RESEARCH ARTICLE

BoLA - DRB3 Gene Polymorphism and its Association with Lactation Yield and Milk Constituents in Crossbred Cattle

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Abstract

The present study was undertaken to explore polymorphism at exon 2 of BoLA-DRB3 gene locus and its association with milk yield, fat % and protein % in a total of 40 Holstein-Friesian crosssbred cows using PCR-RFLP method. PCR-RFLP using *Hae III* restriction enzyme revealed presence of 7 genotypes with frequencies 0.475 (AA), 0.225 (AB), 0.050 (AE), 0.100 (BB), 0.050 (DD), 0.050 (EE) and 0.050 (II), respectively and PCR-RFLP using *Bst YI* yielded 3 genotypes with frequencies 0.050 (AA), 0.625 (AB) and 0.325 (BB), respectively. To study the association of BoLA-DRB3.2 genotypes with milk yield, fat and protein %, the data was subjected to analysis of variance employing general linear model using SPSS software package. Effect of farm ($p < 0.01$) and parity ($p < 0.05$) was significant on lactation yield; while the effect of season of calving, *Hae III* genotypes and *Bst YI* genotypes were non-significant. For fat % the effect of parity and season of calving was significant ($p < 0.05$), while the effect of farm and genotype was non-significant. Milk protein % was significantly ($p < 0.05$) influenced only by *Bst YI* genotype. Genotype AB recorded significantly higher protein % as compared to genotype BB.

Keywords: BoLA-DRB3, Crossbred Cows, Fat %, Lactation yield, PCR-RFLP, Protein %.

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Introduction

The bovine major histocompatibility complex (MHC) genes, also called bovine leukocyte (lymphocyte) antigen (BoLA), have recently received attention because of their high degree of genetic polymorphism and association with immunity and milk production. The BoLA genes are located on the short arm of bovine chromosome number 23 (Behl *et al*., 2007). The polymorphic sites of the BoLA-DRB3 gene are mainly located in exon 2 (BoLA- DRB3.2). The BoLA-DRB3.2 polymorphism has been observed to be associated with milk production and its composition (Rupp *et al*., 2007; Pashmi *et al.,* 2009; Zambrano *et al*., 2014) in different cattle breeds. In India, crossbred cattle are gaining much importance being high milk producer compared to indigenous cattle, which could be observed by recent figures of country's milk production revealing that out of total cattle milk production more than half is contributed by crossbred cattle (BAHS, 2017). The paucity of information on BoLA–DRB3 gene polymorphism and its association with milk production and composition traits in Indian crossbred cattle led to conduct the present study to explore the possibility if this gene could be used as marker gene for selection.

Materials and Methods

Blood samples were collected from 40 Holstein Friesian crossbred cows maintained at the Livestock Farm, College of Veterinary Science and A.H., Mhow, Shri Ahilyamata Jeev Dayamandal Trust Gaushala, Indore and Bull Mother Farm Bhadbhada, Bhopal. The genomic DNA was extracted from venous blood as per standard procedure (John *et al*., 1991). Purity and concentration of genomic DNA was determined by using Nanodrop HD2000 spectrophotometer. DNA samples with optical density (OD) ratio1.7-1.9($OD_{260/280}$) diluted with 0.3X TE buffer to contain 30 ng DNA /µl of solution were used for PCR amplification. Quality of genomic DNA was assessed by using 0.8% horizontal submarine agarose gel electrophoresis. Amplification of exon-2 region of BoLA-DRB3 gene was carried out using specific primer (Sigurdardottir *et al*., 1991). Amplification was performed in thermal cycler (Applied Biosystems) programmed for 30 cycles with an

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initial denaturation at 94°C for 2 minutes, denaturation at 92°C for 45 seconds, annealing at 66°C for 45 seconds and extension at 72°C for 45 seconds with a final extension at 72°C for 10 minutes. The 304 bp amplified PCR products were digested with *Hae III* and *Bst YI* restriction endonucleases separately. Subsequently, digested PCR products were electrophoresed on 3% agarose gel containing 1 % ethidium bromide at constant voltage of 80 V for 1 hour using 0.5 x TBE buffer. On completion of electrophoresis, the gel was visualized under UV transilluminator (UVITECH, Cambridge, U.K.) to reveal different restriction patterns and allele types obtained.

