BoLA-DRB3 polymorphism and their association with milk production traits in Indian cattle breeds

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ABSTRACT

The present study was undertaken to investigate the polymorphism in *DRB3* gene in Indian cattle breeds using PCR-RFLP assay. Amplification of 100 DNA samples of Sahiwal and Hariana breeds revealed 304 bp products using specific primer pairs. These amplicons were digested using *Hae* III restriction enzyme. It produced five genotypic patterns viz., AA genotype (170, 82 and 52 bp); BB genotype (222 and 82 bp), EE genotype (170 & 134 bp), heterozygous AB genotype (222, 170, 82 and 52 bp bands) and BD genotype (222, 193, 82 and 29 bp bands). AA genotype was the most frequent among all the screened cattle, followed by the AB, EE, BD and BB genotypes. The frequency of DRB3/*Hae*III A, B, D and E alleles were 0.660, 0.185, 0.035 and 0.120, respectively. Association study revealed no significant difference for milk production traits among all the *DRB3* genotypes in first and second lactation except the dry period (DP), which had significant difference in second lactation. There was significant difference (*P = 0.0106*) for somatic cell count (SCC) among all the genotypes in screened Hariana cattle, while in sahiwal cattle no association was observed. This study needs further investigation on large population size with the incorporation of other breeds of Indian cattle.

Key words: Sahiwal cattle, Hariana cattle, PCR-RFLP, *DRB3*, Polymorphism, milk production traits.

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INTRODUCTION

BoLA-DRB3 exon 2 alleles in cattle have been found to be associated with resistance or susceptibility to various diseases affecting immunity, somatic cell count (SCC) and mastitis resistance in cattle (Baltian et al. 2012). The *DRB3.2* polymorphism has also been observed to be associated with milk production traits (Starkenburg et al. 1997). Therefore, this MHC gene complex is a potential candidate markers for selection purposes in milch cattle (Parmentier et al. 1999).

Several single nucleotide polymorphisms (SNPs)/ mutations in *BoLA-DRB3* gene and their association with milk production traits have been reported in different exotic cattle (Gelhaus et al. 1995; Miretti et al. 2001; Miltiadou et al. 2003; da Mota et al. 2004; Nassari et al. 2008). This polymorphism has also been studied in various Indian cattle breeds including Ongole (Aravindakshan and Nainar 1999), Gir (Acharya et al. 2002), Rathi (Sharma et al. 2005), Kankrej (Behl et al. 2007) and Sahiwal (Chakravorty et al. 2015); crossbred cattle (Ganguly et al. 2004) and buffalo breed (Arvindakshan et al. 2000; Singh et al. 2004; Stafuzza et al. 2015). Keeping all this in consideration, the present study was undertaken to investigate the status of *BoLA-DRB3* gene polymorphisms in Indian Sahiwal and Hariana cattle breeds using *Hae*III/PCR-RFLP assay and their association with milk production traits and SCC.

MATERIALS AND METHODS

Animal source and DNA Extraction

A total of 100 females of Sahiwal (n = 50) and Hariana (n = 50) cattle maintained at Instructional Livestock Farm Complex (ILFC), DUVASU, Mathuta (Uttar Pradesh), were utilized in the present investigation.

Genomic DNA was isolated from venous blood using the standard protocol of Sambrook and Russel (1991). An amplicon of 304 bp PCR product of exon 2 of *DRB3* gene was amplified by using the primer (Siguardardtottir et al. 1991; 'F': 5 - GAT GGA TCC TCT CTC TGC AGC ACA TTT CCT - 3 & 'R': 5 - CTT GAA TTC GCG CTC ACC TCG CCG CTG -3). The cycle conditions included an initial denaturation at 94°C for 2 min followed by 35 cycles of denaturation at 94°C for 60 sec, annealing at 60°C for 60 sec and extension at 72°C for 60 sec and a final extension at 72°C for 10 min. The PCR product was checked by agarose gel electrophoresis in 1x TAE buffer after staining with ethidium bromide and visualized under UV light.

HaeIII/PCR-RFLP assay and calculation of gene and genotypic frequency

The restriction digestion was carried out at 37°C for 14-16 hr in a total volume of 15µl containing 5.0 µl of PCR product, 1.5 µl of 10X RE buffer and 10 Units (1.0 µl) *Hae*III enzyme (New England Biolabs). The data was generated by estimating the frequency of different RFLP pattern. The allelic frequency and genotypic frequencies of *DRB3* gene was estimated by standard procedure (Falconer and Mackay 1996). The chi square (χ^2) test ($P \leq 0.05$) was performed to test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium (Snedecor and Cochran, 1989).

