

## Full Length Research Paper

# Association of single nucleotide polymorphism of Hsp90ab1 gene with thermotolerance and milk yield in Sahiwal cows

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Heat shock proteins play a critical role in the development of thermotolerance and protection from cellular damage associated with heat stresses. The study was undertaken to investigate the association of single nucleotide polymorphisms (SNPs) of Hsp90ab1 gene with thermo-physiological parameters viz, respiration rate (RR), rectal temperature (RT), heat tolerance coefficient (HTC) and total milk yield in Sahiwal cows. The RR and RT were recorded once in different seasons, viz., winter, spring, and summer, at the probable extreme hot hours of the day. Polymorphism of Hsp90ab1 gene, evaluated by comparative sequencing revealed five SNPs, viz., T17871421C, C17871485del, C17872061T, T17872112C and T17872148G. Individuals with CT genotype recorded significantly ( $P \leq 0.01$ ) lower RT ( $^{\circ}\text{C}$ ) than CC genotype in Sahiwal cows. The CT genotype animals also had better production parameter in terms of total milk yield (TMY) ( $P < 0.01$ ). Therefore, our results inferred that CT genotype in Sahiwal cows may be an aid to selection and breeding to enhance thermo-tolerance.

**Key words:** Hsp90ab1, SNPs, respiration rate, rectal temperature, total milk yield, Sahiwal.

## INTRODUCTION

Climate change is likely to be a major threat to the viability and sustainability of livestock production systems in many regions of the world. Increased in temperature impairs production and reproduction performance, metabolic, health status and immune response (St-Pierre et al., 2003; Rosenzweig et al., 2007). The negative effects of heat stress will become more apparent in the future if climate change continues. Moreover, due to the

close relationship between metabolic heat generation and production level, development in the genetic programs that enhance production traits may increase an animal's susceptibility to high environmental temperatures (Nardone et al., 2010). One possible approach for reducing the impact of heat stress on cattle productivity is to exploit the genetic variability underlying relative thermotolerance (Hoffmann, 2010).

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Cellular tolerance to heat stress is mediated by a family of proteins named heat shock proteins (HSPs). HSPs play essential roles in the immunity of organisms, particularly in relation to heat-resistance (Song et al., 2006; You et al., 2013). The chaperone, Hsp90 is one of the most abundant proteins in eukaryotic cells, comprising 1–2% of cellular proteins under non-stress conditions (Craven et al., 1996). There are two major cytoplasmic Hsp90 isoforms, the inducible (Hsp90 $\alpha$ / Hsp90aa1) and the constitutive (Hsp90 $\beta$ / Hsp90ab1), which have arisen by gene duplication (Chen et al., 2006). A fully functional Hsp90 protein normally associates with other cochaperones, playing an important role in the folding of newly synthesized proteins or stabilizing and refolding denatured proteins after stress (Richter and Buchner, 2001). Recently novel single nucleotide polymorphisms (SNPs) were identified at different positions of the bovine heat shock protein 90 kDa alpha (cytosolic), class member 1 (Hsp90ab1) in *Bos taurus* (crossbred) and *Bos indicus* cattle. High prevalence of this gene in tropical cattle (*Bos indicus*) perform better as compared to temperate breeds or their zebu crossbreds due to higher level of heat tolerance (Charoensook et al., 2012; Deb et al., 2013; Mcmanus et al., 2014; Sajjanar et al., 2015)

The objective of this study was to identify SNPs in the targeted regions of Hsp90ab1 gene in Sahiwal cows and to analyze the association of genetic variants with thermo-physiological parameters and milk yield.

## MATERIALS AND METHODS

### Experimental animals

The study was conducted on randomly selected 50 lactating Sahiwal cows maintained at Livestock Research Centre of National Dairy Research Institute (Karnal). The animals were different age group, clinically healthy and kept under the same conditions. The experimental design and procedure were carefully planned and approved by the Institutional Animal Ethics Committee.

### Recording physiological parameters

Respiration rate (RR) of each animal was recorded by visual observation of inward and outward flank movement. Rectal temperature (RT) was recorded in centigrade with a digital thermometer by keeping the thermometer in contact with *rectal mucosa* for about 2 min. RR and RT were recorded at 6-8 am, 12-02 pm, and 12-02 pm during winter (January), spring (March) and summer (June) respectively. Recording of each of the parameters was done once in each of the three seasons at the probable extreme hours of day.

