

Genetic profiling of *HSP70* gene in local Iraqi goats

Perfil genético do gene *HSP70* em cabras iraquianas locais

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Abstract

Animals display numerous physiological and behavioral responses that reduce the effects of heat stress. Moreover, genetic variance is strongly associated with responses to heat stress, including variants of heat shock proteins (HSPs) that are necessary for thermoregulation and stress resistance. Herein, we performed the molecular profiling of the *HSP70* gene, and its polymorphism was demonstrated as a possible factor in the stress tolerance of local Iraqi goats. A number of different mutations were found owing to seven main polymorphisms. Results indicated the occurrence of silent and missense mutations in sequences obtained for Iraqi local goats. Genetic diversity was observed in the *HSP70* gene of Iraqi local goats on the basis of phylogenetic-tree analysis as some mutations occurred once whereas others occurred multiple times. The polymorphisms LC616787, LC616788, and LC616791 were combined with the reference gene in the same branch, whereas polymorphisms (LC616785 and LC616786) and (LC616789 and LC616790) met in different branches, respectively. Moreover, all studied proteins had mismatches in their three-dimensional structures. Therefore, the presence of specific genetic differences within the *HSP70* gene in Iraqi goats can increase the possibility of selecting animals more suitable to various levels of stress.

Keywords: *HSP70* gene, goats, polymorphism, molecular analysis.

Resumo

Os animais apresentam uma série de respostas fisiológicas e comportamentais que reduzem os efeitos do estresse térmico. Além disso, a variância genética está fortemente associada às respostas ao estresse térmico, incluindo variantes de proteínas de choque térmico (HSPs) que também são necessárias para a termorregulação e resistência ao estresse. O perfil molecular do gene *HSP70* foi realizado neste estudo e o polimorfismo desse gene foi demonstrado como um possível fator na tolerância ao estresse de caprinos iraquianos. Várias mutações diferentes foram encontradas devido a sete polimorfismos principais. Os resultados indicam a ocorrência de mutações silenciosas e sem sentido em sequências obtidas para caprinos iraquianos. A diversidade genética pode ser vista no gene *HSP70* de cabras locais iraquianas com base na análise da árvore filogenética, já que algumas mutações ocorreram uma vez, enquanto outras ocorreram várias vezes. Os polimorfismos LC616787, LC616788 e LC616791 foram combinados com o gene de referência no mesmo ramo, enquanto os polimorfismos (LC616785 e LC616786) e (LC616789 e LC616790) se encontraram em diferentes ramos, respectivamente. O estudo também revelou que todas as proteínas estudadas tinham incompatibilidade sem suas estruturas tridimensionais. De acordo com nossas descobertas, a presença de diferenças genéticas específicas dentro do gene *HSP70* em caprinos iraquianos aumentaria a possibilidade de seleção de animais mais adequados a vários níveis de estresse.

Palavras-chave: gene *HSP70*, caprinos, polimorfismo, análise molecular.

Introduction

Goats are important livestock making up a significant part of the agricultural economy in Iraq (Food and Agriculture Organization of the United Nations, 2018). Climate change, specifically the rise in temperature, has negatively affected the production of farm animals, especially goats (Hassan et al., 2018). This negative impact may develop further as global temperatures increase (Pachauri et al., 2014). In general, animals have many physiological and behavioral responses to withstand heat-stress conditions that result in reducing the impact of stress (Collier et al.,




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2019; Rashamol et al., 2018). These responses are significantly related to genetic variance (Rashamol et al., 2018).

Heat shock proteins (HSPs) play a role in the cell response to heat stress (Richter et al., 2010). They are divided into different members of the same family according to their molecular weight (Karademir & Sari-Kaplan, 2018). HSPs play an important role as molecular chaperones (Sharma et al., 2013) in thermoregulation and stress resistance (Ravaschiere et al., 2017; Shende et al., 2019), as well as in protein folding or unfolding, apoptosis, and immune response (Chatterjee & Burns, 2017; Edkins et al., 2018). Under different stress conditions, HSP70 is the most productive member of the HSP family (Dang et al., 2018).

