISM IN DRB3.2 GENE AND ITS ASSOCIATION WITH MASTITIS IN MURRAH BUFFALOES

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## ABSTRACT

Genetic polymorphism in DRB3.2 gene was studied by polymerase chain reaction-restriction fragment length polymorphism technique along with its association with mastitis in 60 Murrah buffaloes. When 304 bp PCR products of DRB3.2 gene were digested with restriction enzyme *Pst*I, 6 different genotypes viz. *s/s, y/y, z/z, y/z, s/z* and *s/y* with frequencies 0.08, 0.20, 0.53, 0.02, 0.03 and 0.13, respectively were detected. Three allele's viz. *s, y* and *z* were found with frequencies 0.17, 0.27 and 0.56, respectively. Genotype *z/z* (53%) and allele *z* (0.56) were most frequent in the population. Genotype *y/z* found only in healthy animals. This might be playing role in resistance to mastitis. However, there was no significant difference in the genotypic frequencies of this genotype in healthy and mastitic animals, which might have occurred due to small sample size. These observations need to be confirmed on large sample size.

**Key words:** BuLA, DRB3.2, Murrah, PCR-RFLP

Mastitis continues to be an economically important infectious disease of bovine throughout the world. The estimated annual losses due to clinical and sub-clinical mastitis in India are Rs. 1683.89 crores and Rs. 4369.32 crores, respectively (Dua, 2001). Many countries have recently updated their breeding programme to include or increase the weightage for nonproductivity traits, with special attention given to mastitis resistance (Rupp and Biochard, 2003).

Major Histocompatibility complex (MHC) of vertebrates comprises of a group of closely linked genes, which plays central role in the immune surveillance and response. The buffalo lymphocyte antigen system termed BuLA is the MHC of buffalo (Kumar *et al.*, 1993). The genetic polymorphism of class II α and β chain genes occur predominantly in exon 2 encoding the antigen binding site (Brown *et al.*, 1993). Established associations have been found between MHC and various disease conditions in animals (Zanotti *et al.*, 1996). Among class II genes, DRB3 is highly expressed and polymorphic locus. The DRB3.2 alleles *11, *16, *23 and *24 have been found to be associated with somatic cell count (SCC) and mastitis resistance. Allele *8 of DRB3.2 gene has been found to be significantly associated with higher risk of clinical mastitis while allele *3 with lowest risk of clinical mastitis (Rupp *et al.*, 2007).

Of the various methods used to investigate the genetic polymorphism of MHC loci, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) has been found useful for DRB3 typing in cattle. However, there is paucity of information on MHC polymorphism in buffaloes. The MHC is known to control resistance or susceptibility to many infectious diseases (Klein and Figueroa, 1986). Considering the facts mentioned above, the present investigation was undertaken to study polymorphism in exon 2 of DRB3 gene using PCR-RFLP and its association with mastitis in Murrah buffaloes.

## MATERIALS AND METHODS

Blood samples were collected from sixty unrelated Murrah buffaloes (including 30 animals suffering/ suffered from mastitis) from local organized farms and veterinary clinics of the university. Approximately 10 ml of venous blood was collected in 15 ml sterile polypropylene centrifuge tube containing 0.5 ml of 0.5M EDTA and kept at 4°C until the extraction of genomic DNA.

Genomic DNA was extracted from blood samples

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as per the method reported by Sambrook and Russel (2001) with minor modifications. The purity of DNA was evaluated by taking the ratio of optical densities (OD) at 260 nm to that of 280 nm, by spectrophotometer (Biorad Smart Spec™ Plus).

The 304 bp fragment of the DRB3.2 gene comprising 20 bp of the 5' intron, 267 bp of exon 2 and 17 bp of the 3' intron was amplified by PCR using the primers pair as described by Sigurdardottir *et al.* (1991). The amplification of BuLA-DRB3.2 gene segment was carried out using the primers pair LA31 and LA32 in an optimized PCR. The master mix was prepared by adding 18.7 µl nuclease free water, 2.5 µl Taq DNA polymerase buffer (MBI fermentas), 1.5 µl of 25mM $Mgcl_2$, 0.5 µl of 100 µM dNTPs (Biogene), 0.25 µl (40 ng) each primer and 0.3 µl (1.5 U) Taq DNA polymerase (MBI fermentas) for each sample and after proper mixing 1 µl of genomic DNA was added. The 25 µl reaction mixture was kept for amplification in programmed thermocycler (BioRad). After an initial denaturation at 94°C for 2 min, 35 cycles were given, each comprising of denaturation at 94°C for 1 min, annealing at 61°C for 1 min and extension at 72°C for 1 min, followed by final extension at 72°C for 7 min. After final extension, PCR products were resolved in 1.8% agarose gel and stored at 4°C for further analysis. For RFLP analysis, 15 µl of the amplified PCR products were digested with 10 units of *Pst*I restriction enzyme (New England Biolabs) in a final volume of 20 µl at 37°C overnight. The restriction fragments were resolved by electrophoresis on 4% agarose gel (Sigma, USA) in 1X TAE buffer at 80V for 3 h. As a DNA size marker, 50 bp ladder (New England Biolabs) was used. After staining with ethidium bromide, the fragments were visualised by UV transilluminator and documented by photography.

