Electronic Journal of Biotechnology 53 (2021) 61–70

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage:

Research Article

In silico genomic and proteomic analyses of three heat shock proteins (HSP70, HSP90-α, and HSP90-β) in even-toed ungulates

Sayed Haidar Abbas Raza [a], Abdallah A. Hassanin [b], Aya I.M. Dhshan [c], Sameh A. Abdelnour [d], Rajwali Khan [e], Chugang Mei [a], Linsen Zan [a,f,*]

[a] State Key Laboratory of Animal Genetics Breeding & Reproduction, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, 712100, PR China
[b] Genetics Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt
[c] Ministry of Health and Population, Health Affairs Directorate in Sharkia, Zagazig, Egypt
[d] Department of Animal Production, Faculty of Agriculture, Zagazig University, Zagazig, Egypt
[e] Department of Livestock Management, Breeding and Genetic, The University of Agriculture Peshawar, Pakistan
[f] National Beef Cattle Improvement Center, Northwest A&F University, Yangling, Shaanxi, 712100, PR China

ARTICLE INFO

Article history:
Received 10 February 2021
Accepted 5 July 2021
Available online 13 July 2021

Keywords:
Animal
Even-toed ungulates
Genomic analyses
Heat shock protein
Hsp70
Hsp90-α
Hsp90-β
In silico
Molecular adaptation
Proteomic analyses
Thermotolerance

ABSTRACT

Background: Heat shock proteins (HSPs) play important roles in the responses to different environmental stresses. In this study, the genomic and proteomic characteristics of three HSPs (*HSP70*, *HSP90-α* and *HSP90-β*) in five even-toed ungulates (sheep, goats, water buffalo, Zebu cattle and cattle) were analyzed using Multiple sequence alignment, SWISS modeling and phylogenetics analysis tools.

Results: The bioinformatic analysis revealed that the *HSP70* gene in cattle, Zebu cattle, and goat is located on chromosome 23, and is intronless, while in water buffalo and sheep it is located on chromosomes 2 and 20, respectively, and contains two exons linked by one intron. The *HSP90-α* gene is located on chromosome 21 in cattle, Zebu cattle, and goat, while in water buffalo and sheep it is located on chromosomes 20 and 18, respectively. The *HSP90-β* gene is located on the same chromosome as the *HSP70* gene and contains 12 exons interspersed by 11 introns in all studied animals. *In silico* Expasy translate tool analysis revealed that *HSP70*, *HSP90-α* and *HSP90-β* encode 641, 733, and 724 amino acids, respectively. The data revealed that goat *HSP70* protein has seven variable amino acid residues, while in both sheep and cattle only one such amino acid was detected.

Conclusions: This study will be supportive in providing new insights into HSPs for adaptive machinery in these studied animals and selection of target genes for molecular adaptation of livestock.

How to cite: Raza SHA, Hassanin AA, Dhshan AIM, et al. *In silico* genomic and proteomic analyses of three heat shock proteins (HSP70, HSP90-α, and HSP90-β) in even-toed ungulates. Electron J Biotechnol 2021;53. https://doi.org/10.1016/j.ejbt.2021.07.002

1. Introduction

Climate change has a wide range of effects, including reducing both the quality and the quantity of pasture and water supplies, intensifying the outbreak of new pests and diseases during drought periods, and reducing the quality and quantity of products such as milk and meat, ultimately leading to economic losses in the livestock industry [1,2,3]. Animals have adapted to adverse environmental conditions by modifying their phenotypic and genotypic features over long periods [3,4]. Animals exhibit many adaptive mechanisms to survive unfavorable climatic conditions [5,6,7]. The adaptivity of animals is assessed in terms of their competence to mate in addition to their ability to survive harsh climatic conditions [8]. In terms of the adaptive ability of animals, this has been reported to involve various mechanisms, such as anatomical, physiological, behavioral, morphological, biochemical, cellular, and molecular attributes, which enable the animal to survive in a specific environment [9,10].

It has been well documented from a broad range of studies that heat stress (HS) has negative effects on animal productivity, such as growth, milk production, feed intake, fertility, and health [11]. Recently, increased concerns have been raised around the effects of HS given our growing understanding of the influence of global warming on animal production systems [6,12]. However, how and why stress has such negative effects on animals at the cellular

Peer review under responsibility of Pontificia Universidad Catœlica de ValparaÚso
* Corresponding author.
E-mail address: zanlinsen@163.com (L. Zan).

https://doi.org/10.1016/j.ejbt.2021.07.002
0717-3458/

and molecular levels has remained unclear. Identifying and understanding heat tolerance-related genes and the proteins they encode could be effective for reducing the negative impact of HS [13]. Furthermore, understanding genomic and proteomic variation could be fundamental to identifying and interpreting the genetic components of complicated adaptive traits [14].

Heat shock proteins (HSPs) are a group of molecular chaperones that avoid the aggregation of non-specific proteins and help cellular proteins to attain their native structure to maintain cellular homeostasis [5,15]. HSPs are a large protein family, which allow cells to adapt progressively to the changing environment, thereby significantly impacting on thermal adaptation and stress tolerance [16]. The cellular response to HS includes the activation of heat shock factors, improved expression of HSPs, increased levels of amino acid oxidation and glucose, reduced fatty acid metabolism, and the stimulation of immune and endocrine systems through the extracellular secretion of HSPs [5,9]. HSPs are ubiquitous proteins in almost all prokaryotic and eukaryotic organisms. Among those, *HSP70*s play housekeeping roles in protein quality control and protein folding, leading to the prevention of protein accumulation and repair of misfolded proteins [17]. The mammalian HSP90 family of proteins is a cluster of highly conserved molecules that participate in various cellular activities [18]. Their distribution in various cellular locations highlights their crucial roles in cellular homeostasis. HSP90 and its co-chaperones orchestrate essential physiological pathways such as cell cycle control, cell survival, hormone homeostasis, autophagy, and apoptosis [19]. Additionally, literature survey also shown that HSP gene expression in hair follicles in beef calves [20], peripheral blood mononuclear cell in cattle [21] provides accurate and precise facts for assessing HS and can be considered a novel indicator of HS in cattle. It was supposed that HSP genes may be expediently employed as biomarkers for assessing stress response in cattle and buffalo and the expression is species and breed-specific [21,22]. Besides, the variation in expression of HSPs is related with heat resistance and adaptation to different climatic conditions. It has been revealed that the differences in transcripts pattern of *HSP70* family and other *HSP* genes during various seasons may be mainly significant mechanism for better adaptability in Indian zebu cattle [22].

Comparative genomics and proteomics of heat shock genes and proteins between closely related animal species provide a chance to understand the evolutionary relationship of HSPs and the selective pressures that control the evolution of these genes. This work is a step towards understanding the genetic polymorphism between some species of even-toed ungulates that have undergone various types of adaptation to heat stress. This may facilitate future improvement and help to understand how organisms respond to environmental stresses.

