Animal Biotechnology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/labt20

Identification of SNP in HSP90AB1 and its Association with the Relative Thermotolerance and Milk Production Traits in Indian Dairy Cattle

Basavaraj Sajjanar [a], Rajib Deb [b], Umesh Singh [b], Sushil Kumar [b], Manoj Brahmane [a], Avinash Nirmale [a], Santanu Kumar Bal [a] & P. S. Minhas [a]

[a] School of Atmospheric Stress Management, National Institute of Abiotic Stress Management, Pune, MH, India

[b] Molecular Genetics Laboratory, Animal Genetics and Breeding Section Project Directorate on Cattle, Meerut Cantt, UP, India

Published online: 25 Aug 2014.

To cite this article: Basavaraj Sajjanar, Rajib Deb, Umesh Singh, Sushil Kumar, Manoj Brahmane, Avinash Nirmale, Santanu Kumar Bal & P. S. Minhas (2015) Identification of SNP in HSP90AB1 and its Association with the Relative Thermotolerance and Milk Production Traits in Indian Dairy Cattle, Animal Biotechnology, 26:1, 45-50, DOI: 10.1080/10495398.2014.882846

To link to this article: http://dx.doi.org/10.1080/10495398.2014.882846

Animal Biotechnology, 26:45–50, 2015

ISSN: 1049-5398 print/1532-2378 online
DOI: 10.1080/10495398.2014.882846

Taylor & Francis
Taylor & Francis Group

Identification of SNP in *HSP90AB1* and its Association with the Relative Thermotolerance and Milk Production Traits in Indian Dairy Cattle

Basavaraj Sajjanar,[1] Rajib Deb,[2] Umesh Singh,[2] Sushil Kumar,[2] Manoj Brahmane,[1] Avinash Nirmale,[1] Santanu Kumar Bal,[1] and P. S. Minhas[1]

[1]*School of Atmospheric Stress Management, National Institute of Abiotic Stress Management, Pune, MH, India*
[2]*Molecular Genetics Laboratory, Animal Genetics and Breeding Section Project Directorate on Cattle, Meerut Cantt, UP, India*

Heat shock proteins (Hsp) play crucial role in cellular thermotolerance and heat stress response. In the present work, Allele specific PCR (AS-PCR) was standardized to detect the nucleotide polymorphism within the *HSP90AB1* gene (SNP g.4338T>C) in Indian breeds of dairy cattle. The identified genotypes were associated with relative thermotolerance in terms of physiological parameters and milk production traits. The results of the experiments revealed that the genotype frequency of CC, CT, and TT for Sahiwal were 0.05, 0.78, and 0.17, respectively, and in Frieswal, the frequencies were 0.20, 0.70, and 0.10, respectively. The average rectal temperature (ART) and average respiration rates (ARR) were recorded during peak summer stress and heat tolerance coefficient (HTC) was calculated. The association studies indicated that TT genotypes had significantly ($P < 0.01$) higher HTC and lower ARR values than CT and CC in both the breeds. The TT genotype animals also had better production parameter in terms of total milk yield (TMY) ($P < 0.01$). These findings may partly suggest the role of *HSP90AB1* polymorphisms in the regulation of heat stress response and consequent effect on production traits. Nevertheless, involvement of other regulatory mechanisms cannot be overruled.

Keywords AS-PCR; Frieswal; *HSP90AB1*; HTC; Sahiwal; SNP

The environmental changes due to predicted global warming are likely to become the major threats to the agricultural and livestock production systems (1). These changes will necessarily demand the adaptation of animals to harsher environmental conditions (2, 3). In this context, the study of genetic basis of traits linked to resilience and stress tolerance has great importance. Heat is one major stressor that can impact livestock production. Heat stress affects the efficiency of dairy cattle by means of reduced food intake, growth, milk yield, and lower reproductive success rates (4, 5). One approach to overcome the effect of heat stress on dairy cattle is to exploit the genetic variability underlying relative thermotolerance. Suitable breeding programs can help to achieve animal population that could cope with effects of heat stress (6). The physiological and genetic mechanisms in cattle response to heat stress are documented in previous studies (7–10). Under thermoneutral conditions, being homoeothermic in nature, cattle can maintain their body temperature within the normal range by balancing the metabolic heat production with heat flow from its body to the surrounding environment (11). Hyperthermia is result of high environmental temperature above the comfort zone, high relative humidity, and intense solar radiation, which alone or together makes heat flow from animals less effective (12).

