

Research paper

Contrasting pattern of allelic variation in heat shock factor-1 (HSF-1), a major candidate gene regulating heat stress response across Indian native (*Bos indicus*), crossbred and exotic (*Bos taurus*) cattle

Amandeep Jast, Monika Sodhi, Rakesh Ranjan, Preeti Verma, Nampher Mashing, Prince Vivek, Manish Tiwari, Parvesh Kumari, and Manishi Mukesh*

ICAR-National Bureau of Animal genetic Resources, Karnal-132001 (Haryana) India

ABSTRACT

The aim of this study was to assess the frequency distribution of selected SNPs in heat shock factor-1 (*HSF-1*) gene across indigenous, exotic and crossbred cattle. For this study, a total of 1594 genomic DNA samples of 15 Indian native cattle breeds; 2 exotic (Holstein Friesian and Jersey); and 1 crossbred cattle (Karan Fries) were screened for 2 SNPs at locus 909 in intron 3 and locus 4693 in 3'-UTR. The digestion of 129bp product corresponding to 909 locus revealed 3 different genotypes CC (129bp), TC (129bp, 88bp and 41bp) and TT (88bp and 41bp). The allele C was predominant in all the native cattle with frequency of 0.935, whereas the frequency of allele C in Holstein Friesian (HF) and Karan Fries (KF) was 0.463 and 0.503 respectively. The restriction digestion of 287bp amplicon corresponding to 4693 locus revealed 3 different genotypes GG (178bp, 61bp, 41bp and 7bp), GT (178bp, 68bp, 61bp, 41bp and 7bp) and TT (178bp, 68bp and 41bp). The allele T was predominant in all indigenous cattle and Jersey with frequency of 0.744 and 0.643, respectively. However, in HF and KF cattle, allele G was predominant with frequency 0.563 and 0.545, respectively. The data suggested sequence variability in *HSF-1* gene and such genetic variation might be playing crucial role during heat stress response in different cattle populations.

Keywords: Heat shock factor-1 gene, heat stress, single nucleotide polymorphism, Indian native cattle breed.

***Corresponding author:** mmukesh26@gmail.com

INTRODUCTION

Thermo-tolerance is the ability of animals to regulate body temperature by balancing heat gain and heat loss efficiently (Paula-Lopes et al., 2013; Sunil et al., 2011). The optimal performance of livestock species occurs in the thermo neutral zone (TNZ) which is the ambient environmental temperature and it decreases at temperature above this range due to thermal stress in animals (Roefeldt, 1998). The TNZ of the dairy animal's ranges from 16 to 25°C, within which they maintain a physiological body temperature of 38.4 to 39.1°C. Indian native cattle, like other tropical cattle, are more tolerant to increased atmospheric temperature than those from temperate regions (Mwai et al., 2015; Paula-Lopes et al., 2013). The cellular response to thermal stress in mammals is controlled at the transcription level and is mediated by a family of heat shock transcription factors (HSFs), which regulate the inducible expression of HSF genes by acting as transcriptional activators. The coordinated effort of multiple HSFs (HSF-1, HSF2, HSF3 and HSF4) provides chaperonic coverage to the cellular activities and protects the unfolded proteins. Among the different isoforms, HSF-1 holds importance due to its pivotal role in cellular homeostasis. It is the major regulator of heat shock protein transcription. It is activated by the hydrophobic domain of the unfolded

proteins during heat stress, undergoes trimerization and is translocated to the cell nucleus. After activation, it binds with the heat stress elements (HSEs) present in the promoter region leading to enhanced transcription of HSP mRNA. HSF-1 regulates cellular response to oxidative stress, hypoxia and metabolic imbalance (Akerfelt et al., 2010; Fulda et al., 2010; Martindale and Holbrook, 2002; Morimoto, 1998). Thus, HSF-1 plays a key role in the regulation of core cellular processes in cells under stress where it control genes encoding for transcription factors, cell-cycle regulators and components of the translational apparatus and allows for reactivation of the cellular machinery upon restoration of conditions compatible with proliferation (Vihervaara and Sistonen, 2014). Although the major role of HSF is linked to the transactivation of genes encoding HSPs, recent genome-wide studies have revealed that HSF-1 can reprogram gene expression more extensively, being involved both in physiological processes including metabolism and aging, as well as pathological conditions such as neurodegenerative diseases and cancer. Hence, HSF-1 gene is one of the candidate genes associated with thermal tolerance due to its important role in stress-induced HSP expression and other biological functions. Further due to different biological roles of HSF-1 gene, it can be used as marker to select the animals with high thermal tolerance traits. The variations in the HSF-1 gene