2. Material and methods

2.1. Animal selection

To obtain a comprehensive overview of the diversity of *HSP70*, *HSP90-α* and *HSP90-β*, the sequences of these three types of heat shock protein for *Bos taurus* (cattle), *Bubalus bubalis* (water buffalo), *Bos indicus* (Zebu cattle), *Capra hircus* (goat), and *Ovis aries* (sheep) were obtained from the NCBI database for bioinformatic analysis (https://www.ncbi.nlm.nih.gov/genome/annotation_euk/all/).

2.2. Genome size, median GC%, and median protein count of selected animals

Information on the genomes of cattle, buffalo, Zebu cattle, goat, and sheep was obtained from the NCBI database by selecting Eukaryotic Genome Annotation and then Even-toed ungulates and whales (Cetartiodactyla) (Table 1).

2.3. Genomic locations of HSP genes

The genomic locations of HSP genes were determined using the NCBI database by choosing the gene ID of a certain protein to view the genomic and chromosomal position of the gene that encodes it (Table 2).

2.4. Alignment and phylogenetic analysis

For further analysis, all *HSP70*, *HSP90-α* and *HSP90-β* protein sequences were subjected to multiple alignment using the Clustal Omegadatabase [CLUSTAL O (1.2.4)] (https://www.ebi.ac.uk/Tools/msa/clustalo/) [23], assisted by some manual adjustments to indicate the regions of similarity, identifying probably functional, structural, and evolutionary relationships between sequences. A phylogenetic tree was also generated using the UniProt database (http://www.uniprot.org).

2.5. Protein modeling

Three-dimensional (3D) structures of *HSP70*, *HSP90-α* and *HSP90-β* protein sequences were predicted after submitting the protein sequence to an online tool (https://swissmodel.expasy.org/interactive) [24].

2.6. Protein molecular weight calculation

The molecular weight of the three studied heat shock proteins was calculated using an online tool (https://www.bioinformatics.org/sms/prot_mw.html) that analyzes the sequence of a protein and calculates its molecular weight by pasting the FASTA sequence into the specific text area. We can also use this method of calculating the molecular weight of proteins to predict their locations on gels in relation to a set of other proteins.

2.7. FASTA format conversion

Because most databases and programs accept only Fasta format, we used the phylogeny.fr tool (http://phylogeny.lirmm.fr/phylo_cgi/data_converter.cgi) to convert and unify the protein sequences to Fasta format. This tool accepts both nucleotide and protein sequences. (The format of the input file will be automatically detected in most cases. If an error message appears stating that the format cannot be recognized, the input format can be specified instead of choosing "Automatic" or all blank spaces can be removed from the sequence names).

Table 1
Genome size, GC% and protein count of five even-toed ungulate animals.

Organism	Genome size (Mb)	GC%	Proteins count
Bos taurus (Cattle)	2715.85	41.8685	42,497
Bubalus bubalis (Water buffalo)	2836.17	41.9	58,204
Bos indicus (Zebu cattle)	2707.15	42.1399	35,992
Capra hircus (Goat)	2932.26	42.0742	42,687
Ovis aries (Sheep)	2767.82	42.2777	42,391

Table 2
Genomic and proteomic characterization of heat shock protein 70, heat shock protein 90-alpha and heat shock protein 90-beta of five even-toed ungulate animals.

Organism	Genomic properties					Proteomic properties		
	Protein type	Genomic locus	Gene ID	Exons	Introns	Protein length (aa)	Molecular weight (kDa)	Accession No.
Cattle	HSP70	Chromosome 23-NC_037350.1	282254	1	-	641	70.27	NP_976067.3
	HSP90-alpha	Chromosome 21-NC_037348.1	281832	11	10	733	84.74	NP_001012688.1
	HSP90-beta	Chromosome 23-NC_037350.1	767874	12	11	724	83.26	NP_001073105.1
Water buffalo	HSP70	Chromosome 2-NC_037546.1	102409533	2	1	641	70.28	XP_006041955.2
	HSP90-alpha	Chromosome 20-NC_037564.1	102409833	11	10	733	84.76	XP_025127285.1
	HSP90-beta	Chromosome 2-NC_037546.1	102389823	12	11	724	83.26	XP_006069362.2
Zebu cattle	HSP70	Chromosome 23-NC_032672.1	109577026	2	1	641	70.28	QGW08889.1
	HSP90-alpha	Chromosome 21-NC_032670.1	109575457	11	10	733	84.74	XP_019839158.1
	HSP90-beta	Chromosome 23-NC_032672.1	109577125	12	11	724	83.26	XP_019841450.1
Goat	HSP70	Chromosome 23-NC_030830.1	100860849	1	–	641	70.37	AEX55800.1
	HSP90-alpha	Chromosome 21-NC_030828.1	100860851	11	10	733	84.74	XP_017921728.1
	HSP90-beta	Chromosome 23-NC_030830.1	100861006	12	11	724	83.26	XP_005696415.1
Sheep	HSP70	Chromosome 20-NC_040271.1	100913152	1	–	641	70.31	NP_001254803.1
	HSP90-alpha	Chromosome 18-NC_040269.1	100127209	11	10	733	84.74	XP_027813217.1
	HSP90-beta	Chromosome 20-NC_040271.1	101117797	12	11	724	83.26	XP_004018903.1

3. Results

3.1. Genome characteristics of even-toed ungulates

According to data published on the NCBI database, the genome of *Bos taurus* (cattle) is 2715.85 Mbin size, distributed over 29 pairs of somatic chromosomes and two sex chromosomes, with a median protein count of about 42,497 and a median GC% of 41.86% (Table 1). The genome of *Capra hircus* (goat) is 2932.26 Mb in size, distributed over 29 pairs of somatic chromosomes and two sex chromosomes, with a median protein count of about 42,687 and a median GC% of 42.0742% (Table 1). The genome of *Ovis aries* is 2767.82 Mb in size, organized in 26 somatic chromosomes and two sex chromosomes, with a median protein count of about 42,391 and a median GC% of 42.27% (Table 1). The genome of *Bubalus bubalis* (water buffalo) is 2836.17 Mb in size, organized in 24 pairs of somatic chromosomes and two sex chromosomes, with a median protein count of about 58,204 and a median GC% of 41.9% (Table 1). The genome of *Bos indicus* (Zebu cattle) is 2707.15 Mb in size, distributed over 29 pairs of somatic chromosomes and two sex chromosomes, with a median protein count of about 35,992 and a median GC% of 42.1399% (Table 1).

3.2. Genomic analysis of HSP70, HSP90-α and HSP90-β genes

The genomic locations (Fig. 1) and numbers of exons and introns were determined using bioinformatic tools in the five studied even-toed ungulates (Table 2). The bioinformatic analysis illustrated that the *HSP70* gene in cattle, Zebu cattle, and goat is located on chromosome 23 and lacks introns in cattle, goat, and sheep, while this gene in water buffalo is located on chromosome 2 and contains two exons. The analysis also showed that the *HSP70* gene in sheep is located on chromosome 20 and lacks introns.

Regarding *HSP90-α*, the bioinformatic analysis showed the presence of 11 exons and 10 introns in all studied animals. The *HSP90-α* gene is located on chromosome 21 in the case of cattle, Zebu cattle, and goat, while it is located on chromosome 20 in water buffalo and on chromosome 18 in sheep. Interestingly, the *HSP90-β* gene is located on the same chromosome as the *HSP70* gene, but contains one more exon (12 exons and 11 introns) in all studied animals.