In goats, the gene coding for the HSP70 protein comprises 1926bp nucleotide sequences corresponding with 641 amino acids and is located on chromosome 23 (Gade et al., 2010). Although HSP70 genes are highly conserved molecules (Pawar et al., 2013), several studies suggest the possibility of genetic polymorphisms of the HSP70 gene [Fatima et al. (2019) and Nikbin et al. (2014) in goat; Habib et al. (2017) in bulls; Habib et al. (2018a) in rams; Habib (2020) in buffaloes and Habib et al. (2020) in poultry]. Iraqi local goats generally suffer from many problems directly affecting their production. Perhaps one of the most important problems is the different stress conditions, especially high temperatures. Therefore, searching for effective mechanisms to select animals with higher resistance to stress conditions, including the selection of molecular markers resistant to stress conditions, is necessary. One of these markers is the HSP70 gene. Molecular characterization of the HSP70 gene has not been previously conducted in Iraqi goats. Accordingly, the present study aimed to fill this gap through a detailed molecular study of the gene.

Materials and methods

Experimental design

This study was conducted in the laboratories of the College of Agriculture, University of Basrah, Iraq. From December 2020 to April 2021, 125 adult females of a local breed raised by local breeders from different areas of Basrah were used. The current study was approved according to the rules and guidelines of the General Committee of the Animal Use and Welfare, College of Veterinary Medicine, University of Basrah, Iraq.

Sampling and DNA extraction

Samples (blood samples) were collected from the jugular vein in 10 mL tubes containing EDTA as an anticoagulant. They were stored at -20 °C until DNA extraction, which was conducted according to Najafi et al. (2014). It was run on a gel electrophoresis, and the quality and quantity of the extracted DNA was determined using Nano-Drop as described by Desjardins and Conklin (2010).

PCR amplification

Amplification was performed using the primer HSP70-F ⁵TGGCGAAAAACATGGCTATC⁻³ and HSP70-R ⁵CTAATCCACCTCCTCAAT⁻³ (Kõressaar et al., 2018) at the laboratories of "MacrogenInc"/South Korea. The amplification reaction was 25 µL, including 1µL of template DNA (75 ng), 1 µL of from each forward and reverse primer, 12.5 µL of master (Promega M7502) mix (2x) and 9.5 µLDNase (free water). The PCR conditions were as described by Fatima et al. (2019): initial denaturation at 95 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 64 °C for 30 s (determined gradually), extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min, with a ladder (**EnzyQuest**/SKU: NMO18S) of 8 kb base pairs of DNA. Ethidium bromide (1%) was used as a detection method for the PCR products. The ratio of 260/280 was approved in 1.75-1.85 as the best quantification ratio for DNA samples (Dauphin et al., 2011; García-Alegria et al., 2020).

Sequences analysis

The PCR products were purified and sequenced at the laboratories of "MacrogenInc"/South Korea. HSP70 gene sequences were compared with the reference sequence in GeneBank

(accession number JN656104). Based on Kumar et al. (2018), multiple sequence alignment and phylogenetic-tree extraction were performed using Mega-X software. The 3D structure of the protein was predicted using Swiss Model software (Waterhouse et al., 2018).

Results

The size of the PCR product of *HSP70* gene was 1926 bp (Figure 1). Multiple sequence-alignment analysis of the obtained sequences with the reference gene (accession number JN656104) revealed seven polymorphisms as a result of numerous different mutations (Table 1). The obtained polymorphisms were all registered in the GeneBank under the following accession numbers: LC616785 (17 animals), LC616786 (11 animals), LC616787 (9 animals), LC616788 (11 animals), LC616789 (38 animals), LC616790 (33 animals), and LC616791 (6 animals). Results indicated the occurrence of silent and missense mutations in the sequences obtained for Iraqi local goats. Some of them occurred in one polymorphism, and others occurred more than one.

