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CONTENTS

Genetic polymorphism of leptin gene in Badri cattle of Uttarakhand

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ABSTRACT: The present study was undertaken on 50 unrelated Badri (hill cattle) of Kumaon region of Uttarakhand to explore polymorphism in intron 1 and 2 as well as exon 3 regions of leptin gene by PCR-RFLP technique and to find out the frequencies of different restriction fragment patterns for different regions. To reveal polymorphism in intron 1, a 340bp fragment within leptin gene was amplified and then digested with DraI restriction enzyme and study revealed three distinct types of genotypes or restriction patterns were observed. The genotype and gene frequencies were found to be 0.14, 0.40 and 0.46 for GG, GA and AA genotypes and 0.34 and 0.66 for G and A alleles, respectively. Restriction digestion of 422bp amplicons of intron 2 with Sau3AI revealed three genotypes. The genotype and gene frequency were found to be 0.56, 0.30 and 0.14 for AA, AB and BB genotypes and 0.71 and 0.29 for A and B alleles, respectively. The exon 3 fragment of 430bp size was digested with MspI and three distinct genotypes were observed. The genotype and gene frequency were found to be 0.26, 0.58 and 0.16 for CC, CT and TT genotypes and 0.55 and 0.45 for C and T alleles, respectively. The AA genotype in intron 1 was found to be relatively higher than GG genotype. The A allele of Sau3AI digestion within intron 2 was predominant and frequency of mutant allele T in exon 3 was found to be low.

Key words: Badri cattle, exon, intron, leptin gene, polymorphism, PCR-RFLP

Leptin is thought to be an important regulator of appetite, energy metabolism, body composition and reproduction (Campfield *et al.*, 1995; Caro *et al.*, 1996). This makes it of interest to explore the polymorphism at leptin gene loci by a molecular technique, polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) because PCR-RFLP is one of the best methods of studying polymorphism in the sense that it is fast, simple, economical and does not require hazardous radioactive materials (Denincourt *et al.*, 1990).The candidate genes for milk production in Badri cattle PRKCE, ABCG2, GHR, EPS8, CAST and NRXN1 were found to harbour maximum high confidence variance (Rahman *et al*., 2023). The present study was undertaken to explore polymorphism in intron 1 and 2 as well as exon 3 region of leptin gene by PCR-RFLP technique and to find out the frequencies of different restriction fragment patterns for different regions of Leptin gene.

MATERIALS AND METHODS

A total of 50 unrelated individuals of Badri cattle of Kumaon region of Uttarakhand were considered for the study from different pockets including those maintained at Instructional Dairy Farm (IDF), G.B. Pant University of Agriculture and Technology, Pantnagar. Blood samples were collected from jugular vein into EDTA containing vacutainer tubes. Genomic DNA was extracted from whole blood following standard phenol–chloroform extraction method described by Sambrook and Russell (2001). The quality of the extracted DNA was checked by performing horizontal electrophoresis on 0.7% (w/v) agarose gel at 4 volt/cm for 2 hour and documented through gel documentation system. Purity and concentration were checked in spectrophotometer. The PCR amplification was performed with corresponding set of forward and reverse primers (Table 1).

For introns 1 and 2, PCR was carried out in a final volume of 25µl containing 100uM dNTPs mix, 20 pmol of each primer, 2.5 µl 10X PCR buffer containing 1.5 mM $MgCl_2$, 1.5 unit of *Taq* DNA polymerase and 100-150ng of purified genomic DNA whereas for exon 3, the concentration of primer was 25 pmol. The amplification was carried out in thermocycler (Biorad, USA) pre-programmed for the