have been associated with differential thermo-tolerance. Two single nucleotide polymorphism (SNP) at locus 909 and 4693 of HSF-1 gene have been observed to be associated with thermal tolerance traits in Chinese Holstein Cattle (Li et al. 2011). Study by Baena et al. (2018) also identified 12 SNPs in the HSF-1 gene, which are correlated to adaptability in Angus cattle raised in a subtropical climate.

Although few studies have deciphered the association of HSF-1 gene polymorphism with thermo regulation in exotic cattle (Li et al., 2011; Baena et al., 2018), no such work has been carried out in the indigenous cattle breeds. Considering the importance of HSF-1 gene and association of different allelic variants with thermo regulation, the present study was undertaken, to find out polymorphism at HSF-1 909 and 4693 loci located respectively in intron 3 and 3'-UTR of the gene, in indigenous cattle breeds adapted different agro-climatic zones of India along with exotic and crossbred cattle populations.

MATERIALS AND METHODS

Animals and DNA Extraction

Blood samples were collected (8-9 ml) from 1594 animals belonging to Indian native cattle (15 breeds) adapted to different agro-climatic zones, 2 exotic (Holstein Friesian

and Jersey) and 1 crossbred cattle (Karan Fries) cattle. The samples were collected either by visiting different villages/talukas or from organized farms located in the breeding tract of respective breeds. The information about the breeds analyzed and the sampling location is given in Table 1. The blood samples were collected in EDTA coated vacutainers tubes and transported to the laboratory in ice for DNA extraction. Genomic DNA was isolated by phenol-chloroform extraction method (Sambrook and Russel, 2001). The quality and quantity of the genomic DNA was checked on 0.6% agarose gel and NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA).

PCR-RFLP based genotyping of HSF-1 locus

The primers described by Li et al. (2011), were used to amplify 129 bp and 287 bp amplicon covering *HSF-1* 909 and 4693 loci respectively (Table 2). PCR amplification was carried out in a final volume of 25µl containing 50-100ng of genomic DNA, 200µM dNTPs, 1.5mM MgCl₂, 1U of *Taq polymerase* (BioLab, England) and 5 pmol of each forward and reverse primer. The PCR cycling conditions included denaturation step at 94°C for 2 min, followed by 35 cycle of denaturation at 94°C for 30 sec, annealing temperature at 56°C and 53°C for 1 min for locus 909 and 4693 of *HSF-1* gene, respectively, and extension at 72°C for

Table: 1 Details of the cattle breeds undertaken in the present study

S No	Breed	Utility Type	Geographical location	Agroclimate region	Coat colour
1	Rathi	Milch	NWR	AR	Red/Brown
2	Tharparkar	Milch	NWR	AR	Grey/White
3	Nagori	Draught	NWR	AR	Grey/White
4	Red Sindhi	Milch	NR	AR	Dark Red
5	Malvi	Draught	CR	TWDR	White/Grey
6	Kankrej	Dual	NWR	SAR	Grey/White
7	Hariana	Dual	NR	SAR	Grey/White
8	Red Kandhari	Draught	CR	TWDR	Red
9	Nimari	Draught	CR	TWDR	Red
10	Dangi	Draught	CR	TWDR	Grey/White
11	Khillar	Draught	CR	TWDR	Grey/White
12	Sahiwal	Milch	NR / NWR	SAR	Red
13	Gir	Milch	NWR	SAR	Red
14	Ladakhi	Milch	NR	HADR	Black/Brown
15	Gaolao	Dual	CR	HSTR	Grey/White
16	Holstein Friesian	Milch	NWR	SAR	Black and White
17	Jersey	Milch	NR	HADR	Black/Brown
18	Karan Fries	Milch	NWR	SAR	Black and White

NR—Northern region; NWR—North western region; CR—Central region; SR—Southern region; SAR—Semi arid region; AR—Arid region; TWDR—Tropical wet and dry region; HSTR—Humid sub-tropical region; HADR—High altitude cold desert region

