RESEARCH ARTICLE

Study of the Heat Shock Protein 70 Polymorphism on Thermotolerance in Kankrej Cattle

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Abstract

Heat shock protein (HSP) 70 is a key player in the development of thermotolerance among the several members of the HSP superfamily, which function as a molecular chaperon by attaching to denaturing proteins and stabilising them while maintaining their activity. The current study intended to screen the HSP70 gene in cattle of the Kankrej breed for polymorphisms and potential variations in thermotolerance. A 295 bp fragment of HSP70 gene was subjected to PCR - Single Strand Conformation Polymorphism (SSCP) in 100 Kankrej cattle. Association of thermolerance traits, *viz.,* rectal temperature, respiration rate and pulse rate with identified genotypes were analyzed for different seasons. Three SSCP patterns, *viz.,* AA, AB and BB and consequently two alleles namely A and B were documented in fragment of HSP70 gene. Comparative association of identified genotypes with different heat tolerance traits were found non-significant. However, it is anticipated that the polymorphism at HSP70 may be a strong predictor of cow heat tolerance, which could help with thermotolerance selection screening large number of samples and future genetic improvement in breeding.

Key words: HSP 70 polymorphism, Kankrej cattle, SSCP, Thermo-tolerance traits.

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Introduction

India is a large country that includes several agroecological zones with a variety of climates, natural vegetation resources and topographies, harboring nearly all domesticated animal species and crops with various production systems. The summer time highs in a tropical nation like India reach between 40 and 48°C, which is clearly uncomfortable and stressful for milch animals. Stress is the body's response to stimuli that upset homeostasis. This can have negative consequences, such as failure to develop traits to the fullest extent possible genetically, which can have an adverse effect on an animal's growth (Tao *et al*., 2012), reproduction (Avendaño-Reyes *et al*., 2010), lactation (Bernabucci *et al*., 2014) and maintaining its immune system (Khansari *et al*., 1990).

HSP 70 is a crucial molecular chaperon that is essential to every mammalian cell. Substantial studies show that HSP 70 functions as a molecular chaperon and protects the cell from heat shock, which may damage cells and denaturize proteins. Once specific genes responsible for thermotolerance in zebu have been identified or mapped, breeding strategies such as marker assisted selection can be applied to further the exploitation of the zebu genotype for cattle production systems (Hansen, 2004). Keeping above aspects in view, the present study was focused on Kankrej (zebu) cattle in the tropical to subtropical region of North Gujarat, to explore the genetic variability (genetic polymorphism) with association of thermotolerance traits, *viz.,* rectal temperature, respiration rate and pulse rate during different season.

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Materials and Methods

Experimental Animals

A total of one hundred un-related adult cattle of Kankrej breed were selected from the Livestock Research Station, SDAU, Dantiwada and nearby areas of Banaskantha district of North Gujarat (India). Rectal temperatures (RT), respiration rate (RR) and pulse rate (PR) of each animal were recorded in each season, *i.e.,* winter, spring and summer.

Genotyping of Animals

Blood samples (5 mL) were collected by jugular vein puncture from all experimental animals asceptically in

vacutainers containing K_3EDTA as an anti-coagulant and stored at -20°C till further use. Genomic DNA was isolated from blood as per standard phenol: chloroform extraction protocol described by John *et al*. (1991) with required modification.

PCR Amplification

Published forward and reverse primers used for amplification of HSP70 gene length of 295 bp (fragment I) at initial coding region for identification of various allele variants present in Kankrej cattle were 5'AAACATGGCTATCGGCATCGACCT3'and 5'AGGCTTGTCT CCGTCGTTGATGA3', respectively (Bhat *et al.,* 2016).

PCR was carried out in a final reaction volume of 20 µL using 2X PCR Master mix (Emerald, TaKara) containing 0.05 U/µL Taq DNA polymerase in reaction buffer ($MgCl_2$ 4 mM, dNTPS 0.4 mM). A master mix was prepared and aliquoted 17 µL in each of PCR tube. Three µL sample DNA was added in each to make the final volume of 20 µL. All the reactions were carried in thermal cycler (Ependorf) and subjected to PCR with 5 min of initial denaturation at 95°C followed by 30 cycles of 60 s denaturation at 95°C, 45 s annealing at 65°C for Fragment I, 60 s extension at 72°C and 10 min of final extension at 72°C.