3.3. Proteomic analysis of HSP70, HSP90-α, and HSP90-β proteins

Proteomic analysis of the HSPs showed that all *HSP70* proteins consist of 641 amino acids in all of the studied even-toed ungulates. The analysis showed that *HSP70* proteins differ a little in their molecular weight: 70.27, 70.28, 70.28, 70.37, and 70.31 kDa in cattle, water buffalo, zebu cattle, goat, and sheep, respectively. This variation in molecular weight results from variation in particular amino acids, especially in goat, cattle, and sheep *HSP70*s (Table 2, Fig. 2). Proteomic analysis of *HSP90-α* showed that all *HSP90-α* proteins consist of 733 amino acids in all studied even-toed ungulates.

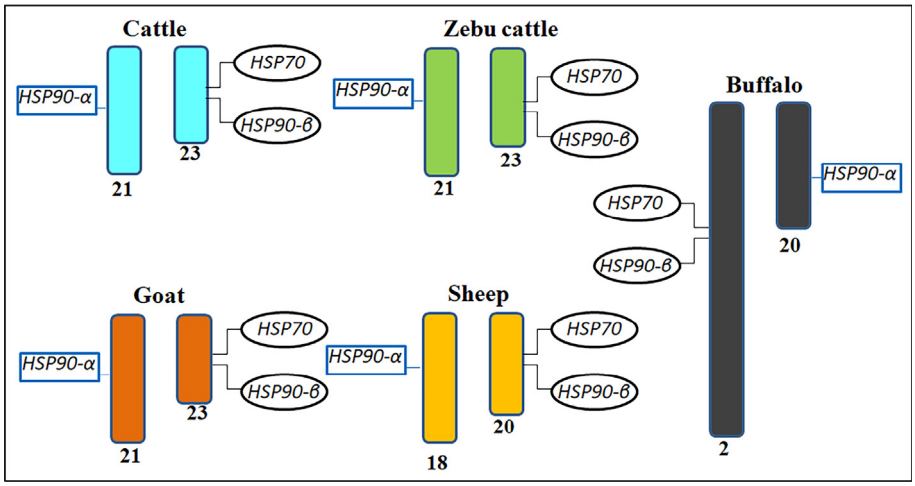

Fig. 1. Genomic location of *HSP70*, *HSP90*-alpha and *HSP90*-beta genes on autosomes 2, 18, 20, 21 and 23 of even-toed ungulate animals.

The analysis showed that *HSP90-α* proteins have a molecular weight of84.74 kDa, except for *HSP90-α* in water buffalo, which has a molecular weight of 84.76 kDa because of the presence of one more carbon atom in the variable amino acid glutamic acid instead of aspartic acid (Table 2, Fig. 3). Moreover, the computational analysis of *HSP90-β* showed that all *HSP90-β* proteins consist of 724 amino acids in all of the studied even-toed ungulates, with a molecular weight of 83.26 kDa (Table 2, Fig. 4).

3.4. Alignment and phylogenetic analysis

Heat shock proteins from the same family were aligned together to determine the genetic polymorphism among the five even-toed ungulates (Fig. 2). The alignment showed that all *HSP70* protein sequences had mostly conserved amino acid residues, along with some variable amino acids, especially in *HSP70* of goat, sheep, and cattle. Goat *HSP70* contains seven variable

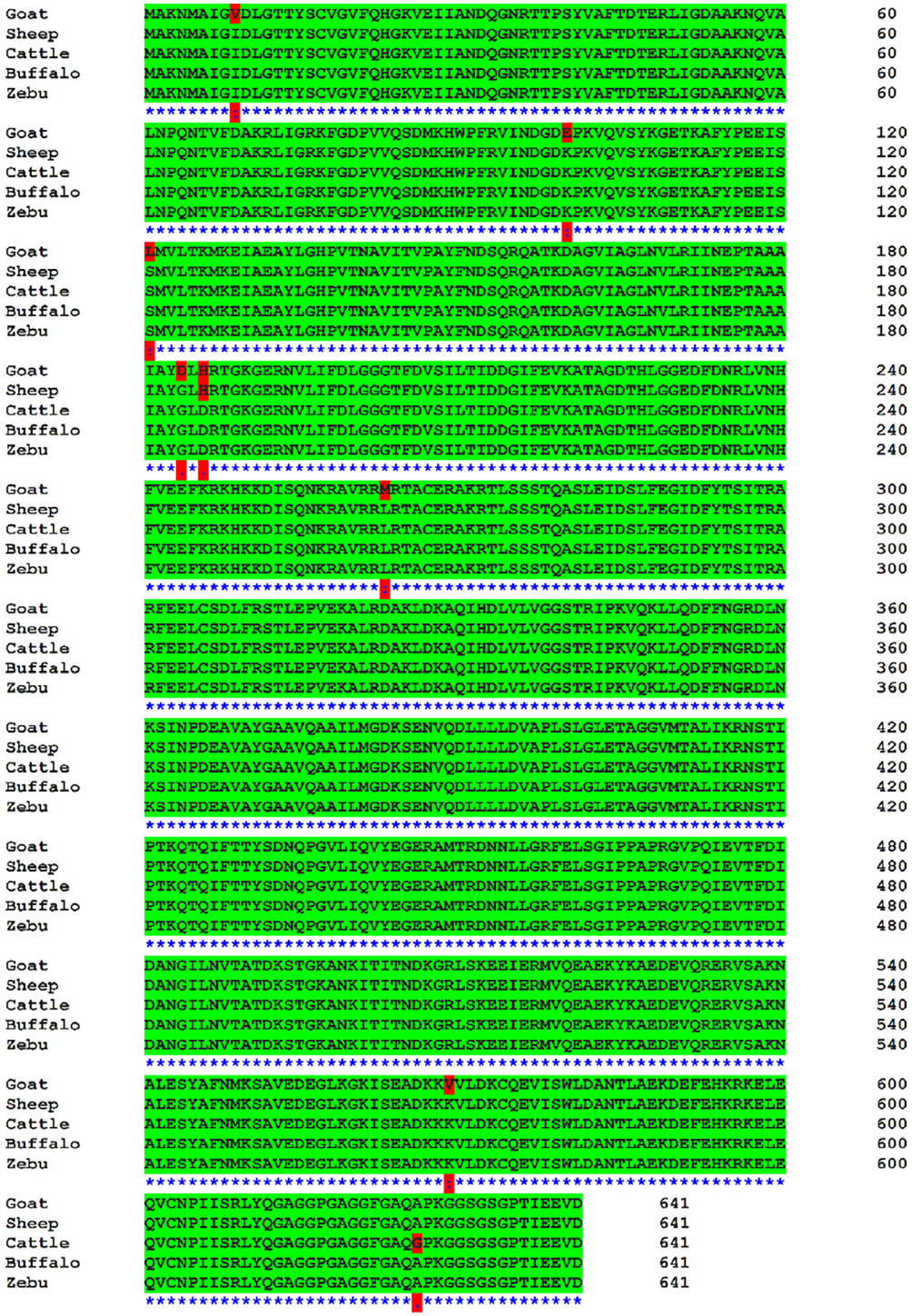

Fig. 2. Multiple sequence alignment of HSP70 protein in five Even-toed ungulate animals indicating the conserved (green) and variable (red) amino acids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

