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Research Article

VALIDATION OF INSILICO DETECTED POINT MUTATION IN HSP70 GENE IN HARDHENU CROSSBRED CATTLE

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ABSTRACT

The aim of this study was to characterize and validate the insilico mutation in HSP70 gene in Hardhenu (*Bos taurus* x *Bos indicus*) and Sahiwal cattle. A total of 100 cows 65 Hardhenu and 35 Sahiwal were used to screen point mutation. The HSP70 gene reported as one of the potential candidate gene influencing thermotolerance in livestock. The polymorphism of HSP70 gene in exonic region on bovine chromosome 23 was genotyped by using PCR-RFLP. A PCR product of 1926 bp was digested with seven different restriction enzymes to screen out reported point mutation. A monomorphic banding pattern with genotype AA was found in all screened animals. The result indicates highly conserved sequence in Hardhenu cattle.

Keywords: HSP70, Hardhenu, Monomorphic, PCR-RFLP, Sahiwal

Heat stress is one of the most unfavourable factors, known to alter the productive and reproductive performance in farm animals. Heat shock protein is highly conserved protein which is activated in response to cellular insults that cause protein unfolding, misfolding and improper aggregation which hampers animal production through series of complex mechanisms. Cellular response to stress includes synthesis of proteins belonging to a subgroup of molecular chaperones called heat shock proteins (HSPs), classified into different families on the basis of molecular weight (Pawar *et al*., 2014). Among all the HSPs, HSP70 is of particular interest since over expression of HSP70 during heat stress (Mishra *et al.,* 2011; Pawar *et al.,* 2014; Bharati *et al.,* 2016) signifies its role as a rescue marker for cells that are susceptible to stress (Hyder *et al.,* 2017). Bovine HSP 70 gene is an intron less gene mapped on chromosome 23 (Gade *et al.,* 2010).

Significant differences in thermotolerance lie between breed, hence animals with specific genotype adapted to specific location needs to be identified (Hoffmann, 2010), which can be exploited to a large extent for the genetic improvement of the animals for effective thermoregulation. Although there are many previous studies on the expression of HSP70 protein and polymorphism in 5' and 3' UTR, there is no report about possible polymorphisms in coding region of HSP70 gene and its association with performance traits in Hardhenu and Sahiwal cattle. With this background, present investigation was aimed to detect possible polymorphisms in the HSP70 gene by PCR RFLP technique and their association with performance traits.

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MATERIALS AND METHODS

The cattle resource population of this study consisted of randomly selected lactating crossbred Hardhenu (HF × Sahiwal × Hariana) and indigenous Sahiwal cows maintained at cattle breeding farm of LUVAS, Hisar, India. A total of 100 animals were taken under this study, to screen for the presence of polymorphism in exonic region of HSP70 gene. Blood samples were collected and genomic DNA was isolated using conventional phenol chloroform method.

Insilico SNP Detection and PCR amplification: In this study, candidate SNPs in bovine HSP70 gene were retrieved from dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) (Table 1). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to verify the screened SNPs. Based on reference sequence ENSBTAG00000025441 of the bovine HSP70 gene, specific PCR primers were designed using Premier 5.0 software to amplify the specific genomic sequence and to validate point mutations. PCR amplification was performed in 25 µl reaction mixture containing 100 ng DNA template, Dream Taq Green PCR Master Mix (2x), 10 pmol/µl each of forward and reverse primer. PCR amplification was carried out in thermal cycler (BIO-RAD T-100). The cycling conditions involved an initial denaturation at 95 °C for 3 min., followed by 34 cycles of 94 °C for 30 s, annealing temperature 61 °C for 30 s, 72 °C for 1 min and a final extension at 72 °C for 5 min. The PCR products were separated on 2 % agarose gel stained with 0.5 µg/ml of ethidium bromide, prior to visualization under gel documentation system (BIO-RAD Gel Doc EZ imager).

PCR-RFLP: Genotype analyses were performed using PCR-RFLP method. The PCR amplified product of HSP70 gene were digested with seven different restriction enzymes *viz*., *AluI*, *HindIII*, *DdeI*, *TaqI*, *EcoRV*, *DraI*, *HaeIII* in a total volume of 20 µl containing 10 µl of PCR product, 2 µl of RE buffer and 0.5 µl of each restriction enzyme to obtain restriction fragments. The reaction mixture for RE digestion was kept for incubation at 37 °C for 12 hours. The digestion products were separated by horizontal electrophoresis in 2 % agarose gels in 1 × TBE buffer at 70 V till complete separation of all fragments of RE digested fragments and marker. The restriction digested gene fragments were visualized and photographed with a gel documentation system.