SSCP (Single Strand Conformational Polymorphism)

Polymorphism was screened using PCR-single strand conformation polymorphism (SSCP) technique. About 10 µL of PCR product was taken with 10 µL formamide solution and mixed properly followed by denaturation at 95°C for 10 min then snap chilling on ice for 15 min. The mixture was loaded on 8% polyacrylamide gel (acrylamide/bis-acrylamide 29:1, w/w) and electrophoresis was carried out using ×0.5 tris-borate EDTA (45 mM tris-borate/1 mM EDTA) at 150 V for 5 h. Distinct band patterns were detected by silver staining of the SSCP product (Bassam *et al*., 1991). Each animal showing different conformation banding pattern was assigned a specific genotype. The frequency of HSP 70 genotypes and their allelic frequencies were estimated by standard procedure.

Association of Genotypes with Thermotolerance Traits

Association study between thermotolerance traits with the HSP 70 genotypes was analyzed by the univariate general linear model of SPSS v 26 according to the following statistical model:

$$Yijkl = \mu + Gi + BFk + \varepsilon ik$$

Where, Yijkl= Observation for rectal temperature, respiration rate and pulse rate, µ= Overall mean for each trait, Gi= Fixed effect of Genotype, BFk= Fixed effects of breed, and εik= Random environment effect.

Results and Discussion

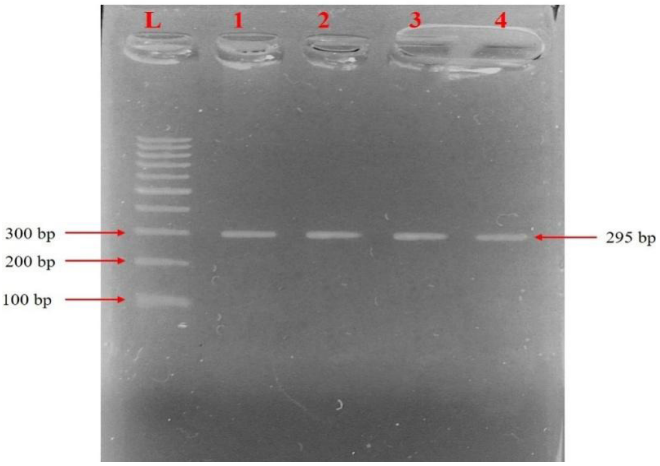

Fig. 1: Amplified HSP 70 fragment I (L: 100 bp ladder, 1-4 sample)

Amplification of 295 bp fragment I was done and horizontal submarine 2 % agarose gel electrophoresis was used to check the amplification (Fig. 1).

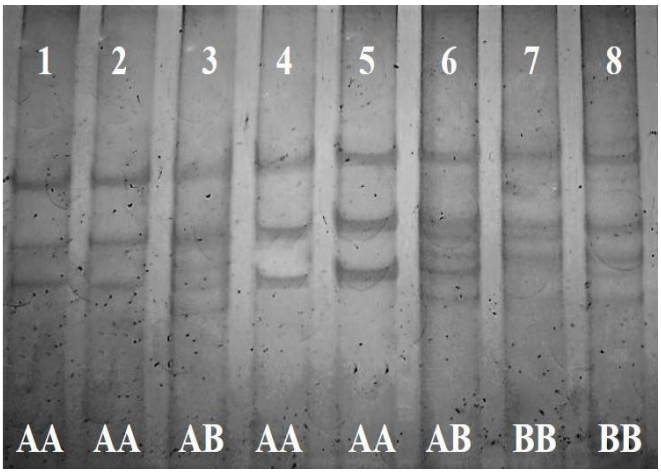

Fig. 2: SSCP genotype of fragment I - HSP70 gene in Kankrej cattle

Following SSCP technique, three genotypes were observed for this fragment of HSP 70 gene and arbitrarily assigned as AA, AB and BB genotypes (Fig. 2). It was found that out of total 100 Kankrej cattle studied, 31 animals had AA genotype, 33 had AB genotype and rest 36 animals had BB genotype. Gene frequencies for A and B alleles were 0.475 and 0.525, while genotypic frequencies of AA, AB and BB genotypes were 0.31, 0.33 and 0.36, respectively (Table 1). This finding indicated that the Kankrej cattle in this herd were found polymorphic for HSP 70 gene.