In the goats from Iraqi localities, genetic diversity was shown in the phylogenetic tree of the *HSP70* gene. On one hand, the polymorphisms LC616787, LC616788, and LC616791 combined with the reference gene were in the same branch, whereas (LC616785 and LC616786) and

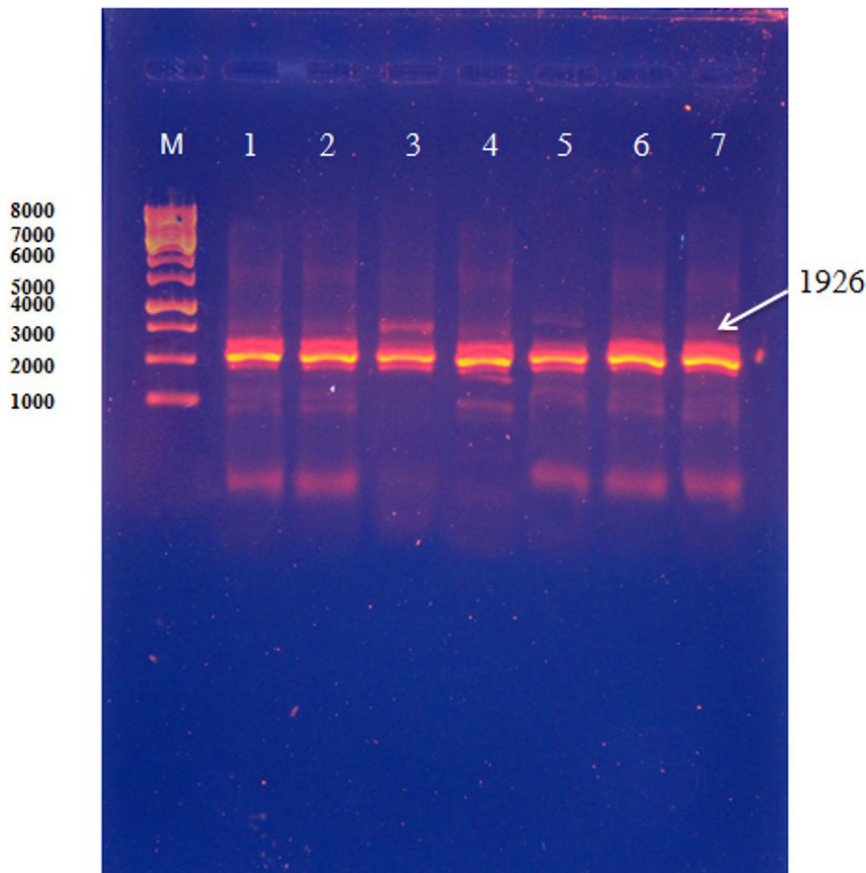


Figure 1. Gel electrophoresis of PCR product for *HSP70* gene in local Iraqi goat. M: ladder of DNA 8kb; 1-7: template of DNA.

(LC616789 and LC616790) polymorphisms were in different branches, respectively (Figure 2). On the other hand, owing to the occurrence of different mutations that can affect the protein's structure, the three-dimensional structure of the protein displayed mismatches for all studied sequences (Figure 3).

Table 1. The polymorphisms of *hsp70* gene in local Iraqi goat.

	No.	Reference Gene	Polymorphism	Position	Mutations	
					Type	Amino acid
Polymorphism (LC616785)	1	A	T	9	Missense	Lysine to Asparagine
	2	T	A	31	Missense	Tryptophan to Arginine
	3	G	A	1131	Silent	
	4	C	T	1878	Silent	
Polymorphism (LC616786)	1	G	A	66	Silent	
	2	C	A	67	Missense	Histidine to Asparagine
	3	T	A	1346	Missense	Methionine to Lysine
	4	G	T	1473	Silent	
Polymorphism (LC616787)	5	C	T	1878	Silent	
	1	A	T	9	Missense	Lysine to Asparagine
	2	C	T	69	Silent	
	3	C	T	195	Silent	
Polymorphism (LC616788)	1	C	T	24	Silent	
	2	C	T	69	Silent	
	3	C	A	128	Missense	Alanine to Aspartic Acid
	4	G	C	219	Silent	
	5	G	A	1479	Silent	
	6	C	T	1749	Silent	
	7	C	G	1904	Missense	Proline to Arginine
Polymorphism (LC616789)	1	T	A	31	Missense	Tryptophan to Arginine
	2	A	T	170	Missense	Asparagine to Isoleucine
	3	C	A	171	Missense	Asparagine to Isoleucine
	4	C	A	271	Missense	Proline to Threonine
	5	C	T	537	Silent	
	6	G	C	1539	Missense	Glutamic Acid to Aspartic Acid
Polymorphism (LC616790)	1	G	C	55	Missense	Glycine to Arginine
	2	A	T	151	Missense	Isoleucine to Phenylalanine
	3	A	T	170	Missense	Asparagine to Isoleucine
	4	C	A	171	Missense	Asparagine to Isoleucine
	5	G	A	309	Silent	
Polymorphism (LC616791)		G	A	1155	Silent	
		C	A	1818	Silent	
	1	C	T	24	Silent	
	2	A	C	739	Silent	
	3	G	A	1114	Missense	Glycine to Arginine

Reference gene in GenBank: JN656104.

