The Pharma Innovation Journal 2021; SP-10(11): 947-949

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(11): 947-949
© 2021 TPI
www.thepharmajournal.com
Received: 19-09-2021
Accepted: 21-10-2021

Status of heat shock protein 27 (HSP27) polymorphism in Hariana cattle

Ramkaran, Sandeep Gera, Ravi Kumar, Pradeep Kumar, Ankit Magotra and Sandeep Kumar

Ramkaran
Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Sandeep Gera
Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Ravi Kumar
Department of Livestock Production Management, College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Pradeep Kumar
Department of Veterinary Parasitology, College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Ankit Magotra
Department of Animal Genetics & Breeding, College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Sandeep Kumar
Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Corresponding Author
Sandeep Kumar
Department of Veterinary Physiology and Biochemistry, College of Veterinary Sciences Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar, Haryana, India

Abstract
The heat shock protein (HSP) family is known for its role in cell protection. HSP 27, also known as heat shock beta 1, is involved in cellular organization (Creagh *et al*., 2000) and interacts with myofilaments (Blunt *et al*., 2007; Fischer *et al*., 2002). The present study was undertaken to to identify point variations in HSP 27 gene in 50 Hariana cattle. Genomic DNA was extracted from whole blood of lactating Hariana cattle. PCR amplification yielded products of 631bp for HSP 27 gene. Amplicons were subjected to direct sequencing and multiple sequence alignment using Clustal W revealed one nucleotide sequence variation in HSP 27 gene. In HSP 27 gene G225A was polymorphic with three genotypes AA, AB and BB in our resource population. Allele and genotype frequencies of HSP 27 gene were calculated using POPGENE software package. Genotype AA, BB and AB had frequencies 0.64, 0.28 and 0.08, respectively whereas, allele frequency for A and B allele were 0.78 and 0.22, respectively.

Keywords: cattle, HSP 27, Hariana, PCR, polymorphism

Introduction
Heat stress (HS) shows negative impact on all aspects of dairy cattle and buffalo reproduction (Rensis and Scaramuzzi, 2003; Hansen, 2007; Marai and Habeeb, 2010) [8, 9], milk production (West, 2003) [15] and immune function (Elvinger *et al*., 1992) [5]. Length of oestrous cycle and degree of expression of oestrus in buffaloes are affected by various factors, such as season, climate, photoperiod, temperature and nutrition (Beg and Totey, 1999). Final Report of the Network Project on Climate Change (2004-07) predicted the annual loss of about 3.2 MT in milk production of cattle and buffaloes due to thermal stress by the year 2020 costing more than 5000 crore rupees (Upadhyay, 2008) [13]. Stress/heat shock protein genes are important candidates for this purpose. There are number of candidate genes for heat tolerance trait like HSP families, ATP1A1, ATP1B2, Uncoupling Protein, Osteopontin, Slick Hair gene, HSF1 etc. HSP 27 is a chaperone of the sHSP (small heat shock protein). HSPB1 gene synthesize HSP 27 located on chromosome 25 in Bos Taurus and spans 2670 bp comprising of 3 exons. The common functions of HSP 27 are chaperone activity, thermo tolerance, inhibition of apoptosis, regulation of cell development, and cell differentiation. Heat shock proteins function as intra-cellular chaperones by helping to stabilize partially unfolded proteins, HSPs aid in transporting proteins across membranes within the cell (Walter and Buchner, 2002; Borges and Ramos, 2005) [2]. The phosphorylated form of HSP 27 inhibits DAXX apoptotic protein and prevents the association of DAXX with FAS and ASK (Charette *et al*, 2000). A well-documented function of HSP 27 is the interaction with actin and protects actin filaments from fragmentation (Sarto *et al*, 2000) [12]. HSP 27 enhances the activation of NF-κB pathway (Parcellier *et al*., 2003) [10]. Objective of this study is to identify single nucleotide polymorphism (s) in HSP 27 gene in Hariana cattle.

Materials and Methods
Experimental Animals & DNA Isolation
The present study was conducted on Hariana (50) cows maintained at Shri Krishan Gaushala Avam Anusandhan Kender, Kabrel, Hisar,with prior permission from Institutional Animal Ethics Committee. A total of 50 randomly chosen animals were used for the present study. Collection of Blood and DNA Isolation: Ten ml of blood was aseptically collected from the jugular vein in vacutainer tube containing EDTA as anticoagulant. The samples were properly labeled and were stored in deep freezer at -200C temperature till the isolation of genomic

DNA. Genomic DNA was isolated by phenol-chloroform method following standard protocol (Sambrook and Russell, 2001) [11].

Evaluation of Quality and Concentration of DNA

Genomic DNA quality was checked to ensure the presence of intact DNA by running the isolated DNA on agarose gel [0.8% (w/v)]. The purity of the genomic DNA was assessed by UV spectrophotometer by checking the optical density (OD) value at 260 and 280 nm. The samples having OD ratio (260 nm/ 280 nm) 1.7 to 1.9 were used for further experiment. The concentration of DNA was calculated by using the following formula: DNA concentration (µg/µl) = OD260 x (Dilution factor) x 50/ 1000. Finally, DNA was diluted in distilled water at the concentration of 50ng/µl. In the PCR reaction 2 µl diluted DNA was used. The genomic DNA samples having good quality DNA (intact bands without smearing in gel) were used for further analysis.

