Insilico analyses of heat shock protein (HSP 70) variation in Asian buffaloes

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Abstract. Heat shock proteins (HSPs) constitute a category of molecular chaperones that play a crucial role in preventing non-specific protein aggregation and facilitating the refolding of cellular proteins to maintain homeostasis. This study analyzed the genomic and proteomic characteristics of HSP70 in six Asian buffalo breeds—Murrah, Diara, Iraqi, Kalahandi, Paralakhemund, and Banni—using the NCBI database, Clustal Omega for multiple sequence alignment, SWISS modeling, and phylogenetic analysis tools. Bioinformatics analysis indicated that the HSP70 gene in buffalo is localized on chromosome 2, comprising two exons separated by a single intron. In silico analysis using the Expasy translate tool identified that HSP70 encodes a protein consisting of 641 amino acids, with molecular weights ranging from 70.28 to 70.43 kDa. The findings further revealed that the HSP70 protein in Iraqi buffalo possesses 25 variable amino acid residues, while Diara and Banni buffalo exhibit seven and three variable amino acids, respectively. This study successfully identified and characterized HSP70 across six Asian buffalo breeds, highlighting that amino acid polymorphisms in Iraqi buffaloes may be associated with phylogenetic divergence and their adaptation to distinct climatic and geographical environments.

1 Introduction

Buffalo has been a part of animal farming for hundreds of years. Buffalo are primarily categorized into two major groups: the African buffalo (*Syncerus caffer*) and the Asian buffalo (Bubalus spp.). Among them, the Asian water buffalo (Bubalus bubalis) has undergone extensive domestication and is currently distributed across 42 countries, spanning regions from the Indian subcontinent to parts of South and Central America [1]. Thermal stress poses significant challenges to the health, welfare, and productivity of water buffalo [2]. Due to their low heat tolerance, these animals are particularly vulnerable to heat stress, primarily attributed to their anatomical characteristics. The combined impact of elevated

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temperatures and high ambient humidity can lead to adverse physiological alterations, ultimately affecting their overall biological functions [3].

Heat stress negatively affects various aspects of animal productivity, including growth, milk yield, feed intake, fertility, and overall health [4]. Heat shock proteins (HSPs) function as molecular chaperones that play a crucial role in ensuring proper protein folding, repairing damaged proteins, preventing abnormal protein aggregation, and promoting the degradation of misfolded proteins via the proteasome pathway [5]. Among various heat shock proteins, heat shock protein 70 (HSP70) is crucial for fertilization and early embryonic development in mammals, especially in livestock species like pigs and cattle [6].

The HSP70 gene is responsible for encoding a family of proteins that have molecular weights between 68 and 73 kDa. In bovine species, the HSP70 protein has an approximate molecular weight of 70,190.56 Da and is composed of a total of 641 amino acids. Among these, there are 92 fundamental residues and 82 residues that are highly acidic. Furthermore, 151 amino acids in the protein exhibit hydrophilic characteristics, whereas 220 amino acids are hydrophobic [7]. The primary heat shock proteins involved in livestock thermotolerance include HSP70, HSP90, and HSP27, with HSP70 recognized as the most reliable biomarker for assessing heat stress [8]. Moreover, HSP70 serves as a critical physiological and cellular indicator of heat and humidity stress in buffaloes.

Understanding genes related to heat tolerance and the proteins they encode is essential for mitigating the adverse effects of heat stress [9]. Furthermore, genomic and proteomic analyses play a crucial role in identifying genetic variations and elucidating the hereditary basis of adaptive traits, ultimately enhancing livestock resilience to extreme environmental conditions. This study aims to characterize the genomic and proteomic properties of HSP70 and identify genetic polymorphisms across six Asian buffalo breeds that have adapted to varying degrees of heat stress. The findings may contribute to future genetic improvement strategies and provide deeper insights into the physiological mechanisms underlying buffalo adaptation to environmental challenges.