The association study of different genotypes with the milk production traits viz., lactation period (LP), total milk yield (TMY), milk yield in 300 days (MY300), dry period (DP) and calving interval (CI) was carried out. Statistical analysis of milk production traits and SCC was carried out using the General Linear Model (GLM) using SPSS software. The following linear model was applied:

$$Yij = \mu + Gi + eij$$

Where: Yij– observed trait value in animal; µ – mean trait value; Gi – effect of genotype; eij– random error. Significant differences among least square means of different genotypes were calculated using Duncan's multiple-range test, and $P \leq 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

*Hae*III/PCR-RFLP assay of the 304 bp of amplified products of *DRB3.2* gene revealed five types of genotypes; one of them was of 170, 82, 52 bp (AA genotype); second of 222 and 82 bp (BB genotype), third of 170 and 134 bp (EE genotype) and heterozygous pattern have 222, 170, 82 and 52 bp bands (AB genotype) and 222, 193, 82 and 29 bp bands (BD genotype) (Fig. 1). This revealed that the screened cattle used in the present study were polymorphic in nature with four types of alleles A, B, D and E. The genotypic and allelic frequencies of DRB3/*Hae*III gene are given Table 1. Chi square analysis revealed the screened cattle population was not in Hardy- Weinberg equilibrium (Table 1).

Arvindakshan and Nainar (1999) observed seven restriction patterns in Jersey crossbred animals and four patterns in Ongole cattle where three restriction

Table 1. Genotypic and allelic frequencies of DRB3/HaeIII gene in cattle.

Breed	Genotypic frequency (%)			Allelic frequency					
	AA	AB	BB	BD	EE	A	B	D	E
Sahiwal	48.0	20.0	6.0	12.0	14.0	0.58	0.22	0.06	0.14
(N = 50)	(n = 24)	(n = 10)	(n = 3)	(n = 6)	(n = 7)				
Hariana	64.0	20.0	4.0	2.0	10.0	0.74	0.15	0.01	0.10
(N = 50)	(n = 32)	(n = 10)	(n = 2)	(n = 1)	(n = 5)				
Total	56.0	20.0	5.0	7.0	12.0	0.66	0.185	0.035	0.12
(N = 100)	(n = 56)	(n = 20)	(n = 5)	(n = 7)	(n = 12)				
Observed	56	20	5	7	12	$\chi2$ cal = 133.65			
						$\chi2$ tab = 16.81 (P<0.01)			
Expected	43.56	24.42	3.42	1.29	1.44	$\chi2$ cal > $\chi2$ tab, df = 6, P<0.01			

Where; N = Sample size, n = Number of animals in particular genotype

Table 2. Association of DRB3/ HaeIII genotypes with milk production traits in Indian cattle

Lactation	Genotype	n	LP	TMY	MY300	DP	CI
First							
(N=73)	AA	45	360.0 ± 14.33	1710.0 ± 104.0	1400.0 ± 71.92	283.0 ± 19.68	525.0 ± 22.31
	AB	14	407.0 ± 32.60	2230.0 ± 231.0	1750.0 ± 136.0	304.0 ± 51.27	506.0 ± 23.54
	BB	4	325.0 ± 20.61	1740.0 ± 71.72	1560.0 ± 105.0	368.0 ± 49.26	470.0 ± 35.58
	BD	3	350.0 ± 49.31	1690.0 ± 41.13	1410.0 ± 122.0	281.0 ± 3.17	413.0 ± 116.0
	EE	7	350.0 ± 33.91	1920.0 ± 273.0	1600.0 ± 219.0	200.0 ± 37.68	539.0 ± 64.61
Second	AA	35	345.0 ± 20.19	1710.0 ± 131.0	1390.0 ± 85.53	291.0 ± 12.19b	454.0 ± 19.96
(N= 57)	AB	11	321.0 ± 34.94	1900 ± 211.0	1610.0 ± 166.0	253.0 ±28.57 b	476.0 ± 34.72
	BB	4	302.0 ± 33.57	1790.0 ± 145.0	1640.0 ± 39.78	206.0 ± 2.98 b	457.0 ± 47.97
	BD	2	377.0 ± 20.5	2300.0 ± 54.00	1840.0 ± 143.0	95.0 ± 30.0a	328.0 ± 129.0
	EE	5	388.0 ± 50.16	2030.0 ± 365.0	1530.0 ± 195.0	282.0 ± 45.91 b	493.0 ± 54.95

Different letters in superscript of a given row indicates significant (P<0.05) difference between genotypes, n — number of individuals in particular genotype, N — total number of individual in particular lactation.

patterns, *viz*., D, E and G were seen in Jersey crossbred cattle but not observed in studied Ongole cattle. Out of the seven *Hae*III patterns observed in their study, six patterns, viz. AA (170, 82, and 52 bp), BB (222 and 82 bp), DD (193, 82 and 29 bp), EE (170, 82, 46 and 6 bp) were also reported by Gelhaus et al. (1995) in cattle. The fragment patterns CC (170, 82 and 49 bp) and FF (170, 82, 48 and 4 bp) reported by Gelhaus et al. (1995). However, a new pattern, tentatively named as 'I' characterized by the absence of any *Hae*III site was observed with frequencies 0.15 and 0.17 in Jersey crossbred and Ongole breeds, respectively. The presence of this apparently zebu-specific pattern is quite understandable in the light of the highly polymorphic nature of the *BoLA-DRB3* gene. In the present investigation, the frequency of BD genotype was very low (2.0%) in the Hariana cattle and this might be due to the random selection of animals for polymorphism study.

