



## Polymorphism in the promoter region of HSP70 gene and its association with performance traits in Deoni cattle

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Received: 24 March 2016; Accepted: 6 June 2016

**Key words:** Deoni cattle, HSP70, PCR-SSCP, Polymorphism, Reproductive traits

Heat shock protein-70 (HSP70), one of the most abundant members of the HSP family, is present in all cells, and increase, when cells are exposed to elevated temperatures or to a variety of other stresses. The heat stress to livestock results in reduced feed consumption and decreased milk production (Bernabucci *et al.* 2010). There are several physiological mechanisms (sweating, high respiratory rate, rising rectal temperature, increased water consumption, reduced metabolic rate and a decreased dry matter intake) to cope with heat stress, at the same time reveal a negative impact on the production and reproduction performance of the cattle (Beatty *et al.* 2006). Heat tolerance coefficient (HTC) using appropriate formula is generally calculated for each cow to assess its heat adaptability. Heat stress produces oxidative stress and affects the alternation of plasma potassium (K<sup>+</sup>) and sodium (Na<sup>+</sup>). The present study was undertaken with the objective to analyse genetic variation in promoter region of HSP70 gene and to evaluate the association between HSP70 haplotypes with heat tolerance and performance in *Bos indicus* (Deoni) cattle.

DNA was isolated from blood samples (10 ml) collected from Deoni cows (59) maintained at SRS of ICAR-NDRI, Bengaluru, by the high salt method as per Miller *et al.* (1988). PCR amplification was performed (Ramesha *et al.* 2015) using primers (Forward- HSP-Pro749F-GCCAGGAAACCAGAGACAGA; Reverse-HSPPro126 8R CCTACGCAGGAGTAGGTGGT) reported by Banks *et al.* (1999). The amplified PCR products were resolved on 10% acrylamide:bisacrylamide (29:1) and the gels were silver-stained (Sambrook and Russel 2001). Different SSCP band patterns were sequenced and sequence data were analysed using Bio-edit and CLUSTAL W multiple alignments software's (Hall 1999). The BLAST analysis was performed to study the homology of our sequences with available sequences from other breeds of indigenous cattle

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and *Bos taurus* cattle. The lactation length, lactation yield, age at first calving (AFC) and calving interval (CI) data of Deoni cows was collected from records. The respiratory rate (diaphragm movements/minute) (RR) and rectal temperature (RT) (°C) of Deoni cows were recorded in the morning (8.00 AM) and in the afternoon (2.00 PM) after their 6 h exposure to sun. Heat tolerance coefficient (HTC) for individual cows was calculated using Iberia heat tolerance test formula (Rhoad 1944),  $HTC = 100 - 10(ART - 38.3)$ , where HTC is the heat-tolerance coefficient, ART is the average rectal temperature, 38.3 is the physiological 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. The index of HTC was calculated for each cow to assess its heat adaptability.

Temperature humidity index (THI) was calculated using the formula:

$$THI = (T_{db} + T_{wb}) \times 0.72 + 40.6 \text{ (NRC1971)}$$

where, T<sub>db</sub>, dry bulb temperature and T<sub>wb</sub>, wet bulb temperature. The potassium content in erythrocytes (PCE) was calculated as per Barani *et al.* (2015).

Statistical analysis were carried out using SAS software (Statistical Analysis System 9.2) to study the association of SSCP patterns with performance traits, physiological parameters and PCE using the model

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

where, Y<sub>ij</sub>, observation of i<sup>th</sup> genotype; μ, overall mean; G<sub>i</sub>, effect of i<sup>th</sup> pattern and e<sub>ij</sub>, random error.

Table 1. SSCP patterns in promoter region of HSP70 gene in Deoni cattle

	SSCP Pattern		
	Pattern AA	Pattern AB	Pattern BB
Genotypic frequency	0.712 (42)	0.254 (15)	0.034 (2)
Gene frequency	A allele = 0.161 B allele = 0.839		

The figures in parenthesis indicate the number of animals.

Table 2. Association of SSCP patterns on performance traits in Deoni cattle

Trait	Band pattern	LSM±SE
Lactation length (days)	AA	309.59±94.90
	AB	207.70±61.79
Lactation yield (kgs)	AA	1224.80±417.38
	AB	744.04±271.76
AFC (years)	AA	4.10±0.32
	AB	3.52±0.21
Calving interval (months)	AA	15.74±2.29
	AB	15.66±1.49
		F=2.01; P=0.94 F=0.19; P=0.70 F=2.91; P=0.23 F=1.92; P=0.30