Since the milk yield and milk constituents are influenced by various genetic and non-genetic factors such as breed, farm/location, parity and season of calving, to study the effect of various polymorphic variants of BoLA-DRB3.2 gene on lactation yield, fat % and protein % the data were subjected to analysis of variance by SPSS software package using following general linear model.

$$Y_{ijklmn} = \mu + F_{i} + P_{j} + S_k + H_l + B_m + e_{ijklmn}$$

Where, Y_{ijklmn} is the observed value of trait, µ is the population mean, F is the effect of farm (i = 1, 2, 3), P_j is the effect of parity (j = 1, 2, 3, 4), S_k is the effect of season of calving (k = 1, 2, 3, 4), H_l is the effect of *Hae III* BoLA-DRB3.2 genotype (l = 1, 2, …., 7), B_m is the effect of *Bst YI* BoLA-DRB3.2 genotype (m = 1, 2, 3) and e_{ijklmn} is the random error.

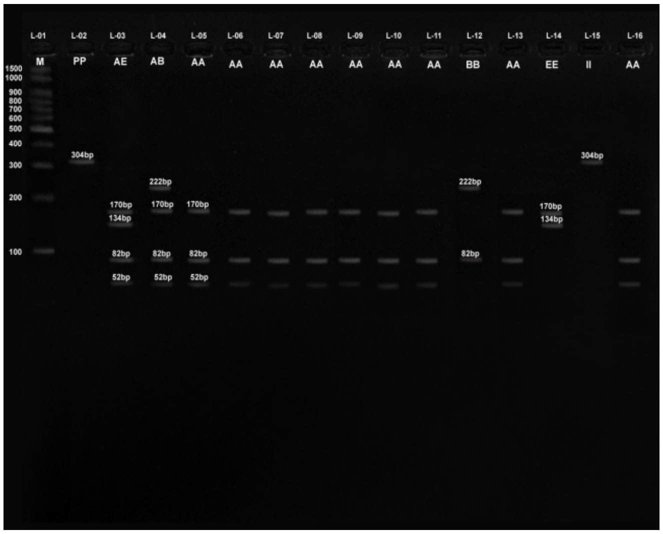

Fig. 1: PCR-RFLP pattern of BoLA DRB 3.2 gene using *Hae III* in Holstein crossbred cows

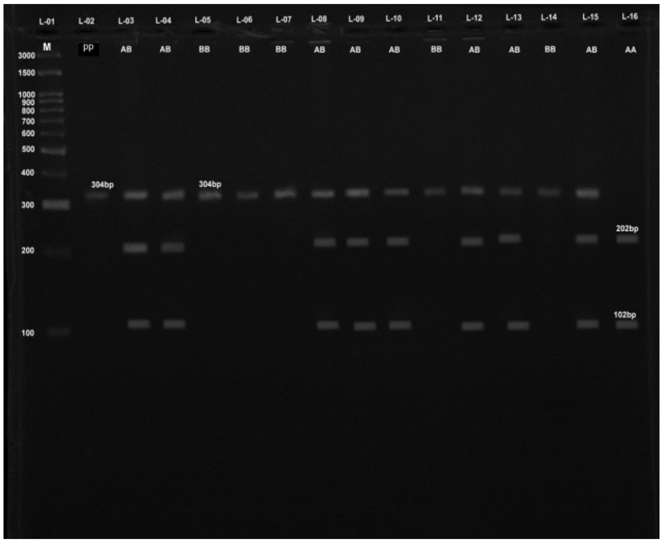

Fig. 2: PCR-RFLP pattern of BoLA DRB 3.2 gene using *Bst YI* in Holstein crossbred cows

RESULTS AND DISCUSSION

BoLA-DRB3.2 gene polymorphism: The different genotypes and alleles obtained by using two restriction endonucleases along with their frequencies have been presented in Table 1. The RFLP patterns for *Hae III* (Fig.1) were AA (170, 82 & 52 bp), AB (222, 170, 82 & 52 bp), AE (170, 134, 82 & 52 bp), BB (222 & 82 bp), DD (193, 82 & 29 bp), EE (170 & 134 bp) and II (304 bp). The genotypic frequencies were found to be 0.475, 0.225, 0.050, 0.100, 0.050, 0.050 and 0.050 respectively for AA, AB, AE, BB, DD, EE and II genotypes. The frequencies of alleles A, B, D, E and I were 0.6125, 0.2125, 0.050, 0.075 and 0.050. Similarly, digestion of amplified gene fragements with Bst YI restriction enzyme yielded three restriction patterns (Fig.2): AA (202 & 102), AB (304, 202 & 102) and BB (304 bp) and alleles A and B. The genotypic frequencies for AA, AB and BB were 0.050, 0.625 and 0.325, respectively. The frequencies of alleles A and B were 0.3625 and 0.6375, respectively. The results indicated that BoLA DRB3.2 gene locus under study in Holstein crossbreds was polymorphic for both the restriction endonucleases viz., *Hae III and Bst YI* used in the present study. These findings on RFLP patterns for *Hae III* and *Bst YI* are in congruence with the findings of Behl *et al.* (2007) in Kankrej and Darshan Raj *et al.* (2012) in

