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Genetic polymorphism of MHC class II DRB exon2 gene in Salem black goats by PCR-RFLP

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Abstract
The study was conducted to explore the polymorphism of CLA-DRB3 gene in Salem black goat by using PCR-RFLP technique. A region of exon 2 encompassing 285 bp fragment of DRB gene was amplified by polymerase chain reaction. The restriction digestion by *BsaI* revealed three genotypes AA (285 bp), AB (285/182/103 bp) and BB (182/103bp) with frequencies 0.930, 0.060 and 0.010, respectively and two alleles A and B with frequencies 0.96 and 0.04, respectively. The polymorphic information content (PIC) value and expected heterozygosity were 0.074 and 0.060, respectively which were low in both cases. The present study showed polymorphic nature of MHC Class II DRB exon2 Gene in Salem black goats at this locus and the frequencies of homozygote were greater than heterozygote.

Keywords: *BsaI*, MHC class II DRB, PCR, RFLP, Salem black goats, SNPs technique

Introduction
In Caprine, Major Histocompatibility Complex (MHC) is located on chromosome number 23 and which has three subgroups, MHC Class I, MHC Class II, MHC Class III. Among these, the Class II molecule plays a pivotal role in the initiation of the immune response by presenting exogenous antigens to helper T-lymphocytes specially elicit the antibody production against the pathogen or parasites (Klein, 1986) [7]. MHC Class II isotypes such as DQ and DR have been already expressed in cattle (BoLA) (Takada *et al*., 1998) [13].
Among these two subgroups, the DRB locus is the most polymorphic and considered functionally to be responsible for the differences among individuals in the immune response to infectious agents. So that, the research groups for association studies in sheep had greatest attention on this study.
The high degree polymorphism at MHC loci is intended to be an outcome of balancing selection at this locus (Garrigan D, *et al*. 2003) [3]. The exon 2 of caprine MHC class II DRB3 gene polymorphic variation of allele reported in Rohilkhandi goats (Shrivastava *et al*., 2015) [12], Raeini Cashmere goats (Baghizadeh *et al*., 2009) [2] and Chinese indigenous goats (Li MH *et al*., 2006) [8] at different SNP loci. Genetic polymorphism study at MHC locus facilitates the identification of specific allelic variations that may be affecting disease resistance and susceptibility traits. Salem black goats are innate resistance to the harsh climatic conditions prevailing in its original habitat. There is a lack of information on genetic characterization of Salem black goats at MHC Class II loci. Therefore, current study was planned to estimate the polymorphism of the most critical regions of the MHC class II DRB gene in Salem black goats.

Materials and Methods
Sample Collection and Isolation of genomic DNA
The study was undertaken at Molecular Genetics Laboratory, Division of Animal Genetics, Indian Veterinary Research Institute (IVRI), Izatnagar, Bareilly (UP). A total of 100 blood samples were collected from a randomly mating population of Salem black goats maintained at Government Sheep and Goat farm, Chinnasalem, Villupuram District of Tamil Nadu, India. About 5 ml of anticoagulated blood was collected under sterile conditions from the jugular vein of goats by using 2.7% EDTA. All the blood samples were kept in -20 °C till further processing for DNA extraction.

Genomic DNA was isolated from whole blood by phenol-chloroform extraction and ethanol precipitation method as per standard protocol (Sambrook and Russell, 2001) [11].

Locus under investigation

The locus under investigation was selected from NCBI GenBank database. The SNP (T/C) at *BsaI* position at 103bp position was chosen from 285 bp fragment. Then, single cutter restriction enzyme (*TaqI*) was selected by using NEB cutter V2.0 online available software. The *TaqI* enzyme digests the 285 bp fragment of MHC class II gene and creates sticky ends at 103/105bp position.

Polymerase Chain Reaction (PCR)

To get the desired 285 bp fragment, PCR was performed using primers with the sequence of the forward and reverse primers were 5′-TAT CCC GTC TCT GCA GCA CAT TTC-3′ and 5′-TCG CCG CTG CAC ACT GAA ACT CTC-3′, respectively (Amills *et al*., 1990) [1].

The PCR reaction was performed in 25µl reaction mixture that included 10 pmol of each primer, 12.5 µl of 2X PCR master mix (Thermo Scientific) and 1 µl of 50 to 100 ng/µl of goat genomic DNA as a template. The PCR conditions includes initial denaturation at 95 °C for 5 minutes followed by 40 cycles each of denaturation at 95 °C for 1 minute, annealing at 59.5 °C for 45 seconds, extension at 72 °C for 1 minute and then a final extension at 72 °C for 5 minutes. The 5 µl PCR products were checked by 1.5% agarose gel electrophoresis in order to check the quality and specificity of PCR product using ethidium bromide staining. Finally, the gels were photographed under UV light with a gel documentation system (Syngene).