PCR-SSCP analysis of 539 bp segment of bovine HSP70 gene promoter region revealed three SSCP band patterns, viz. AA, AB and BB with frequencies of 0.712, 0.254 and 0.034, respectively (Table 1). The sequence analysis revealed 4 transitions (A859G, A1046G, C1257T and G1294A) (Fig. 1) and 3 transversions (A897C, A1127C and G1166T). The BLAST analysis showed that the obtained sequence had 99.1% homology with *Bos indicus* sequence (Kankrej, Ongole sequence) and 98.9% homology with *Bos taurus* cattle. The productive and reproductive performances among cows with different SSCP patterns were similar (Table 2). The rectal temperature of Deoni cattle during summer in Bengaluru ranged from 38.33 to 38.90°C with an overall least squares mean of 38.47±0.03°C. The respiratory rate ranged from 26.22 to 31.22 breaths/min with an overall least squares mean of 28.87±0.35 breaths/min. The pulse rate ranged from 69 to 71 beats/min with an overall least squares mean of 70.57±0.17 beats/min. The calculated HTC in Deoni cows ranged from 94.27 to 99.67. THI minimum ranged (68.18–70.98) and maximum THI ranged (77.82–78.04) in the experimental area during the summer season (March–May). The potassium content in erythrocytes (PCE) ranged from 166.5 to 676.00 mg/l with an overall least squares mean of 303.70±24.40 mg/l. There was no significant difference in physiological parameters as well as PCE between the animals belonging to different SSCP patterns.

The study revealed high degree of genetic variability in the promoter region of HSP70 gene and lack of association of observed genetic variants in the promoter region with performance traits in Deoni cattle. Further, our findings showed no significant difference in physiological parameters as well as potassium content in erythrocytes between the animals belonging to different SSCP patterns and supported the earlier findings of higher heat tolerance of indigenous cattle. Earlier researchers reported that genetic polymorphisms in heat stress proteins were associated with decreased fertility (Collier *et al.* 2008, Rosenkrans *et al.* 2010). Rosenkrans *et al.* (2010) reported that SNPs in promoter region of HSP70 is associated with pregnancy rate in *Bos taurus* cattle. The observed lack of difference

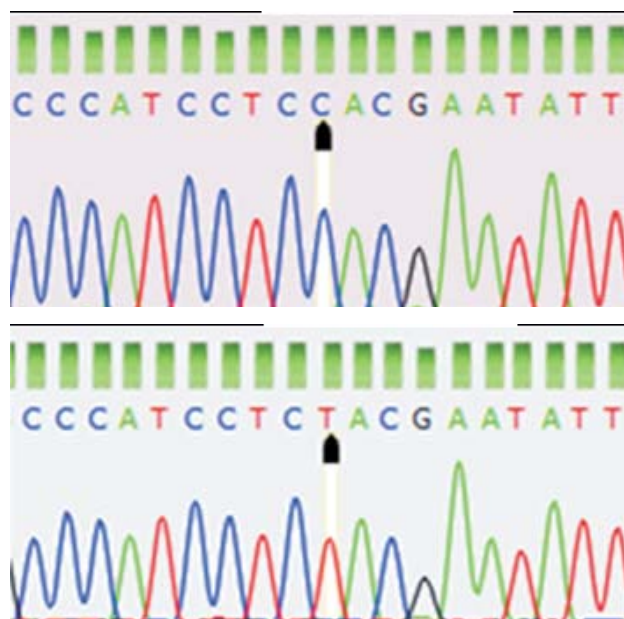


Fig. 1. Sanger's trace figure showing comparison of AA and BB of bovine HSP 70 gene. The arrow indicates C→T transition at 1257 position in Deoni cattle.

among cows with different SSCP patterns in production and reproduction performance could be due to higher heat tolerance of Deoni cattle. Further, there was less variability in THI during different seasons in Bengaluru. The studies on physiological parameters and calculation of Heat Tolerance Coefficient (HTC) for each cow using Iberia heat tolerance test indicated that even after 6 h of exposure to sun, Deoni cows maintained at Bengaluru were not under heat stress during summer though THI exceeds 72. In *Bos taurus* cattle, heat stress begins to occur in dairy cattle when the THI is more than 72 (Freitas *et al.* 2006), however, such a trend was not observed in Deoni cattle. The observed higher degree of thermal tolerance of Deoni cows could be due to its natural adaptability to local climatic conditions.

## SUMMARY

A study was undertaken to analyze associations between HSP70 genetic variants of promoter region with heat tolerance and performance in Deoni (*Bos indicus*) cattle using PCR-SSCP technique. The analysis revealed three SSCP patterns, viz. AA, AB and BB with frequencies of 0.712, 0.254 and 0.034, respectively. Sequence analysis of representative samples of each pattern showed 7 SNPs, which included four transitions (A859G, A1046G, C1257T and G1294A) and 3 transversions (A897C, A1127C and G1166T). Cows with different SSCP patterns showed similar production and reproduction performances. The studies on physiological parameters and calculation of Heat tolerance coefficient (HTC) of individual cows indicated that Deoni cows maintained at Bengaluru were not under heat stress even after 6 h of exposure to sun during summer (March to May).

## ACKNOWLEDGEMENT

The authors are thankful to the Director, NDRI, Karnal and Head, SRS of NDRI, Bengaluru for providing necessary facilities and support.

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