Original Research

Fixation of T Allele in C>T Polymorphism in Intron IV Region of Secreted Phosphoprotein 1 (*SPP1*) Gene in Indian Cattle Breeds

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Abstract

Secreted phosphoprotein 1 (SPP1) is a highly negative phosphorylated glycoprotein, which has potent role in growth, production and reproduction of the animals. In the present study, investigation of SPP1 gene polymorphism was undertaken in 147 Sahiwal and Hariana cattle maintained at Instructional livestock farm complex (ILFC), DUVASU, Mathura using BsrI/PCR-RFLP assay. Amplification of SPP1 intron IV (C>T) region revealed 290 bp product and restriction digestion with BsrI showed only one type of genotypes, namely, TT (290 bp). The frequency of TT genotypes was 100% in all screened samples with T allele (1.0). Association studies of SPP1 gene with milk production traits could not performed because of monomorphic pattern of genotypes.

Key words: Sahiwal, Hariana, *Bsr*I, PCR-RFLP, *SPP1*, Intron IV, Milk Production Traits

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Introduction

Secreted phosphoprotein 1 (*SPP1*) is a highly negative phosphorylated glycoprotein, which has potent roles in growth, production and reproduction of the animals. *SPP1* gene has potential roles in cancer metastasis, cell-mediated immune responses bone mineralization, inflammation and cell attachment. It plays important role in initiation and maintenance of pregnancy, as well as in the development of the fetus (Denhardt *et al*., 2001). Schnabel *et al.* (2005) identified QTL on bovine chromosome 6 (BTA6) and sequenced a 12.3-kb region harboring *SPP1* using 38 microsatellite markers in Holstein bulls. Several SNPs have been reported

in CDS, introns and regulatory region of the *SPP1* gene (Leonard *et al*., 2005; White *et al*., 2007; Khatib *et al*., 2007). *C>T* polymorphisms has been reported in Intron IV of the *SPP1* gene and associated with milk yield, fat yield, fat %, and protein % in several exotic cattle (Leonard *et al*., 2005, Khatib *et al*., 2007, Pareek *et al*., 2008b, Boleckova *et al*., 2012). However, this polymorphism study is lacking in Indian cattle breeds. Therefore, the present study was undertaken to investigate the status of *C>T* polymorphism in intron IV region of *SPP1* gene Indian Sahiwal and Hariana cattle breeds.

Materials and Methods

Animal Source, DNA Extraction and *Bsr*I /PCR-RFLP

A total of 147 females of Sahiwal (n=72) and Hariana (n=75) cattle maintained at Instructional Livestock Farm Complex (ILFC), DUVASU, Mathura (U.P.), were utilized in the present investigation. Genomic DNA was extracted from venous blood using the standard protocol of Sambrook and Russel (2001). The primers used for amplification of Intron IV region of *SPP1* gene (F: 5′-GCA AAT CAG AAG TGT GAT AGA C-3′ and 'R: 5′-CCA AGC CAA ACG TAT GAG TT-3′) were as per method described by Leonard *et al.* (2005). The restriction digestion was carried out at 65ºC with *Bsr*I for overnight in a total volume of 15µl containing 5.0 µl of PCR product, 1.5 µl of 10X RE buffer and 10 units of (1.0 µl).

Sequencing Analysis

The PCR products were sequenced commercially (ILS active, Invitrogen) by automated sequencer using standard cycle conditions by Sanger's dideoxy chain termination method using specific PCR primers. The sequences obtained were subjected to BLAST analysis (www.ncbi.nlm.nih.gov/BLAST) to ascertain that the obtained sequences corresponded to *SPP1* amplified region. Further, the presence of the restriction site in PCR products confirmed by aligning using the ClustalW method of MegAlign programme of Lasergene software (DNASTAR, USA).

Statistical Analysis

The data was generated by estimating the frequency of different *SPP1* genotypes. The allelic and genotypic frequencies were estimated by standard procedure (Falconer and Mackay, 1996).

Results and Discussion

The amplified fragments of the *SPP1* Intron IV region revealed 290 bp product (Fig.1). The *Bsr*I/PCR-RFLP assay revealed monomorphic pattern (uncut genotypes, TT genotype, 290 bp) (Fig. 1). The enzymatic activity of *Bsr*I was confirmed by digesting ϕ x 174 RF I DNA (5386 bp) and that on digestion produced several fragments (Fig. 1). Further, the absence of the restriction site for *Bsr*I in PCR products confirmed by DNA sequencing. The obtained sequence of *SPP1*/*Bsr*I revealed C→T substitution (Fig. 2). For TT genotype; C→T substitution was found in both of the strand *i.e.* 290 bp fragment. This confirmed that all

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the screened cattle used in the present study were monomorphic in nature with only T allele with TT (wild) genotype.

