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Molecular Characterization of the Coding Region and 5' UTR of *HSP70* Gene in Indian Riverine Buffalo Breeds

Ravinder Singh[1,3], Ankita Gurao[1], S.K. Mishra[1], S.K. Niranjan[1], Vikas Vohra[2], Manishi Mukesh[1], C. Rajesh[3], R.S. Kataria[1]

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ABSTRACT

Background: *HSP70* (Heat Shock Protein 70), plays a crucial role in nascent protein folding; the added challenges due to physiological factors demand stringent role-playing of such chaperones for tropical livestock such as water buffalo (*Bubalus bubalis*). Therefore to evaluate the variations at nucleotide level in *HSP70* that could potentially unravel the molecular basis of thermal adaptation in the riverine buffalo breeds of India, the current study was targeted to sequence the CDS (Coding Sequence) and UTR (Untranslated Region) of the gene in a panel of 16 Indian riverine buffalo breeds.

Methods: Blood samples were collected and genomic DNA was isolated followed by PCR standardized for the amplification of different fragments of the *HSP70* gene using different sets of primer pairs covering the entire coding region and 5′UTR. Multiple amplicons generated to cover the entire gene were sequenced. Sequences were further analyzed manually for the identification of heterozygous animals to detect the polymorphic nucleotide sites and variation between breeds documented.

Result: The *HSP70* results suggest, the highly conserved nature of gene in buffalo. The only non-synonymous polymorphic site was found in the Toda buffalo breed (g.SNPC>T at position 14), resulting in amino acid change 5M>T. A total of 7 polymorphic sites were found in the 5′UTR flanking region. Additionally, two insertion/deletions (INDEL) of 30 and 1 nucleotide length were found in the 5′UTR.

Key words: *Bubalus bubalis*, *HSP70* gene, Molecular characterization, Polymorphism, Thermal adaptation.

INTRODUCTION

The livestock sector is an important source of livelihood and income to a major section of the population in developing countries like India, employing directly or indirectly 0.602 billion people (Employment in Agriculture, India data-2019, ILO). Among the dairy animals, buffaloes are the major contributors to the milk production in India, which is evident from the 20th livestock census reporting 1.06% growth in buffalo populations, whereas the dairy cattle population witnessed an increase of 0.85% in the last 8 years (20th Livestock Census-2012, Department of Animal Husbandry and Dairying). Besides the contribution to milk production, draft power, dung for fuel and manure and leather industry, buffalo also significantly contributes to the meat industry, since India has exported 12,36,638.39 metric tons of buffalo meat products worth 3608.72 USD millions across the world during 2018-19, rated as the top animal commodity being exported by the country (http://apeda.gov.in/apedawebsite/index.html), apart from their contribution to the animal (Singh *et al.,* 2020; Singh *et al.,* 2018; Singh *et al.,* 2017).

The distribution of domestic water buffaloes (*Bubalus bubalis*) is limited to the tropical region of Southeast Asia. Hence, they are well adapted to different agro-climatic conditions of India and India possesses 57% of the world's buffalo population. India is home to 17 different registered breeds very well adapted to their respective agro-ecological production system. Despite the black coat colour and poorly developed sweat glands, buffalo has very well acclimatized itself to the hot and humid climate, emerging as a major source of income for the resource-poor farmers. Understanding the genetics of heat stress has led to the identification of various genes responsible for climatic adaptation, among which the heat shock protein (HSP) family members play a major role. One of the members, Heat Shock Protein *70* (*HSP70*) is a highly evolutionary conserved protein, expressed in both stress and non-stressed conditions (Rosenzweig *et al*., 2019; Jolly and Morimoto, 2000; Morimoto, 1998). Since HSP70 also acts as a molecular chaperone and assists in the folding of other proteins, under stressful conditions, the highest levels of *HSP70* among the major HSPs were reported (Singh *et al.,* 2019). It plays a major role in the folding of nascent peptide chains on ribosomes thus preventing the aberrant folding by protecting the hydrophobic surface, which would normally be exposed to solvent (Gaviol *et al.,* 2008). The *HSP70* gene of buffalo is encoded by a single

[1]ICAR-National Bureau of Animal Genetic Resources, Karnal-132 001, Haryana, India.
[2]ICAR-National Dairy Research Institute, Karnal-132 001, Haryana, India.
[3]Sri Guru Granth Sahib World University, Fatehgarh Sahib-140 407, Punjab, India.

Corresponding Author: R.S. Kataria, ICAR-National Bureau of Animal Genetic Resources, Karnal-132 001, Haryana, India.
Email: katariaranji@yahoo.co.in

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coding-exon with a transcript length of 1.93 kb and has not been extensively explored, therefore this study was designed to characterize the promoter and coding region of different Indian buffalo breeds by sequencing and document the polymorphism.