Discussion

According to the result of PCR product size, this gene (*HSP70*) was usually short as it contained only exons and had no introns. This finding was consistent with many previous ones (Gade et al.,

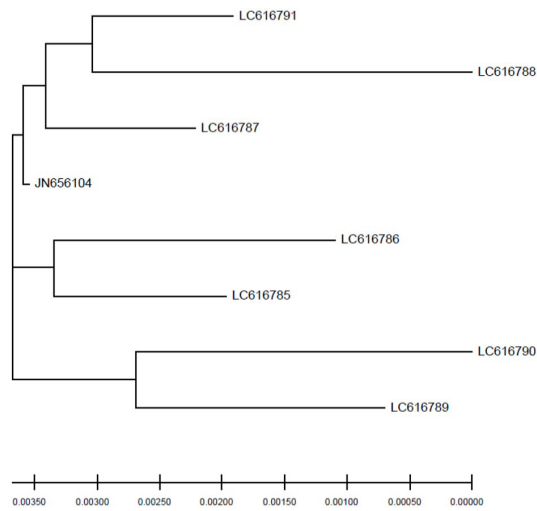


Figure 2. Phylogenetic tree of *HSP70* gene in local Iraqi goat. LC616785, LC616786, LC616787, LC616788, LC616789, LC616790, and LC616791: *HSP70* gene sequences in Iraqi local goats. JN656104: reference gene in GenBank.

