Molecular characterization of heat shock protein 70 gene in Deoni cattle (*Bos indicus*)

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Abstract

The present study was conducted in 60 Deoni cattle raised under semi-arid, hot tropical climatic conditions of Marathwada region of Maharashtra with the objectives to identify single nucleotide polymorphism (SNPs) in targeted promoter regions of HSP70 gene and to analyse the association of different genotypes with physiological parameters. The PCR amplification was performed using primers available in the NCBI database and revealed size of 539 bp for the amplified fragment promoter region 1 of HSP70 gene. PCR-RFLP analysis with *HindIII* restriction enzyme of targeted primer produced two different banding patterns, resulting in their identification as homozygotes AA and BB genotypes. Sequence analysis revealed single nucleotide polymorphism in coding promoter region of HSP70 gene at 284th position A to G, the A284G SNP resolved into two genotypes i.e. AA and BB, showed non-significant association with physiological parameters (rectal temperature and respiration rate) with a change in the amino acid from valine to isoleucine. However, there was no any change observed for physiological parameters in the studied population. Associations between HSP70 gene polymorphism in Deoni cattle with physiological parameter i.e. respiration rate and rectal temperature were analysed and rectal temperature in genotype AA was 100.52±0.023, in genotype BB it was 100.65±0.019. Similarly, 19.07±0.117 respiration rate in genotype AA while 19.17±0.108 in genotype BB were observed. The observed rectal temperature and respiration rate across different genotypes were found to be non- significantly ($p > 0.05$) differ. Therefore, there is scope of considering genetic information of HSP70 gene in breeding and management strategies for the improvement of Deoni cattle by considering large number of Deoni population.

Key words: Deoni cattle; Genotypes; PCR-RFLP; Polymorphism

Introduction

The indigenous cattle are well known for better disease resistance, survive better in local climatic condition, suitable for draught purpose, more suitable for low input management system and heat tolerance property as they have been evolved over time to survive in specific environments and as a result, they often possess genetic variants that make them well-adapted to those environments (Das *et al.,* 2011). Many indigenous breeds of cattle have developed unique features for adaptations to cope with adverse climatic conditions. For example, some indigenous cattle have a higher density of sweat glands, which allows them to cool themselves more efficiently. There are many genes responsible for thermoregulation / heat stress in cattle i.e. additionally, research has shown that some indigenous cattle possess a unique variant of the HSP70 gene, which plays important role in heat stress response. Deoni is an important dual-purpose cattle breed originated from Deoni taluka of Latur district in Maharashtra state and distributed all over Latur district, parts of Parbhani, Osmanabad and Nanded district of Maharashtra state and also found in adjoining areas of Karnataka, Telangana and Andra Pradesh (Singh *et al.,* 2006). The heat shock proteins family consists of many proteins which are classified as HSP110, HSP100, HSP90, HSP 72, HSP70, HSP60, HSP40, HSP10, and small HSP families (Gade *et al.,* 2010). An increase in HSP 72 mRNA in dairy cattle during peak hot seasons of the year and the level of induction varied depending upon the duration of exposure to stress during natural environment (Vaidya et al 23). The HSP70 is one of the most abundant members of the HSP family and is present in all cells and increases when an individual is exposed to various stressors. Among Heat Shock Proteins, HSP70 is an essential molecular chaperone of primary importance. Heat Shock Protein (HSP70) is reported to protect cells, tissue and organs from stress (Kiang and Tsokos, 1998). HSP gene family have been broadly discussed as candidate genes for heat resistance (Hoffmann *et al.,* 2003) and few studies have shown association between Single Nucleotide Polymorphisms (SNPs) at HSP genes and stress resistance in different species (Sun *et al.,* 2007; Li *et al.,* 2009). Considering these facts, there is a need to find out the genetic polymorphism and its association with various heat stress related parameters in Deoni cattle breed. Identification of SNPs in targeted region of HSP70 gene and study association between genetic variants of HSP70 gene with rectal temperature and respiration rate in Deoni cattle.

Methodology

The present study was undertaken to identify HSP70 gene polymorphism in Deoni cattle. A total of 50-60 unrelated Deoni animals was selected for the study of promoter region 1 of HSP70 gene in Deoni cattle. Sample was collected from organized farm i.e., Instructional Livestock Farm Complex (ILFC) of College of Veterinary and Animal Sciences, Udgir and sample were also collected from field i.e. breeding tract of Deoni cattle.