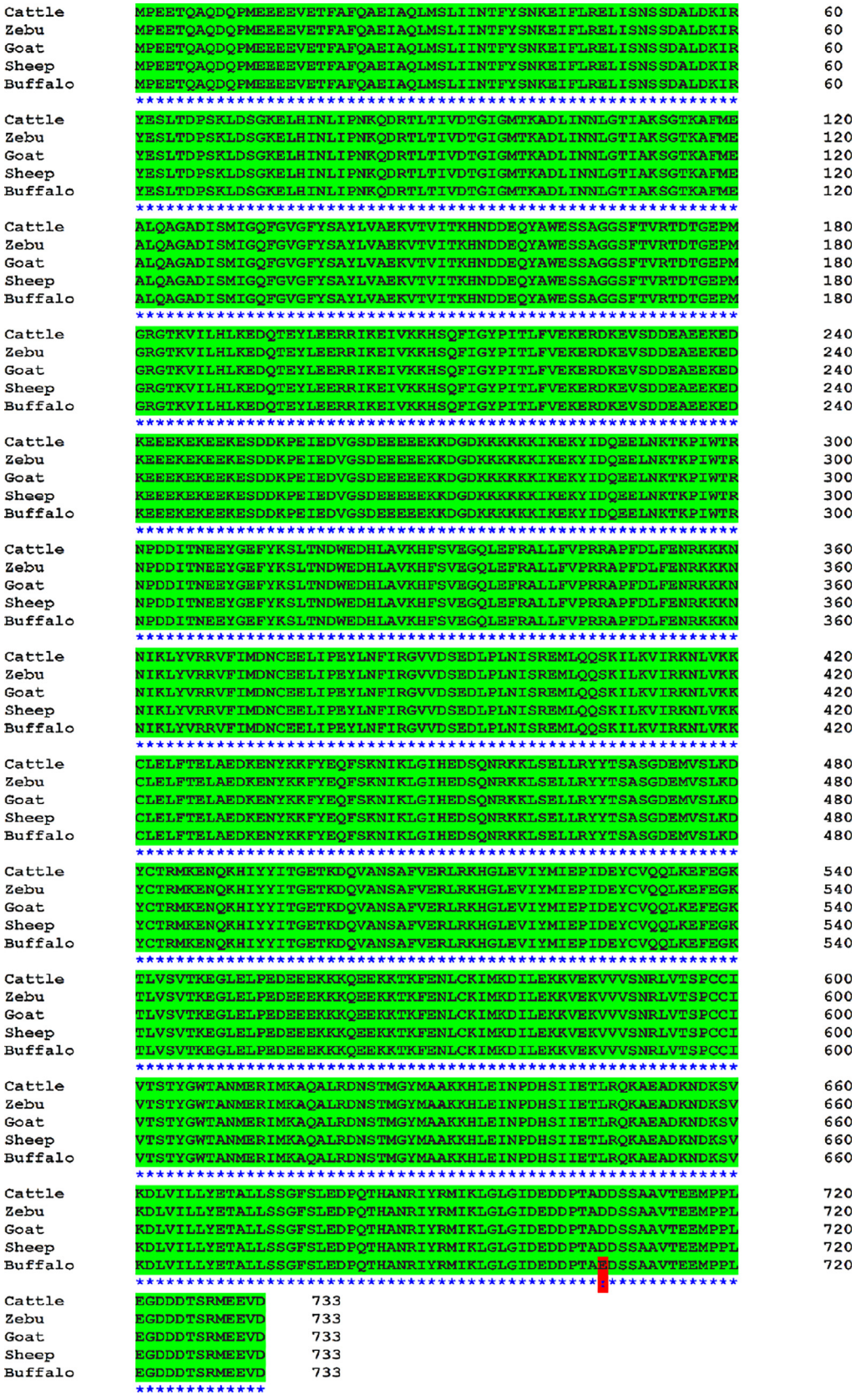

Fig. 3. Multiple sequence alignment of HSP90-alpha protein in five even-toed ungulate animals indicating the conserved (green) and variable (red) amino acids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