MATERIALS AND METHODS

Blood samples of Chilika, Kalahandi and Paralakhemundi buffaloes were collected from the different parts of their breeding tracts in Odisha state. Murrah buffalo blood samples were collected from National Dairy Research Institute, Karnal, Haryana, maintaining at 4°C until transferred to the Buffalo Genomics Lab, at National Bureau of Animal Genetic Resources, Karnal, where the work has been carried out during the year 2018. Genomic DNA was isolated from blood samples by the standard protocol of SDS-Proteinase-K described by Sambrook and Russell (2001). The assessment of quantity and quality of DNA was done using NanoDrop (ND-1000 Thermo Scientific) and agarose gel electrophoresis respectively. PCR was standardized for the amplification of different fragments of the *HSP70* gene using different sets of primer pairs, reported by Sodhi *et al.,* (2013), in overlapping fragments covering the entire coding region and 5′UTR.

The various amplicons were electrophoresed on 1.5% agarose gel and single-band PCR products of 1.2 kbp were used for dideoxy sequencing after exonuclease and alkaline phosphatase treatment. Sequences were further analyzed by using different software. The chromatograms were screened manually for the identification of heterozygous animals to detect the polymorphic nucleotide sites using Chromas Lite 2.0 software (http://chromas-lite.software.informer.com/2.0). The amplicons were edited and trimmed using the Editseq software and aligned using MegAlign, both of which are part of Lasergene 12 package of DNASTAR (https://www.dnastar.com/software/lasergene/).

RESULTS AND DISCUSSION

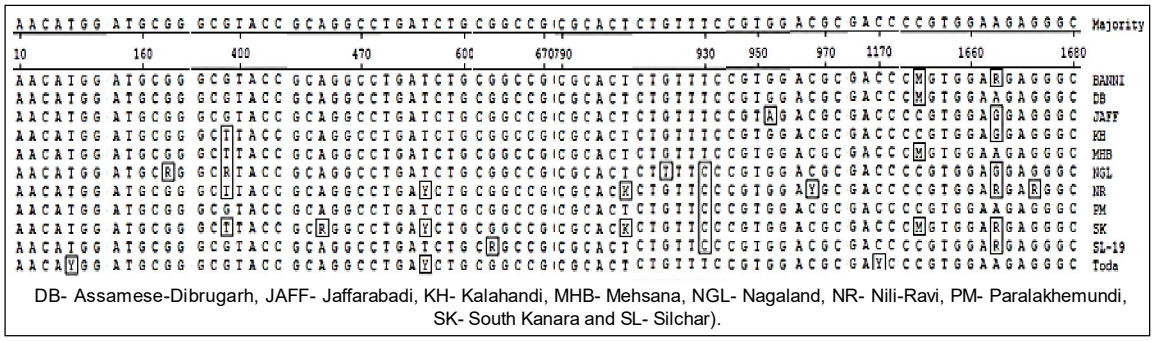
The sequences were aligned and analyzed for polymorphism by comparing with the *Bos taurus's* and *Bos indicus's* sequences (Table 1). *HSP70* gene of buffalo showed 98.9% identity with the *Bos indicus* and *Bos taurus.* The complete open reading frame (ORF) and 5′UTR of 1926 and 204 bp respectively, were amplified for each animal in the panel of 16 different buffalo breeds/populations (Chilika, Kalahandi, Murrah, Assamese-Dibrugarh, Jaffarabadi, Kalahandi, Mehsana, Nagaland, Nili-Ravi, Paralakhemundi, South Kanara, Toda, Banni and Silchar). The subsequent sequencing helped in the identification of 22 polymorphic sites (Table 1), amongst which 15 were present in the exonic region and 7 were identified in the 5′UTR. Among the exonic region's polymorphic sites, only a single polymorphic site was found to be non-synonymous in the Toda buffalo breed (g.SNP C>T at position 14), resulting in amino acid change 5M>T. This suggests the highly conserved nature of *HSP70* in buffalo (Fig 1,2). An earlier study has reported 15 single nucleotide polymorphic (SNPs) sites, out of which 9 SNPs were exonic and 6 were located in the 5′UTR of the *HSP70* gene in *Bubalus bubalis* (Sodhi *et al.,* 2013).