Geographical and Climatic description

Deoni breed is distributed in the climatic conditions which are generally hot throughout the year except during winter season. The breeding tract of Deoni cattle is located at latitude between 17°35′to 20º05′N and longitude of 75º16′to 78º15′E. The maximum temperature in the tract varied from 29 to 44°C while the minimum temperature varied from 9 to 27°C.The annual rainfall varies from 750mm to 890mm.

Physiological parameters recorded

Respiration rate per minute was recorded once in a fortnight consecutive day during May, June, July, August, September, October and average was taken as final reading for association analysis. The observations were recorded in degree Fahrenheit (°F). Respiration rate and rectal temperature were recorded once in a fortnight consecutively.

Blood Collection

About 4-5 ml of blood sample was collected from Deoni animals once at the beginning of experiment using aseptic conditions from jugular vein in vaccuteiner containing EDTA. The genomic DNA was isolated from blood samples using traditional Phenol: Chloroform: Isoamyl alcohol (P: C: I) method as described by Sambrook and Russell (2006). Quality of DNA was checked by loading 5 µl DNA on 1% agarose gel electrophoresis in horizontal mini electrophoresis unit in 1X TBE. The DNA was quantified using UV spectrophotometer, and Optical Density (O.D.) will be determined at 260 nm and 280 nm wavelengths against distilled water as blank.

Quantity of DNA in µg/ml = O.D. 260 x 50 (dilution factor) x 50

The ratio between OD260 and OD280 was calculated. The samples were subjected to further analysis. The DNA was quantified using the convention that one absorbance unit at 260 nm wavelength equals 50 µg DNA per ml. Sequence specific primers reported by Ramesha, *et al.,* 2016 were utilized to amplify the region of interest. i.e. promoter region 1. The details of the primers are as follows:

PCR amplification

The optimization of PCR conditions for genomic DNA with appropriate $MgCl_2$ concentration and annealing temperature was done to obtain a specific amplified product in sufficient quantity. The reaction volume was kept constant at 25 µl. Thermal Cycler was programmed accordingly to carry out the PCR amplification. Taq gold fast PCR Master mix 25 rxn (Thermofisher) was used in the reaction. Total 25.0 µl reaction was setup containing 9.0 µl Nucleus free water, 12.5 µl PCR Master Mix, 1.0 µl Forward Primer, 1.0 µl Reverse Primer, 1.5 µl DNA Template. The restriction enzyme Hindlll was used to digest the amplicons of HSP70 gene for promoter region 1.

Table 1. Description of primers used and the amplified products

Locus	Primer sequence 5'-3'	Tm	Primer length in bp	Primer source	Amplified product length
HSP70 Promoter region 1	F'5-GCCAGGAAACCAGAGACAGA-3 R'5-CCTACGCAGGAGTAGGTGGT-3	59 ^{0}c	20	Ramesha *et al.,* 2016	539 bp

Table 2: Frequencies of different patterns of Promoter region 1of HSP 70 gene in Deoni cattle

Promoter region	Genotypic Frequency	
	AA	BB
Promoter region-1	0.406 (28)	0.593(32)

Note: The values in the parenthesis indicate the number of animals

Table 3: Association between HSP70 genotypes and physiological parameters in Deoni cattle

Physiological Parameter	Genotypic Frequency		Chi-test (P value)
	AA (Mean±SE)	BB (Mean±SE)	
Rectal Temperature	100.52±0.023	100.65±0.019	0.04^{NS}
Respiration rate	19.07±0.117	19.17±0.108	0.06^{NS}

*NS = Non-significant difference

Results and Discussion

The Promoter region 1 of HSP70 gene of 539 bp was amplified from genomic DNA, using specific primers. Oligonucleotide primers for amplification of the desired region of gene (Fig.1). PCR-RFLP analysis with *HindIII* restriction enzyme of targeted primer produced two different banding patterns, resulting in their identification as homozygotes AA and BB genotypes (Fig.2). The genotypic frequency for AA allele was found to be 0.406 while genotypic frequency for BB allele was observed as 0.593 which was found to non-significant as shown in Table No 2.

Sequence analysis revealed single nucleotide polymorphism in coding promoter region of HSP70 gene at 284th position A to G. Further it was observed that there is a change in the amino acid from valine to isoleucine. However, there was no any change observed for respiration rate and rectal temperature in the studied population of Deoni cattle (Fig. 3). In accordance with the present finding, Cai *et al.* (2005) reported nucleotide substitution (A→G) in the ac genotype at the 33rd site compared with that in the AA genotype in dairy cattle. Further, Rosenkrans *et al*., (2010) reported 11 single nucleotide polymorphisms were detected; 1 deletion at base position 895, 7 transitions (G1013A, G1045A, C1069T, A1096G, G1117A,T1134C, and T1204C), and 3 transversions (A1125C, G1128T, and C1154G).