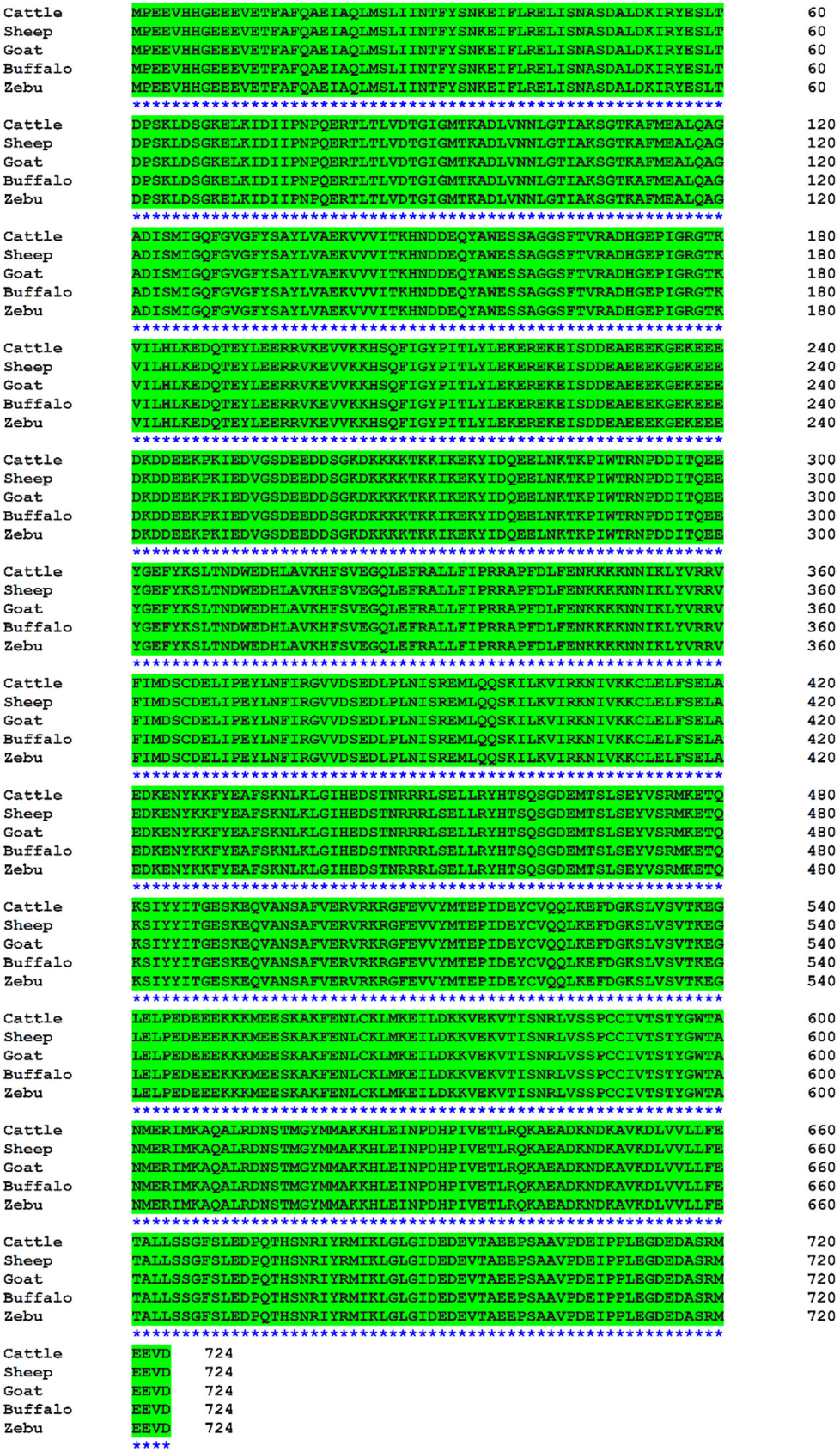

Fig. 4. Multiple sequence alignment of HSP 90-beta protein in five even-toed ungulate animals indicating the conserved (green) amino acids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

amino acids: valine at position 9 instead of isoleucine, glutamic acid at position 100 instead of lysine, leucine at position 121 instead of serine, aspartic acid at position 184 instead of glycine, histidine at position 186 instead of aspartic acid, methionine at position 263 instead of leucine, and valine at position 569 instead of lysine. *HSP70* of sheep contains one variable amino acid: histidine at position 186 instead of aspartic acid. Finally, *HSP70* of cattle contains one variable amino acid: glycine at position 626 instead of alanine (Fig. 2). All *HSP90-α* protein sequences showed conserved amino acid residues with only one variable amino acid, namely, glutamic acid at position 707 instead of aspartic acid in *HSP90-α* of water buffalo (Fig. 3). All *HSP90-β* protein sequences showed completely conserved amino acid residues (Fig. 4). Phylogenetic analysis of *HSP70*, *HSP90-α*, and *HSP90-β* was performed based on the full length of protein sequences of *Bos taurus, Bubalus bubalis, Capra hircus, Ovis aries,* and *Bos indicus* to investigate their potential evolutionary relationships. Phylogenetic analysis based on protein sequences of HSPs classified the total HSPs into three families (Fig. 5A).

3.5. Heat shock protein modeling

Three-dimensional (3D) structures of *HSP70*, *HSP90-α*, and *HSP90-β* proteins were generated using the SWISS-MODEL database. The three-dimensional (3D) structure of HSPs includes two main domains: a domain that binds nucleotides located at the N-terminal (NBD) and a domain that binds substrate located at the C-terminal (SBD) (Fig. 5B). The NBD forms a V-shaped structure, in the center of which nucleotides (ATP or ADP) are bound. The SBD consists of a hydrophobic peptide and an α-helical subdomain (SBD-α) that regulates the binding of the substrate to the SBD-β. Both the NBD and the SBD are bound by a conserved hydrophobic linker.

4. Discussion

With the increasing temperature and scarcity of water resources across the planet in the context of climate change, farmers are searching for different livestock species with suitable

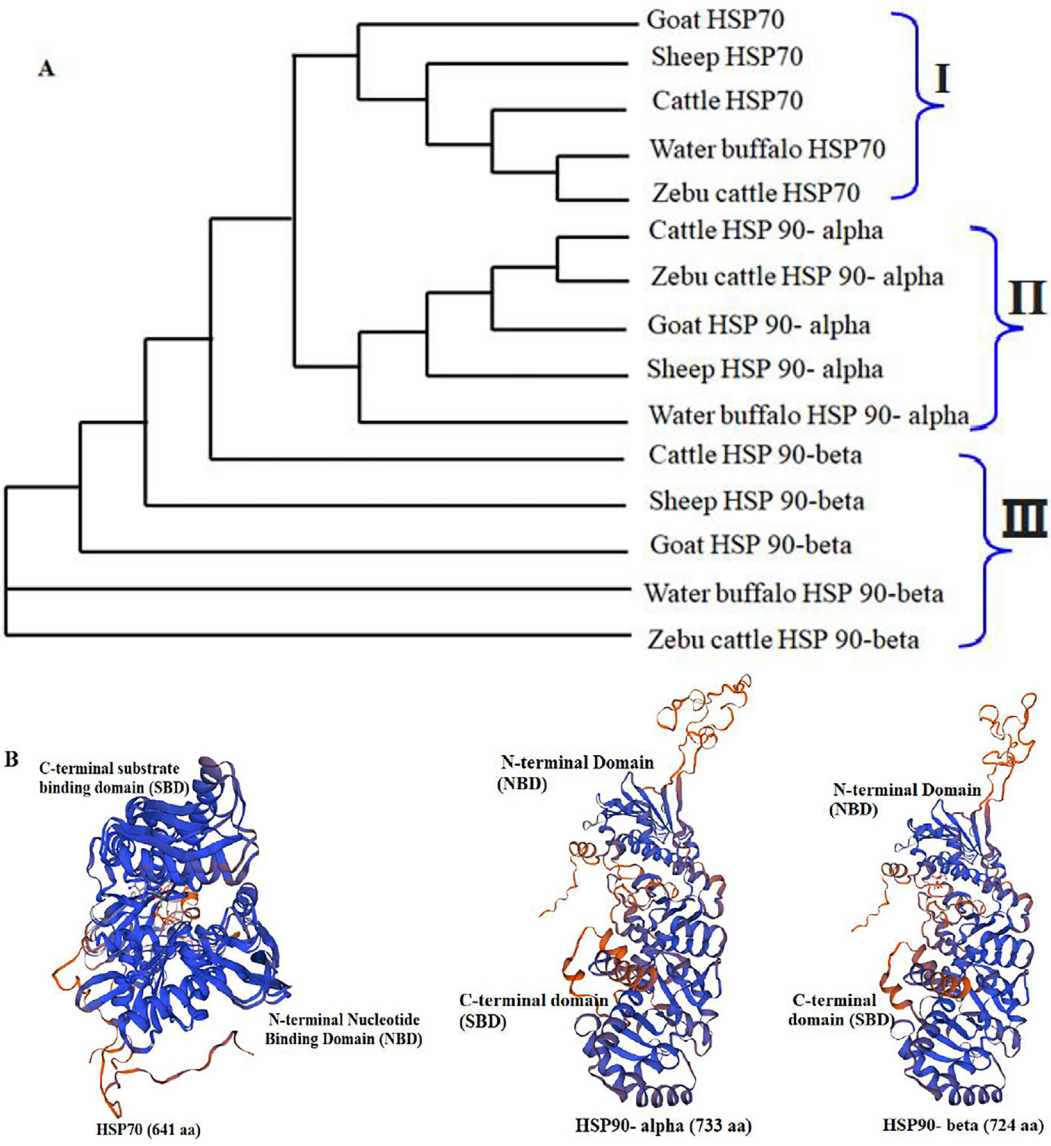

Fig. 5. Phylogenetic tree and modeled 3D structures of heat shock proteins of five even-toed ungulate animals. A. Phylogenetic tree based on the sequence of heat shock protein 70, heat shock protein 90-alpha and heat shock protein 90-beta. B. The 3D structure of HSP70, HSP90-alpha and HSP90-beta proteins.