In this study, 21 variations were identified when *Bubalus bubalis* compared with *Bos taurus*, amongst 11 were

Table 1: Distribution of SNPs found in *HSP70* gene in *Bubalus bubalis.*

Location	Location (CDS)	Variation	Type of SNP
Exon-1	14	T/C	Non-Synonymous
Exon-1	162	G/A	Synonymous
Exon-1	399	T/G	Synonymous
Exon-1	467	A/G	Synonymous
Exon-1	597	T/C	Synonymous
Exon-1	666	G/A	Synonymous
Exon-1	795	T/G	Synonymous
Exon-1	927	G/T	Synonymous
Exon-1	930	T/C	Synonymous
Exon-1	951	G/A	Synonymous
Exon-1	969	C/T	Synonymous
Exon-1	1170	C/T	Synonymous
Exon-1	1656	C/A	Synonymous
Exon-1	1662	G/A	Synonymous
Exon-1	1677	G/A	Synonymous
5′UTR	-174	G/C	NA
5′UTR	-172	C/T	NA
5′UTR	-167	G/T	NA
5′UTR	-141	G/T	NA
5′UTR	-108	A/C	NA
5′UTR	-107	G/T	NA
5′UTR	-106	A/C	NA

NA- Not applicable.



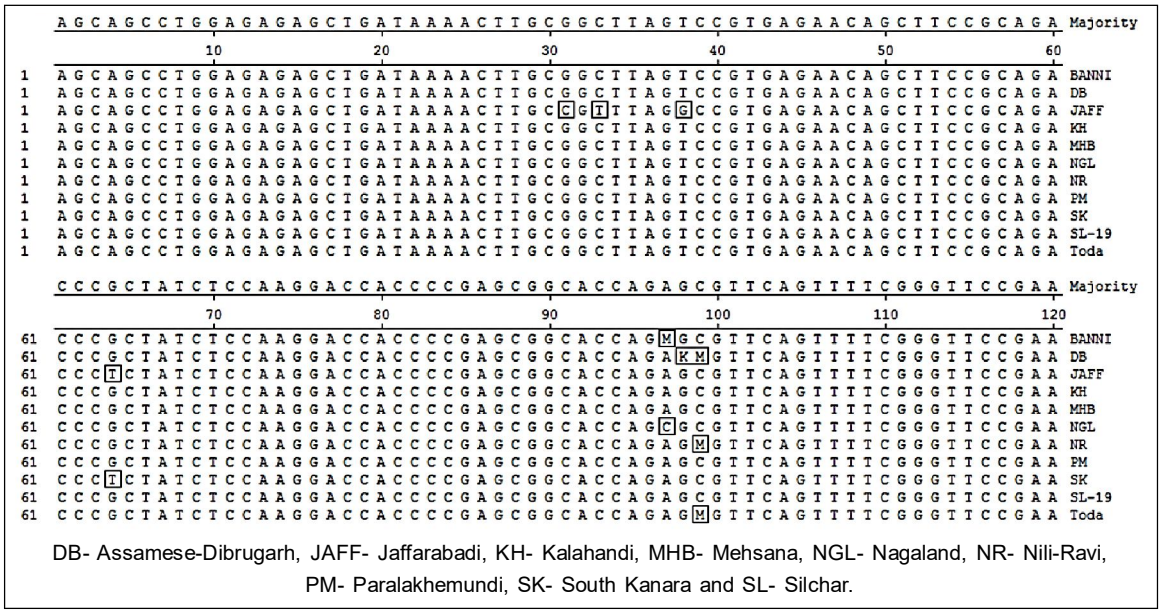
DB- Assamese-Dibrugarh, JAFF- Jaffarabadi, KH- Kalahandi, MHB- Mehsana, NGL- Nagaland, NR- Nili-Ravi, PM- Paralakhemundi, SK- South Kanara and SL- Silchar).

Fig 1: Polymorphism analysis of *HSP70* gene sequences between different buffalo breeds/populations.

transversions and the rest were translation type of variations. Whereas only 18 nucleotide variations were reported by Sodhi and Coworkers (2013) when comparing the *HSP70* of *Bubalus bubalis* with *Bos taurus*, since the study analyzed the panel with a lesser number of breeds than the present study. Similarly, higher numbers of nucleotide transversions (11 out of 22 variations) were found, when compared *Bubalus bubalis* with *Bos indicus*. Higher numbers of variable sites were identified in *Bubalus bubalis* vs *Bos taurus* compared to *Bubalus bubalis* vs. *Bos indicus,* which shows similarity with the previous reports (Sodhi *et al.,* 2013).

Important findings were extracted related to the 5' untranslated region (5′UTR) in the buffalo *HSP70* gene. As 5′UTR is known for regulating gene expression, therefore, variation in this region plays a significant role by governing the rate of transcription. Within 16 different breeds of buffaloes, a total of 5 polymorphic sites were found in the 5′ flanking region (Table 1). As compared to cattle, nucleotide sequences in the 5′UTR of *Bubalus bubalis* showed significant variation concerning nucleotide changes, but 5′UTR of buffalo was 32 nucleotides longer (204 nucleotides) compared to *Bos taurus* (172 nucleotides, accession number- NM_174550). Two insertions/deletions (INDEL) of 30 and 1 nucleotide at positions -105 to -75 and -17, respectively were the reason for the longer length of 5'UTR in the *Bubalus bubalis* (Fig 3). Three transversions and two transitions along with the INDELs were also identified in this comparative analysis between the two species. These findings are similar to those reported by Sodhi *et al*. (2013).

