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Short Communications

Genetic analysis of MHC Class II *DRB* gene in an endangered Jamunapari breed of goats

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Present investigation was aimed to analyze the polymorphism of Class II *DRB* gene region of MHC, using PCR-RFLP with *Pst*I and *Taq*I restriction enzymes, in endangered Jamunapari goat breed. A total of 203 Jamunapari goats (male-77 & female-126) were included in the study to know the distribution of different genotypes in the population. *Pst*I revealed three genotypes, i.e., PP, Pp and pp, with genotype frequency as 0.054, 0.222 and 0.724, respectively. *Taq*I also showed TT, Tt and tt genotypes with frequencies of 0.241, 0.527 and 0.232, respectively. The frequencies of P and p alleles were 0.165 and 0.835, while frequencies of T and t alleles were 0.505 and 0.495, respectively. Marked variations were observed in the distribution of *Pst*I and *Taq*I generated genotypes in the Jamunapari goat population.

Keywords: *DRB* gene, Jamunapari goat, *MHC Class II* gene, PCR-RFLP

High degree of polymorphism for MHC genes in a population is itself an explanation of the capability of host immune systems to attack such a wide variety of antigens by recognizing them as not of their own. MHC class II molecules are of different kinds, where *DQ* and *DR* subtypes are the most polymorphic both in human and domestic species, and probably play a major role in the development of MHC restricted immune response[1]. The most polymorphic among the MHC gene is *DRB* locus[2]. Amills *et al*[3] has developed a *Pst*I and *Taq*I based PCR-restriction fragment length polymorphism (RFLP) method to characterize the goat MHC Class II *DRB* region. Furthermore, *Taq*I and *Pst*I restriction sites are found to be associated with amino acid substitutions at important antigen recognition site (ARS). The genomic variation at such critical point may potentially be responsible for susceptibility or resistance to certain diseases and this information may be of great concern to conserve any species that is being counted endangered due to higher disease susceptibility.

Jamunapari is a majestic, large, dual-purpose breed of Indian goats recognized for its milk yield. Unfortunately, population of pure Jamunapari goats has, recently, declined fast and, at present, less than 5000 goats are left in their native tract[4]. This breed has recently been put under 'highly threatened' category due to changes in the demography of the native tract, farming and practices, social structure, diminishing returns and high susceptibility to diseases, particularly to paratuberculosis[5]. A nucleus flock of Jamunapari goats is being maintained at Central Institute for Research on Goats (CIRG), Makhdoom under genetic improvement programme as an All India Coordinated Research Project (AICRP) component. However, cases of paratuberculosis have been encountered at this farm despite of adequate control measures[6]. The disease has been adversely affecting the genetic progress of the flock and improvement in genetic resistance has been considered as the best long term strategy for disease control. MHC has been known as the key locus for disease resistance/susceptibility. Considering the highly polymorphic nature of *DRB* region of MHC and its importance in antigen recognition, the present study was planned to characterize *DRB* region of MHC using PCR-RFLP with *Pst*I and *Taq*I enzymes. The gene and genotype frequencies were also estimated in Jamunapari flock maintained at CIRG, Makhdoom.

A total of 203 Jamunapari goats (male-77 & female-126) were included in the study. The blood samples (1 mL) were collected from Jugular vein of each goat in vacutainer (with anti-coagulant) tube and subjected to isolation of DNA as per method of Singh *et al*[6]. Second exon of MHC Class II *DRB* region was amplified through nested PCR method using the primers and cyclic condition as described by Amills *et al*[3]. PCR products (from two goats) were sequenced and nucleotide sequence reads were subjected to global BLAST (www.ncbi.nilm.nih.gov/BLAST) for confirming the amplification of target region.