2 Material and methods

Animal selection and HSP70 properties

The data used in this study were obtained from publicly accessible and open-access databases, which means that ethical approval was not required. We conducted a comprehensive analysis of the sequence diversity of the HSP70 gene in six Asian buffalo breeds: Murrah, Diara, Iraqi, Kalahandi, Paralakhemund, and Banni. This analysis was performed using bioinformatics methods and was based on data retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/all/). The NCBI database was specifically utilized to determine the genomic locations of the HSP genes. This process involved selecting the gene ID of a target protein to identify its corresponding genomic and chromosomal positions, as detailed in Table 1.

Alignment and phylogenetic analysis

The HSP70 protein sequences were analyzed through multiple sequence alignment using the Clustal Omega database [CLUSTAL O (1.2.4)] (https://www.ebi.ac.uk/Tools/msa/clustalo/). Manual modifications were made to enhance the alignment and emphasize regions of similarity.

Protein modeling and molecular weight calculation

The SWISS-MODEL online platform (https://swissmodel.expasy.org/interactive) was used to predict the three-dimensional (3D) structure of HSP70 protein sequences, where the corresponding protein sequences were submitted. Additionally, the molecular weight of HSP70 was calculated using an online platform (https://www.bioinformatics.org/sms/prot_mw.html), which analyzes protein sequences in

FASTA format to determine their molecular weight. This method also allows for the estimation of protein positions on gels by comparing them to a reference set of proteins.

FASTA format conversion

The Phylogeny.fr tool (http://phylogeny.lirmm.fr/phylo_cgi/data_converter.pl) was employed to transform and standardize protein sequences into FASTA format, a widely accepted format in numerous databases and bioinformatics applications. Furthermore, this tool supports the processing of both nucleotide and protein sequences. In most instances, the input file format is automatically detected. However, if an error occurs indicating an unrecognized format, users can manually specify the input format instead of selecting "Automatic" or remove any blank spaces from sequence names to resolve the issue.

3 Results and discussions

The insilico analysis of genomic and proteomic of the HSP70 gene in buffaloes are located on chromosome 2 contains simple genomic structure with two exons and one intron and the GenBank reference (NC_059158.1), indicating a highly conserved genomic locus (Table 1). Proteomic analysis showed that HSP70 protein in buffaloes consists of 641 amino acids acid residues, reinforcing the fact that this protein sequence is highly conserved at the species evel. [10] stated that the HSP70 protein in river buffalo is found on chromosome 2, with a molecular weight of 70.28 kDa and made up of 641 amino acids.

Table 1. Insilico analysis of genomic and proteomic properties of HSP70 in buffaloes

Buffaloes	Genome Properties			Proteome properties		
	Protein type	Locus (Genbank)	Gene ID	Length (aa)	MW (kDa)	Accession No.
Murrah	HSP70	Chromosome 2-(NC_059158.1)	102409533	641	70.28	XP_006041955.2
Diara	HSP70	Chromosome 2-(NC_059158.1)	MH814762.1	641	70.32	AYN80160.1
Iraqi	HSP70	Chromosome 2-(NC_059158.1)	LC496272.1	641	70.43	BBN20953.1
Kalahandi	HSP70	Chromosome 2-(NC_059158.1)	MF061305.1	641	70.28	AVI01404.1
Paralakhemundi	HSP70	Chromosome 2-(NC_059158.1)	KY912034.1	641	70.28	AVI00622.1
Banni	HSP70	Chromosome 2-(NC_059158.1)	GU183098.1	641	70.37	ADQ27308.1

The molecular weight (MW) of HSP70 varies slightly, ranging from 70.28 kDa (Murrah, Kalahandi, Paralakhemundi) to 70.43 kDa (Iraqi). This small variation is likely due to differences in post-translational modifications or minor variations in the amino acid sequence. Each buffalo breed has a unique GenBank accession number for HSP70, reflecting minor differences in the characterized nucleotide or protein sequences. According to [11], HSP70 (Heat Shock Protein 70) is essential for maintaining protein homeostasis by facilitating the correct folding of newly synthesized proteins. Moreover, the physiological challenges faced by tropical livestock, including water buffalo (Bubalus bubalis), necessitate a more stringent function of this chaperone to ensure cellular homeostasis and protein stability.