genotypes that confer greater adaptability and higher productivity despite the associated climatic stresses. The selection of animals based on their genetic adaptability might improve sustainability in livestock systems in order to secure a food supply that can cope with the increasing global population [4,5]. In the present study, we focused on animals making significant contributions to the food supply globally, namely, cattle, Zebu cattle, buffalo, sheep, and goat. Various attempts have been made to understand how these animals adapt to and withstand dehydration, heat stress, and other harsh environmental conditions [25,26]. At the anatomical level, several adaptations have been identified in these animals [25]. However, in terms of molecular adaptation, there is a scarcity of data regarding the molecular mechanisms behind the adaptation in these animals. To obtain a clearer picture of this molecular adaptation, we selected key molecules (HSPs) that play a major role in cellular stress resistance and are broadly expressed in many cell and tissue types.

Based on the genomic screening of these animals, the present analysis revealed that even-toed ungulates differ in their genome size and GC content, which means that they may have variable characteristics. A GC-rich genome is considered a good marker for thermostable DNA. In eukaryotes, GC-rich regions are gene-rich, thermostable, and actively transcribed. The substitution pressure in the human genome is generally AT-biased [27,28], which may explain why most of the genome is AT-rich. The analysis performed here revealed that sheep has the highest GC content, while water buffalo has the lowest; this may explain the wider spread of sheep across a range of climatic and geographical conditions compared with that of buffalo, which is widespread in swamps. Recently, it has been confirmed that the thermostability of genes in animals increases more slowly with increasing GC content than that in random sequences, whereas their bendability increases faster [29].

An important finding of this study is that the *HSP70* gene lacks introns in all studied even-toed ungulates, except in water buffalo. The absence of non-coding regions (introns) has been considered as a typical characteristic for the majority of *HSP* genes, but some exceptions to this have been reported [26]. As reported by Evgen'ev et al. [26], because the mechanism of RNA splicing is affected by various stresses, the transcripts of intron less HSP genes can easily be transported from the nucleus through the nuclear membrane to the cytoplasm without splicing, resulting in selection for intron less genes over the course of evolution. In contrast, some studies reported that the presence of introns in genes allowed greater mRNA accumulation than their absence [30]. In this study, we did not find introns in *HSP70* genes in cattle, zebu cattle, goat, and sheep, while we found that the *HSP70* gene in buffalo had one intron and the other *HSP90-α* and *HSP90-β* genes had 10–11 introns (Table 2). Although we have not explored the association of genetic variants of the HSP genes with traits related to environmental adaptability, based on *in silico* analysis, we can predict that the polymorphism in *HSP70* and *HSP90-α* protein sequences among even-toed ungulates may reflect the self-protection mechanism present in livestock under adverse environmental conditions. It has been revealed that the differences in transcripts pattern of *HSP70* family and other *HSP* genes during various seasons may be mainly significant mechanism for better adaptability in Indian zebu cattle [22].

The alignment of *HSP70* and *HSP90-α* proteins revealed that there is complete conservation between these two proteins in zebu cattle, which may explain why this species may have unique characteristics in its adaptability to environmental conditions [22,31]. In addition, it has been proven that *Bos indicus* has unique thermo tolerance in comparison to cattle breeds. *Bos indicus* is known to be able to survive in arid areas and can adapt to a high temperature and harsh climatic conditions [4,32,33]. The most important genetic adaptations that have developed over the course of Zebu cattle evolution include the acquisition of genes for thermo tolerance. Cattle from Zebu breeds are better able to regulate their body temperature in response to heat stress than are cattle from a variety of B. taurus breeds of European origin [25]. Moreover, the differences in anatomy such as body size, color, number of sweat glands, and physiological parameters, for example, skin, body temperatures pulse and respiration rates in animals showed variable response to environmental stressors [34,35,36]. It was reported that adverse environmental conditions could induce mis- or unfolded proteins in cells; however, HSPs can eliminate or limit this. Hence, understanding the genetic architecture of HSPs in different animals could clarify the variation in adaptability and survivability under harsh conditions among species [16,18]. Here, we demonstrated a small difference in the molecular weight of *HSP70* among all studied species. Variation in the *HSP70* profile among the studied animals might be responsible for their differences in thermo tolerance, especially in cattle, sheep, and goats (Fig. 2).

Nellore cows derived from *Bos indicus* have unique characteristics at the anatomical and molecular levels regarding heat loss under heat stress conditions [32]. It has been demonstrated that the efficiency of heat dissipation is principally related to sweating capacity and the cellular adaptation was approved by the cellular maintenance of *HSP70* in Nellore cows [37]. Studies have revealed that *HSP70* is an important molecular chaperone for the development of mammalian cells and protects cells upon exposure to cold stress, which can denature proteins [16,18].

Two isoforms of *HSP90* (*HSP90-α* and *-β*) were identified in all studied animals in the present computational analysis. Regarding the molecular weight of *HSP90-α*, the results revealed that *HSP90-α* in the water buffalo (84.74 kDa) has a higher molecular weight than that in the other animals. This variation is associated with the presence of one more carbon atom in the variable amino acid glutamic acid instead of aspartic acid (Fig. 3). It has been reported that *HSP90* is present in the cytoplasm of male germinal cells and could protect them against the harmful effects of HS in rabbits [38]. *HSP90* has also been demonstrated to play a pivotal role in providing heat tolerance to livestock [39]. In addition, *HSP90* modulates the cell response by being a key part of signal transduction and interacting with protein kinases and transcription factors [40].The dynamics of *HSP90* involving its elevation under stress conditions showed that it is involved in the first step of protection of cells and the body [5,39]. It was also suggested that *HSP70* and *HSP90* collaborate and undergo crosstalk in order to protect against burdensome environmental conditions in cattle [39]. In Nigerian zebu cattle Onasanya et al. [41] reported that the heterozygotic SNP genotypes at distinct spots within the nucleotide sequences of exon 3 of HSP 90 gene conceivably contributed to the lower thermoregulatory response. Likewise, studies shown that the genetic variability in cattle at HSP 90 has been reported elsewhere [42].

Moreover, these results reveal the presumed impacts of selective selection (both artificial or natural selection) on the *HSP70* gene to eventually favor animals with superior thermo tolerance, and stress resilience. Our outcomes are in line with several studies on buffalo that described evidence for the selection of genes associated with digestive physiology, social behavior, and heat tolerance [43,44].

The present computational analysis of *HSP90-β* showed that all *HSP90-β* proteins consist of 724 amino acids in all studied even-toed ungulates, having a molecular weight of 83.26 kDa (Fig. 4). This might reflect the insignificant role of the isoform *HSP90-β* in the regulation and response of cells to adverse environmental impacts. Buffaloes are native of tropical areas and it is accepted that riverine buffalo (*B. bubalis*) is not particularly heat-tolerant.