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Neighbor-joining phylogeny based analysis was also performed with the *DRB* nucleotide sequences of Jamunapari and other breeds of goat and other animal species using LASERGENE software (DNASTAR, version4.0, Inc., Madison, USA). Amplicons were digested separately with two restriction enzymes, namely, *Taq*I and *Pst*I (Fermentas, USA) as per manufacturer instructions and digested products were electrophoresed to get *Pst*I and *Taq*I restriction profile of each goats. The distribution of different genotypes in male and female goats was also estimated by direct counting method. Allelic as well as genotypic frequencies of MHC Class II *DRB* region were estimated as per the method described by Falconer and Mackey[7].

PCR based amplification of *DRB* region using nested primers resulted in an amplicon of 285 bp size, which was similar to that reported earlier[3,8]. Sequencing of amplicon could read only 229 bp nucleotides that was submitted to GenBank database vide Acc.No. JF416295. Further, global BLAST and multiple sequence alignment confirmed the amplification of targeted region. In phylogenic analysis, sequences of Jamunapari *DRB* region with those of other caprine breeds, ovines and bovines were included. In the constructed phylogenetic tree, Jamunapari breed along with Angora, Changthangi and *Capra aegagrus* constituted a separate major cluster; while Chinese, Spanish and Indian Chegu breed of goats were present in another major cluster along with different breeds of ovine and bovine (Fig. 1). Interestingly, low evolutionary distances were recorded between domesticated goats (*C. hircus*) of China and Spain. Similarly, taurine and zebu cattle also clustered closely. The phylogenetic analysis of DRB gene sequences revealed that most of the goat breeds are phylogenetically more related among themselves, and similarly most of the ovine breeds have also higher relationship among themselves. Therefore, the inter-generic distances in general were more than intra-generic distances. However, some the domesticated goat showed evolutionary relationship with cattle. This shows a continuum in evolutionary pattern of *DRB* genes over various genera of mammals.

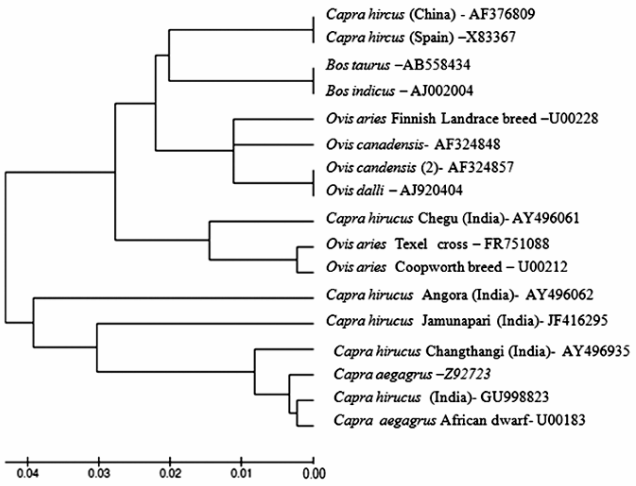

Fig. 1—Neighbor-joining phylogenetic analysis of caprine, ovine and bovine MHC Class II *DRB* region. The GenBank accession numbers are shown between parentheses.

Amills *et al*[1] observed co-amplification in single step PCR or two step nested-PCR under low stringent condition, indicating the presence of at least two *DRB* genes. Presence of multi *DRB* genes (3) was also reported by Schwaiger *et al*[9]. The existence of several *DRB* genes in other artiodactyla species reinforces this hypothesis. However, in present study, no co-amplification was observed in one step Nested-PCR (data not shown) and it may be due to the presence of single *DRB* gene or existence of mismatches between primers and their complementary regions of the other *DRB* gene. Therefore, further studies are required to clarify whether Jamunapari or others Indian breeds have single or multiple DRB genes.