This data suggests that HSP70 is a highly conserved protein among buffalo breeds, highlighting its critical role in cellular protection mechanisms, such as the thermal stress response and protein folding. Suhendro et al. [12] reported the HSP70 gene is crucial for

cellular defense mechanisms against stressors, as it regulates gene expression and prevents apoptosis, which contributes to cell survival under adverse conditions.

Although the molecular weight difference of HSP70 is relatively small in this study, the observed variation is likely related to local or environmental adaptations experienced by different buffalo breeds. For example, the Iraqi buffalo has the highest molecular weight, which may reflect genetic adaptations to specific environmental conditions.

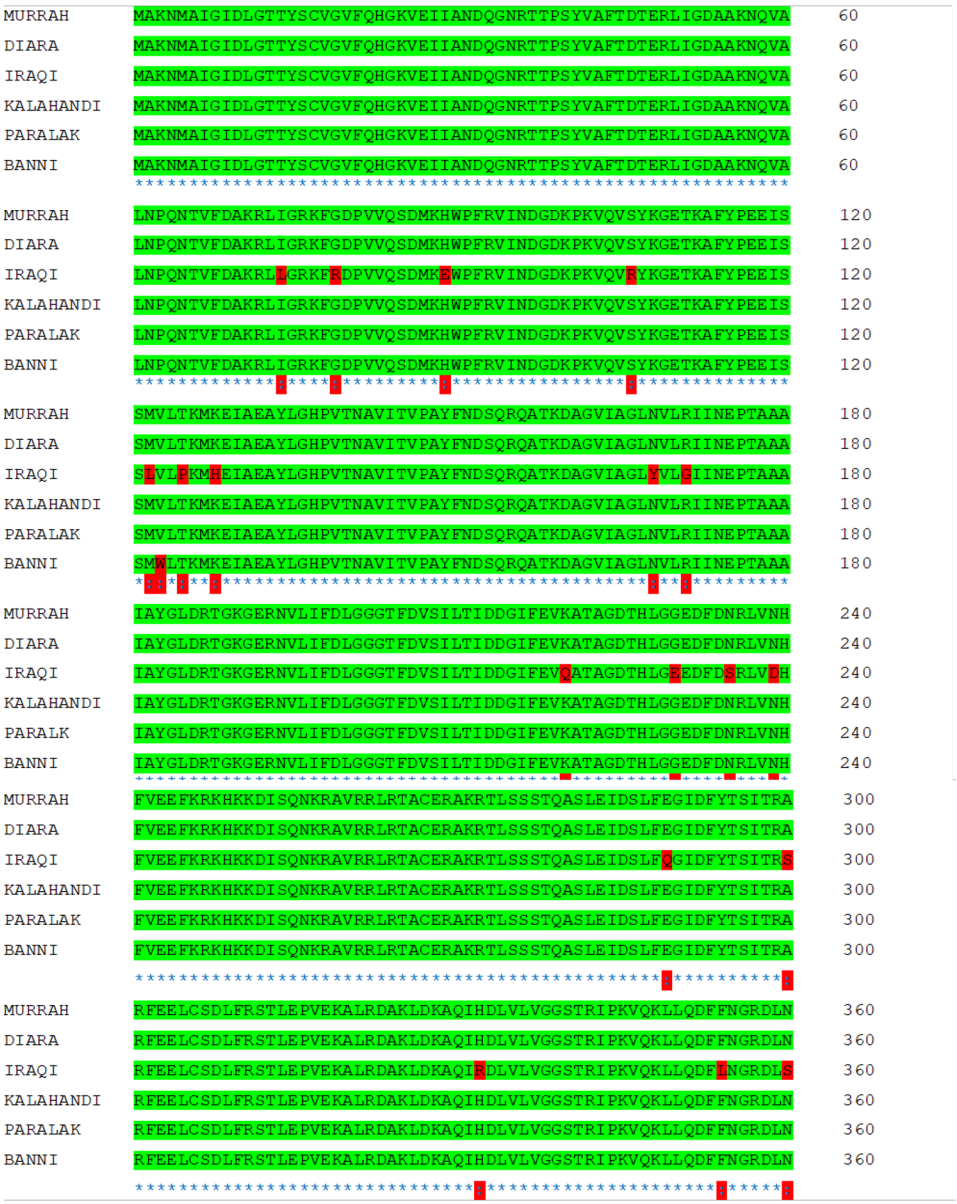

Fig. 1a. The multiple sequence alignment of HSP70 protein across six buffalo breeds highlights conserved amino acids in green and variable amino acids in red.