PCR to Explore HSP 27 Polymorphism

The PCR reaction was carried out in 0.2 ml PCR tubes in a thermal cycler (Applied Biosystem, USA). PCR was carried out in a final reaction volume of 25 µl containing approximately 100 ng DNA template, 12.5 ml of 2X PCR master mix (Thermo Fisher Scientific), 0.5 µl (10 pM/ µl) of each primer. Primer sequences were as follows: forward - 5'-CCCGTCATTGCCATTAATAGAG -3' and reverse 5'-CGTCCTCGGCTAAGTTTTCA-3'. PCR amplification protocol included an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C (30 s), 59 °C (30 s) and 72 °C (45 s) and a final extension at 72 °C for 10 min. PCR amplification was confirmed by running 10 µl of PCR product mixed with 2 µl of 6X gel loading dye (Thermo Fisher Scientific) on 1% agarose gel (depending on the expected size of amplified product) at a constant voltage of 70 V for 30 minutes in 1X TBE buffer. Ethidium bromide (0.5 µg/ml) was added @ 5µl of 1% solution /100 ml of gel solution. The amplified product was visualized as a single compact fluorescent band of expected size under UV light and documented by gel documentation system (AlphaImager, USA). Amplified PCR products from 2 sets of primers were subjected to sanger sequencing from both ends (5' and 3' ends).Nucleotide sequences were visualized and edited using Bio Edit software. The forward and reverse sequences for each PCR fragments were assembled to form complete sequence for the respective region of HSP 27 gene. The multiple sequence alignments of the edited sequence with corresponding reference sequences (*Bostaurus*: NC_037350.1) were performed with Clustal W software to identify SNPs.

Calculation of gene and genotypic frequencies

Gene and genotypic frequencies were calculated as given by Falconer and Mackay (1996) [6]

$$\text{Genotype frequency} = \frac{\text{Total number of individuals of a particular genotype}}{\text{Total number of individuals of all genotypes}}$$

$$\text{Gene frequency} = \frac{(2D + H)}{2N}$$

Where

D = No. of homozygotes of particular genotypes,

H = No. of heterozygotes having that gene and

N = Total no. of individuals

Results and Discussion

Allele and Genotype frequencies

Mutation g.G225A was confirmed in HSP 27 gene of Hariana cattle resolved into three genotype i.e. AA, BB and AB. Allele and genotype frequencies of HSP 27 gene were calculated using POPGENE software package. Genotype AA, BB and AB had frequencies 0.64, 0.28 and 0.08, respectively whereas, allele frequency for A and B allele were 0.78 and 0.22, respectively.

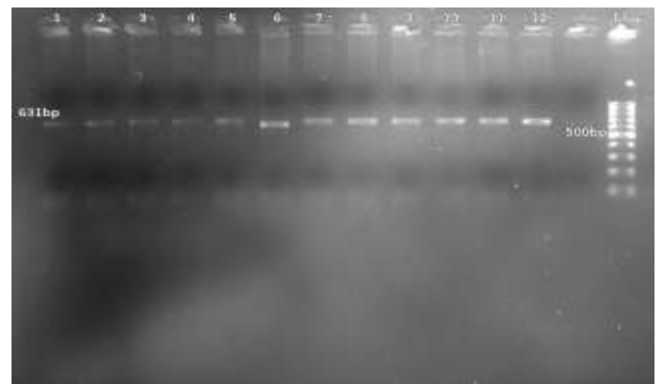

Plate 1: Resolution of PCR product of HSP 27 gene in Hariana cattle Lane 1-12 : PCR product (631 bp) Lane M : 100 bp DNA ladder

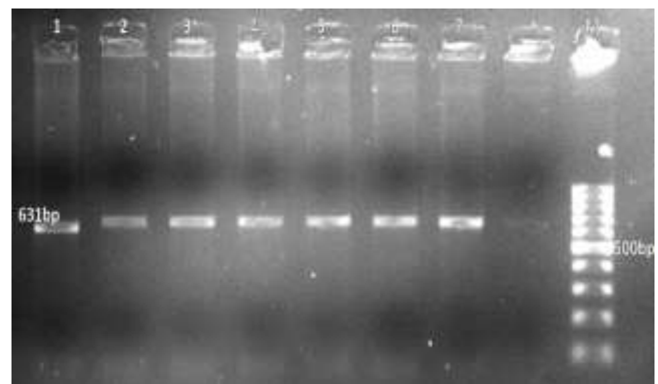

Plate 2: Resolution of PCR product of HSP 27 gene in Hariana cattle Lane 1-7 : PCR product (631 bp) Lane M : 100 bp DNA ladder