Table 1: Frequency of genotypes and alleles at BoLA-DRB3.2 locus in HF crossbreds using *Hae III* and *Bst YI*

Hae III (40)		*Bst YI (40)*	
Genotype/Allele	*Frequency*	*Genotype/Allele*	*Frequency*
AA	0.475 (19)	AA	0.050 (02)
AB	0.225 (09)	AB	0.625 (25)
AE	0.050 (02)	BB	0.325 (13)
BB	0.100 (04)	A	0.3625
DD	0.050 (02)	B	0.6375
EE	0.050 (02)	-	-
II	0.050 (02)	-	-
A	0.6125	-	-
B	0.2125	-	-
D	0.050	-	-
E	0.075	-	-
I	0.050	-	-
Chi square value	99.56**	Chi square value	4.96*

* Significant (P <0.05); ** significant (P < 0.01)

Amrithmahal, Amrithmahal crosses, Jersey crossbred and Red-Dane x Red-Sindhi crossbreds using same primer. Since, the size of amplification product was same (304 bp) for all the animals of different breeds included in present study and earlier studies quoted above suggesting that this region is conserved in cattle.

Association of BoLA-DRB3.2 gene polymorphic variants with lactation yield and milk constituents:

The results of least squares analysis of variance for different milk performance traits have been presented in Table 2 and least squares means have been presented in Table 3.

Lactation yield: The least squares analysis of variance (Table 2) revealed that the effect of BoLA-DRB3.2 genotypes (both *Hae III* and *Bst YI*) was non-significant on lactation yield. This is in agreement with the findings of Sharif *et al*. (1998) in Jersey, Pashmi *et al*. (2009) in Iranian Holstein and Gowane *et al*. (2013) in Crossbred cows. However, Starkenburg *et al*. (1997) reported that allele *8 was associated with significantly decreased milk yields in Holstein. Contrary to this Sharif *et al*. (1998) observed that allele *8 was significantly associated with increased milk yield and allele *22 was significantly associated with decreased milk yield in Holstein cows. This discrepancy is indicative of variations among breeds/ populations, environments and management practices. The significant effects of farm ($P < 0.01$) and parity ($P < 0.05$) on lactation yield was observed in present study. Pashmi *et al*. (2009) and Zambrano *et al*. (2014) have also reported significant ($p \leq 0.01$) effect of calving sequence on lactation yield in Holstein and Holstein crossbreds.

Milk fat percent: The results of least squares analysis of variance (Table 2) indicated that the effect of parity were significant ($P<0.05$). Farm did not exert significant effect on this trait. Genotype also (both *Hae III* and *Bst YI*) did not affect this trait significantly. Contrary to our findings, Pashmi *et al*. (2009) reported significant positive effect of alleles *22 ($P<0.01$) and *11 ($P<0.05$) on milk fat percentage in Holstein cows. Some reports of association of BoLA DRB3.2 alleles with milk fat yield are available. The significant ($P<0.05$) effect of parity on milk fat % as observed in this study is in agreement with the findings of Pashmi *et al*. (2009), Zambrano *et al*. (2014) and Tarun (2018) in pure Gir, HF and crossbred cattle.

Milk protein percent: The results of least squares analysis of variance for milk protein% have been presented in Table 3. It is evident from this table that the effect of *Bst YI* genotype was significant ($P<0.05$). Mean milk protein % was significantly higher for cows possessing *Bst YI* genotype AB (3.02 ± 0.04) as compared to cows of genotype BB (2.80 ± 0.06) which recorded the lowest milk protein % (Table 3). Farm, parity and season of calving did not exert significant effect on this trait. *Hae III* genotype also did not affect this trait significantly. Significant effect of *Bst YI* genotype on milk protein % observed in this study is in conformity to the findings of Pashmi *et al*. (2009), who have reported significant positive effect of alleles *24 and *22 ($P<0.05$) on milk protein percentage in Holstein cows. They further reported significantly negative association of allele *22 with protein yield in Holstein cows which in contrary to the findings of Pashmi *et al*. (2009) in Holstein. Further, Nascimento *et al*. (2006) have reported significant association of DRB3*54 allele ($P<0.05$) with lower protein yield. These contrary observations in different studies can probably be pointing to some factors such as sample size, varied frequencies of BoLA-DRB3.2 variants in different breeds, method of recording and estimating performance traits and other uncontrolled factors. The non significant effects of farm, parity and season on milk protein % as observed in this study is in close agreement with the findings of Tarun (2018) in pure Gir and HF crossbreds. In contrast to the present findings, Pashmi *et al*. (2009) reported significant ($P< 0.05$) effect of season, herd (farm) and parity on milk protein percentage in Iranian Holstein cows.