Table 1:Primer used for amplification of different regions of Leptin gene

Region	Primer code	Primer Sequence(5'→3')	Product length (bp)	Reference
Intron 1	LEP-I1F	ACATCCGTTGTTCACTGTGG	340	Dubey *et al.* (2012)
	LEP-I1R	TGCAGGCATATCCCATAACC		
Intron 2	LEP-I2F	TGGAGTGGCTTGTTATTTTCTTCT	422	Liefers *et al.* (2002)
	LEP-I2R	GTCCCCGCTTCTGGCTACCTAACT		
Exon 3	LEP-E3F	GCTCTTGCTCTCCCCTTCCT	430	Dubey *et al.* (2012)
	LEP E3R	GGTTTCTTCCCTGGACTTTGG		

following conditions: for intron 1, initial denaturation for 5 min at 95^{0}C followed by 35 cycles (denaturation at 95^{0}C for 1min, annealing at 60^{0}C for 1min and extension at 72^{0}C for 1 min) and a final extension at 72^{0}C for 10 min. However, for intron 2 and exon 3, the best result was obtained at annealing temperature of 64^{0}C and 58^{0}C, respectively. The amplicons were analyzed by running on 2% agarose gel by electrophoresis. To digest the PCR product of introns 1 and 2, and exon 3, 10 units each of DraI, Sau3AI and MspI restriction enzymes were used in 20µl aliquot. The reaction was stopped by adding a drop of 0.5M EDTA (pH 8.0). The digested PCR products were subjected to 4 per cent (W/V) agaros gel electrophoresis and stained with ethidium bromide, the banding being visualized and documented using a gel documentation system. The gene and genotype frequencies were calculated according to the method as suggested by Falconer and Mackey (1998).

RESULTS AND DISCUSSION

For intron 1, the digestion of the PCR product with DraI restriction enzyme revealed three patterns, one with 340bp i.e., no DraI site (GG genotype); second with 340bp, 232bp and 108bp (GA genotype) and third with 232bp and 108bp (AA genotype) in Badri cattle (Fig. 1). The genotype and gene frequencies for intron 1were found to be 0.14, 0.40 and 0.46 for GG, GA and AA genotypes and 0.34 and 0.66 for G and A alleles, respectively. Dubey *et al.* (2013) reported the genotype frequencies for GG, GA and AA to be 0.5297, 0.3663 and 0.1040, respectively. The genotype frequencies are not in agreement with the present study. In contrast to the present findings, lower frequency of the mutant allele (A) was reported in Sahiwal cattle 0.2871 by Dubey *et al.* (2013).

For intron 2, the digestion of the 422bp PCR product with Sau3AI restriction enzyme revealed three patterns, one with 390bp and 32bp (AA genotype); second with 390bp, 303bp, 87bp and 32bp (AB genotype) and third with 303bp, 87bp and 32bp (BB genotype) (Fig. 2). The genotype and gene frequency in Badri cattle were found to be 0.56, 0.30 and 0.14

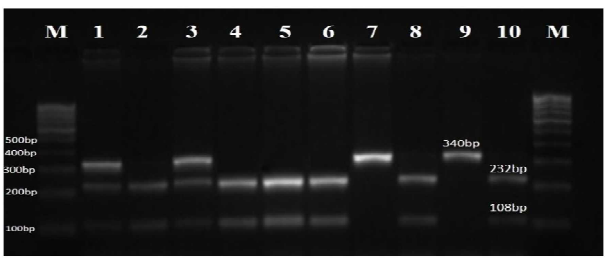

Fig. 1: DraI-RFLP genotypes of 340bp fragment of intron 1 within Leptin gene; Lane 7 and 9: GG genotype (340bp); Lane 1 and 3: GA genotype (340bp, 232bp and 108bp); Lane 2, 4, 5, 6, 8 and 10: AA genotype (232bp and 108bp); Lane M: Molecular size marker (100bp)

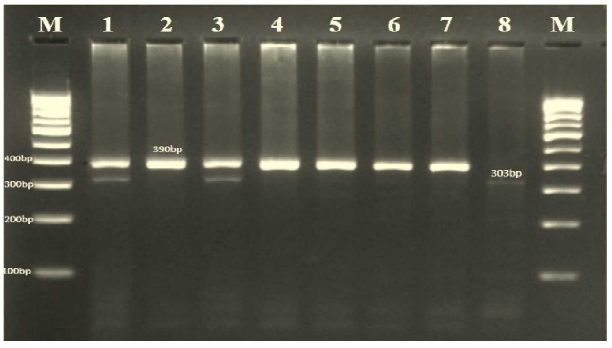

Fig. 2: Sau3AI-RFLP genotypes of 422bp fragment of intron 2 within Leptin gene; Lane 2, 4, 5, 6 and 7: AA genotype (390bp and 32bp); Lane 1 and 3: AB genotype (390bp, 303bp, 87bp and 32bp); Lane 8: BB genotype (303bp, 87bp and 32bp); Lane M: Molecular size marker (100bp).