Heat tolerance coefficient (HTC) was calculated by Benezra Coefficient of Heat Adaptability (Benezra, 1954) with the following formula:

$$HTC = RR/23 + RT/38.33$$

The denominators 23 and 38.33 in the equation represent the normal RR and RT (°C) of cattle, respectively, under ideal conditions.

### Recording milk production data

Data for total milk yield was obtained from the data sheets of the farm records. Milk production records from the entire lactation length of all the individuals were utilized.

### Blood collection and DNA extraction

Ten ml blood was collected aseptically from the cows in a sterile Beckton-Dickinson vacutainer containing 0.5 per cent (10  $\mu$ l/ml of blood) anticoagulant ethylene diamine tetraacetic acid (EDTA). Genomic DNA was extracted using Phenol-chloroform method described by Sambrook and Russel (1989) with minor modifications, and detected by 0.7% agarose gel electrophoresis. The content of DNA was estimated by Biospec-nano spectrophotometer, and the genome DNA was diluted to a final concentration of 50 ng/ $\mu$ l, and stored at -20°C for PCR amplification.

### PCR amplification

Two sets of forward and reverse gene-specific oligonucleotide primers were designed using DNASTAR software and gene specific sequence (ENSBTAT00000001034) available at ensemble genome browser ([www.ensembl.org](http://www.ensembl.org)). The working solutions of both forward and reverse primers were prepared to obtain final concentration of 10pmol for each primer.

Final reaction mix (25  $\mu$ l) comprised of forward primer (0.5  $\mu$ l), reverse primer (0.5  $\mu$ l), PCR Master Mix (12.5  $\mu$ l), water (8.5  $\mu$ l) and template DNA (3.0  $\mu$ l). PCR amplification was performed using Thermal cycler (MJ research and Biorad T100). Each tube, containing 25  $\mu$ l PCR reaction cocktail was kept in Thermal Cycler for amplification of target region of bovine Hsp90ab1 gene. PCR conditions involved initial denaturation at 95°C for 1 min, followed by 34 cycles with denaturation at 94°C for 30 s, primer specific annealing temperature of 59°C and 61°C for 30 s to specifically amplify target region 1 and 2 respectively, extension at 72°C for 25 s followed by final extension at 72°C for 6 minutes and 30 s.

### Temperature humidity index

The outdoor temperature and the relative humidity (RH) (%) were recorded daily during the experiment to ascertain Temperature humidity index (THI) value. THI was calculated as per National Research Council (NRC, 1971). THI of winter (49.7), spring (64.65), and summer (86.44) obtained from ICAR-CSSRI, Karnal were the three subclasses of THI considered in association analysis.

$$THI = 0.72 (Wb + Db) + 40.6$$

Where, Wb is wet bulb temperature and Db is dry bulb temperature in °C.

### Purification and Sequencing

Amplified PCR products of both sets of primers Table 1 were custom sequenced from both ends, i.e. 5' and 3' ends. DNA sequencing results for the respective region of bovine Hsp90ab1 gene were visualized and edited using Chromas Lite Software. Each edited sequence was aligned with corresponding reference sequence of *Bos taurus* using ClustalW multiple sequence alignment program to identify single nucleotide polymorphism ([www.ebi.ac.uk/Tools/msa/clustalw2](http://www.ebi.ac.uk/Tools/msa/clustalw2)).

**Table 1.** Sequence of primers, targeted regions and amplicon sizes of bovine Hsp90ab1 gene.

Primer set	Primer sequence (5'-3')	Targeted regions	Amplicon size (bp)
1	F: AGTGAGTATCTTTTGGCCCTAATG (23) R:TCTCCTCTAACCGAATGAAAAA (22)	17871343-17871801	459
2	F: GCTGCTGCGCTATCACACG (19) R: GCCCTCCTTGGTCACAGA (18)	17871892-17872278	387

1The number within parenthesis indicates base pairs. 2Targeted region 1 includes part of intron 7, exon 8, and part of intron 9, Targeted region 2 includes part of exon 10, intron 10, and exon 11.