The high conservation of HSP70, both at the genomic and proteomic levels, suggests its potential as a universal biomarker for stress and reproductive health across all buffalo breeds.

Fatmilla *et.al.* [13] reported that the HSP70 gene is significantly associated with fertility and has the potential to be a biomarker for assessing fertility in Bali bulls. Furthermore, the small differences in the molecular weight of HSP70 could serve as a basis for further studies on functional variations among buffalo populations.

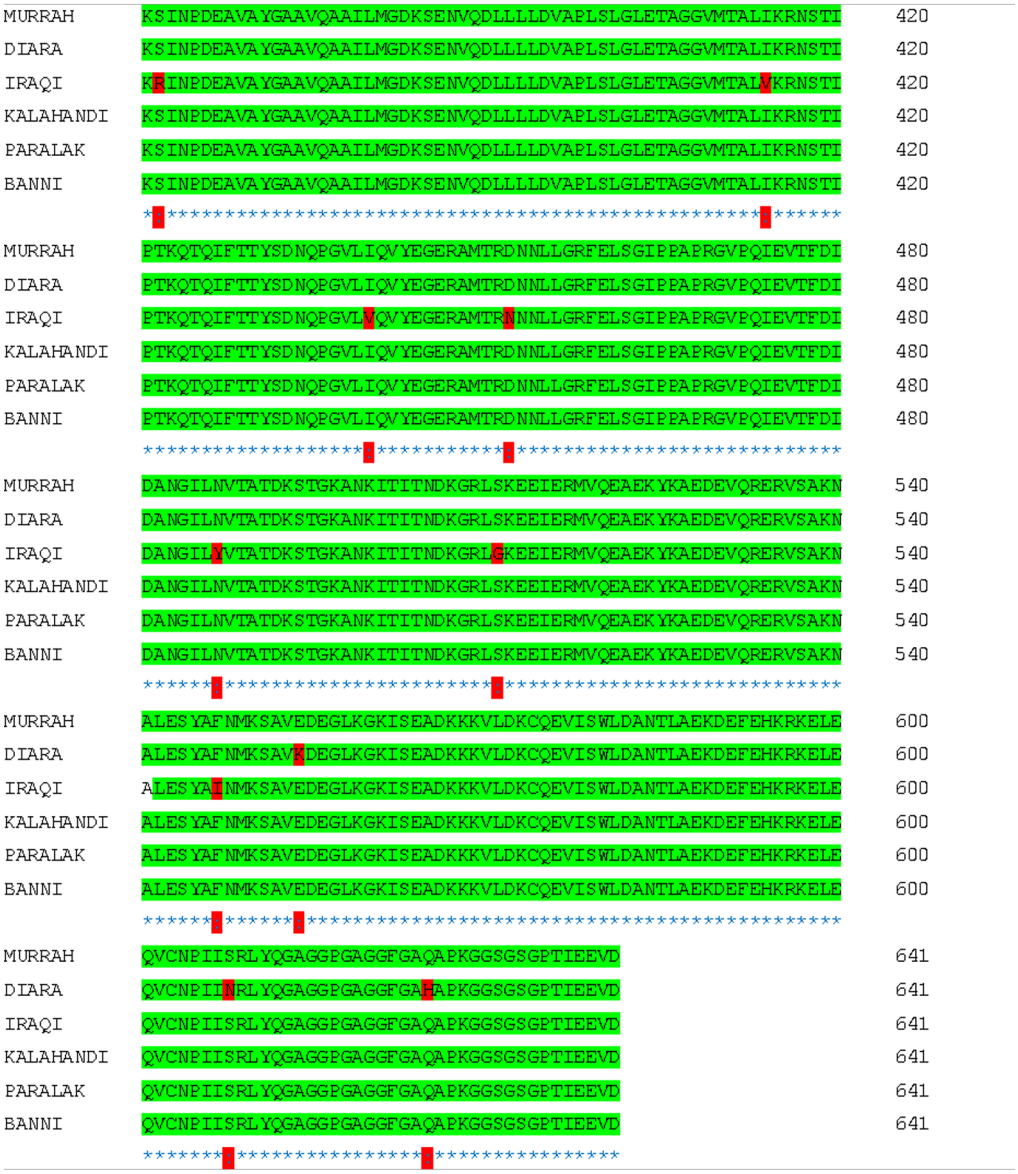

Fig. 1b. The multiple sequence alignment of *HSP70* protein across six buffalo breeds highlights conserved amino acids in green and variable amino acids in red

This study produced results similar to those of [14], who found that the HSP70 protein in bovines has a molecular weight of 70,190.56 Da. They identified 92 out of a total of 641 amino acids as highly essential. Additionally, 82 of these amino acids were classified as highly acidic. Furthermore, 151 amino acids displayed hydrophilic properties, while 220 amino acids showed no affinity for water. To identify genetic polymorphisms, HSP70 protein

sequences from six buffalo breeds were aligned. The alignment revealed that HSP70 was largely conserved, with some variations observed, particularly in the Diara, Iraqi, and Banni breeds. For instance, in Diara buffalo, three amino acid variations were identified: glutamic acid at position 559 replacing lysine, asparagine at position 608 replacing serine, and glutamine at position 625 replacing histidine (Fig. 1b). Meanwhile, in Banni buffalo, a single variation was found, with tryptophan at position 123 replacing valine (Fig. 1b).