RESULTS AND DISCUSSION

In present study, twenty eight SNPs were screened in bovine HSP70 gene using online genome database. All were synonymous mutation except four i.e., g.14A/G, g.15C/T, g.692T/C and g. 1877C/G which lead to amino acid changes: methionine to threonine, methionine to isoleucine, glutamate to glycine and glycine to alanine, respectively. In present study, PCR amplification generated 1926 bp fragment covering complete exonic region of HSP70 gene (Fig.1). Seven different restriction enzymes based on insilico analysis of cutting sites were used to detect polymorphism of HSP70 gene. The PCR-RFLP demonstrated the existence of only one allele A, showing single band consisting of 1926 bp, was assigned as the AA genotype. The results of this study are in close confirmation with Basirico *et al*., 2011, who reported homomorphism in 446 Italian Holstein cows. Similarly, Sodhi *et al*. (2013) found that the 253 bp region of the 3' UTR of HSP70 was monomorphic in both the *Bos taurus* and *Bos indicus* cattle breeds. Oner *et al.* (2017) detected 13 SNPs (G63T, A101G, T110A, T167A, T174G, C176T, T184G, T184A, A202G, T209C, A210G, A215G and T226A) and one indel in native Turkish cattle breeds (n=199) in 3'UTR whereas same region was found to be monomorphic in Holstein Friesian (n=50) cattle. There have been a few reports of genetic polymorphism in HSP70 gene that have association with thermotolerance in cattle. Kerekoppa *et al*. (2015) found high variability in the HSPA1A gene in Deoni and HF crossbred cattle by using single strand conformation polymorphism analysis, which revealed 14 band patterns in Deoni cattle and 8 band patterns in HF crossbreds. Sequence analysis showed 12

Table 1

Sequence of the primers used for amplification of complete exon of HSP70 gene

Sequence (5'-3')	No. of base pairs	Amplicon size (bp)
F: ATGGCGAAAAAC ATGGCTATCGGC	24	1926 bp
R: CTAATCCACCTCC TCAATGGTGGGGCC	27	

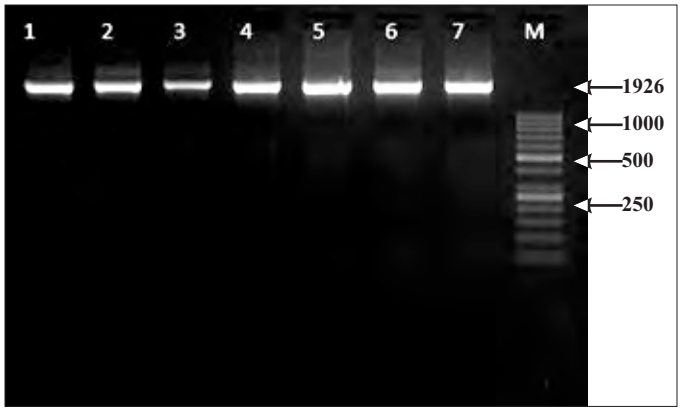

Fig. 1. Resolution of PCR amplified product of complete exon of HSP70 in Hardhenu and Sahiwal cattle on 2% Agarose gel. Lane 1-7: PCR product (1926 bp), Lane M: 50 bp ladder molecular marker

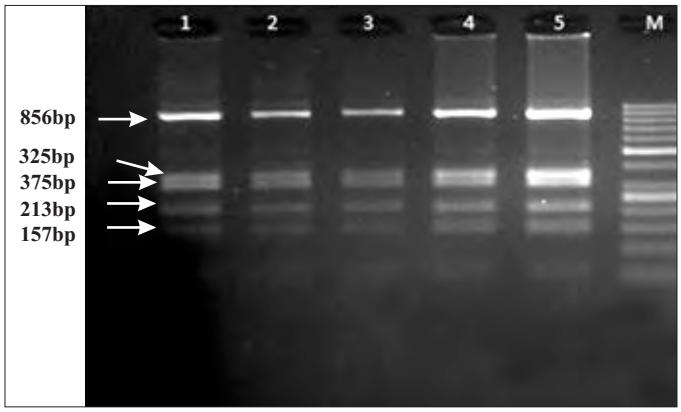

Fig. 2. PCR RFLP genotyping of HSP 70 in Hardhenu cattle using DdeI restriction enzyme. Lane 1-5: RE digested product, Lane M: 50 bp ladder molecular marker