Similar to present findings, Bhat *et al*. (2016) also demonstrated three genotypes AA, AB and BB genotypes with frequencies of 0.328, 0.359 and 0.312, respectively, and allelic frequencies for the A and B alleles as 0.5078 and 0.4922 for Fragment I (295 bp) of the HSP70 gene in Tharparkar cattle. Cai *et al*. (2005) also examined the polymorphism at the 5'-flanking region of the cow HSP70 gene with polymorphic

Table 1: Genotype and allelic frequencies of fragment I of HSP 70 gene in Kankrej cattle

Locus	Genotype	Observed no. of Genotype	Expected no. of Genotype	Genotypic frequency	Gene frequency	
					A	B
HSP 70 (Fragment I)	AA	31	23.04	0.31	0.475	0.525
	AB	33	49.92	0.33		
	BB	36	27.04	0.36		

four different genotypes AA, BB, AB and AC using PCR-SSCP. The frequencies of different genotypes were 58.9%, 11.1%, 7.78% and 22.22% respectively. The frequencies of alleles A, B and C were 0.739, 0.150 and 0.111 respectively. Adamowicz *et al.* (2005) used the MBOII restriction enzyme to perform a restriction study on a 253 bp fragment of the 3'UTR region of the HSP70.1 gene and found three genotypes: GG, GT, and TT. They opined that both *Bos taurus* and *Bos indicus* have the highest frequency of the G allele. They also reported that digestion of the same product with AluI resulted in AA, AG and GG genotype indicating the presence of HSP 70.1 gene diversity in these two sub-species of cattle.

Thermo-Humidity Index:

During the study period, THI was found to be highest in summer and lowest in winter season (Table 2). Heat stress levels are frequently classified into normal, alert, danger and emergency categories using the Livestock Weather Safety Index (LCI, 1970) as a standard. In this index, THI ≤74 is classified as normal, 75-78 as alert and 79-83 as danger and THI ≥84 as emergency. Therefore, based on this indicator, cattle under study raised throughout the summer may fall into the danger category which indicates that the animals were under stress.

Table 2: Ambient temperature and thermo-humidity index (THI)

Season	Max Temp	Min Temp	RH	THI
Winter	21.9	7.0	56	58.07
Spring	27.6	8.8	49	62.86
Summer	43.1	24	53	83.43

Effect of Season on Rectal Temperature, Respiration Rate and Pulse Rate:

The effect of different seasons, *viz.,* spring, summer and winter on rectal temperature, respiration rate and pulse rate of Kankrej cattle measured during experimental period are presented in Table 3.

Statistical analysis of data revealed that season had significant effect ($p<0.05$) on both morning and evening rectal temperature, respiration rate and pulse rate of Kankrej cattle, the values for all being maximum in summer and minimum in winter season (Table 3). These results of the current study agreed with those of Singh *et al.* (2014), where they also observed a substantial seasonal influence on the rectal temperature of Murrah buffalo. Similarly, Bhat *et al.* (2016) observed a considerable rise in rectal temperature and respiration rate in the morning and evening during the summer in Tharparkar cattle. Kumar (2019) also found significant effect of seasons (winter, spring and summer) on both morning and evening respiration rate and pulse rate, though increase in rectal temperature was non-significant in summer in Murrah buffalo. When animals are exposed to ambient temperatures over the thermoneutral zone, their initial response is an increase in respiration rate. Das *et al.* (1999) reported that when Murrah buffalo calves were exposed to direct sun light for six hours in the month of June showed an increase in respiratory rate. Rao *et al.* (2014) also investigated positive association in thermotolerance parameters in Tarai buffaloes.

Association of HSP 70 Genotypes with Thermotolerance Traits during Different Season:

Effect of genotypes on physiological parameters like rectal temperature, respiration rate and pulse rate of Kankrej cattle in winter, spring and summer season were measured. The findings are presented in Table 4. The data clearly show that genotypes did not significantly affect the rectal temperature, respiration rate and pulse rate of Kankrej cattle in the morning or the evening in any of the seasons of the year including overall mean values.