The HSP70 protein sequence of Iraqi buffalo exhibits 25 amino acid substitutions, demonstrating genetic variations. These modifications include leucine replacing isoleucine at residue 74, glycine substituting arginine at residue 79, glutamic acid replacing histidine at residue 89, and arginine taking the place of serine at residue 106. Additional differences involve leucine replacing methionine at residue 122, proline taking the place of threonine at residue 125, histidine replacing lysine at residue 128, tyrosine substituting asparagine at residue 167, and glycine replacing arginine at residue 170. Moreover, aspartic acid is observed in place of asparagine at residue 239, serine replacing asparagine at residue 235, and glutamic acid taking the place of glycine at residue 230.

Further alterations include glutamine replacing lysine at residue 220, glutamine taking the place of glutamic acid at residue 289, serine replacing alanine at residue 300, and arginine substituting histidine at residue 331. Additionally, leucine is found in place of phenylalanine at residue 354, serine replacing asparagine at residue 360, and arginine taking the place of serine at residue 362. Other modifications include valine replacing isoleucine at residues 414 and 440, asparagine substituting aspartic acid at residue 452, tyrosine taking the place of asparagine at residue 487, glycine replacing serine at residue 511, and isoleucine substituting phenylalanine at residue 547. These amino acid variations may indicate specific genetic adaptations in Iraqi buffalo (Fig. 1ab).