At the cellular level, mammals respond to heat stress by transcriptional activation of a set of proteins known as heat shock proteins (Hsps) (13). In many of the model organisms studied, these proteins have been frequently referred to as Hsp27, Hsp60, Hsp70, and Hsp90 based on their molecular weight (14). Hsp90 exists as two cytoplasmic isoforms; Hsp90α (inducible) and Hsp90β (constitutive) (15). Apart from function of molecular chaperones, the Hsp90 isoforms contributes in several cellular processes including signal transduction, apoptosis (16). It was found that the mammalian Hsp90 ortholog, Hsp83 of drosophila, and its genomic region was a potential quantitative trait loci for heat stress resistance (17, 18). Similarly, Hsp90 in higher animals may play significant role in influencing heat stress response.

Address correspondence to Basavaraj Sajjanar, School of Atmospheric Stress Management, National Institute of Abiotic Stress Management, Pune, MH 413115, India. E-mail: bsajjanar@niam.res.in

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/labt.

Recently novel single nucleotide polymorphisms (SNP) were identified at different positions covering 5664 bp of the bovine heat shock protein 90 kDa alpha (cytosolic), class member 1 (*HSP90AB1*). Among different *HSP90AB1* SNPs identified, SNP03 (g.4338T>C) was found significantly associated with the physiological parameters indicating relative thermotolerance in Thai native cattle (19).

The aim of the present study was to develop an allele specific PCR (AS-PCR) protocol to detect the presence of nucleotide polymorphism within the *HSP90AB1* gene (SNP g.4338T>C) among Sahiwal (*B. indicus*) and Frieswal (*B. indicus* × *B. taurus*) cattle of Indian origin. Furthermore, the polymorphisms were associated with heat tolerance coefficient (HTC), average respiratory rate (ARR), and milk production traits.

MATERIALS AND METHODS

Experimental Animals and Blood Sample Collection

A total of 200 animals including 80 Sahiwal and 120 Frieswal cows, from a Military Farm, Meerut, UP, India were utilized as blood donors. The animals were kept under natural condition in open housing conditions according to animal welfare rules. The experiments were undertaken after due permission from the institute's animal ethics committee. Blood samples were obtained by jugular venepuncture using sodium heparin (10 IU/mL) as an anticoagulant. Immediately after collection, blood samples were stored in a portable refrigerator at 4°C, transported to the laboratory, and stored at −80°C until DNA extraction.

DNA Isolation and AS-PCR for Genotyping

The genomic DNA was extracted from blood using a QIAamp DNA Blood Mini Kit following manufacturer's instructions. A set of two forward primers were designed for allele specific amplification (AS-PCR), one with the C at 3′ end and the other forward primer with T at 3′ end (Acc. No. NW001494158). Based on the earlier reports, a mismatch at 3rd base from 3′ end of both forward primers were included to improve the specificity of the AS-PCR (25). A single reverse primer was included in the procedure (Fig. 1) (Table 1). The PCR was carried out to amplify 560 bp fragment from a starting template of approximately 50 ng of genomic DNA in a final reaction volume of 25 µL containing 1X Taq DNA polymerase buffer (Sigma), 1.5 mM MgCl2 (Sigma), 200 µM dNTPs (Sigma), 0.5 µM of each primer and 1U Taq polymerase (Sigma). PCR conditions were: initial denaturation at 94°C for 5 minutes; followed by 5 cycles of 94°C for 30 seconds, 66°C for 30 seconds, and 72°C for 30 seconds; thereafter 30 cycles of 94°C for 30 seconds, 64°C for 30 seconds and 72°C for 30 seconds and a final extension 72°C for 5 minutes. PCR products were visualized in 1.0% agarose gels. The amplicon was sequenced using automated DNA sequencer by Sanger's dideoxy chain termination method. PCR was carried out in two sets of reaction: one set with primer 1 and 3 and another set with primer 2 and 3 (Fig. 2). Gene (allele) and genotype frequencies were calculated as per Falconer and Mackay (20).