for AA, AB and BB genotypes and 0.71 and 0.29 for A and B alleles, respectively. The presence of A allele in high frequency was also reported by Liefers *et al.* (2002) who genotyped 613 Holstein Friesian heifers and found that the genotype frequencies were 0.813, 0.185 and 0.002 for AA, AB and BB, respectively. Also, Madeja *et al.* (2004) genotyped 117 Polish Black- and White-bulls and reported the frequencies of alleles as 0.86, 0.11 and 0.03 for A, B and C alleles, respectively.

For exon 3, the digestion of the PCR product with MspI restriction enzyme revealed three patterns, one with 197bp, 132bp, 51bp and 50bp (CC genotype); second with 197bp, 132bp, 51bp and 50bp (CT genotype) and third with 197bp, 132bp and 101bp (TT genotype) in Badri cattle (Fig. 3). The genotype and gene frequency in Badri cattle were found to be 0.26, 0.58 and 0.16 for CC, CT and TT genotypes and 0.55 and 0.45 for C and T alleles, respectively. Dubey *et al.* (2013) reported the genotype frequencies for CC, CT and TT to be 0.4059, 0.4505 and 0. 1436, respectively which is in agreement with the present study. They also observed the novel C→T mutation in Sahiwal cattle and reported the allele frequencies of C and T to be 0.6312 and 0.3688, respectively. The present findings in Badri cattle were in agreement with Sahiwal cattle as lower frequency of the mutant allele (T) was also reported in Sahiwal cattle (0.3688) by Dubey *et al.* (2013). The low frequency of TT genotype in Badri cattle may probably be due to absence of T allele in Badri cattle.

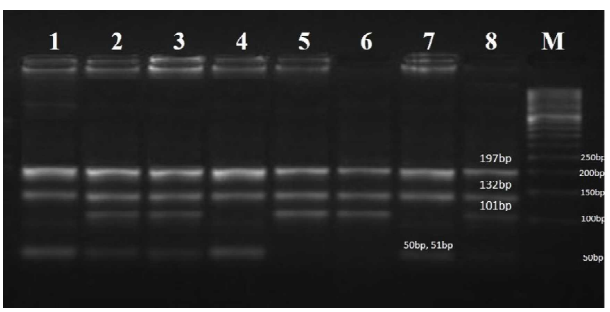

Fig. 3: MspI-RFLP genotypes of 430bp fragment of exon 3 within Leptin gene; Lane 1, 4 and 7: CC genotype (197bp, 132bp, 51bp and 50bp); Lane 2 and 3: CT genotype (197bp, 132bp, 101bp, 51bp and 50bp); Lane 5 and 6: TT genotype (197bp, 132bp and 101bp); Lane M: Molecular size marker (50bp)

The present study was conducted to characterize leptin gene and to explore polymorphism in this gene in Badri cattle. This is the first report on the identification of polymorphism in the leptin gene in Badri cattle. The leptin gene was found to be polymorphic with respect to the locus studied in a sample size of 50 animals. The introns 1and 2 and exon 3 of leptin gene were polymorphic for DraI, Sau3AI and MspI restriction sites, respectively. The frequency of AA genotype in intron 1 was found to be relatively higher than GG genotype. A allele obtained by Sau3AI digestion within intron 2 was found to be predominant and it was present in much higher frequency than B allele. The frequency of mutant allele T in exon 3 was found to be low in this investigated population of Badri cattle.

CONCLUSION

The introns 1 and 2 as well as exon 3 of leptin gene are polymorphic for DraI, Sau3AI and MspI restriction sites, respectively. The AA genotype in intron 1 was found to be relatively higher than GG genotype and the A allele of Sau3AI digestion within intron 2 is predominant in Badri cattle and frequency of mutant allele T in exon 3 was found to be low. This genetic information on leptin gene could be helpful in selecting superior animals using marker assisted selection.

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