Table 2: Primer sequences for Polymerase Chain Reaction of HSF-1 gene

Primer	Locus	Sequence of primers	Amplicon Length (bp)	Tm (°C)
P1	909 Locus	F: 5'-GCCATGAAGCAGTAGGTC-3' R: 5'-GGGGTAAGTCTGGTTACGAC-3'	129	55
P2	4693 Locus	F: 5'-CCCACTGTCTCGTAGAAGCC-3' R: 5'-TCATCGGTCTGTTTGTCCAT-3'	287	53

30sec. The PCR amplified products were confirmed by running ethidium bromide stained 1.5% agarose gel electrophoresis. In order to assess the polymorphism at 909 locus, 15µl of PCR product was digested with 2.5U of *ApaI* enzyme. For assessing variation at 4693 locus, PCR product was digested with 0.5U *NlaIV* enzyme. In both the cases, the PCR products were incubated at 37°C for overnight. The digested fragments were checked for genotypes on ethidium bromide stained 3.5% agarose gel and visualized the band pattern on gel documentation system.

Statistical analysis

Genotype of individual animal at two different loci was documented by direct counting. Allelic frequencies for both the loci were calculated as the sum of the frequency of the homozygous plus half of the frequency of heterozygous genotype as proportion of the total animals.

RESULTS AND DISCUSSION

In this study, an effort was made to screen allelic variations at locus 909 located in intron 3 and locus 4693 located in 3'-UTR of *HSF-1* gene across Indian native, exotic and crossbred cattle. The digestion of 129bp product for locus 909 revealed 3 genotypes; CC (129bp), TC (129bp, 88bp and 41bp) and TT (88bp and 41bp) as shown in Figure 1.

Genotype CC was predominant in all the indigenous cattle

breeds, ranging from 1.00 to 0.579. The draft breeds Nagori, Malvi, Nimari and Khillar were monomorphic for CC genotype. The frequency of CC genotype in Jersey, Holstein Friesian and crossbred KF was comparatively lower with values of 0.389, 0.168 and 0.153 respectively. In contrast to CC genotype, the frequency of heterozygous TC genotype was highest in Karan Fries (0.7) followed by Holstein Friesian (0.589) and Jersey cows (0.556) (Table 3). Interestingly, the TT genotype was absent in almost all the Indian native cattle animals. Only few animals of Sahiwal cattle showed TT genotype with an overall frequency of only 0.008. On the other hand, the frequency of TT genotype was comparably higher in Holstein Friesian, Jersey and cross-bred KF cows with values of 0.242, 0.056, and 0.147 respectively. Overall allele C at locus 909 was predominant and almost fixed in all the native cattle breeds with frequency ranging from 0.778 (Sahiwal) to 1.00 (Nagori, Malvi, Nimari and Khillar). The frequency of C allele was on lower side in two exotic cattle (HF and Jersey) and crossbred cattle (KF) with a value of 0.667, 0.537 and 0.503 respectively. The breed wise allele frequencies for locus 909 are summarized in Figure 2. The predominance of C allele in Indian native cattle breeds could be attributing to their heat stress tolerance as this allele was reported to be associated with superior thermo tolerance in Chinese cattle (Li et al., 2011).