Based on the results of present study, it could be concluded that there is possibility of using *Bst YI* genotype at BoLA-DRB 3.2 locus as marker for selection to increase milk protein percent. But results need to be validated on large population before generalization.

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Table 2: Least squares analysis of variance for lactation yield, milk fat % and protein % (Mean Square values) in Holstein Friesian crossbred cows.

Source of variation	*Degree of freedom*	*Lactation yield*	*Fat %*	*Protein %*
Farm	2	6588693.12^{**}	0.151^{NS}	0.009^{NS}
Parity	3	519017.65^{*}	0.345*	0.012^{NS}
Season of calving	3	381242.05^{NS}	0.425*	0.022^{NS}
Hae III genotype	6	286975.74^{NS}	0.258^{NS}	0.217^{NS}
Bst YI genotype	2	528454.33^{NS}	0.344^{NS}	0.385*
Error		188733.69 (73)	0.11 (23)	0.035 (23)

*Significant ($P<0.05$); ** Significant ($P<0.01$); NS-Non Significant
Figure in parentheses are the error degree of freedom.

Table 3: Least squares means of different milk performance traits in Holstein Friesian crossbred cows

Effect	*Lactation Yield (Kg)*	*Milk Fat %*	*Milk Protein %*
Farm			
College farm	1850.76 ± 98.27^{b} (32)	3.22 ± 0.06 (13)	2.90 ± 0.05 (13)
Gaushala farm	1686.42 ± 97.55^{b} (17)	3.15 ± 0.07 (08)	2.88 ± 0.05 (08)
Bull Mother farm	2402.25 ± 87.64^{a} (41)	3.20 ± 0.05 (19)	2.92 ± 0.04 (19)
Parity			
1	1755.28 ± 90.32^{b} (12)	3.22 ± 0.08^{ab} (09)	2.88 ± 0.05 (09)
2	1955.20 ± 138.05^{ab} (29)	3.29 ± 0.07^{a} (11)	2.91 ± 0.05 (11)
3	2086.17± 88.68^{a} (32)	3.15 ± 0.07^{b} (12)	2.92 ± 0.04 (12)
4	2122.59 ± 105.92^{a} (17)	3.10 ± 0.11^{b} (08)	2.89 ± 0.06 (08)
Season of calving			
Spring	1880.76 ± 129.58 (11)	3.29 ± 0.10^{a} (05)	2.95 ± 0.08 (05)
Summer	2050.52 ± 94.71 (25)	3.28 ± 0.07^{a} (11)	2.90 ± 0.05 (11)
Rainy	1973.16 ± 88.87 (22)	3.08 ± 0.05^{b} (14)	2.87 ± 0.04 (14)
Winter	2014.80 ± 91.57 (32)	3.11 ± 0.07^{b} (10)	2.88 ± 0.06 (10)
Hae III Genotype			
AA	2098.70 ± 73.52 (40)	3.24±0.07 (19)	2.91 ± 0.06 (19)
AB	1950.92 ± 78.11 (21)	3.21±0.08 (09)	2.92 ± 0.07 (09)
AE	1898.36 ± 105.20 (9)	3.16±0.10 (02)	2.88 ± 0.09 (02)
BB	2011.49 ± 89.81 (4)	3.22±0.09 (04)	2.95 ± 0.09 (04)
DD	1979.36 ± 109.70 (6)	3.14±0.10 (02)	2.89 ± 0.10 (02)
EE	1925.27 ± 121.40 (5)	3.17±0.11 (02)	2.91 ± 0.09 (02)
II	1994.57 ± 113.03 (5)	3.19±0.11 (02)	2.84 ± 0.10 (02)
Bst YI Genotype			
AA	1888.82 ± 103.22 (06)	3.19± 0.06 (02)	2.88 ± 0.08^{ab} (02)
AB	1987.60 ± 75.15 (55)	3.21 ± 0.07 (25)	3.02 ± 0.04^{a} (25)
BB	2063.01 ± 83.87 (29)	3.17 ± 0.05 (13)	2.80 ± 0.06^{b} (13)

a, b : Values with a common alphabet as superscript do not differ significantly
Figures in parentheses are the number of observations

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