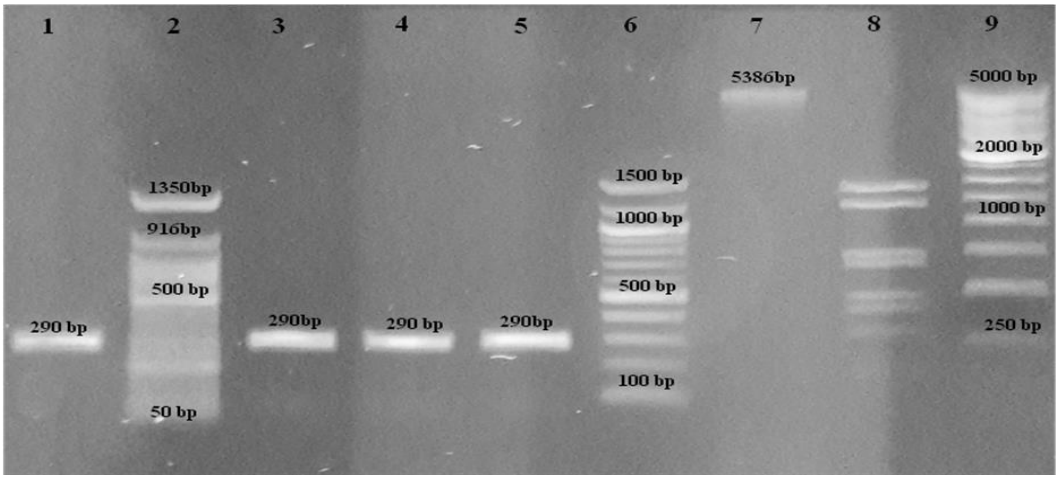

Fig. 1: SPP1/*Bsr*I PCR-RFLP assay in 1.5% agarose gel showing monomorphic pattern Lane 1: Undigested PCR product (290 bp), 2: Marker (50 bp DNA ladder, New England Biolabs, Cat No. N3236S), 3-5: TT genotype (uncut band, 290 bp), 6: Marker (100 bp DNA ladder, New England Biolabs, Cat No. N3231S), 7: ϕ x 174 RF I DNA (5386 bp), New England Biolabs, Cat No. N3021G, 8: *Bsr*I digested ϕ x 174 RF I DNA, 9: Marker (250 bp DNA ladder, Banglore Genei, Cat No. 61265307050A).

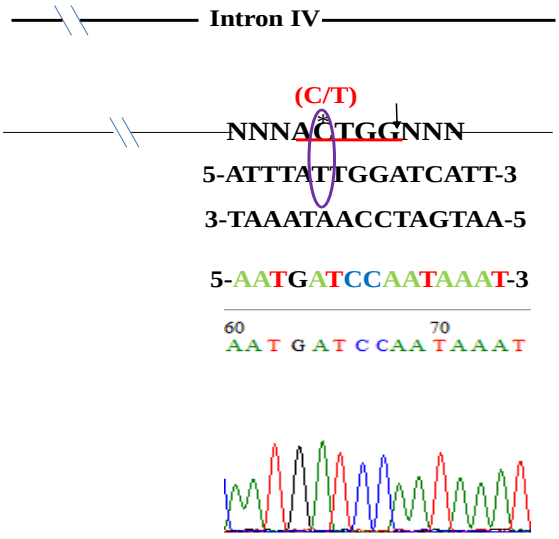

Fig. 2: Sequencing of *SPP1* Intron IV region revealed that C→T substitution and absence of *Bsr*I recognition site.

In present investigation, the CT and CC genotype were absent (0.0) and the genotypic frequency of TT genotype was 100% with the allelic frequency of allele T and C as 1.0 and 0.0, respectively in all the screened Hariana and Sahiwal animals. Similar results were observed by Rahmatalla *et al.* (2015) in Butana and Kenana Sudanese cattle. In contrast, the genotypic frequency of TT genotype ranged from 1.10 % to

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68.0 % with frequency distribution of T allele ranged from 0.22 to 0.84 in different cattle breeds as presented in Table 1.

