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Status of heat shock protein 90 (HSP90AB1) polymorphism in Hariana cattle

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Abstract
The 90 kDa heat shock protein family (HSP90) is one of the most abundant cytosolic proteins in eukaryotes. The present study was taken to explore the point variations in HSP90AB1 gene in 50 lactating Hariana cattle. A 459bp PCR fragment of HSP 90AB1 gene was amplified and amplicons were subjected to direct sequencing for characterization and mutation detection. Multiple sequence alignment using ClustalW of sequenced samples revelated but no SNP for HSP90AB1 gene in our studied population which indicates highly conserved DNA sequence in Hariana cattle. Since present study has formulated the results based on a relatively small sample, further studies are required to validate these results in large samples.

Keywords: HSP90AB1, Hariana, PCR, polymorphism, heat shock protein

Introduction
One of the greatest challenges being faced by producers and livestock across the world today is heat stress. Global warming and climate change are the major threats to the sustainability of livestock production systems in the future (Moss *et al*., 2000; Gaughan *et al*., 2010; Naqvi and Sejian, 2011) [7, 6, 8]. Climatic change causes heat stress in livestock characterized by reduced feed consumption rate, decreased milk production and lower reproductive success rate (Cavestany *et al*., 1985; Sharma *et al*., 1988; Bernabucci *et al*., 1999) [1, 2, 10]. 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), milk production (West, 2003) and immune function (Elvinger *et al*., 1992).
There are number of candidate genes for heat tolerance trait like HSP families, ATP1A1, ATP1B2, uncoupling protein, Osteopontin, Slick Hair gene, HSF1 etc. HSP90 gene is one of the excellent candidate genes for this trait. HSP90 is a chaperone protein that assists other proteins to fold properly and stabilizes them against heat stress (Csermely *et al*., 1998) [4]. HSP90AB1 gene has been significantly associated with heat tolerance in *Bostaurus* (crossbred Holstein Friesian) and *Bosindicus* (Thai native cattle *viz*., White Lamphun and Mountain cattle) animals in Thailand as reported by Charoensook *et al*., 2012 [3]. Therefore, identified SNPs and information on their association with adaptive response will be an aid to selection of native cow breeds, so that we can propagate thermo adaptable cows as future generation to achieve optimum profits under global warming scenario. Objective of this study is to identify single nucleotide polymorphism (s) in HSP90AB1 gene in Hariana cattle.

Materials and Methods
Resource population and DNA isolation
The present study was conducted on 50 Harianacattle maintained at Shri Krishan Gaushala Avam Anusandhan Kender, Kabrel, Hisar. 10 ml of blood was aseptically collected from the jugular vein in sterile vacutainer containing EDTA and samples were properly labeled and stored in deep freezer for further processing. Genomic DNA was extracted by phenol-chloroform method (Sambrook and Russell, 2001) [9]. Quality and quantity of the isolated genomic DNA was evaluated using UV-vis spectrophotometer.

PCR amplification
The self-designing primer sequences were used to amplify the targeted HSP90AB1 gene.The sequence of primers, their respective nucleotide numbers and amplicon size is given in Table 1.

PCR amplification was carried out in a final volume of 25 µl containing 100 ng DNA template, 12.5 ml of 2X PCR master mix (Thermo Fisher Scientific), 0.5 µl (10 pM/ µl) of each primer and reaction was carried out in 0.2 ml PCR tubes in a thermal cycler (Applied Bio system, USA) in following stages: initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C (30 s), 59 °C (30 s) and 72 °C (30 s) and a final extension at 72 °C for 5 min. The amplified product was visualized under UV light and documented by gel documentation system (Alphalmager, USA).

Sequence Data Analysis and SNP Detection

Amplified PCR product was 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 HSP90AB1 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.