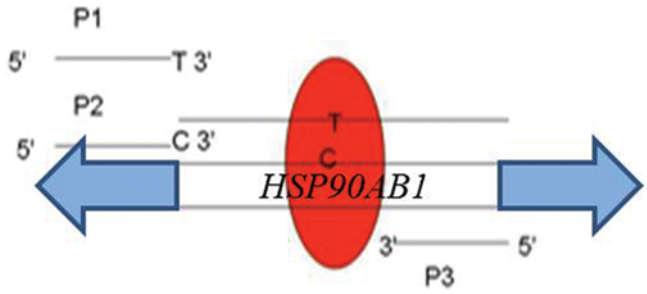

FIG. 1. Allele specific PCR strategy for SNP (4338T>C) within HSP90AB1. P1: Forward primer (T at 3′ end), P2: Forward primer (C at 3′ end), P3: Reverse primer (Common for both). The highlighted region indicates nucleotide changes at position of SNP.

Recording Physiological Parameters

Rectal temperatures (RT) were measured for the genotyped animals in the morning (7.00 a.m.) and in the afternoon (4.00 p.m.) using digital clinical thermometer and taking care that the thermometer bulb was in close contact with mucous membrane. The respiration rates were recorded by observing the flank movements from a distance before recording rectal temperature to avoid any disturbance to the animals. The measurements were performed during summer consecutively for 3 weeks when temperatures rise more than normal. The outdoor temperature and the relative humidity (RH) (%) were recorded daily during the experiment. Previously, based on heat tolerance experiments, Rhoad (21) developed the following formula to calculate an individual's heat tolerance coefficient (HTC):

$$HTC = 100 - 10(ART - 38.3)$$

where HTC is the heat tolerance coefficient, ART is the average rectal temperature, 38.3°C is the physiological

TABLE 1
Set of allele specific primer designed for the present study

Sl. No.	Primer name	Sequence
1.	HSP90 (C) Forward	5′CTGGAGTCACACTGAG**G**AAC 3′
2.	HSP90 (T) Forward	5′CTGGAGTCACACTGAG**G**AAT 3′
3.	HSP90 Reverse	5′TGTTGGAGATCGTCACCTG 3′

Bold letter indicate mismatch at third position from 3′ end.

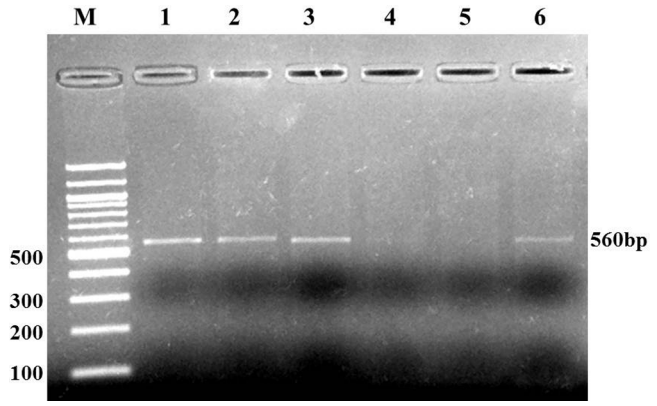

FIG. 2. Allele specific PCR based genotypic analysis of HSP90AB1 (SNP:4338T>C). Legend: M: 100 bp ladder, 1&2: CT genotype, 3&4: CC genotype, 5&6: TT genotype.

bovine body temperature, 10 is a correction factor to convert deviations in body temperature to a unit basis and 100 is the perfect efficiency in maintaining temperature at 38.3°C.

