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Fixation of T allele in G>T Polymorphism in Exon 7 Region of Secreted Phosphoprotein 1 (*SPP1*) Gene in Indian Cattle Breeds

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ABSTRACT

Secreted phosphoprotein 1 (*SPP1*) is popularly known as osteopontin (*OPN*), which plays an important role in initiation and maintenance of pregnancy, as well as in the development of the fetus and milk production. In the present study, investigation of G>T polymorphism in exon 7 region of *SPP1* gene was undertaken in 147 Sahiwal and Hariana cattle maintained at Livestock Farm Complex (LFC), DUVASU, Mathura using *HpyCH4*IV/PCR-RFLP assay. Amplification of *SPP1* exon 7 region revealed 204 bp product and *HpyCH4*IV restriction digestion screening showed monomorphic pattern. Only one type of genotype, namely, TT (204 bp) was observed in population. The frequency of TT genotypes was 100% in all screened samples with T allele (1.0). The results revealed that *SPP1* T allele seems to be fixed in screened cattle population. Consequently, we could not perform the association study of this substitution with milk production traits.

Keywords: SPP1, Exon 7, PCR- RFLP, Sahiwal, Hariana

In cattle, one major specific gene secreted phosphoprotein1 (*SPP1*) plays an important role in milk production. *SPP1* gene is popularly known as osteopontin (*OPN*). It was identified independently, as well as together with bone sialoprotein (*BSP*) which is a major sialoprotein in the extracellular matrix of bone. OPN protein is found in milk, plasma, urine and expressed in several tissues (Salehi *et al.,* 2015). The presence of *SPP1* protein in milk and its high level of expression in mammary epithelial cells may cause proliferation and differentiation of the mammary gland. *SPP1* gene has potent roles in growth, production and reproduction of the animals. It plays important role in initiation and maintenance of pregnancy, as well as in the development of the fetus (Leonard *et al*., 2005).

Bovine OPN is located on chromosome 6 composed of seven exons and five introns spanning about 7 Kb of genomic DNA (Chakraborty *et al.*, 2010). Several single nucleotide polymorphisms (SNPs) have been reported in coding sequence (CDS), introns and regulatory region of the *SPP1* gene. This gene has been reported to be associated with various traits like milk yield, milk composition, lactation persistency, growth, body weight, stillbirth, dystocia, mastitis infections and twinning rate etc. in bovine (Leonard *et al*., 2005; Khatib *et al*., 2007; Alain *et al.*, 2009; Boleckova *et al.*, 2012; Salehi *et al.*, 2015; Bissonnette, 2018). G>T polymorphisms has been reported in exon 7 of the *SPP1* gene and associated with milk yield, fat yield, fat %, and protein % in dairy cattle (Cohen-Zinder *et al*., 2005; Kowalewska-Luczak and Kulig, 2013). However, till date this polymorphism study is not reported in Indian cattle breeds. Considering lack of such information in Indian cattle breeds, the present study was undertaken to investigate the status of G>T polymorphism in *SPP1* exon 7 of Sahiwal and Hariana cattle breed.

MATERIALS AND METHODS

Animals and DNA isolation

For PCR-RFLP study, a total of 147 females of Sahiwal (n = 72) and Hariana (n = 75) cattle maintained at Livestock Farm Complex (LFC), DUVASU, Mathura (U.P.), were utilized in the present investigation. Blood samples were collected from jugular vein of animals and genomic DNA was isolated using the standard phenol–chloroform extraction method (Sambrook and Russel, 1991). The concentration and purity of genomic DNA was determined by nanodrop (Bio-Rad, USA). The integrity of the DNA was analyzed by agarose gel (0.7%) electrophoresis followed by examination of the gel under UV light after staining with ethidium bromide (EtBr).

Amplification of *SPP1* exon 7 region

An amplicon of 204 bp consisting of *SPP1* exon 7 region was amplified using a specific primer pairs SPP1EX_F: 5′-ACC CTG CTT TAA TGT ATC CTT TAC -3′ and SPP1EX_R: 5′-GTC AGG AAA ATT CCA AAC TCA GCC -3’; Cohen-Zinder *et al*., 2005). PCR amplification was carried out in a total volume of 25 µl that contained 2.0 µl of genomic DNA (50 nmoles/ µl), 1X PCR buffer (10 mM Tris–HCl, pH 8.8 at 25ºC, 50 mM KCl), 2.5 mM of $MgCl_2$, 2.5 mM of each dNTPs, 5 pmoles of each primer and one unit of *Taq* DNA polymerase (Fermentas, USA). The cycle conditions included an initial period of denaturation at 95ºC for 2 min, followed by 35 cycles of denaturation at 94ºC for 30 s, annealing at 53ºC for 50 s and extension at 72ºC for 50 s, and a final extension at 72ºC for 10 min. The PCR product was checked by agarose gel (1.0%) electrophoresis.