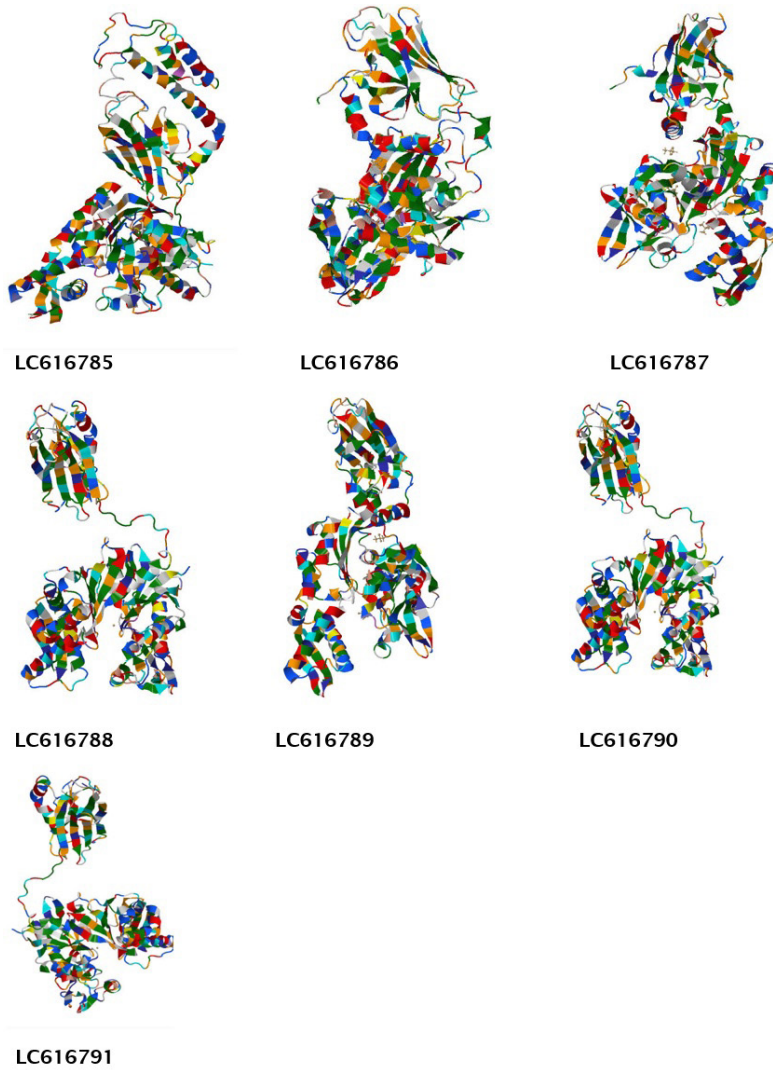


Figure 3. Three-dimensional structure of the *HSP70* protein in local Iraqi goat. LC616785, LC616786, LC616787, LC616788, LC616789, LC616790, and LC616791: *HSP70* gene sequences in Iraqi local goats.

2010; Nikbin et al., 2014). Thus, it is a highly conserved gene (Tripathy et al., 2021), explaining its rapid expression and production during stress (Silver & Noble, 2012). Several studies have reported that polymorphisms may exist in the *HSP70* gene (Abhijith et al., 2021; Mohalik et al., 2021; Raza et al., 2021). However, some studies suggest that polymorphisms could be associated with gene performance, whereas other studies claim that *HSP70* nucleotide polymorphisms may indicate resistance to stress (Bhat et al., 2016; Habib et al., 2018b; Mohalik et al., 2021). Depending on their type, mutations may affect transcription, translation, and mRNA transportation (Goymer, 2007). Conversely, missense mutations can damage the protein, make it nonfunctional (Minde et al., 2011), or sometimes enhance its action, indicating that they are beneficial to the animal as a whole (Sallam, 2021).

Given the mismatch (caused by various mutations) in some polymorphisms on the same branch (Novick & Fuselier, 2019), phylogenetic-tree analysis showed genetic diversity for the *HSP70* gene in local Iraqi goats. The genetic distance was 0.0035 (Figure 2), indicating that it showed a strong affinity with the reference gene. This finding confirmed the aforementioned result about this gene being highly conserved. All polymorphisms of the current study showed differences in protein structure, indicating differences in the performance of these proteins (Simoncini & Zhang, 2019). Indeed, Rodrigues et al. (2018) and Chen et al. (2020) agreed that changes in protein structure can affect protein function.

According to Sodhi et al. (2013) and based on the genetic variations in Iraqi goats, the polymorphism in the *HSP70* gene-coding region impacts goats' ability to tolerate stress (Afsal et al., 2021; Mohalik et al., 2021). Therefore, animals more tolerant to different forms of stress including heat stress can be selected based on variations in the polymorphisms of *HSP70* genes as molecular biomarker (Abbas et al., 2020; Kumar et al., 2015).

Overall, these results may contribute to explaining the apparent variations in production of the local Iraqi goat breed (Juma & Alkass, 2005) because of mutations in the *HSP70* gene. These mutations may contribute to an increase in the susceptibility of animals to resistance under various stress conditions (Rong et al., 2019), in agreement with Singh et al. (2020) that mutations can result in SNPs either resistant or susceptible to heat stress.

Conclusion

We determined whether the presence of specific genetic differences within the *HSP70* gene in Iraqi goats contributed to the selection of animals more suitable for different stress situations. The role of this gene in the development of different physiological and reproductive characteristics needs to be further explored in detail.

Acknowledgements

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Ethics statement

The current study was designed according to the rules and guidelines of the College of Veterinary Medicine, University of Basra, Iraq. The study was approved by the animal care and used committee of the College of Veterinary Medicine, University of Basra, Iraq.

Financial support

None.

Conflict of interests

No conflict of interest.

Authors' contributions

HNH, WMMS, and QJG - drafted the manuscript and approved the version to be published. HNH, WMMS, and QJG - performed the samples collection and laboratory diagnosis, with data analysis.

Availability of complementary results

All information obtained as a result of the study is included in the manuscript. More information is available through WMMS, email: Wessam.Mohammed@uobasrah.edu.iq; wessamgm@gmail.com.

The study was carried out at Laboratory of Molecular Genetics and Genetic Engineering/ College of Agriculture/University of Basrah/Iraq and laboratories of “MacroGenInc”/South Korea.

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