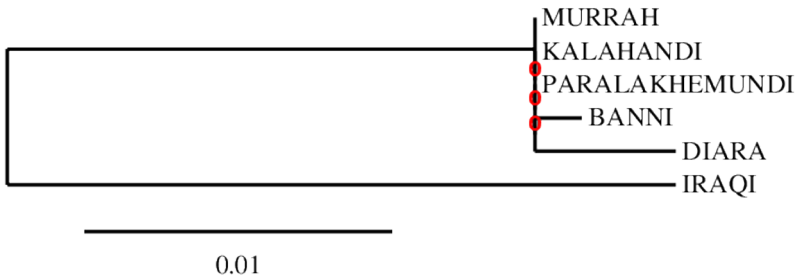

Fig. 2. The phylogenetic tree constructed using the HSP70 protein sequences

A phylogenetic analysis of HSP70 was conducted using the full-length protein sequences from six Asian buffalo breeds (Murrah, Diara, Iraqi, Kalahandi, Paralakhemund, and Banni) to explore their evolutionary relationships. The classification based on HSP70 protein sequences grouped these buffalo breeds accordingly, with Iraqi buffalo exhibiting distinct sequence variations compared to the others (Fig. 2). The findings are consistent with the study conducted by [15], which identified unique polymorphisms in the HSP70 gene of Iraqi buffalo. The phylogenetic tree analysis provides additional support for the hypothesis that Iraqi buffalo have developed genetic adaptations to suit their specific environmental conditions.

The three-dimensional (3D) structures of the HSP70 proteins were predicted using the SWISS-MODEL database. The structural modeling revealed variations between the HSP70 protein of Murrah buffalo and that of Iraqi buffalo, indicating potential differences in their structural conformation (Fig. 3). The 3D structural models of HSP70 proteins from Murrah and Iraqi buffaloes further elucidate the conserved nature of this protein while also highlighting subtle breed-specific differences. The Murrah buffalo HSP70 model exhibits a

highly ordered arrangement of α-helices and β-sheets, reflecting its conserved role in protein folding and stress response. Similarly, the Iraqi buffalo HSP70 model displays a comparable structure, but with minor variations in spatial configuration.

These structural differences may be linked to the higher molecular weight of the Iraqi HSP70 (70.43 kDa) compared to the Murrah variant (70.28 kDa). Such variations could influence the protein's functionality, potentially reflecting adaptations to different environmental stressors. The three-dimensional structure of heat shock proteins (HSPs) consists of two main domains. The nucleotide-binding domain (NBD) is located at the N-terminal and is responsible for binding nucleotides, specifically ATP and ADP. In contrast, the substrate-binding domain (SBD) is found at the C-terminal and interacts with target substrates. The SBD features a hydrophobic peptide-binding region and an α-helical subdomain, which are connected by a conserved hydrophobic linker [10].

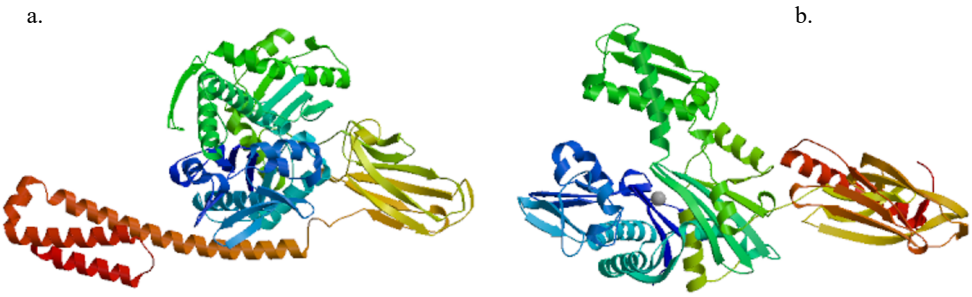

Fig. 3. The predicted three-dimensional (3D) structures of heat shock protein 70 (HSP70) in Murrah and Iraqi buffaloes were generated through computational modeling. a. HSP70 3D protein model of Murrah buffalo; b. HSP70 3D protein model of Iraqi buffalo

Overall, these findings confirm the evolutionary conservation of HSP70 across buffalo breeds and suggest that subtle structural and functional differences may play a role in breed-specific adaptations to thermal stress and reproductive challenges. The structural differences between the two proteins, including the prominence and distribution of alpha-helices and beta-sheets, as well as the presence of a potential cofactor in the right protein, suggest distinct functional roles. These variations underline the unique adaptations of each protein to its specific biological context and interaction requirements.

4 Conclusion

This study successfully identified and characterized HSP70 across six Asian buffalo breeds, highlighting that amino acid polymorphisms in Iraqi buffaloes may be associated with phylogenetic divergence and their adaptation to distinct climatic and geographical environments.

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