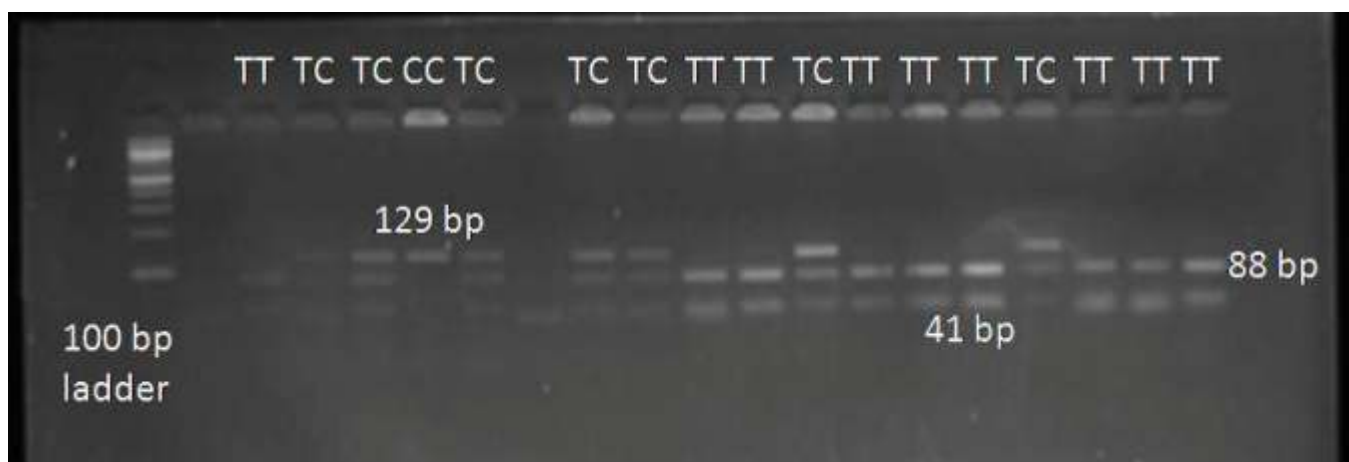


Fig. 1. Representative restriction pattern at HSF-1/*ApaI* 909 locus on 3.5% agarose gel. The gel picture depicts 3 genotypes: CC (129 bp), TC (129+88+41 bp), TT (88+41 bp). M-100bp molecular weight ladder.

Table: 3 Genotypic and allelic frequency for HSF-1 909 locus across different cattle breeds

S.No.	Breeds	Sample Size	No of Genotype			Allele frequency		Genotypic frequency			
			TT	TC	CC	f(T)	f(C)	f(TT)	f(TC)	f(CC)	
1	Rathi	32	0	5	27	0.078	0.922	0.000	0.156	0.844	
2	Tharparkar	84	0	1	83	0.006	0.994	0.000	0.012	0.988	
3	Nagori	32	0	0	32	0.000	1.000	0.000	0.000	1.000	
4	Red Sindhi	32	0	1	31	0.016	0.984	0.000	0.031	0.969	
5	Malvi	32	0	0	32	0.000	1.000	0.000	0.000	1.000	
6	Kankrej	30	0	1	29	0.017	0.983	0.000	0.033	0.967	
7	Hariana	32	0	3	29	0.047	0.953	0.000	0.094	0.906	
8	Red Kandhari	32	0	5	27	0.078	0.922	0.000	0.156	0.844	
9	Nimari	32	0	0	32	0.000	1.000	0.000	0.000	1.000	
10	Dangi	27	0	4	23	0.074	0.926	0.000	0.148	0.852	
11	Khillar	30	0	0	30	0.000	1.000	0.000	0.000	1.000	
12	Sahiwal	99	5	34	60	0.222	0.778	0.051	0.343	0.606	
13	Gir	59	0	4	55	0.034	0.966	0.000	0.068	0.932	
14	Ladakhi	30	0	2	28	0.033	0.967	0.000	0.067	0.933	
15	Gaolao	19	0	8	11	0.211	0.789	0.000	0.421	0.579	
Indian Native (Bos indicus)			602	5	68	529	0.065	0.935	0.008	0.113	0.879
1	Holstein Friesian	95	23	56	16	0.537	0.463	0.242	0.589	0.168	
2	Jersey	36	2	20	14	0.333	0.667	0.056	0.556	0.389	
Exotic (Bos taurus)			131	25	76	30	0.481	0.519	0.191	0.580	0.229
Karan Fries(Crossbred)			150	22	105	23	0.497	0.503	0.147	0.700	0.153