Recording Milk Production Data

Data for milk production traits, namely, total milk yield and fat% were obtained from the data sheets of the farm records. Milk records from the entire lactation length of each animal were utilized in the present study.

Statistical Analysis

Data are presented as mean ± SEM and analyzed by using SPSS statistical program (SPSS 10.0 for Windows; SPSS, Inc., Chicago, IL, USA). Significant differences were determined by one-way analysis of variance (ANOVA) using the SPSS program. Data pertinent to physiological parameters (rectal temperature, respiration rate, and heat tolerance coefficient) as well as milk production traits (total milk yield and fat %) of different genotypes were considered. The parameters were subjected to ANOVA using the general linear model (GLM) applying SPSS (Statistical Package for Social 89 Sciences) according to the following statistical model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

where Y_{ij} is the analyzed trait of each cow; μ is the overall mean; G_i is the fixed effect of the ith genotype; and e_{ij} is the random error.

RESULTS

Detection of SNP Using AS-PCR

AS-PCR detected the nucleotide polymorphism (SNP g.4338T>C) in *HSP90AB1* gene among Frieswal and Sahiwal cattle breeds. In heterozygous genotypes (CT) both the forward primers along with the common reverse primer amplified the 562 bp HSP90AB gene fragment. In homozygous genotypes (CC & TT) either one of the forward primer along with the reverse primer amplified the same gene fragment (Fig. 2). The nucleotide sequencing of the purified amplicon had also confirmed the utility of AS-PCR in identifying the genotypes at this SNP position. The standardized protocol of AS-PCR was used for genotyping 200 dairy animals. The results revealed that the genotype frequencies of CC, CT, and TT for Sahiwal were 0.05 (n = 4), 0.78 (n = 62), and 0.17 (n = 14), respectively, and in Frieswal, the frequencies were 0.20 (n = 24), 0.70 (n = 80), and 0.10 (n = 16), respectively. The calculated allele frequency indicated that the T allele was more predominant than C allele in indigenous breed Sahiwal. The opposite was true in the cross breed Frieswal (Table 2). The tested population was not in Hardy-Weinberg equilibrium when observed genotypes values were tested against expected values using Chi-square test.

Association of Physiological Parameters with *HSP90AB1* SNP

Table 3 shows the results of the association studies performed for the identified genotypes with HTC. It was found that HTC of TT genotype are significantly ($P < 0.01$) higher than CT and CC in both Sahiwal and Frieswal breeds. Furthermore, the TT genotype animals had significantly lower ARR ($P < 0.01$) compared to the CT and CC genotypes in both the breeds (Table 3). Taken together, the animals (TT) with the higher HTC and lower ARR indicate better physiological response to summer heat stress compared to those of lower HTC and higher ARR (CC and CT).

TABLE 2
Gene and genotype frequency

	Genotype frequency			Allele frequency	
Breeds	CC	CT	TT	C	T
Frieswal (n = 120)	0.20 (n = 24)	0.70 (n = 80)	0.10 (n = 16)	0.54	0.46
Sahiwal (n = 80)	0.05 (n = 4)	0.78 (n = 62)	0.17 (n = 14)	0.44	0.56

TABLE 3
Association of physiological parameters with different genotypic groups of *HSP90AB1* among Sahiwal and Frieswal cows

Breeds	Genotypes	ART(°C) ± SEM	HTC ± SEM	ARR(times/ min) ± SEM
Sahiwal	CC	39.29 ± 0.34^{a}	90.12 ± 0.43^{a}	33.54 ± 0.21^{e}
	CT	39.10 ± 0.31^{a}	92.11 ± 0.48^{a}	33.37 ± 0.25^{e}
	TT	38.71 ± 0.36^{b}	95.93 ± 0.46^{b}	32.16 ± 0.29^{f}
Frieswal	CC	39.61 ± 0.38^{c}	86.93 ± 0.47^{c}	34.41 ± 0.26^{g}
	CT	39.22 ± 0.33^{c}	90.89 ± 0.43^{d}	34.21 ± 0.24^{g}
	TT	38.96 ± 0.34^{d}	93.41 ± 0.42^{d}	33.28 ± 0.29^{h}

Means with different lower superscript letters indicate significant difference at $P < 0.01$.