**Table 2.** Genotypic and allelic frequency at each SNP locus of Hsp90ab1 gene in Sahiwal cows.

SNPs	Genotype	Genotypic frequency	Allele	Allelic frequency
SNP1 T17871421C (Exon 8)	TT	-	T	-
	TC	-	C	1
	CC	1(50)		
SNP2 C17871485del (Exon 8)	CC	-	C	-
	Del	1(50)	Del	1
SNP3 C17872061T (Intron 10)	CC	0.34(17)	C	0.67
	CT	0.66(33)	T	0.33
	TT	-		
SNP4 T17872112C (Intron 10)	TT	0.78(39)	T	0.89
	TC	0.22(11)	C	0.11
	CC	-		
SNP5 T17872148G (Exon 11)	TT	-	T	0.06
	TG	0.12(04)	G	0.94
	GG	0.88(36)		

The number within parenthesis indicates number of animals.

### Statistical analysis

Genotypic and allelic frequencies were calculated using POPGENE software package (Yeh et al., 1999). The association of SNP genotype with rectal temperature (RT), respiration rate (RR), heat tolerance coefficient and total milk yield (TMY) was analyzed using GLM procedure of SAS. The effect of SNP genotype on physiological parameters was analyzed using the following model:

$$Y_{ijklmn} = \mu + T_i + G_j + G_k + G_l + e_{ijklm}$$

$Y_{ijklmn}$  = nth observation on RR/RT/HTC/TMY of cows in ith THI, jth genotype, kth genotype, lth genotype and mth genotype;  $\mu$  = overall mean;  $T_i$  = effect of ith THI;  $G_j$  = Fixed effect of jth genotype;  $G_k$  = Fixed effect of kth genotype;  $G_l$  = Fixed effect of lth genotype;  $e_{ijklmn}$  = random error associated with  $Y_{ijklmn}$  observation and assumed to be NID (0,  $\sigma^2_e$ ).

## RESULTS AND DISCUSSION

The present study targeted 846 bp of bovine Hsp90ab1 gene from each DNA sample. The first targeted region was found to be monomorphic for allele C at SNP locus

T17871421C and deletion (-) at SNP locus C17871485del. These were not considered for subsequent analysis. The frequencies of genotypes in the population was in accordance with Hardy-Weinberg equilibrium ( $P > 0.05$ ). Allele and genotype frequencies are displayed in Table 2. The calculated allele frequency indicated that allele "T" (89%) was more predominant than mutant allele "C" at T17872112C locus. The frequency of mutated allele "G" (0.94%) was more than the wild type at SNP locus T17872148G.

### Association between the polymorphism of Hsp90ab1 gene with thermo-physiological parameters

Several studies have shown significant association of SNPs with physiological parameters (RR, PCV, RT and HTC) to evaluate the heat tolerance/stress in cattle (Liu et al., 2010; Liu et al., 2011; Charoensook et al., 2012; Sajjanar et al., 2015). Heat tolerance traits such as RR, RT and HTC of Sahiwal cows differed significantly ( $p < 0.01$ ) in all three THI subclasses of different seasons.

**Table 3.** Association of physiological parameters with different genotypic groups of Hsp90ab1 among Sahiwal cows.

Effect	Subclass	RR	RT	HTC
THI	49.7 (winter)	15.74±0.79 <sup>c</sup>	37.30±0.09 <sup>c</sup>	1.66±0.04 <sup>c</sup>
	64.5 (Spring)	18.16±0.79 <sup>b</sup>	38.18±0.09 <sup>b</sup>	1.79±0.04 <sup>b</sup>
	86.44(Summer)	29.82±0.79 <sup>a</sup>	38.81±0.09 <sup>a</sup>	2.31±0.04 <sup>a</sup>
SNP3 (C1787061T)	CC (N=17)	20.93±0.79	38.18±0.09 <sup>a</sup>	1.91±0.04
	CT (N=33)	21.55±0.69	38.01±0.08 <sup>b</sup>	1.93±0.03
(SNP4) T17872112C	TT (N=39)	21.14±0.92	38.09±0.11	1.91±0.04
	TC (N=11)	21.34±0.82	38.09±0.09	1.92±0.04
(SNP5) T17872148G	TG (N=6)	21.27±1.22	38.06±0.15	1.92±0.05
	GG (N=44)	21.21±0.67	38.13±0.08	1.92±0.03

Means within a factor with different superscript differ significantly ( $p < 0.01$ ).