Further, comparative sequence analysis for bubaline *HSP70* gene coding region with other twelve different species revealed maximum homology of 98.8% with taurine cattle



DB- Assamese-Dibrugarh, JAFF- Jaffarabadi, KH- Kalahandi, MHB- Mehsana, NGL- Nagaland, NR- Nili-Ravi, PM- Paralakhemundi, SK- South Kanara and SL- Silchar.

Fig 2: Polymorphism analysis in 5′UTRs of *HSP70* gene sequences of different buffalo breeds/populations.

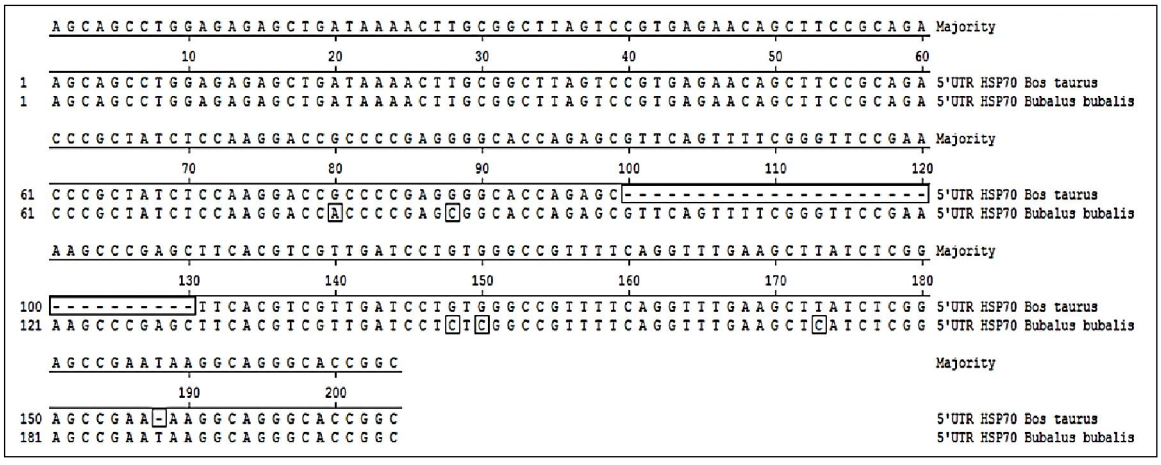


Fig 3: Comparative analysis of 5′UTRs of *HSP70* gene sequences between *Bubalus bubalis* and *Bos taurus*.

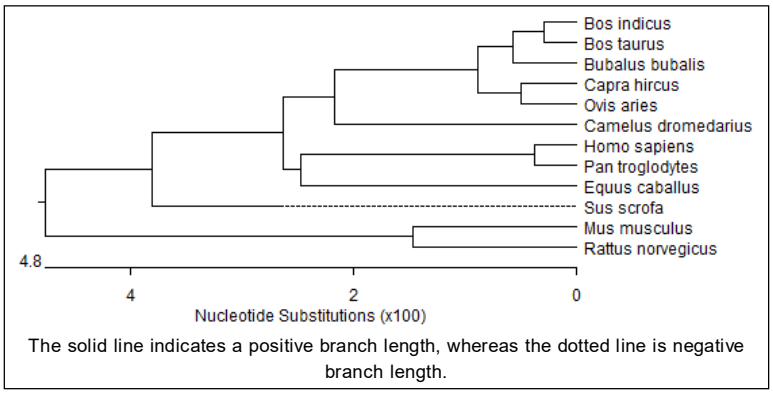


Fig 4: Phylogenetic analysis of buffalo *HSP70* gene compared with other reported species on the basis of nucleotide sequence using MegAlign by ClustalW (weighted) method.

indicating the overall high similarity of the gene among the mammalian species. Phylogenetic analysis of bubaline *HSP70* gene with different species showing the closeness of bubaline with the *Bos indicus* and *Bos taurus* (Fig 4). All ruminant species are grouped in a single major clade, while two other livestock species horse (*Equus cabalus*) and pig (*Sus scrofa*) being placed distantly.

CONCLUSION

We conclude with observing 15 and 7 SNPs in the coding region and 5′ untranslated region respectively in bubaline *HSP70*. The gene sequences showed maximum percent identity and the gene is highly conserved across all swamp and riverine buffalo breeds. The identified genetic variant adds to the current knowledge about *HSP70* variations in *Bubalus bubalis*. This work is preliminary and further research on a large number of populations can help in understanding the impact of global warming on this production-oriented livestock.

Conflict of interest

All authors declared that there is no conflict of interest.

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