TABLE 4
Determination of the relationships between genotypes and its association with total milk yield and fat percentage among Frieswal and Sahiwal cows

Breed	Genotypes	Total milk yield (kg) ± SEM	Milk fat (%) ± SEM
Frieswal	CC	$2630.21 \pm 219.28_{a}$	$4.26 \pm 0.22_{ns}$
	CT	$2662.00 \pm 186.01_{a}$	$4.46 \pm 0.27_{ns}$
	TT	$2783.87 \pm 187.94_{b}$	$4.41 \pm 0.29_{ns}$
Sahiwal	CC	$2113.27 \pm 227.23_{c}$	$4.13 \pm 0.23_{ns}$
	CT	$2365.21 \pm 197.92_{c}$	$4.11 \pm 0.27_{ns}$
	TT	$2687.82 \pm 194.98_{d}$	$4.17 \pm 0.25_{ns}$

Note: Different lower case subscripts letters of the least squares mean indicate significant difference at $p < 0.01$; ns: non-significant.

Association of Milk Production Traits with *HSP90AB1* SNP

Table 4 shows the genotype effect on the relative milk production traits in 200 randomly selected cows. TT genotypes show significantly higher levels of total milk yield ($P < 0.01$) than CC and CT genotypes. Differences were also observed in milk fat% between different genotypes but were not statistically significant. The better relative thermotolerance of animals (TT) in terms of their physiological parameters observed earlier reflected in the less detrimental effect of heat stress on milk production.

DISCUSSION

Our experiments have shown that AS-PCR can successfully be used to genotype the previously reported SNP in *HSP90AB1* (19). The need for detection of SNP in large samples led to development of several methods (22). Though these protocols are highly efficient but involve expensive molecular probes, microchips, and instruments. In contrast, AS-PCR is conceptually simple SNP genotyping strategy that introduces mismatch at 3′ end of forward primer that renders Taq polymerase unable to extend the annealed primer (23). The benefit of AS-PCR is that it combines amplification with detection events without involvement of additional probes or enzymes making it most cost effective to identify nucleotide polymorphism (24). In order to overcome the false positive results of AS-PCR, various strategies had been tried including addition of artificial mismatches within the three bases from 3′ end of the primers (25). Based on these reports, the mismatch at third base from the 3′ end of the forward primer was included in the present experiments. This modification has improved the specificity and reliability of detecting genotype for the *HSP90AB1* SNP (g.4338T>C). The SNP g.4338T>C lies within the intron 9 of *HSP90AB1*, even though it does not code for an amino acid of *HSP90AB1* but may be involved in other regulatory mechanisms including the post-transcriptional modifications, alternative splicing, and generation of transcription variants.

Our results indicate that 'T' allele was more common in indigenous breed, Sahiwal compared to the cross breed, Frieswal (HF × Sahiwal). The proportion of 'C' allele in Frieswal was more than 50% in genotyped animals. Earlier Charoensook et al. (19) found that HF also had significantly higher percentage of 'C' allele than the indigenous Thai native cattle at this position, SNP03 (g.4338T>C). The explanation for similar results lies in the fact that Frieswal is a cross of HF and Sahiwal with 62.5% and 37.5% blood, respectively. Even though it is well known that the allele frequency of specific SNP is not repeatable in the different population of the same breed or different breeds but the pattern can be observed for the presence of higher frequency of particular allele type in native tropical breeds (*B. indicus*) compared to European breed (*B. taurus*). These results may hint association of allele type at this *HSP90AB1* SNP with relative thermal stress tolerance, as it is observed that compared to *B. taurus, B. indicus* is generally better adapted to heat stress and detrimental effects of heat stress on production traits are lesser in *B. indicus* (12, 26). Earlier heat tolerance experiments led to the development of HTC (21) and the same was used as

