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Polymorphism in MHC Class II DYA EXON 2 and 3 of Deoni cattle

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Abstract
Sixty DNA samples of Deoni cattle maintained at College of Veterinary Science, Rajendranagar were studied for polymorphism (PCR- RFLP) in Exon 2 and Exon 3 of Cattle MHC Class II (BoLA) DYA gene. The two fragments of the gene were amplified by specific cattle sequence primers and digested each with two enzymes. On digestion of exon 2 with *Alu I* resulted in two fragments of 179bp and 74bp whereas, *Hae III* produced 210bp and 43bp fragments in all samples. Exon 3 (296bp) digested with *AvaII* and *Hinf I* each produced two fragments of sizes 180bp, 116bp and 171bp, 125bp respectively in all samples indicating absence of polymorphism.

Keywords: MHC, class II DYA gene, PCR-RFLP, polymorphism

Introduction
The MHC of vertebrates includes class I and class II genes which encode cell surface glycoproteins with a key function in the immune regulation (Klein 1975) [7]. The distribution of class II molecules is limited mainly to B-lymphocytes and certain other lymphoid cells. These molecules contain two covalently linked polypeptide chains called α and β. Each chain consists of five domains and coded by A, B genes. The bovine MHC (BoLA) is located on chromosome 23 (Fries *et al*. 1989) [5] and divided into two regions and the class II genes designated as BoLA –D (Usinger *et al.* 1977) [13]. In this region, there are evidences of presence of one DRA, three DRB, one or two DQA, DQB, single DOB and DNA and non-orthologous genes DY and DI (Andersson *et al*. 1986 and Stone 1990) [1]. The sequence polymorphisms located in the peptide-binding domain (PBD) in the exon2 of the polypeptide (De *et al.* 2002; Sigurdardottir *et al.* 1988; Sena *et al*. 2003; Russell and Gallagher 1996 and Skow and Nall 1996) [3, 11, 10, 9, 12]. It is speculated that DY genes may also express a unique class II molecule with a specialized function in ruminants (Wright *et al*. 1994 and Mann *et al*. 1993) [16, 8] suggesting that they are restricted to ruminants. The study was undertaken to explore polymorphism in the genomic sequences of exon 2 and 3 of MHC class II DYA gene in the cattle.

Materials and Methods
A total of 60 Deoni cattle maintained at College of Veterinary Science, Rajendranagar were utilized for the present study. Genomic DNA was isolated from blood by standard Phenol-Chloroform method (Sambrook and Russel 2001). The samples were run on 0.8 % agarose gel electrophoresis to verify DNA quality and quantified by spectrophotometry for PCR amplification.
The two exons of DYA gene were amplified (exon 2 and 3) separately with a set of region specific primers designed based on available cattle sequences (Gen Bank. Acc No AJ- 58058) for each exon. The primer sequences are given in table I.

PCR amplification
The two genes were amplified in 25 µl reaction mix containing 70ng of genomic DNA, 30pM of each primers (1µl), dNTPs 2.5 µl (2mM), 10x Buffer 2.5µl and 1U of *Taq* polymerase and Mg^{+2} at the rate 1.5mM concentration.

Cycling conditions
Initial denaturation step at 94° C for 3 min, followed by 34 cycles of denaturation at 94° C for 45 sec, primer annealing at 61° C (Exon 2) and 62.5° C (Exon 3) for 40 sec, extension at 72° C for 45 sec, with a final extension at 72° C for 5 min. The products were confirmed by visualizing on 2% agarose.

Restriction enzyme digestion of PCR Products (RFLP)

*Alu*I and *Hae III* restriction enzymes were used to digest the 253 bp fragment of exon 2 of DYA gene. The digestion was carried out overnight at 37ºC in a total volume of 15µl reaction mix containing, restriction enzyme (10u/µl) 1.0µl, 10x assay buffer for RE 1.5µl, PCR product 10.0µl, and autoclaved distilled water 2.5µl. Exon 3 was digested with *AvaII* and *Hinf I* enzymes with similar conditions applied to exon 2 digestion. The digested products were run on a 4% agarose gel and visualized on gel doc system for analysis.