Table 1: Genotypic and allelic frequencies of SPP1/*Bsr*I gene in different cattle breeds as observed by other authors

Breed	Genotypic Frequency			Allelic Frequency		References
	TT (%)	CT (%)	CC (%)	T	C	
Holstein bull (CDDR)	23.2	53.5	23.3	0.48	0.52	Leonard *et al*., 2005
Holstein cows (UW)	27.57	46.73	25.7	0.51	0.49	Leonard *et al*., 2005
Holstein cows (UW)	26	51	24	0.515	0.485	Khatib *et al*., 2007
South Anatolian Red (SAR)	55	37.5	7.5	0.74	0.26	Oztabak *et al*., 2008
East Anatolian Red (EAR)	67.5	32.5	0	0.84	0.16	Oztabak *et al*., 2008
Polish Holstein	25	60.7	14.3	0.554	0.446	Pareek *et al*., 2008a
Polish Red	39.1	56.6	4.3	0.674	0.326	
Hereford	21.7	65.2	13.1	0.543	0.457	
Limousine	33.4	54.1	12.5	0.604	0.396	
Polish Holstein	25.26	50	24.74	0.503	0.497	Pareek *et al*., 2008b
Girolando cattle	52.53	38.71	8.76	0.72	0.28	Mello *et al*., 2012
Czech Fleckvieh cattle	68	28	4	0.82	0.18	Boleckova *et al*., 2012
Jersey cow	1.65	42.54	55.8	0.23	0.77	Luczak and Kulig, 2012
Jersey cow	1.1	41.1	57.8	0.22	0.78	Luczak and Kulig, 2013
Holstein Frisien	24	57	19	0.53	0.47	Pasandideh *et al*., 2015
Iranian Holstein bulls	34.69	48.62	16.69	0.59	0.41	Salehi *et al*., 2015

CDDR = Cooperative Dairy DNA Repository, UW = University of Wisconsin herd, SAR = South Anatolian Red, EAR = East Anatolian Red

In the present study, only TT genotype was found in all the screened animals for SPP1/*Bsr*I locus, so association analysis could not be performed with milk production traits. However, several authors (Schnebel *et al*., 2005; Leonard *et al*., 2005 and Khatib *et al*., 2007) studied the association of *SPP1 C>T* variant with milk production traits in dairy cattle. Mello *et al.* (2011) investigated SPP1/*Bsr*I polymorphism in Girlando cattle and found no significant association between the alleles and milk yield on 305 days and predicted transmitting ability for milk yield (PTAM), although the highest milk production was observed in animals with at least one copy of the T allele. Leonard *et al.* (2005) observed that C allele was associated with an increase in milk protein percentage and milk fat percentage in repository Holstein bull population. Significant effect of the C allele with fat percentage ($P < 0.0001$), protein percentage ($P < 0.0001$) and fat yield ($P = 0.014$) was also observed in Holstein cows (Khatib *et al*., 2007). Boleckova *et al.* (2012) reported that *C* allele was significantly associated with protein percentage and breeding value for this trait. Luczak and Kulig (2012) observed significant highest SCC value for the TT genotype in Jersey cows of Wielkopolska region of Poland. However, no significant association was found with milk performance traits in Jersey cows (Luczak and Kulig, 2013). Pasandideh *et al.* (2015) found significant association

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between the *C>T* genotypes and FATP2X (milk fat content adjusted for two milking per day (%)) and PROPER305 (milk protein content adjusted for 305 days (%) traits. CT genotype cows had higher FATP2X ($P < 0.05$) and more PROPER305 ($P < 0.01$) than those carrying other genotypes. No associations were observed between the studied SNPs genotypes and the other traits ($P > 0.10$). However, CC genotype had higher fat yield, protein yield, fat and protein percent but lower milk yield than TT genotype (Salehi *et al*., 2015). Pareek *et al.* (2008b) reported that favourable allele T showed significant effect on body weight in young Holstein bulls aged 3, 6 and 12 months and in 6 and 12 months aged heifers, indicating that, the investigated *SPP1 C>T* SNP marker could be a suitable choice simultaneously for milk production traits (favouring *C* allele) and growth traits (favouring allele *T*) in Polish Holstein Frisien cattle.

Conclusion

In the present study, we observed absence of *C>T* polymorphism in Intron IV region of *SPP1* gene in screened Sahiwal and Hariana cattle, consequently we could not perform association with milk production trait because in these screened cattle *SPP1* T allele was found fixed. Further, investigations in large population of these cattle may be useful for studying the status of this allele/SNP in order to exploit it for marker assisted selection for milk traits in cattle.

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