*Hpych4*IV PCR-RFLP Assay

The PCR product of 204 bp was digested using restriction enzyme *Hpych4*IV (New England Biolab, USA). The restriction digestion was carried out at 37ºC for 14 hr in a total volume of 15µl containing 5µl PCR products, 1.0 µl *Hpych4*IV (10U/µl) and 1.5µl 10X RE buffer. For restriction fragment analysis, digested products were checked on 1.5% agarose gel electrophoresis. Restriction analysis indicates that the mutant-type homozygote (G/G) possessing a polymorphic *Hpych4*IV site generates 2 fragments (161 and 43 bp) which is referred as genotype GG. While absence of polymorphic *Hpych4*IV site, the wild homozygote (T/T) yields uncut fragments (204 bp) which is referred as genotype TT.

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* gene revealed 204 bp product by performing 1.5% agarose gel electrophoresis. The *HpyCH4*IV/PCR-RFLP assay revealed monomorphic pattern (uncut genotypes; TT genotype; 204 bp) (Fig. 1).

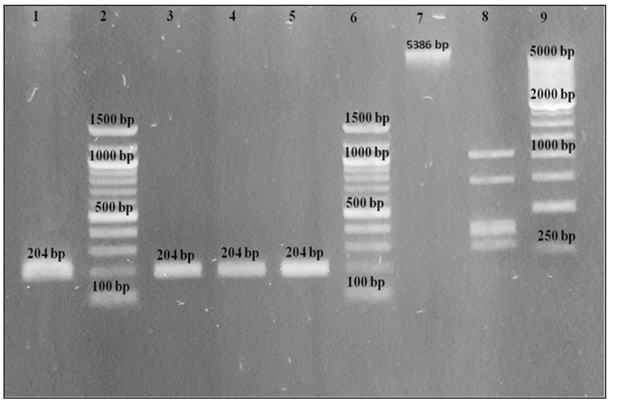

Fig. 1: SPP1/*HpyCH4*IV PCR-RFLP assay in 1.5% agarose gel showing monomorphic pattern Lane 1: Undigested PCR product (204 bp), 2: Marker (100 bp DNA ladder, New England Biolabs, Cat No. N3231S), 3-5: TT genotype (uncut band; 204 bp), 6: Marker (100 bp DNA ladder, New England Biolabs, Cat No. N3231S), 7: ϕ × 174 RF I DNA (5386 bp), New England Biolabs, Cat No. N3021G, 8: *HpyCH4*IVdigested ϕ × 174 RF I DNA, 9: Marker (250 bp DNA ladder, Banglore Genei, Cat No. 61265307050A)

The enzymatic activity of *HpyCH4*IV was confirmed by digesting ϕ × 174 RF I DNA (5386 bp) which produced several fragments (Fig. 1). This revealed that all the screened cattle used in the present study were monomorphic in nature with only T allele with TT (wild) genotype. Genetic polymorphism of SPP1/*HpyCH4*IV gene had been observed in other exotic cattle breeds (Cohen-Zinder

et al., 2005; Kowalewska-Luczak and Kulig, 2013), which was not in accordance to the findings of present study. The SPP1/*HpyCH4*IV assay revealed only TT genotype (204 bp) in screened cattle population.

The genotypic frequency of TT genotype was 100% in all the screened animals. In contrast, the genotypic frequency of TT genotype was 53.3% and 24.5% in Israeli Holstein (Cohen-Zinder *et al*., 2005) and Jersey (Kowalewska-Luczak and Kulig, 2013) cattle breeds, respectively. In present study, the genotypic frequencies of GG and GT were 0.0%. However, Cohen-Zinder *et al*. (2005) observed the frequency of GG genotype as 7.3% and for GT genotype as 41.4% in Israeli Holstein cattle population, while, Kowalewska-Luczak and Kulig (2013) observed 0.0 and 75.5% genotypic frequencies of GG and GT, respectively in Jersey cattle.

The allelic frequency observed for allele T in the screened animals of Sahiwal and Hariana was 1.0 and for allele G was 0.0 in the present work. In contrast, Cohen-Zinder *et al*. (2005) observed allelic frequency of T and G allele as 0.729 and 0.271, respectively in Israeli Holstein. Similarly, Kowalewska-Luczak and Kulig (2013) also found allelic frequency of T and G alleles as 0.62 and 0.38, respectively in Jersey cattle breeds. These studies in exotic cattle breeds indicate that the allelic frequency of T allele was higher than G allele.

Cohen-Zinder *et al*. (2005) investigated SPP1/*HpyCH4*IV polymorphism in Israeli Holstein cattle and found significant association between the alleles and milk yield, fat % and protein %, indicating that T allele is favourable for high milk yield, fat% and protein%. While, in the present study, only TT genotype was found in all the screened animals for SPP1/*HpyCH4*IV locus, so any association analysis could not be performed with milk production traits.

CONCLUSION

The present study is the first report on *G >T* polymorphism in exon 7 region of *SPP1* gene in Indian cattle breeds, in which we identified the absence of *G >T* polymorphism in *SPP1* exon 7 region with only 100% TT genotype. The results revealed that *SPP1* T allele seems to be fixed in screened cattle population. Consequently, we could not establish any association between genotype and milk production traits. It would be interesting to further examine the SNP status of this polymorphism in large diversified population for better exploitation of the marker assisted selection for milk traits in cattle.

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