A wide range of research has indicated that physiological, productive, and reproductive features are decreased up on exposure to a high environmental temperature in buffalo [10,34]. It has been suggested that the relative susceptibility of buffalo to HS is due to their dark skin and fewer hair follicles and sweat glands compared with the levels in cattle [45]. It was observed that the *HSP90* gene family of buffalo had aliphatic index greater than 65, demonstrating the stability of proteins at high temperatures [44]. This might be associated with the extent of thermostability of HSPs in buffalo [46]. Furthermore, the genomic identification for HSPs of buffalo, Nadeem et al. [44] found that a highly negative grand average of hydropathicity index values of *HSP90* indicating the superior hydrophilic nature of its members. It can assist to boost the functional actions of protein oligomerization and binding. Results regarding the functional properties of HSPS in different animals need further investigation for enhancing our knowledge about the molecular mechanisms to select the thermo-tolerance of animals against the upcoming climate change years. However, the genetic mechanisms responsible for thermo tolerance and the physiological structures resisting thermal stress remain incompletely understood.

5. Conclusion

This study successfully identified and characterized three types of heat shock protein (*HSP70, HSP90-α,* and *HSP90-β* in five even-toed ungulates. The variation in *HSP70* and *HSP90-α* proteins observed in most studied animals, the presence or absence of introns in HSP genes, and the GC content in their genomes may correlate with and explain the distribution of some animals in different climatic and geographical conditions and/or provide an advantage in response to the selective pressure imposed by particular environmental conditions. Building on the computational analysis of these HSPs, there is a need for more in-depth exploration of the mechanisms of molecular adaptation in these animals in order to enhance the genetic breeding to counteract the negative influences of elevated temperature associated with climate change.

Ethical approval

The data used in this study were from an open-access and publicly available database, so the need for approval from an ethics committee was waived.

Financial support

This work was supported by Key R&D Program of Shaanxi Province [2019NY-098], the National Key R&D Program of China [2018YFD0501700] and the National Natural Science Foundation of China [31301937].

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

All authors acknowledge and thank their respective institutes and universities.

References

[1] Wolfenson D, Roth Z, Meidan R. Impaired reproduction in heat-stressed cattle: basic and applied aspects. Anim Reprod Sci 2000;60:535–47. https://doi.org/10.1016/S0378-4320(00)00102-0.

[2] Adams F, Ohene-Yankyera K. Socio-economic characteristics of subsistent small ruminant farmers in three regions of northern Ghana. Asian J Appl Sci Eng 2014;3:351–64. https://doi.org/10.15590/ajase/2014/v3i8/54489.

[3] Aleena J, Sejian V, Bagath M, et al. Resilience of three indigenous goat breeds to heat stress based on phenotypic traits and PBMC HSP70 expression. Int J Biometeorol 2018;62:1995–2005. https://doi.org/10.1007/s00484-018-1604-5 PMid: 30178111.

[4] Sejian V, Bhatta R, Gaughan JB, et al. Adaptation of animals to heat stress. Animal 2018;12:s431–44. https://doi.org/10.1017/S1751731118001945 PMid: 30139399.

[5] Abdelnour SA, Abd El-Hack ME, Khafaga AF, et al. Stress biomarkers and proteomics alteration to thermal stress in ruminants: A review. J Therm Biol 2019;79:120–34. https://doi.org/10.1016/j.jtherbio.2018.12.013 PMid: 30612672.

[6] Abdelnour SA, El-Saadony MT, Saghir SAM, et al. Mitigating negative impacts of heat stress in growing rabbits via dietary prodigiosin supplementation. Livest Sci 2020;240:104220. https://doi.org/10.1016/j.livsci.2020.104220.

[7] Sheiha AM, Abdelnour SA, Abd El-Hack ME, et al. Effects of dietary biological or chemical-synthesized nano-selenium supplementation on growing rabbits exposed to thermal stress. Animals 2020;10:430. https://doi.org/10.3390/ani10030430 PMid: 32143370.

[8] McManus C, Paludo GR, Louvandini H, et al. Heat tolerance in Brazilian sheep: Physiological and blood parameters. Trop Anim Health Prod 2009;41 (1):95–101. https://doi.org/10.1007/s11250-008-9162-1 PMid: 19052907.

[9] Collier RJ, Collier JL, Rhoads RP, et al. Genes involved in the bovine heat stress response. J Dairy Sci 2008;91:445–54. https://doi.org/10.3168/jds.2007-0540 PMid: 18218730.

[10] Das R, Sailo L, Verma N, et al. Impact of heat stress on health and performance of dairy animals: A review. Vet World 2016;9:260–8. https://doi.org/10.14202/vetworld.2016.260-268 PMid: 27057109.

[11] Sharma AK, Rodriguez LA, Wilcox CJ, et al. Interactions of climatic factors affecting milk yield and composition. J Dairy Sci 1988;71(3):819–25. https://doi.org/10.3168/jds.S0022-0302(88)79622-8.

[12] West JW. Effects of heat-stress on production in dairy cattle. J Dairy Sci 2003;86:2131–44. https://doi.org/10.3168/jds.S0022-0302(03)73803-X.

[13] Gaughan JB, Mader TL, Holt SM, et al. Heat tolerance of Boran and Tuli crossbred steers. J Anim Sci 1999;77:2398–405. https://doi.org/10.2527/1999.7792398x PMid: 10492446.

[14] Hayes BJ, Bowman PJ, Chamberlain AJ, et al. A validated genome wide association study to breed cattle adapted to an environment altered by climate change. PLoS ONE 2009;4:e6676. https://doi.org/10.1371/journal.pone.0006676 PMid: 19688089.

[15] Shinozaki K, Yamaguchi-Shinozaki K. Molecular responses to drought stress. In: Satoh K, Murata N eds. Stress Responses of Photosynthetic Organisms 1998:149-163. ISBN 9780444828842 https://doi.org/10.1016/B978-0-444-82884-2.50013-3.

[16] Hoter A, Amiri M, Prince A, et al. Differential glycosylation and modulation of camel and human HSP isoforms in response to thermal and hypoxic stresses. Int J Mol Sci 2018;19:402. https://doi.org/10.3390/ijms19020402 PMid: 29385708.

[17] Mayer MP, Bukau B. Hsp70 chaperones: cellular functions and molecular mechanism. Cell Mol Life Sci 2005;62:670–84. https://doi.org/10.1007/s00018-004-4464-6 PMid: 15770419.

[18] Hoter A, El-Sabban ME, Naim HY. The HSP90 family: structure, regulation, function, and implications in health and disease. Int J Mol Sci 2018;19:2560. https://doi.org/10.3390/ijms19092560 PMid: 30158430.

[19] Buchner J, Li J. Structure, function and regulation of the hsp90 machinery. Biomed J 2013;36:106. https://doi.org/10.4103/2319-4170.113230 PMid: 23806880.

[20] Kim WS, Nejad JG, Peng DQ, et al. Identification of heat shock protein gene expression in hair follicles as a novel indicator of heat stress in beef calves. Animal 2020;14:1502–9. https://doi.org/10.1017/S1751731120000075 PMid: 32038000.