Results and Discussion

A PCR product of 253bp corresponding to exon2 and 296bp of exon 3 of DYA gene were amplified from the genomic DNA of all the 60 Deoni cattle by specific primers. The products were subjected to restriction digestion. *Alu I* digestion of exon 2(253bp) yielded two fragments of size 179bp and 74bp and *Hae III* digestion yielded two fragments of 210bp and 43bp uniformly in all the sixty samples of PCR products digested, indicating no polymorphism in the exon2. Restriction digestion of exon 3(296bp) with *Ava II* produced fragments of 180bp and 116bp and with *HinfI* produced 171bp and 125bp in all samples studied, suggesting no polymorphism in exon 3 also.

Table I: Primer sequences, restriction digestion pattern of cattle MHC class II exon 2 and 3 of DYA gene

Primer Name	Primer sequence (5' to 3')	Restriction enzymes	Recognition sequence	Digested fragments (bp)
DYA exon 2 (253bp)	Forward: GCACTTACGGCACAAATG Reverse: GAGAGGCCACACTTACCACT	*Alu*I	5′ AGCT 3′	179,74
		Hae III	5′ GGCC 3′	210,43
DYA exon 3 (296bp)	Forward: GAAGCCTCCCCTAAACTGATT Reverse: ACTGGTGGGAAGAAAGGTGAG	*AvaII*	5′ GGACC 3′	180,116
		Hinf I	5′ GATC 3′	171,125

The results indicate that the MHC Class II exon 2 and exon 3 which were supposed to be more variable and form peptide binding site (PBS) of a MHC class II molecule and thereby regulate immune response by binding antigenic peptides were not much variable as in the other MHC class II molecules such as DQA and DQB and DRB (Andersson *et al.*, 1996; Dutia *et al.* 1995 [4]; Groenen *et al.* 1989 [6]; Van-der-poel *et al.* 1990 [14] and Sigurdordottir *et al.* 1988) [11]. Sequencing of exon 2 revealed 2 alleles (Van eijk *et al.* 1992; Skow and Nall 1996) [15, 12]. Ballingall *et al*. (2004) [2] reported that these genes are expressed and substituted to the DP locus in human beings and restricted to the dendritic cells in the ruminants with some specific function but they are not polymorphic. These results are also in agreement with the findings of Sakaram *et al.* (2013). Although extensive screening of diverse cattle populations may be required to find out low level of polymorphism.

Fig 1: PCR product of DYA exon 2 (253bp)

Lane M: 50bp ladder used as marker

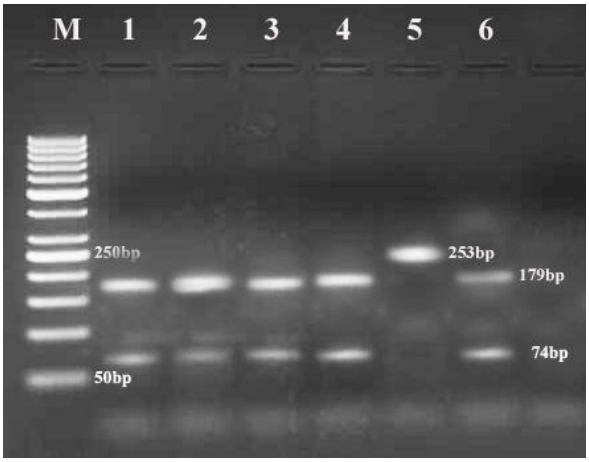

Fig 2: *Alu I* digestion of 298bp fragment of DYA exon 2 gene

Lane 5: Undigested PCR product (253bp)
Lane 1- 6: *Alu I* digested fragments of DYA exon 2 gene
Lane M: 50 bp ladder used as marker

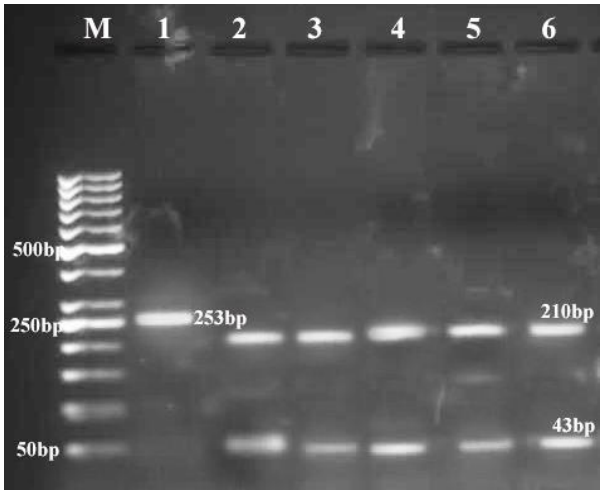

Fig 3: *Hae III* digestion of 298bp fragment of DYA exon 2 gene

Lane 1: Undigested PCR product (253bp)
Lane 2- 6: *Hae III* digested fragments of DYA exon 2 gene
Lane M: 50 bp ladder used as marker