Total two homozygous genotypes (AA and BB) were observed by amplifying targeted sequence of HSP70 gene in Deoni cattle. The association of HSP70 genotypes i.e AA and BB genotypes in Deoni cattle was done with rectal temperature and respiration rate (Fig 4 and Fig 5).

It was observed that rectal temperature in genotype AA was 100.52±0.023. However, in genotype BB it was 100.65±0.019. Similarly, 19.07±0.117 respiration rate in genotype AA while 19.17±0.108 in genotype BB were observed. The observed rectal temperature and respiration rate across different genotypes were found to be non- significantly ($p > 0.05$) different from each other (Table 3).

Conclusions

The genotypic frequency for AA allele was found to be 0.406 while genotypic frequency for BB allele was observed as 0.593 which was found non-significant with single nucleotide polymorphism in coding promoter region of HSP70 gene at 284th position A to G which leads to change in the amino acid from valine to isoleucine. However, there was no any change observed for respiration rate and rectal temperature. The rectal temperature in genotype AA was 100.52±0.023, in genotype BB it was 100.65±0.019. Similarly, 19.07±0.117

respiration rate in genotype AA while 19.17±0.108 in genotype BB were observed rectal temperature and respiration rate across different genotypes were found to differ non- significantly (p >0.05).

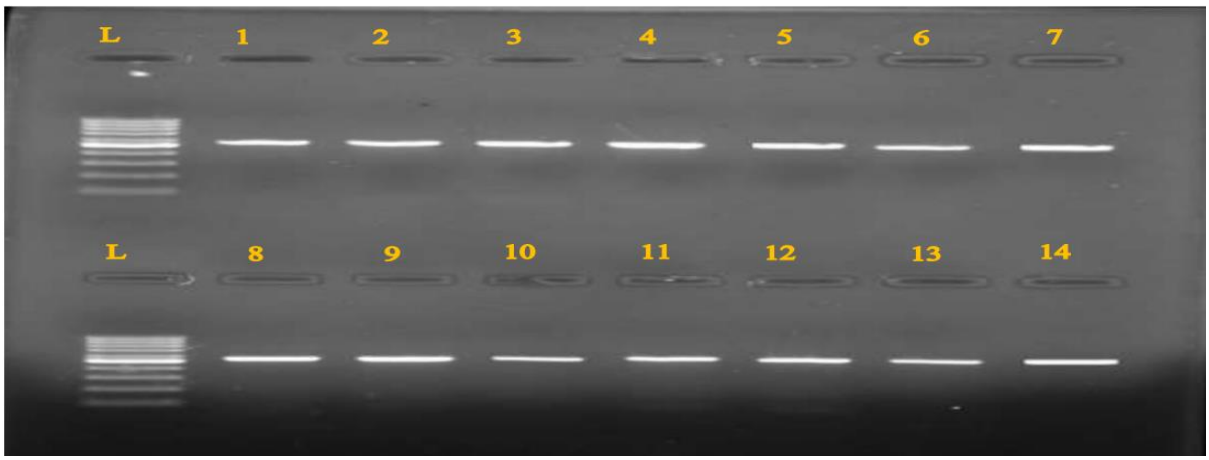

Fig 1: Resolution of primer 1 (promoter region 1) PCR products of HSP70 gene 1.5%agarose gel (539 bp PCR product)