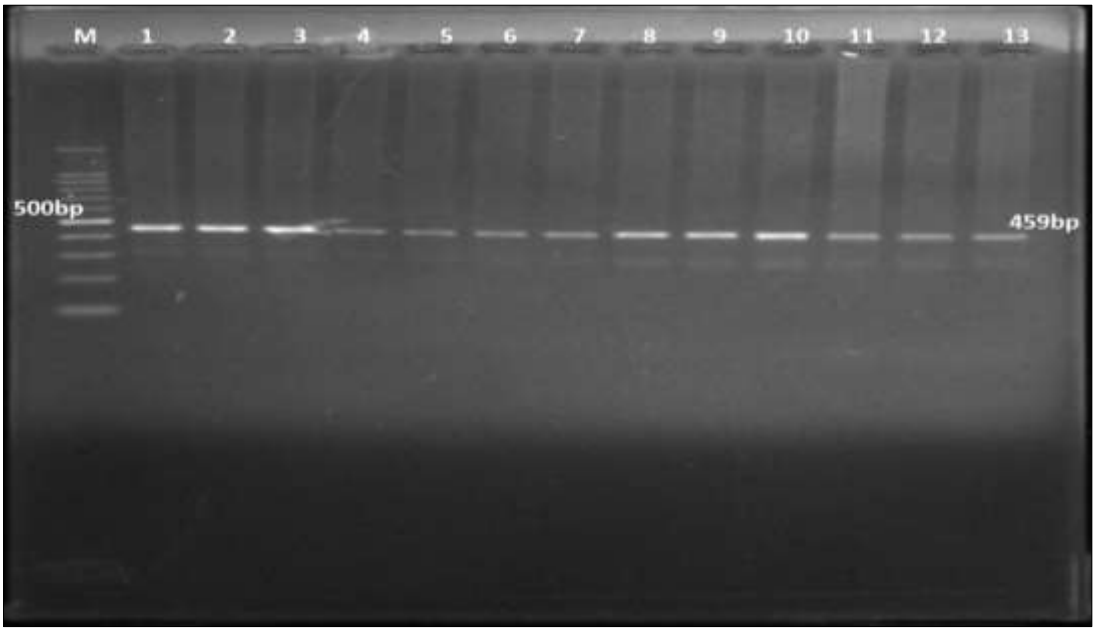
Table 1: Sequence of primers for amplification of HSP 90AB1 gene

Primer		Sequence (5'-3')	No. of base	Amplicon size (bp)
1	F	AGTGAGTATCTTTTGCCCTAATG	23	459
	R	TCTCCTCTAACCGAATGAAAAA	22	

Results and Discussion

Heat stress has been one of the major concerns in reducing animal's productivity in tropical, sub-tropical and arid areas. The degree to which an animal resists change in body temperature varies with different species because of differences in their heat regulating mechanisms Genetic differences in thermo tolerance at the physiological and cellular levels are documented by number of studies on *Bosindicus* and *Bostaurus* cattle breeds. In a research conducted at CIRC, allele frequencies of C and T alleles of HSP90 gene in Sahiwal and Frieswal cattle have been reported as 0.44, 0.56 and 0.5 and 0.5 respectively (PDC, Annual Report 2012-13). Bernabucci *et al*. (2013) reported one SNP g4338T/C within intron 3 of HSP 90AB1 gene with frequency of C and T allele as 58.6% and 41.3%, respectively in Italian Holstein dairy cows. The SNP was identified to be associated with thermo tolerance. Deb *et al*. (2014) studied the effect of heat stress on the expression profile of HSP90 among Sahiwal (Bosindicus) and Frieswal (Bosindicus × Bostaurus) breed of cattle and found that in the induced in vitro and environmental stress conditions Sahiwal cattle expressed higher levels of HSP90 than Frieswal cattle to regulate their body temperature and increase cell survivability under heat stressed conditions.

PCR amplification yielded 459bp for targeted HSP90AB1 gene (figure 1). Amplicons were subjected to direct sequencing and multiple sequence alignment using ClustalW analysis which did not reveal any nucleotide variation in our studied population.So, it is prerequisite to explore and validate reported variation in different dairy breeds/species before implementation in selection criteria.



Lane 1-13: PCR product (459 bp)
Lane M: 100 bp DNA ladder

Fig 1: Resolution of PCR product of HSP90AB1gene in Hariana cattle

Conclusion

This study was aimed to charactize and unreveals point mutation in the genomic sequence HSP90AB1 gene in Hariana cattle. But targeted region of HSP90AB1 gene after multiple sequence analysis did not revealed any SNP for HSP90AB1 gene in our studied population that concluded that amplified genomic region was highly conserved. Since present study has formulated the results based on a relatively small sample, further studies are required in large samples to establish the role of SNPs in HSP90AB1 genefor heat stress in cattle.

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