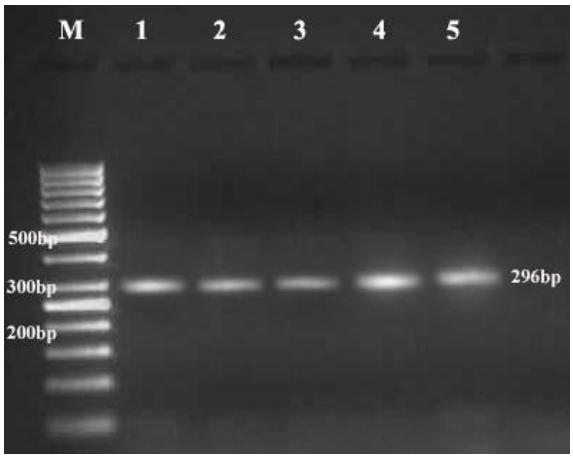

Fig 4: PCR product of DYA exon 3 (296bp)

Lane M: 50bp ladder used as marker

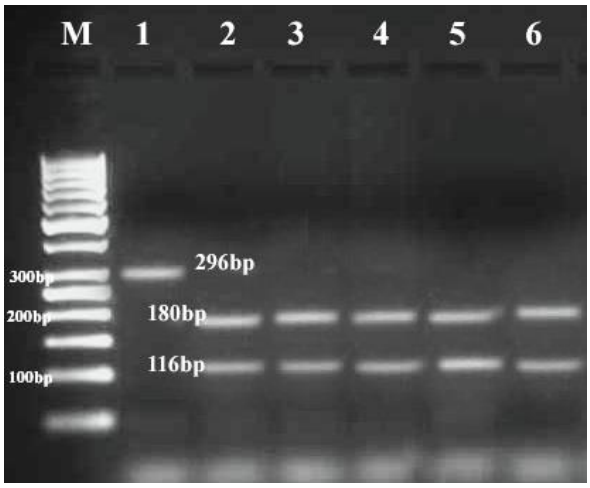

Fig 5: *Ava II* digestion of 296bp fragment of DYA exon 3 gene

Lane 1: Undigested PCR product (296bp)
Lane 2- 6: *Ava II* digested fragments of DYA exon 3 gene
Lane M: 50 bp ladder used as marker

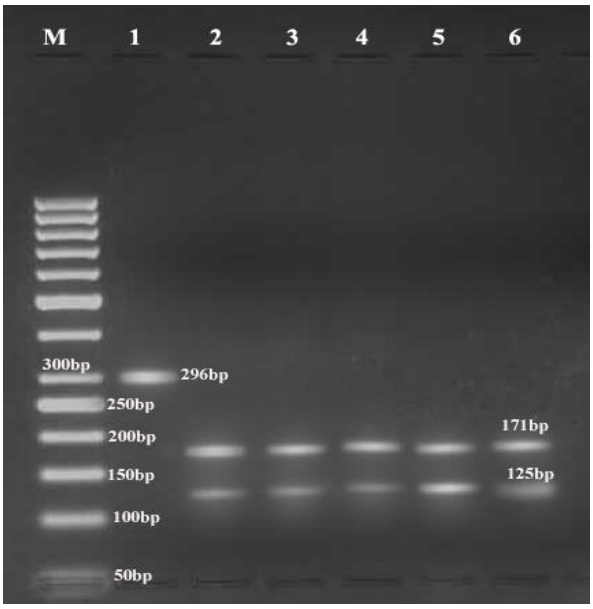

Fig 6: *Ava II* digestion of 296bp fragment of DYA exon 3 gene

Lane 1: Undigested PCR product (296bp)
Lane 2- 6: Hinf I digested fragments of DYA exon 3 gene
Lane M: 50 bp ladder used as marker

Summary
Sixty DNA samples of Deoni cattle were used to study the polymorphism in exon 2 (253bp) and 3 (296bp) of DYA gene. *Alu I* digestion of exon 2(253bp) yielded two fragments of size 179bp and 74bp and *Hae III* digestion yielded two fragments of 210bp and 43bp uniformly in all the samples. *Ava II* produced fragments of 180bp and 116bp and with *HinfI* produced 171bp and 125bp in all samples indicating no polymorphism.

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