[21] Deb R, Sengar GS. Expression pattern of bta-mir-2898 miRNA and their correlation with heat shock proteins during summer heat stress among native vs crossbred cattle. J Therm Biol 2020;94:102771. https://doi.org/10.1016/j.jtherbio.2020.102771 PMid: 33293003.

[22] Kumar A, Ashraf S, Goud TS, et al. Expression profiling of major heat shock protein genes during different seasons in cattle (*Bos indicus*) and buffalo (*Bubalus bubalis*) under tropical climatic condition. J Therm Biol 2015;51:55–64. https://doi.org/10.1016/j.jtherbio.2015.03.006 PMid: 25965018.

[23] Madeira F, Park YM, Lee J, et al. The EMBL-EBI search and sequence analysis tools APIs in 2019. Nucleic Acids Res 2019;47:W636–41. https://doi.org/10.1093/nar/gkz268 PMid: 30976793.

[24] Waterhouse A, Bertoni M, Bienert S, et al. SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res 2018;46:W296–303. https://doi.org/10.1093/nar/gky427 PMid: 29788355.

[25] Hansen PJ. Physiological and cellular adaptations of zebu cattle to thermal stress. Anim Reprod Sci 2004;82:349–60. https://doi.org/10.1016/j.anireprosci.2004.04.011 PMid: 15271465.

[26] Evgen'Ev MB, Garbuz DG, Zatsepina OG. Heat shock proteins and whole body adaptation to extreme environments. Springer, Dordrecht 2014, 218 p. ISBN 978-94-017-9234-9 https://doi.org/10.1007/978-94-017-9235-6.

[27] Eyre-Walker A, Hurst LD. The evolution of isochores. Nat Rev Genet 2001;2:549–55. https://doi.org/10.1038/35080577 PMid: 11433361.

[28] Petrov DA, Hartl DL. Patterns of nucleotide substitution in Drosophila and mammalian genomes. Proc Natl Acad Sci 1999;96:1475–9. https://doi.org/10.1073/pnas.96.4.1475 PMid: 9990048.
[29] Vinogradov AE. Bendable genes of warm-blooded vertebrates. Mol Biol Evol 2001;18:2195–200. https://doi.org/10.1093/oxfordjournals.molbev.a003766 PMid: 11719569.
[30] Le Hir H, Nott A, Moore MJ. How introns influence and enhance eukaryotic gene expression. Trends Biochem Sci 2003;28:215–20. https://doi.org/10.1016/S0968-0004(03)00052-5.
[31] Singh AK, Upadhyay RC, Chandra G, et al. Genomewide expression analysis of the heat stress response in dermal fibroblasts of Tharparkar (zebu) and Karan-Fries (zebu× taurine) cattle. Cell Stress Chaperones 2020;25:327–44. https://doi.org/10.1007/s12192-020-01076-2 PMid: 32062819.
[32] Fernandes Júnior GA, de Oliveira HN, Carvalheiro R, et al. Whole-genome sequencing provides new insights into genetic mechanisms of tropical adaptation in Nellore (*Bos primigenius indicus*). Sci Reports 2020;10. https://doi.org/10.1038/s41598-020-66272-7 PMid: 32523018.
[33] Shandilya UK, Sharma A, Sodhi M, et al. Heat stress modulates differential response in skin fibroblast cells of native cattle (*Bos indicus*) and riverine buffaloes (*Bubalus bubalis*). Biosci Rep 2020;40:2. https://doi.org/10.1042/BSR20191544 PMid: 31994693.
[34] Marai IFM, Haeeb AAM. Buffalo's biological functions as affected by heat stress-A review. Livest Sci 2010;127:89–109. https://doi.org/10.3389/fcimb.2019.00318 PMid: 31572689.
[35] Pegorer MF, Vasconcelos JLM, Trinca LA, et al. Influence of sire and sire breed (Gyr versus Holstein) on establishment of pregnancy and embryonic loss in lactating Holstein cows during summer heat stress. Theriogenology 2007;67:692–7. https://doi.org/10.1016/j.theriogenology.2006.09.042 PMid: 17118436.
[36] Rocha A, Randel RD, Broussard JR, et al. High environmental temperature and humidity decrease oocyte quality in *Bos taurus* but not in *Bos taurus* cows. Theriogenology 1998;49:657–65. https://doi.org/10.1016/S0093-691X(98)00016-8.
[37] Hooper HB, Titto CG, Gonella-Diaza AM, et al. Heat loss efficiency and HSPs gene expression of Nellore cows in tropical climate conditions. Int J Biometeorol 2019;63:1475–86. https://doi.org/10.1007/s00484-018-1576-5 PMid: 30116935.
[38] Pei Y, Wu Y, Qin Y. Effects of chronic heat stress on the expressions of heat shock proteins 60, 70, 90, A2, and HSC70 in the rabbit testis. Cell Stress Chaperones 2012;17:81–7. https://doi.org/10.1007/s12192-011-0287-1 PMid: 21830018.
[39] Kumar J, Yadav B, Madan AK, et al. Dynamics of heat-shock proteins, metabolic and endocrine responses during increasing temperature humidity index (THI) in lactating Hariana (Zebu) cattle. Biol Rhythm Res 2020;51:934–50. https://doi.org/10.1080/09291016.2019.1566986.
[40] Prodromou C. Mechanisms of Hsp90 regulation. Biochem J 2016;473:2439–52. https://doi.org/10.1042/BCJ20160005 PMid: 27515256.
[41] Onasanya GO, Msalya GM, Thiruvenkadan AK, et al. Single nucleotide polymorphisms at heat shock protein 90 gene and their association with thermo-tolerance potential in selected indigenous Nigerian cattle. Trop Anim Health Prod 2020;52:1961–70. https://doi.org/10.1007/s11250-020-02222-9 PMid: 31981054.
[42] Kerekoppa RP, Rao A, Basavaraju M, et al. Molecular characterization of the HSPA1A gene by single-strand conformation polymorphism and sequence analysis in Holstein-Friesian crossbred and Deoni cattle raised in India. Turkish J Vet Anim Sci 2015;39:128–33. https://doi.org/10.3906/vet-1212-3.
[43] Luo X, Zhou Y, Zhang B, et al. Understanding divergent domestication traits from the whole-genome sequencing of swamp-and river-buffalo populations. Natl Sci Rev 2020;7:686–701. https://doi.org/10.1093/nsr/nwaa024.
[44] Nadeem A, Javed M, Hassan F, et al. Genomic identification, evolution and sequence analysis of the heat-shock protein gene family in buffalo. Genes (Basel) 2020;11:1388. https://doi.org/10.3390/genes11111388 PMid: 33238553.
[45] Basu SB. Genetic improvement of buffaloes. Genet Improv Buffaloes J.D. Turton and C.R. Henderson editor New Delhi Kalyani Publishers 1985, pp.187.
[46] Yadav VP, Dangi SS, Chouhan VS, et al. Expression analysis of NOS family and HSP genes during thermal stress in goat (*Capra hircus*). Int J Biometeorol 2016;60:381–9. https://doi.org/10.1007/s00484-015-1035-5 PMid: 26205811.