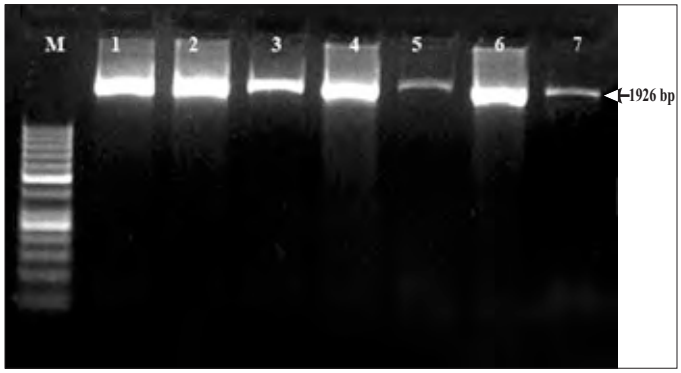

Fig. 3. PCR RFLP genotyping of HSP 70 in Hardhenu, Sahiwal and Hariana cattle using DraI restriction enzyme. Lane 1-7 RE digested product, Lane M: 50 bp molecular marker

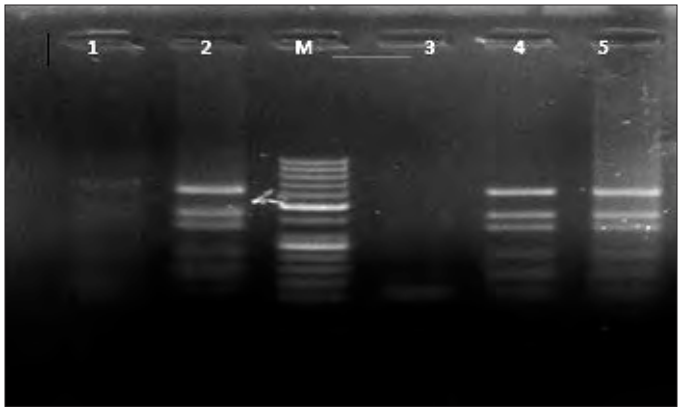

Fig. 4. PCR RFLP genotyping of HSP 70 in Sahiwal and Hariana cattle using AluI restriction enzyme. Lane 1-2 & 3-5: RE digested product of 180 bp, 200 bp, 380 bp, 500 bp and 660 bp. Lane M: 50bp ladder molecular marker

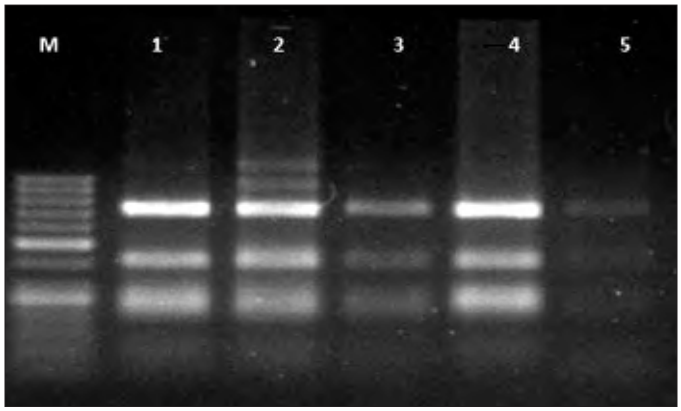

Fig. 5. PCR RFLP genotyping of HSP 70 in Hardhenu and Sahiwal cattle using HaeIII restriction enzyme. Lane 1-5: RE digested product of 192 bp 244 bp 250 bp 450 bp, 790 bp, Lane M: 50bp ladder molecular marker