The mean values (Mean ± S.E.M.) of each trait related to each genotype in the first and second lactation are presented in Table 2. Association study revealed that no significant difference was observed for any milk production traits among all the *DRB3* genotypes in first lactation. In second lactation, only DP showed significant (*P = 0.01*) difference among genotype. BD genotype had lower DP among all the genotypes in second lactation, which might be due to small sample size. Though, no reports were available regarding association studies of exon 2 of *DRB3* locus with milk

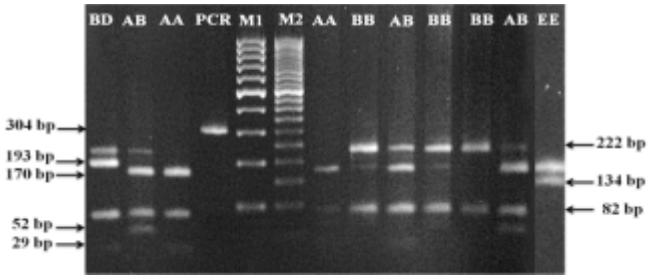

Fig 1. The HaeIII/PCR-RFLP assay in cattle and buffalo revealed five types of banding pattern (genotypes); one of them was of 170, 82, 52 bp (AA genotype); second of 222 & 82 bp (BB genotype), third of 170 & 134 bp (EE genotype) and heterozygous pattern have 222, 170, 82 & 52 bp bands (AB genotype) & 222, 193, 82 & 29 bp bands (BD genotype) M1=100 bp ladder, M2=50 bp ladder

production traits. However, various authors had reported that *DRB3.2* locus with different alleles described by Van Eijk et al. (1992) other than present study had significant association with milk yield and related traits. Starkenberg et al. (1997) found the association of allele *7 with increased milk yield and allele *26 with decreased milk yield in Holsteins cattle and Rupp et al. (2007) observed significant association of increase in milk yield with allele *11 in Canadian Holsteins. *BoLA-DRB3.2**10 has significant association with reduced fat yield in the Jersey population (Ledwidge et al. 2001) and *BoLA-DRB3.2**22 was associated with decreased milk and protein yield in Canadian dairy cattle (Sharif et al. 1998).

The mean values (Mean ± S.E.M.) of SCC for each genotype are presented in Table 3. There was significant difference *(P = 0.0106)* for SCC among AA,

Table 3. Means and S.E.M. of SCC for different DRB3/HaeIII genotypes.

Genotype	Somatic cell count (lakh/ml)	
	Hariana	Sahiwal
AA	3.96 ± 0.26b	3.88 ± 0.32
AB	3.19 ± 0.33ab	3.86 ± 0.54
BB	1.92 ± 0.29a	3.94 ± 1.04
BD	-	5.24 ± 1.17
EE	3.20 ± 0.98ab	3.18 ± 0.73
Overall mean	3.63± 0.21	3.91 ± 0.24

AB, BB, BD and EE genotypes in screened Hariana cattle. The BB genotype had lower SCC than AA, AB and EE genotypes. There was no significant different found for SCC in screened Sahiwal cattle in this investigation, which might be due to small sample size and presence of more number of alleles. Kumar et al. (2008) reported that genotype AA and EE were involved in resistance to mastitis in Nili Ravi breed of buffalo. Significant associations between SCC and various *DRB3* haplotypes and alleles were established by Oddgeirsson et al. (1988) in Canadian Holsteins.

In present study, the genotype AA had higher frequency among all the possible genotypes in screened cattle population. This finding was in concurrence with the reports of Aravindakshan and Nainar (1999) in Ongole, Wojdak-Maksymiec et al. (2010) in Jersey, Sharma et al. (2005) in Rathi. However, in contrast to this finding Aravindakshan and Nainar (1999) observed higher genotypic frequency of BB genotype in Jersey crossbred cattle. In the present study, A allele was the most frequent (0.660). Similar findings were reported by various authors in different cattle breeds including Argentinean Holstein, Caracu, Pantaneiro, Nelore, Gir (Miretti et al. 2001); Rathi (Sharma et al. 2005); Jersey (Wojdak-Maksymiec et al. 2010); Sahiwal (Chakraborty et al. 2015). In contrast, Miretti et al. (2001) and Thiruvaran and Bhushan (2015) observed higher allelic frequency of B in Argentinean creole and Umbalchery cattle, respectively.

In the present study, the *Hae*III/PCR-RFLP assay of 304 bp *DRB3* gene resulted in five genotypes, viz., AA, BB, AB, BD, EE with frequency ranging from 0.07-0.56 and four allele, viz., A, B, D, E with frequency range 0.035-0.660. AA genotype was the most frequent in all the screened cattle, followed by the AB, EE, BD and BB genotypes. Association study revealed no significant difference for milk production traits in first and second lactation except dry period (DP), which had significant difference among genotypes in second lactation. SCC has significant difference among genotypes in Hariana cattle, while in sahiwal cattle association was absent. However, the result showed that this region of *DRB3* gene was highly polymorphic, so this study needs further research in large population size and other different breeds of Indian cattle.

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