In the present study, *Pst*I and *Taq*I based PCR-RFLP analysis in Jamunapari goat population showed different restriction profiles. A total of two restriction patterns, namely, P with 15, 44 and 226 bp fragments, and p with 15 and 270 bp fragments, were detected from *Pst*I RFLP. In case of heterozygotes, four bands of 270, 226, 44 and 15 bp fragments were resolved on the gel, indicating the presence of two *Pst*I enzyme recognition sites on one chromosome and one recognition site on its homologous pair. Thus, Jamunapari goat reveals presence of three genotypes, such as, PP, Pp and pp, and two alleles, such as, P and p at the second exon of *DRB* gene. In *Taq*I RFLP, a total of two restriction patterns, such as, T pattern with 122 and 166 bp fragments, and t pattern with 285 bp, were observed. Heterozygote for *Taq*I PCR-RFLP exhibited three bands of 285, 166 and 122 bp size. Thus, three genotypes, such as, TT, Tt and tt and two alleles such as T and t were recorded in Jamunapari population. The genotypic frequency of PP genotype estimated was 0.054, whereas the frequency of Pp and pp genotype were 0.222 and 0.724, respectively.

Consequently, the frequencies of P and p alleles were found to be 0.0.165 and 0.835, respectively. In *Taq*I RFLP, the observed genotype frequencies were 0.241 for TT, 0.527 for Tt and 0.232 for tt genotype, while the frequencies of T and t alleles were 0.505 and 0.495, respectively in Jamunapari goats. Sex-wise frequencies of each genotype for both polymorphic sites (by direct counting method) were given in Table 1. Results demonstrated higher prevalence of homozygous (pp) and heterozygous (Tt) genotypes.

In the studied region of *DRB* gene, different amino acid substitutions were reported due to highly polymorphic nature of gene[10]. Amills *et al*[3] and Sheikh *et al*[11] have clearly shown that the protein profiles of both *Pst*I and *Taq*I alleles were associated with change in amino acid. The presence of the *Pst*I site was associated with the TAC codon (tyrosine), whereas its absence was associated with the TGT codon (cysteine) at the same nucleotide position. Likewise, *Taq*I site was associated with a TTC codon (phenylalanine), whereas its absence leads to the TAC codon (tyrosine) in the t allele. Moreover, the replacements in this region are thought to be functionally important for antigen recognition by forming the antigen binding site (ABS)[11]. In fact, this protein is primarily responsible for synthesis of antigen presentation domain on the cell surface and the changes of protein structure probably influence the stringency of epitope binding and ultimately affect the host immune response against the causative organism. Therefore, these SNPs may have enormous potential to be used as marker for identifying the animals as susceptible/resistant to the disease. Besides, the potential of single nucleotide polymorphisms (SNPs) of *DRB* regions as a candidate gene marker for various livestock diseases has been summarized by Amills *et al*[3]. The SNPs of *DRB* region were also found to be associated with certain production[8,11,12] and reproduction traits[13,14] in livestock population.

The successful and specific amplification of the second exon of *DRB* gene is the first step for optimizing a PCR-RFLP based method to type this important locus in Jamunapari goats. Similarly, optimized nested-PCR and RFLP analysis would be useful in identifying new variants in the second exon of the *DRB* gene. Since, Redacliff *et al*[15] has reported the association of MHC Class II gene with paratuberculosis susceptibility and this study showed the existence of marked variations in distribution of *DRB* polymorphic sites in targeted Jamunapari population; therefore, it will be interesting for future studies to investigate the association of SNPs of *DRB* region with susceptibility to paratuberculosis in order to conserve this precious germplasm.

Table 1—Sex wise distribution of *Pst*I and *Taq*I genotypes, and genotype and gene frequencies in Jamunapari goat population

Genotypes	Numbers and genotype frequencies			Allele frequency	
	Male	Female	Total		
		*Pst*I		P	p
PP	0*	11	11		
	(0)	(0.054)	(0.054)		
Pp	14	31	45	0.165	0.835
	(0.069)	(0.153)	(0.222)		
pp	52	95	147		
	(0.256)	(0.468)	(0.724)		
		*Taq*I		T	t
TT	16	33	49		
	(0.079)	(0.162)	(0.241)		
Tt	37	70	107	0.505	0.495
	(0.182)	(0.345)	(0.527)		
tt	13	34	47		
	(0.064)	(0.168)	(0.232)		

*Figures in parentheses are genotype frequencies

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