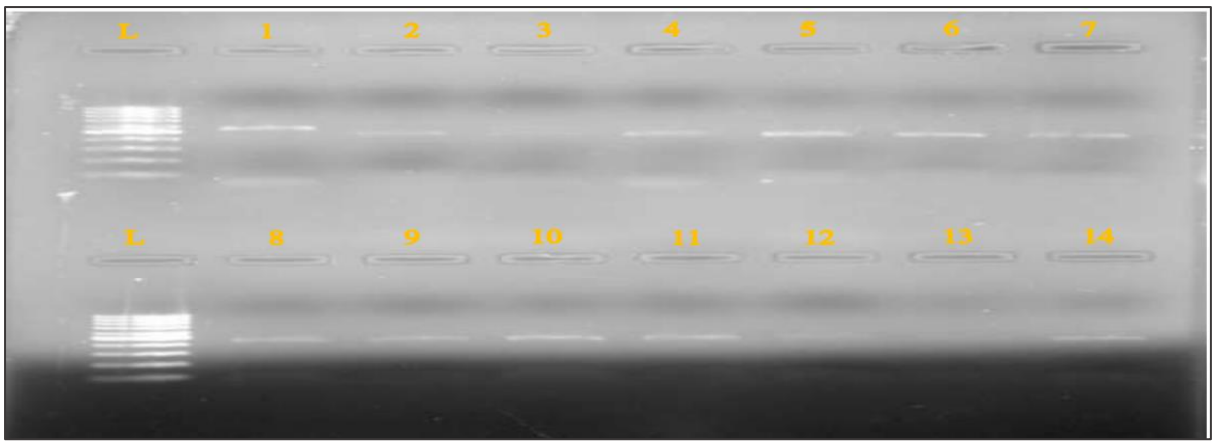

Fig 2: PCR-RFLP of promoter region 1 of HSP70 gene using Hind III RE in Deoni cattle

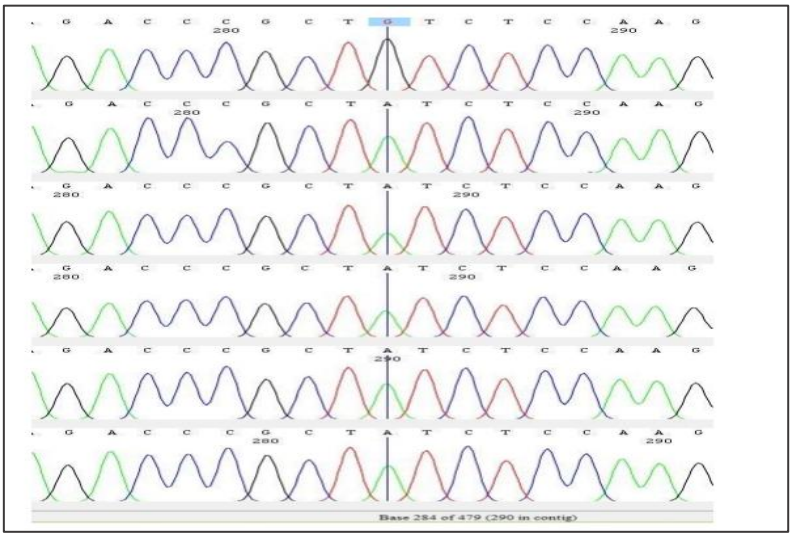

Fig 3: Chromatograph showing SNP at 284th position A to G

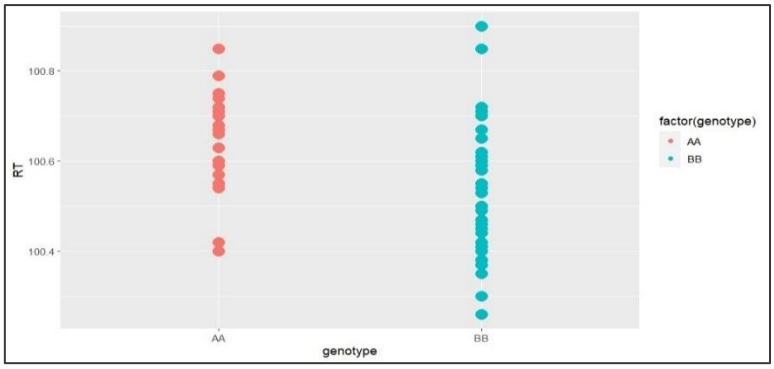

Fig 4: Graphical representation of rectal temperature across different genotypes

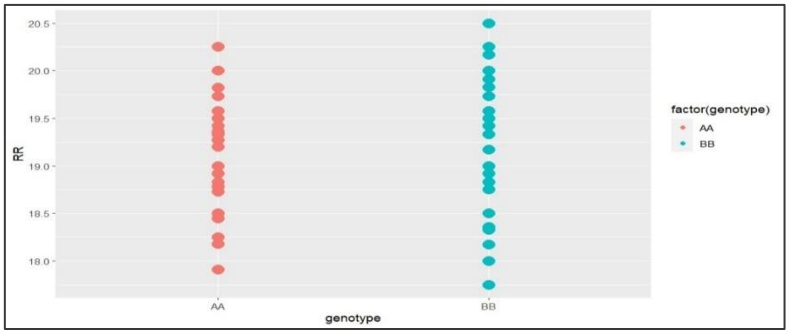

Fig 5: Graphical representation of respiration rate across different genotypes

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