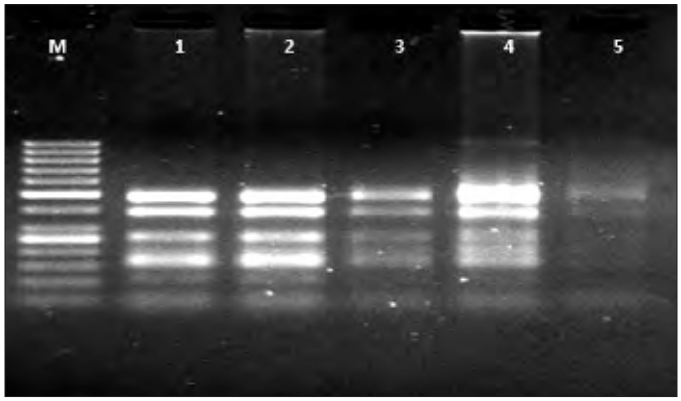

Fig. 6. PCR RFLP genotyping of HSP70 Hardhenu cattle using TaqI restriction enzyme. Lane 1-5: RE digested product of 82 bp, 125 bp, 182 bp, 230 bp, 389 bp, 448 bp, 500 bp, Lane M: 50bp ladder molecular marker

single nucleotide polymorphisms in the coding region of the HSPA1A gene. 7 SNPs including 5 transitions (G456A, A972G, A1098G, C1766T, G1788A) and 2 transversions (C312G, G2033C) in Deoni cattle, and 5 SNPS including 2 indels (C at positions 574-575 and 624-625), 2 transitions (A480G, A1098G) and 1 transversion (C312G) in HF

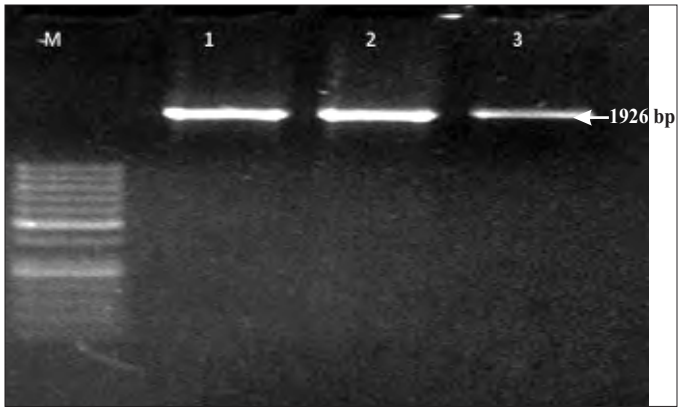

Fig. 8. PCR RFLP genotyping of HSP70 in Sahiwal using HindIII restriction enzyme. Lane 1-3: RE digested product, Lane M: 50bp ladder molecular marker

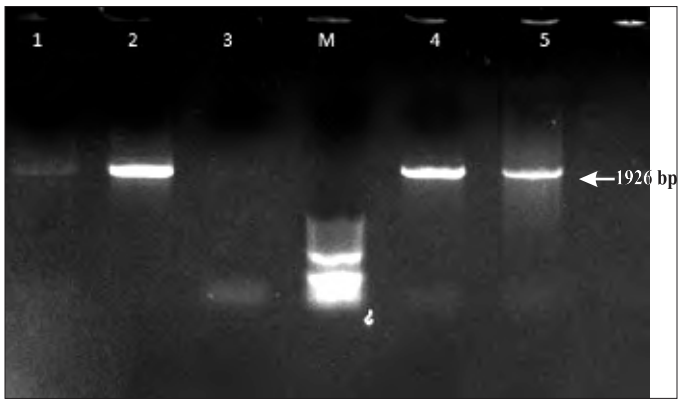

Fig. 7. PCR RFLP genotyping of HSP70 in Hariana cattle using EcoRV restriction enzyme. Lane 1-3 & 4-5: RE digested product, Lane M: 50bp ladder molecular marker

crossbred cattle.

CONCLUSION

Bovine HSP70 gene was amplified with specific primers. PCR amplification yielded an amplified product of 1926 bp comprising of complete exon of HSP70 gene. The HSP70 gene in the Hardhenu and Sahiwal cattle included in present study is monomorphic as revealed by PCR-RFLP analysis using *AluI*, *Hind III*, *DdeI*, *TaqI*, *EcoRV*, *DraI*, *HaeIII* restriction enzymes. Thus, the monomorphic pattern of HSP70 gene in studied population may be a due highly conserved sequence.

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