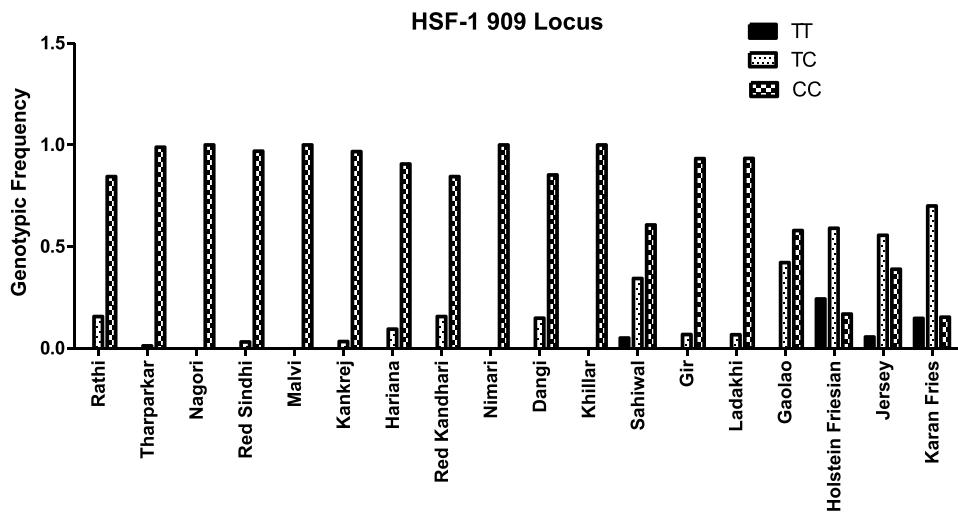


Fig. 2. The distribution of genotypic frequencies of the 909 locus in the HSF-1 gene in indigenous, exotic and crossbred cattle

The digestion of 287bp amplicon for locus 4693, revealed 3 genotypes GG (178bp, 61bp, 41bp and 7bp), GT (178bp, 68bp, 61bp, 41bp and 7bp) and TT (178bp, 68bp and 41bp) as shown in Figure 3. In Indian cattle breeds, the frequency of TT genotype ranged from 0.241(Dangi) to 1.0 (Kankrej) with an overall value of 0.696. The relative frequencies observed in Jersey, HF and KF were 0.643, 0.333 and 0.374, respectively. The heterozygous GT

genotype was observed with a frequency of 0.095, 0.208 and 0.162 in Indicus, exotic and crossbred animals, respectively. Among Indian native breeds, the maximum frequency (0.621) of GT genotype was observed in Dangi cattle while all other breeds showed frequency lower than 0.256. Amongst the three genotypes, GG was least frequent in Indian, exotic as well as crossbred cattle. The homozygous GG genotype showed relatively higher

frequency in HF (0.458) and KF (0.465), whereas in Indian cattle, its frequency was only 0.209. In many of Indian cattle breeds including Rathi, Kankrej, Haryana and Khillar, the GG genotype was absent. The genotypic and allelic frequencies for 4693 locus in different cattle breeds are shown in Table 4. The breed wise allele frequencies for locus 4693 are summarized in Figure 4. With respect to allelic pattern at *HSF-1* locus 4693, allele T was predominant in Indian native cattle with frequency of

0.744. However, in HF and KF cattle, allele G is predominant with frequency of 0.563 and 0.545 respectively. Similar frequency pattern has also been reported by Li et al. (2011) for Chinese Holstein dairy cattle wherein frequency for allele T was 0.625. The authors suggested T allele to be associated with thermo-tolerance in Chinese Holstein dairy cattle. Occurrence of T allele with higher frequency in Indian native cattle might be attributing to their better thermo-tolerance ability.