Restriction Enzyme Digestion and Electrophoresis

About 10 µl of PCR products were digested by restriction endonuclease (2U) with the appropriate buffer supplied with the enzyme and kept for overnight digestion at 65 ºC for *TaqI* (Thermo Scientific). The digested products were run on agarose gel from 2% as expected size of fragments with suitable DNA marker. Finally, the gels pictures were saved with a gel documentation system (Syngene gel doc system).

Statistical Analysis

After enzymatic digestion, the allelic and genotypic frequencies of the locus at CLA-DRB3 gene fragment were estimated by using PROC ALLELE procedure of SAS.9.3. Test for Hardy Weinberg equilibrium and neutrality ratios were done using POP GENE v 1.32.

Results and Discussion

The quality of PCR amplified product of 285 bp MHC class II DRB 3 gene in Salem black goat was examined in agarose gel electrophoresis (Fig. 1). The digestion of PCR product by *BsaI* restriction enzymes showed AA, AB and BB genotypes and presence of two alleles (Fig. 2). The allelic and genotypic frequency at marker loci *BsaI* is shown in Table 1. In Salem black goat population the genotypic frequencies of *BsaI* locus were found to be 0.930, 0.060 and 0.010 AA (285/285 bp), AB (285/182/103 bp) and BB (182/103 bp), genotypes respectively. The frequency of homozygote was greater than heterozygote. The frequency of A allele (0.96) was higher than B allele (0.04) (Table 1).

The loci showed a PIC value of 0.074 with heterozygosity values of 0.060 and the allelic diversity values were estimated to be 0.077. The summary of markers in relation to Polymorphic Information Content (PIC) and test for HWE are given in (Table 2). Test for HWE showed that population was significantly (P=0.023) deviating from HW equilibrium at this locus, this may be due to the use of less number of males for breeding purpose.

The gene and genotypic frequencies of the present study are in concordance with the other reported studies (Shrivastava *et al.*, 2015) [12] and Prakash Om *et al*., 2017) [10]. The population genetic analysis of the genotypic data showed loci to be significantly deviating from HWE with more homozygosity. However, earlier PCR RFLP studies on this gene have been reported heterozygote excess and significant deviations from HWE using multiple restriction enzymes (Jamshidi *et al*., 2011) [5] and (Gruszczynska *et al.*, 2004) [4]. Previous reports have also shown that population that is relatively closed and breeding randomly within the herd tends to be in HWE (Li R Y *et al.*, 2011) [9].

MHC Class II genes are pivotal in conferring resistance/susceptibility to parasitic infestation (Karrow *et al*., 2014) [6]. MHC gene loci polymorphism is one of the major drivers of species survival. The polymorphism was reported in MHC Class II DRB3 gene of Salem black goats by PCR-RFLP technique. The polymorphism of this gene locus has been extensively studied with the association studies and is advocated to be used as a genetic marker for nematode resistance/susceptibility (Jamshidi *et al.*, 2011) [5].

Table 1: Allelic and genotypic frequencies at *TaqI* locus

Locus	Genotype	Count	Frequency	Allele	Count	frequency
TaqI	AA	93	0.93	A	192	0.96
	AB	06	0.06	B	8	0.04
	BB	01	0.01	Total	200	1
	Total	100	1			

Table 2: Chi square test values for HWE at *TaqI* locus

Locus	Count	No of alleles	PIC	Heterozygosity	Allelic diversity	Test for HWE			
						HWE Chi square probability	DF	Pr>ChiSq*	Prob** Exact
TaqI	100	2	0.074	0.060	0.077	4.785	1	0.029	0.139

*p-value for the Chi-square test (*if P value < 0.05 population not consistent with HWE); **an estimate of the exact p-value for the HWE test; HWE=Hardy–Weinberg equilibrium; PIC=Polymorphic information content