one of the important parameter to evaluate the heat stress tolerance of cattle (27). In the present study, HTC of TT of genotypes at SNP g.4338T>C were significantly ($P < 0.01$) higher than CT and CC for both Sahiwal and Frieswal breeds. Furthermore, the 'T' allele at SNP g.4338T>C consistently had low ARR compared with 'C' allele which indicates these animals comparatively had better heat stress response. Increased respiration rate (RR) is an important indicator of heat stress response that aids in heat dissipation (8). Hence, lower RR may indicate an improved thermotolerance. These findings are in accordance with the earlier results obtained for the same genotypes in Thai native cattle (19).

Heat stress affects milk production by its cumulative effect on the feed intake, metabolism, and physiology of dairy cattle (28). Our results indicated that TT genotype animals had better total milk yield than other two genotypes in both the breeds. The animals considered in the present study were in different parity. However, the term parity of the animals was found statistically nonsignificant in terms of total milk production and fat%. Even though the precise mechanism by which the polymorphism described herein affects milk production was not studied, better physiological responses during summer stress (HTC & RR) indicate the ability of animals to overcome the detrimental effects on milk production. The heat stress tolerance is considered as quantitative trait (QT) (26) but at present precise QTL for the heat stress tolerance has not been mapped in bovines. Efforts were done to link between the thermal-stress related phenotypes with genotypes. For example, SNP in ATP1A gene (position 2789) and HSP70.1 (Position, 895) were associated with the heat tolerance trait in dairy cattle (27, 29). Similarly SNP in the 5′ flanking region of *HSP90AA1* was associated with different thermal conditions in ovine species (18). Our present study indicates the 'T' allele at the *HSP90AB1* (SNP g.4338T>C) improved the heat stress tolerance and total milk production. Even though the aforesaid SNP were not responsible for the physiological responses, the same can be utilized as a genetic marker to select appropriate breeds for hot climatic conditions.

DISCLAIMER

Basavaraj Sajjanar and Rajib Deb contributed equally to this work. The authors declared that they do not have any conflict of interest for the present study.

ACKNOWLEDGMENTS

The authors are thankful to the Director, NAISM, Baramati and PDC, Meerut for providing all the necessary facilities to conduct the present work. Authors are also thankful to the In-charge Military Farm for providing the experimental animals.