Apparently in winter, AB genotype had highest rectal temp (100.82±0.13), AA genotype had highest respiration rate (28.55±0.13) and AB genotype had highest pulse rate (57.67±0.08) in evening hours. In spring, AB genotype had

Table 3: Rectal temperature, respiration rate and pulse rate

Season	Rectal temperature (°F)		Respiration rate (per minute)		Pulse rate (per minute)	
	Morning	Evening	Morning	Evening	Morning	Evening
Winter	99.48±0.52^{c}	100.69±0.78^{c}	23.75±0.07^{c}	28.48±0.07^{c}	52.89±0.07^{c}	57.52±0.05^{c}
Spring	100.74±0.01^{b}	101.55±0.13^{b}	24.60±0.05^{b}	29.35±0.07^{b}	55.19±0.07^{b}	60.57±0.05^{b}
Summer	101.48±0.26^{a}	102.63±0.02^{a}	28.90±0.89^{a}	34.69±0.07^{a}	61.60±0.05^{a}	67.52±0.05^{a}

Mean ± SEs with different superscript within the column differ significantly ($p<0.05$).

Table 4: Effect of genotype on thermo-tolerance traits in all season

Season	Physiological parameter	Time of recording	Genotype		
			AA (n=31)	AB (n=33)	BB (n=36)
Winter	Rectal temperature (°F)	Morning	99.50±0.09	99.45±0.09	99.49±0.08
		Evening	100.65±0.14	100.82±0.13	100.63±0.13
	Respiration rate (breaths/min)	Morning	23.64±0.13	23.67±0.12	23.92±0.12
		Evening	28.55±0.13	28.36±0.13	28.53±0.12
	Pulse rate (beats/min)	Morning	53.00±0.13	52.97±0.13	52.72±0.12
		Evening	57.48±0.08	57.67±0.08	57.42±0.08
Spring	Rectal temperature (°F)	Morning	100.76±0.03	100.72±0.03	100.76±0.03
		Evening	101.54±0.02	101.57±0.02	101.55±0.02
	Respiration rate (breaths/min)	Morning	24.65±0.10	24.58±0.09	24.59±0.09
		Evening	29.23±0.12	29.27±0.124	29.53±0.11
	Pulse rate (beats/min)	Morning	55.13±0.14	55.30±0.13	55.14±0.13
		Evening	60.52±0.09	60.61±0.08	60.58±0.08
Summer	Rectal temperature (°F)	Morning	101.45±0.04	101.40±0.04	101.55±0.04
		Evening	102.56±0.04	102.67±0.03	102.67±0.03
	Respiration rate (breaths/min)	Morning	28.77±0.16	28.82±0.15	29.08±0.14
		Evening	34.65±0.13	34.64±0.12	34.78±0.12
	Pulse rate (beats/min)	Morning	61.52±0.10	61.52±0.10	61.75±0.09
		Evening	67.42±0.08	67.45±0.08	67.67±0.08
Overall	Rectal temperature (°F)	Morning	100.57±0.09	100.53±0.09	100.59±0.08
		Evening	101.58±0.09	101.68±0.09	101.62±0.08
	Respiration rate (breaths/min)	Morning	25.69±0.24	25.69±0.23	25.86±0.22
		Evening	30.81±0.29	30.76±0.28	30.94±0.27
	Pulse rate (beats/min)	Morning	56.55±0.31	56.60±0.37	56.55±0.36
		Evening	61.81±0.43	61.91±0.42	61.89±0.40

highest rectal temp (101.57±0.02), BB genotype had highest respiration rate (29.53±0.11) while AB genotype had highest pulse rate (60.61±0.08) in evening. In summer, AB and BB genotypes had highest rectal temp (102.67±0.03), BB genotype had highest respiration rate (34.78±0.12) as well as highest pulse rate (67.67±0.08) in evening hours. Overall, animals having AB genotype had highest rectal temperature (101.68±0.09) than AA (101.58±0.09) and BB (101.62±0.08) in evening, whereas BB genotype had highest respiration rate (30.94±0.27) than AA (30.81±0.29) and AB (30.76±0.28). In case of pulse rate AB genotype had highest value (61.91±0.42) than AA (61.81±0.43) and BB (61.89±0.40) (Table 4).

Conclusion

The findings of the present study in Kankrej cows to identify polymorphism in 295 bp fragment of HSP 70 gene and its association with thermotolerance traits, revealed that the genotypes (AA, AB and BB) did not influence the thermotolerance traits in this breed. Although, more focus on association studies with thermotolerance traits may be useful to develop strategies to implement marker-assisted selection for adaptability traits in breeding programs of cattle in future. Moreover, the research can be expanded to create animals that are resilient to climate change and reduce the negative impacts of climate stress.

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