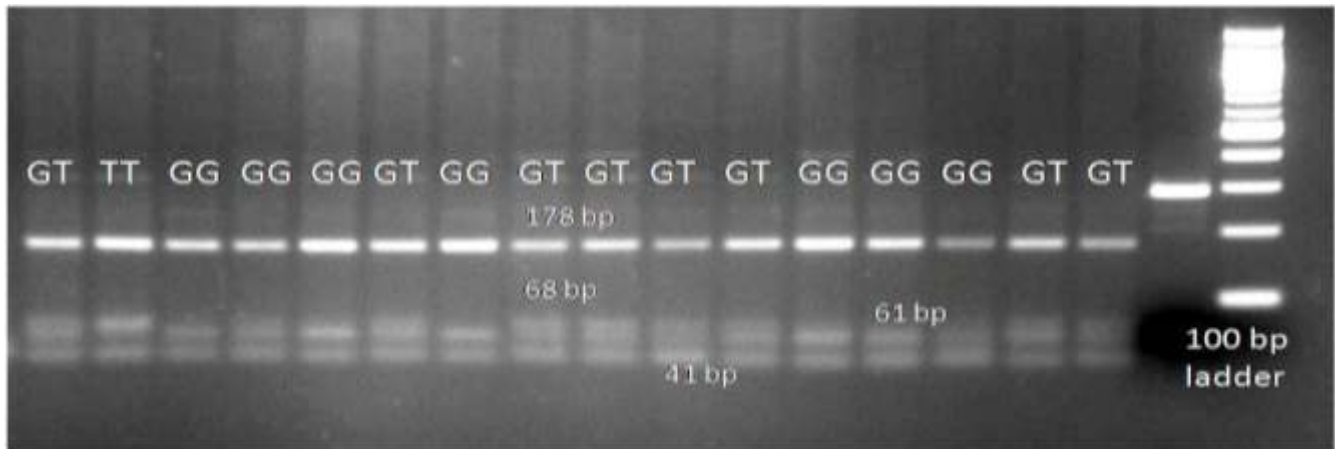


Fig. 3. Representative restriction pattern at *HSF-1*/*NlaIV* 4693 locus on 3.5% agarose gel. The gel picture depicts 3 genotypes GG (7+41+61+178 bp), GT (7+41+61+68+178 bp), and TT (41+68+178 bp). 7 bp band is not visible. M-100bp molecular weight ladder.

Table: 4 Genotypic and allelic frequency for *HSF-1* 4693 locus across different cattle breeds

S.No.	Breeds	Sample Size	No of Genotype			Allele frequency		Genotypic frequency		
			TT	TC	CC	f(T)	f(C)	f(TT)	f(TC)	f(CC)
1	Rathi	31	28	3	0	0.952	0.048	0.903	0.097	0.000
2	Tharparkar	82	47	21	14	0.701	0.299	0.573	0.256	0.171
3	Nagori	32	17	0	15	0.531	0.469	0.531	0.000	0.469
4	Red Sindhi	30	14	2	14	0.500	0.500	0.467	0.067	0.467
5	Malvi	32	27	1	4	0.859	0.141	0.844	0.031	0.125
6	Kankrej	29	29	0	0	1.000	0.000	1.000	0.000	0.000
7	Haryana	27	24	3	0	0.944	0.056	0.889	0.111	0.000
8	Red Kandhari	32	25	0	7	0.781	0.219	0.781	0.000	0.219
9	Nimari	31	28	0	3	0.903	0.097	0.903	0.000	0.097
10	Dangi	29	7	18	4	0.552	0.448	0.241	0.621	0.138
11	Khillar	31	30	1	0	0.984	0.016	0.968	0.032	0.000
12	Sahiwal	71	50	3	18	0.725	0.275	0.704	0.042	0.254
13	Gir	63	32	0	31	0.508	0.492	0.508	0.000	0.492
14	Ladakhi	30	25	0	5	0.833	0.167	0.833	0.000	0.167
Indian Native (Bos indicus)		550	383	52	115	0.744	0.256	0.696	0.095	0.209
1	Holstein Friesian	48	16	10	22	0.438	0.563	0.333	0.208	0.458
2	Jersey	14	9	0	5	0.643	0.357	0.643	0.000	0.357
Exotic (Bos taurus)		62	25	10	27	0.484	0.516	0.403	0.161	0.435
Karan Fries (Crossbred)		99	37	16	46	0.455	0.545	0.374	0.162	0.465