REFERENCES

1. Adams RM, Hurd BH, Lenhart S, Leary N. Effects of global climate change on agriculture: An interpretative review. Clim Res 1998; 11:19–30.
2. Smith B, Skinner MW. Adaptation options in agriculture to climate change: A typology. Mitig Adapt Strateg Glob Change 2002; 7:85–114.
3. Seo SN, Mendelsohn R. An analysis of livestock choice: Adapting to climate change in Latin American farms. World Bank Policy Res Work Paper 2007; 4156:18.
4. Bernabucci U, Bani P, Ronchi B, Lacetera N, Nardone A. Influence of short- and long-term exposure to a hot environment on rumen passage rate and diet digestibility by Friesian heifers. J Dairy Sci 1999; 82:967–973.
5. Hahn GL. Dynamic responses of cattle to thermal heat loads. J Anim Sci 1999; 77:10–20.
6. Hoffmann I. Climate change and characterization, breeding and conservation of animal genetic resources. Anim Gen 2010; 41:32–46.
7. Hansen PJ. Physiological and cellular adaptations of zebu cattle to thermal stress. Anim Reprod Sci 2004; 74:295–303.
8. Beatty DT, Barnes A, Taylor E, Pethick D, McCarthy M, Maloney SK. Physiological responses of *B. taurus* and *B indicus* cattle to prolonged, continuous heat and humidity. J Anim Sci 2006; 84:972–985.
9. Collier RJ, Collier JL, Rhoads RP, Baumgard LH. Invited review: genes involved in the bovine heat stress response. J Dairy Sci 2008; 9:1445–1454.
10. Aguilar I, Misztal I, Tsuruta S. Genetic components of heat stress for dairy cattle with multiple locations. J Dairy Sci 2009; 92:5702–5711.
11. Bernabuccia1 U, Lacetera N, Baumgarda LH, Rhoads RP, Ronchi B, Nardone B. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal 2009; 4:1167–1183.
12. Mader TL, Davis MS, Brown-Brandl T. Environmental factors influencing heat stress in feedlot cattle. J Anim Sci 2006; 84:712–719.
13. Kregel KC. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol 2022; 922:177–2186.
14. Kampinga HH, Hageman J, Vos MJ, et al. Guidelines for the nomenclature of the human heat shock proteins. Cell Stress Chaperones 2009; 14(1):105–111.
15. Chen B, Zhong D, Monteiro A. Comparative genomics and evolution of the HSP90 family of genes across all kingdoms of organisms. BMC Genom 2006; 7:156.
16. Csermely P, Schnaider T, Soti C, Prohaszka Z, Nardai G. The 90-kDa molecular chaperone family: structure, function, andclinical applications. A comprehensive review Pharmacol Therap 1998; 79:129–168.
17. Morgan TJ, Mackay TF. Quantitative trait loci for thermotolerance phenotypes in Drosophila melanogaster. Heridity 2006; 96:232–242.
18. Marcos-Carcavilla A, Mutikainen M, González C, et al. A SNP in the HSP90AA1 gene 5′ flanking region is associated with the adaptation to differential thermal conditions in the ovine species. Cell Stress Chaperones 2010; 15:67–81.

19. Charoensook R, Gatphayak K, Sharifi, AR, Chaisongkram C, Brenig B, Knorr C. Polymorphisms in the bovine *HSP90AB1* gene are associated with heat tolerance in Thai indigenous cattle. Trop Anim Health Prod 2012; 44:921–928.
20. Falconer DS, Mackay TFC. *Introduction to Quantitative Genetics*, 4th edn. Longman Group Ltd, Essex, UK; 1996.
21. Rhoad AO. The Iberia heat tolerance test for cattle. Trop Agr (Trinidad) 1944; 21:162.
22. Tsuchihashi Z, Dracopoli NC. Progress in high throughput SNP genotyping methods. Pharmacogenom J 2002; 2:103–110.
23. Bottema CD, Sommer SS. PCR amplification of specific alleles: rapid detection of known mutations and polymorphisms. Mutat Res 1993; 288:93–102.
24. Bui M, Liu Z. Simple allele-discriminating PCR for cost-effective and rapid genotyping and mapping. Plant Method 2009; 5:1.
25. Drenkard E, Richter BG, Rozen S, et al. A simple procedure for the analysis of single nucleotide polymorphisms facilitates map-based cloning in Arabidopsis. Plant Physiol 2002; 124:1483–1492.
26. Gaughan JB, Mader TL, Holt SM, Sullivan ML, Hahn GL. Assessing the heat tolerance of 17 beef cattle genotypes. Int J Biometerol 2010; 54:617–627.
27. Liu Y, Li D, Li H, Zhou X, Wang G. A novel SNP of the ATP1A1 gene is associated with heat tolerance traits in dairy cows. Molecul Biol Rep 2011; 38:83–88.
28. Rhoads ML, Rhoads RP, VanBaale MJ, et al. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. J Dairy Sci 2009; 92(5):1986–1997.
29. Deb R, Sajjanar B, Singh U, et al. Promoter variants at AP2 box region of Hsp70.1 affect thermal stress response and milk production traits in Frieswal cross bred cattle. Gene 2013; 09:37.