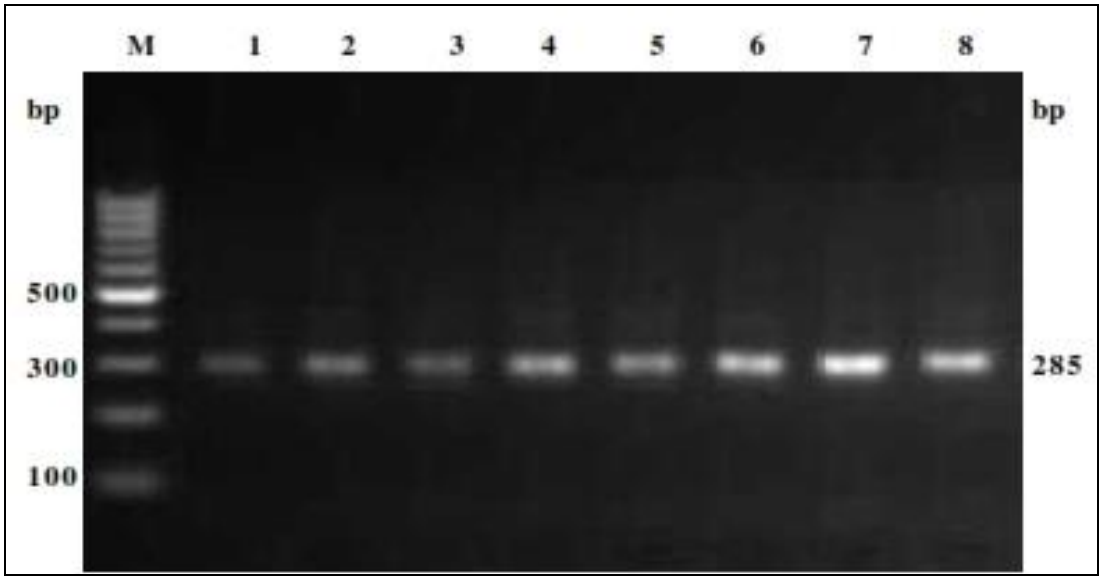

Fig 1: Amplification of 285bp fragment of MHC Class II DRB gene exon 2 in Salem Black goat Lane M: 100 bp DNA marker; Lane 1-8: Amplified products

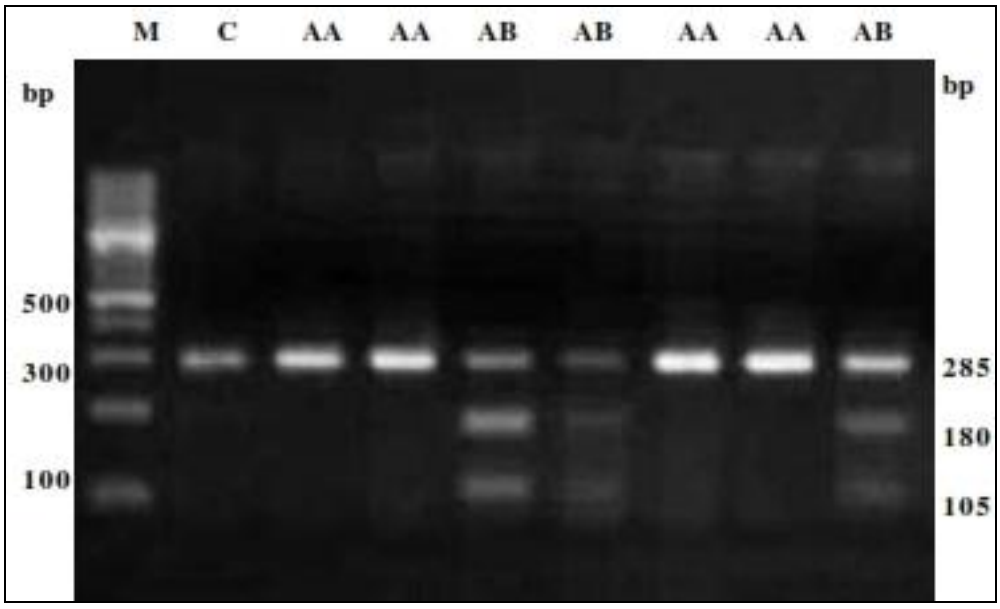

Fig 2: RE digestion of 285bp fragment of MHC Class II DRB gene exon 2 by *BsaI* enzyme in Salem Black goat Lane M: 100bp DNA marker Lane C: Undigested PCR Product Lane 1-7: Genotypes obtained after *BsaI* digestion

Conclusion

The Salem black goat population, the frequency of homozygote was greater than heterozygote genotypic frequencies were documented. At the same time, the frequency of A allele (0.96) was higher than B allele (0.04).

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