Table 1: Genotypic and allelic frequency of different genotype for HSP 27 gene

Breed	Genotype frequency			Total	Allele frequency	
	AA	AB	BB		A	B
Hariana cattle	0.64 (32)*	0.28 (14)*	0.08 (4)*	50	0.78	0.22

*Figures in parenthesis shows no. of animals

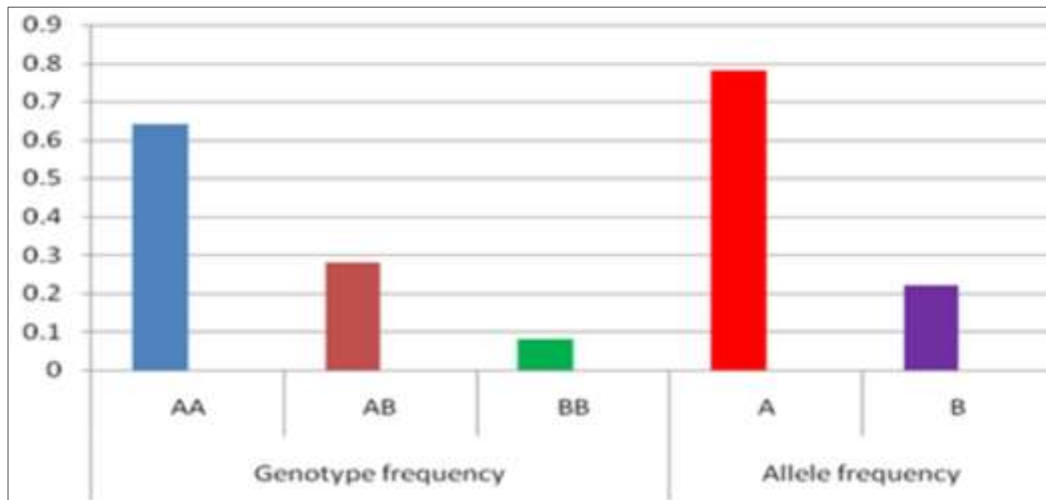

Fig 1: Genotypic and allelic frequency of different genotype for HSP 27 gene

Conclusion

It is concluded from the present investigation that one set of primers yielded amplicon size of 631bp encompassing exon 1 of HSP 27 gene. Mutation g.G225A was confirmed in HSP 27 gene of Hariana cattle resolved into three genotype i.e. AA, BB and AB. Allele and genotype frequencies of HSP 27 gene were calculated using POPGENE software package. Genotype AA, BB and AB had frequencies 0.64, 0.28 and 0.08, respectively whereas, allele frequency for A and B allele were 0.78 and 0.22, respectively. This preliminary information may further help in taking up association studies between HSP 27polymorphisms and production traits in Hariana cattle.

References

1. Blunt BC, Creek AT, Henderson DC, Hofmann PA. H_2O_2 activation of HSP25/27 protects desmin from calpain proteolysis in rat ventricular myocytes. Am J Physiol Heart Circ Physiol 2007;293:H1518–H1525.
2. Borges JC, Ramos CH. Protein folding assisted by chaperones. Protein and peptide letters 2005;12(3):257–261.
3. Charette SJ, Lavoie JN, Lambert H, Landry J. Inhibition of Daxx-mediated apoptosis by heat shock protein 27. Molecular and cellular biology 2000;20(20):7602-7612.
4. Creagh EM, Sheehan D, Cotter TG. Heat shock proteins – modulators of apoptosis in tumour cells. Lukemia 2000;14:1161-1173.
5. Elvinger F, Natzke RP, Hansen PJ. Interactions of Heat Stress and Bovine Somatotropin Affecting Physiology and Immunology of Lactating Cows. Journal of dairy science 1992;75(2):449-462.
6. Falconer DS, Mackay TFC. Introduction to quantitative genetics. Harlow Essex, UK: Longmans Green 1996, 3.
7. Fischer D, Matten J, Reimann J, Bonnemann C, Schroder R. Expression localization and functional divergence of αB-crystallin and heat shock protein 27 in core myopathies and neurogenic atrophy. Acta Neuropathologica 2002;104(3):297-304.
8. Hansen PJ. Exploitation of genetic and physiological determinants of embryonic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress. Theriogenology 2007;68:S242-S249.
9. Marai IFM, Haeeb AAM. Buffalo's biological functions affected by heat stress – A review. Livestock science 2010;127:89-109.
10. Parcellier A, Gurbuxani S, Schmitt E, Solary E, Garrido C. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochemical and biophysical research communications 2003;304(3):505-512.
11. Sambrook J, Russell DW. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
12. Sarto C, Binz PA, Mocarelli P. Heat shock proteins in human cancer. Electrophoresis 2000;21(6):1218-1226.
13. Upadhyay RC, Singh SV, Ashutosh. Impact of climate change on livestock. Indian Dairyman 2008;60(3):98-102.
14. Walter S, Buchner J. Molecular chaperones--cellular machines for protein folding. Angewandte Chemie (International ed. in English) 2002;41(7):1098-1113.
15. West JW. Effects of heat-stress on production in dairy cattle. Journal of dairy science 2003;86(6):2131-2144