**Table 4.** Association of genotypes with total milk yield among Sahiwal cows.

Effect	Subclass	Total milk production
SNP3 (C1787061T)	CC (N=17)	1669.717±130.780 <sup>b</sup>
	CT (N=33)	2162.339±153.452 <sup>a</sup>
(SNP4) T17872112C	TT (N=39)	1978.173±257.474
	TC (N=11)	1653.883±127.443
(SNP5) T17872148G	TG (N=6)	1910.514±188.212
	GG (N=44)	1921.542±162.317

Means within a factor with different superscript differ significantly ( $p < 0.01$ ).

This observed pattern in RR, RT and HTC revealed that with increase in THI level there was increased in these thermoregulatory responses by all the cows. Using GLM procedure of Statistical Analysis System (SAS), we observed a significant association ( $p < 0.01$ ) of SNP3 at locus *C1787061T* with RT trait in Sahiwal cows. RT (°C) for genotype CC was highest (38.182±0.094<sup>a</sup>) compared to CT (38.010±0.083<sup>b</sup>) in Sahiwal cows. Previous studies reported that allele *T* at SNP locus *T4338C* was associated with lower RT in Thai indigenous, Sahiwal and Frieswal cattle in India (Charoensook et al., 2012; Sajjanar et al., 2015). The present study inferred that cows of genotype CT has the least rectal temperature and are able to maintain their body temperature under stress condition compared to other cows (Table 3). Hence, lower RT may indicate an improved thermotolerance. It is also well described that RT and RR of *Bos taurus* is higher than *Bos indicus* cattle and, as a result *Bos taurus* cattle are more sensitive to heat stress than *Bos indicus* (Mayengbam, 2008; Singh and

Upadhyay, 2009).

#### Association between the polymorphism of Hsp90ab1 gene and TMY

Table 4 shows the genotype effect on the relative milk production traits in 50 randomly selected Sahiwal cows. SNP3 at locus *C1787061T* has a significant association ( $p < 0.01$ ) with TMY trait in Sahiwal cows. TMY for genotype CT was highest (2162.339±153.452<sup>a</sup>) compared to CC (1669.717±130.780<sup>b</sup>) in Sahiwal cows. The better relative thermotolerance of CT genotype in Sahiwal cows in terms of their thermo-physiological detrimental effect of heat stress on milk production. Our results inferred that CT genotype in Sahiwal cows had parameters observed earlier reflected in the less better total milk yield than other two genotypes. However, the findings are different from that of Sajjanar et al., 2015 where, they reported TT genotype animals also had better production parameter in terms of total milk yield in both Sahiwal and Frieswal cows

The heat tolerance is a quantitative trait (Gaughan et al., 2010; Liu et al., 2011). Several studies have been conducted to link between the thermal-stress related phenotypes with genotypes. The ortholog of the mammalian Hsp90 gene, Hsp83 of *Drosophila* was considered for potential quantitative trait loci with important effects on heat stress resistance (Morgan and Mackay, 2006). In sheep, association of genetic variants of Hspaa1 gene with different thermal conditions was observed at position -660 in the 5 flanking region (Marcos-Carcavilla et al., 2010). Single Nucleotide Polymorphism at nucleotide position 2789 within ATP1A1 messenger RNA is found to be associated with heat tolerance traits in dairy cows (Liu et al., 2010; 2011). Similarly, association of Single Nucleotide Polymorphisms

in HSP70A1A gene with thermo-tolerance was observed in Chinese Holstein cattle (Liu et al., 2011).

## Conclusion

The present study indicates that genotype CT at locus C1787061T improved the heat stress tolerance and total milk production in Sahiwal. Therefore, the results may hint association of allele type at this Hsp90ab1 SNP with relative thermal stress tolerance and total milk production. This finding may be an aid to selection and breeding to enhance thermo-tolerance in Sahiwal cows.

## Conflict of interests

The author(s) did not declare any conflict of interest.

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