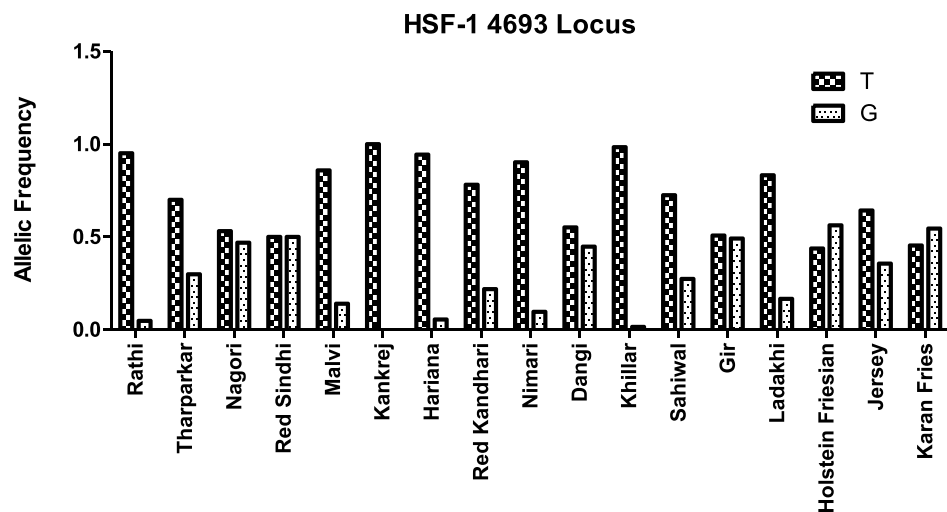


Fig. 4. The distribution of allelic frequencies of the 4693 locus in the HSF-1 gene in indigenous, exotic and crossbred cattle.

Several other reasons have been cited for better regulation of body temperature during heat stress in *Bos indicus* cattle as compared to European breeds (Hansen et al., 2004). It is believed that *Bos taurus* and *Bos indicus* cattle have undergone separate evolution from a common ancestor (Bradley et al., 1996). During the course of evolution, zebu cattle acquired set of certain variation due to which they gained the ability to regulate body temperature in response to heat stress (Gaughan et al., 1999) more effectively and efficiently. Consequently, the effect of heat stress towards production and reproduction traits is less for tropically adapted *Bos indicus* than the breeds from taurine temperate zone. Number of studies have shown variations in heat stress associated genes in different cattle populations. For example, several variants in promoter, 5'-UTR and 3'-UTR and coding region have been identified in *HSP70A1A* gene (Adamowicz et al. 2005; Basirico et al., 2011; Brown et al., 2010; Rosenkrans et al., 2010; Sodhi et al., 2013). Some of these variations have been shown to be associated with better thermo-tolerance ability (Li et al., 2011; Baena et al., 2018). These variants may affect inducibility, degree of expression, stability of important transcripts which may further contributes towards differential response towards stress tolerance. Not only in cattle, but certain SNPs in genes regulating heat stress response have also been shown to provide higher thermo-tolerant in other livestock species like pig, chicken and sheep (Chen et al., 2013; Salces-Ortiz et al., 2013). Further, the variations in heat stress associated genes has also shown to be associated with fertility, sperm quality and reproduction rate (Collier et al., 2008; Nikbin et al., 2014; Rosenkrans et al., 2010) indicating the multi functionality of these genes. For example, a SNP in promoter region of *HSP70* gene in crossbred Brahman cows was reported to be associated

with lower calving percentages (Rosenkrans et al., 2010). Further, polymorphism in coding region having CC genotype at 1623 locus of *HSP70A1A* was observed to be associated with resistance to mastitis in Chinese Holstein cows (Cheng et al., 2009).

In conclusion, polymorphism identified in *HSF-1* gene holds importance as this is a major transcription factor regulating heat stress response by modulating the expression of key heat shock proteins. The distinct pattern of alleles and genotypes at the two *HSF-1* loci might point towards the differential response of different cattle types towards heat stress. In future, the phenotypic and physiological data related to heat stress response across exotic and Indicus cattle may be evaluated across different genetic variants to understand their specific roles in providing thermo-tolerance.

ACKNOWLEDGEMENT

The work was supported by Indian Council of Agriculture Research, New Delhi under National Fellow scheme.

Disclosure statement: No potential conflict of interest was reported by the authors.

REFERENCES

- Adamowicz T, Pers E and Lechniak D. 2005. A new SNP in the 3'-UTR of the HSP70.1 gene in *Bos taurus* and *Bos indicus*. *Biochemical Genetics* 43(11-12): 623-627.
- Akerfelt M, Morimoto RI and Sistonen L. 2010. Heat shock factors: integrators of cell stress, development and lifespan. *Nature reviews Molecular cell biology* 11(8): 545.
- BV SK, Kumar A and Kataria M. 2011. Effect of heat stress in tropical livestock and different strategies for its amelioration. *Journal of Stress Physiology and*