## RESULTS AND DISCUSSION

When genomic DNA extracted from blood samples was evaluated on 0.7% w/v agarose gel electrophoresis, all samples showed single intact distinct band near the wells. The molecular size of the PCR products was estimated to be 304 bp by comparing with DNA size markers (Fig 1). When 304 bp PCR products

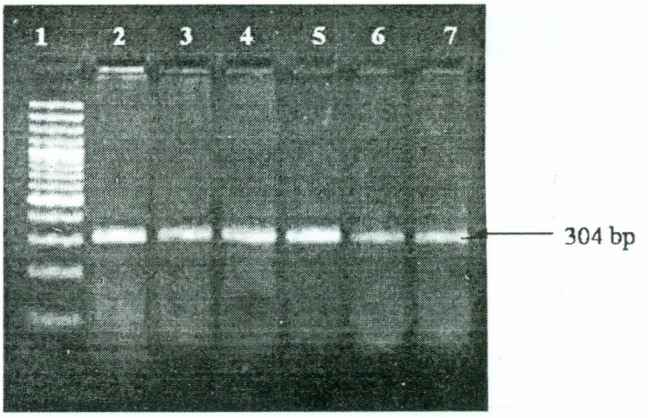


Fig 1. PCR amplification of DRB3.2 gene.
Lane 1: 100 bp ladder (MBI Fermentas), Lane 2: 3, 4, 5, 6 and 7 PCR product of DRB3.2 gene (304 bp).

were digested with *Pst*I, it detected 6 genotypes (Fig 2) viz. *s/s, y/y, z/z, y/z, s/z* and *s/y* with frequencies 0.08, 0.20, 0.53, 0.02, 0.03 and 0.13, respectively (Table 1). Genotype *z/z* was present in 53 % population. Three alleles viz. *s, y* and *z* were found with frequencies 0.17, 0.27 and 0.56, respectively (Table 2) and allele 0.56 *z* was most frequent. It might be due to natural selection favoring the fixation of allele. Few reports are available on PCR-RFLP of DRB3.2 gene with *Pst*I restriction enzyme. Singh *et al.* (2002) observed only three genotypic patterns in Murrah buffalo using *Pst*I which might have occurred due to small sample size. Singh *et al.* (2004) reported restriction site at 216 bp, when 284 bp PCR product of Murrah was digested with *Pst*I. This is in consistent with the findings in

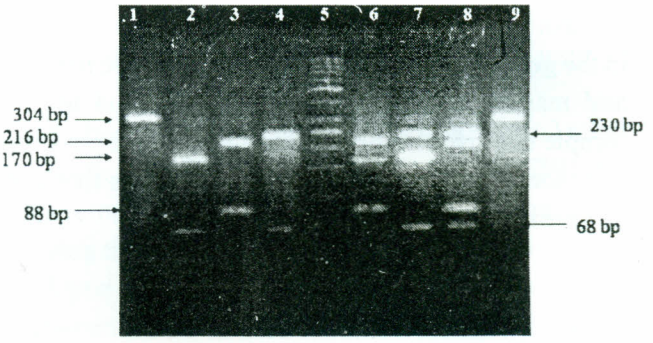


Fig 2. Restriction patterns obtained by digestion of DRB3.2 gene PCR products using *Pst*I.
Lane 1, 9: Uncut, Lane 2: Pattern ss (fragment size: 170, 68, 66), Lane 3: Pattern yy (fragment size: 216, 88), Lane 4: Pattern zz (fragment size: 230, 68, 6), Lane 5: 50 bp ladder, Lane 6: Pattern sy (fragment size: 216, 177, 88, 68), Lane 6: Pattern sz (fragment size: 230, 170, 68), Lane 6: Pattern yz (fragment size: 230, 216, 88, 68).

**Table 1**

**Genotypic frequencies of DRB3.2 gene using *Pst*I enzyme**

| Animals | Genotypic frequencies | | | | | |
|---|---|---|---|---|---|---|
| | *ss* | *yy* | *zz* | *yz* | *sz* | *sy* |
| Mastitic | 0.10 (3)* | 0.17 (5) | 0.57 (17) | 0.00 (0) | 0.03 (1) | 0.13 (4) |
| Healthy | 0.07 (2) | 0.23 (7) | 0.50 (15) | 0.03 (1) | 0.03 (1) | 0.13 (4) |
| Total | 0.08 (5) | 0.20 (12) | 0.53 (32) | 0.02 (1) | 0.03 (2) | 0.13 (8) |

*Figures in parentheses refers to the number of animals

**Table 2**

**Allelic frequencies of DRB3.2 gene using *Pst*I enzyme**

| Animals | Allelic frequencies | | |
|---|---|---|---|
| | *s* allele | *y* allele | *z* allele |
| Mastitis | 0.18 | 0.23 | 0.58 |
| Healthy | 0.15 | 0.32 | 0.53 |
| Total | 0.17 | 0.27 | 0.56 |

present study. The findings of the present study are in consonance with Sudhir *et al.* (2008) who has reported *s, x, y* and *z Pst*I restriction patterns in Murrah buffalo. Except for *x,* all restriction patterns were observed in present study.

When the PCR product of healthy animals of Murrah breed digested with *Pst*I, it revealed 6 different genotypes viz. *s/s, y/y, z/z, y/z, s/z* and *s/y* with frequencies range 0.03 - 0.50 (Table 1) and three alleles viz. *s, y and z* with frequencies range 0.15-0.53 (Table 2). In mastitis cases, only 5 genotypes viz. *s/s, y/y, z/z, s/z* and *s/y* with frequencies range 0.03-0.57 were found. The genotype y/z was found only in healthy animal. It may be associated with resistance to mastitis. However, there are no significant differences in the genotypic frequencies of this genotype in healthy and mastitic animals which might be due to small sample size. Nonetheless, it has initiated the possibility of association between BuLA and mastitis that needs to be explored in a large population size.

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