- Biochemistry, 7(1): 45-54.
- Baena MM, Tizioto PC, Meirelles SLC and Regitano LCDA. 2018. HSF-1 and HSPA6 as functional candidate genes associated with heat tolerance in Angus cattle. *Revista Brasileira de Zootecnia*, 47.
- Basirico L, Morera P, Primi V, Lacetera N, Nardon A and Bernabucci U. 2011. Cellular thermotolerance is associated with heat shock protein 70.1 genetic polymorphisms in Holstein lactating cows. *Cell Stress Chaperones* 16(4): 441-448.
- Bradley DG, MacHugh DE, Cunningham P and Loftus RT. 1996. Mitochondrial diversity and the origins of African and European cattle. *Proc Natl Acad Sci USA* 93:5131-5135.
- Brown A, Reiter S, Brown M, Johnson Z, Nabhan I, Lamb M, Starnes A and Rosenkrans C. 2010. Effects of heat shock protein-70 gene and forage system on milk yield and composition of beef cattle. *Prof Ani Sci* 26(4): 398-403.
- Chen Z, Gan J, Xiao X. et al. 2013. The association of SNPs in Hsp90 β gene 5' flanking region with thermo tolerance traits and tissue mRNA expression in two chicken breeds. *Molecular Biology Report* 40:5295-5306.
- Cheng WJ, Li QL, Wang CF, Wang HM, Li JB, Sun YM and Zhong JF. 2009. Genetic polymorphism of HSP70-1 gene and its correlation with resistance to mastitis in Chinese Holstein. *Yi Chuan* 31(2): 169-174.
- Collier RJ, Collier JL, Rhoads RP and Baumgard LH. 2008. Invited review: genes involved in the bovine heat stress response. *Journal of dairy science* 91(2): 445-454.
- Fulda S, Gorman AM, Hori O and Samali A. 2010. Cellular stress responses: cell survival and cell death. *International journal of cell biology* 2010.
- Gaughan JB, Mader TL, Holt SM, Josey MJ and Rowan KJ. 1999. Heat tolerance of Boran and Tuli crossbred steers. *Journal of Animal Science* 77: 2398-2405.
- Hansen PJ. 2004. Physiological and cellular adaptations of zebu cattle to thermal stress. *Animal Reproduction Science* 82-83:349-360.
- Li QL, Ju ZH, Huang JM, Li JB, Li RL, Hou MH, Wang CF and Zhong JF. 2011. Two novel SNPs in HSF-1 gene are associated with thermal tolerance traits in Chinese Holstein cattle. *DNA and cell biology* 30(4):247-254.
- Martindale JL and Holbrook NJ. 2002. Cellular response to oxidative stress: signaling for suicide and survival. *Journal of cellular physiology* 192(1):1-15.
- Morimoto RI. 1998. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes and development* 12(24):3788-3796.
- Mwai O, Hanotte O, Kwon YJ and Cho S. 2015. African Indigenous Cattle: Unique Genetic Resources in a Rapidly Changing World. *Asian-Australasian journal of animal sciences* 28(7): 911-921.
- Nikbin S, Panandam JM, Yaakub H, Murugaiyah M and Sazili AQ. 2014. Novel SNPs in heat shock protein 70 gene and their association with sperm quality traits of Boer goats and Boer crosses. *Animal Reproduction Science* 146(3-4):176-181.
- Paula-Lopes FF, Lima RSD, Satrapa RA and Barros CM. 2013. Physiology and endocrinology symposium: influence of cattle genotype (*Bos indicus* vs. *Bos taurus*) on oocyte and preimplantation embryo resistance to increased temperature. *Journal of animal science* 91(3): 1143-1153.
- Roefeldt S. 1998. You can't afford to ignore heat stress. *Dairy Herd Management*, 35: 6-12.
- Rosenkrans C Jr, Banks A, Reiter S and Looper M. 2010. Calving traits of crossbred Brahman cows are associated with Heat Shock Protein 70 genetic polymorphisms. *Animal Reproduction Science* 119(3-4): 178-182.
- Salces-Ortiz J, Gonzalez C, Moreno-Sanchez N, Calvo JH, Perez-Guzman MD, Serrano MM. 2013. Ovine HSP90AA1 expression rate is affected by several SNPs at the promoter under both basal and heat stress conditions. *PLoS ONE*, 8(6).
- Sambrook J and Russell DW. 2001. *Molecular cloning: A laboratory manual*, 3rd edition. Cold Spring Harbor Laboratory Press, New York.
- Vihervaara A and Sistonen L. 2014. HSF-1 at a glance. *Journal of Cell Science* 127: 261-266.