

Molecular characterization of heat shock protein 70 gene in Iraqi buffalo

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(Received April 21, 2019; Accepted June 16, 2019)

Abstract

The heat shock protein 70 (HSP 70) has important roles in protecting cells and keeping them alive when exposed to different stress conditions. The polymorphism of the *hsp 70* gene could be linked with the ability of stress tolerance. This study aimed to determine the polymorphism of the *hsp 70* gene in Iraqi buffaloes and study its effects on the resistance to stress. This study was conducted during from November 2018 to February 2019. The number of buffalo females used was 35 at the age of 4 - 6 years, which belonged to the local farmers from Basra city, Iraq. The DNA was extracted from the blood samples then the polymerase chain reaction (PCR) amplification was performed. The DNA sequences were analyzed by using bioinformatics analysis. The results of the molecular analysis showed that there were two groups of the *hsp 70* gene as compared with the same genes in GenBank due to silent and missense mutations. Based on these findings, it can be concluded that the Iraqi buffaloes have adapted to the surrounding environmental conditions as a result of the action of HSP 70 proliferation. The *hsp 70* gene was a useful biomarker of stress tolerance in buffaloes.

Keywords: *hsp 70* gene, Buffalo, Environmental stress, Polymorphism

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التوصيف الجزيئي لجين بروتينات الصدمة الحرارية ٧٠ في الجاموس العراقي

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قسم الإنتاج الحيواني، كلية الزراعة، جامعة البصرة، البصرة، العراق

الخلاصة

تلعب بروتينات الصدمة الحرارية ٧٠ دور مهم في حماية الخلايا وإبقائها على قيد الحياة عند تعرضها لظروف الإجهاد المختلفة. ترتبط التشكلات الوراثية لجين *hsp 70* بقدرة الكائنات على تحمل ظروف الإجهاد المختلفة. هدفت هذه الدراسة إلى الكشف عن التشكلات الوراثية لجين *hsp 70* في الجاموس العراقي. أجريت الدراسة خلال الفترة من تشرين الثاني ٢٠١٨ إلى شباط ٢٠١٩. تم استخدام خمسة وثلاثين من إناث الجاموس في سن ٤-٦ سنوات، والتي كانت تابعة للمزارعين المحليين من مدينة البصرة، العراق. تم استخراج الحمض النووي من عينات الدم ثم تم إجراء تضخيم باستخدام تفاعل السلسلة المتبلعمة من أجل الحصول على تسلسل الحمض النووي. لإجراء بعض تحليلات المعلوماتية الحيوية. أظهرت نتائج التحليل الجزيئي أن هناك مجموعتين من جين *hsp 70* كمقارنة مع بنك الجينات وذلك بسبب حدوث الطفرات الصامتة والمحسوسة. بناءً على هذه النتائج، يمكن أن نستنتج أن الجاموس العراقي قد تكيف مع الظروف البيئية المحيطة إذ إنه يمكن أن يعد جين *hsp 70* واسم جزيئي لتحمل ظروف الإجهاد المختلفة.

Introduction

The HSP 70 is a dominant protein member of the huge HSP family. This family has a diversity of functions in the cells such as protecting living cells under the different

conditions of stress (1). Furthermore, the HSP 70 was specified to be a confirmatory molecular marker for determination the response of different stress conditions in the farm animals (2). The *hsp 70* gene can be used as an elect gene for the election cattle based on the stress

leniency traits (3). The *hsp 70* gene is imperative to generate HSP 70 that influencing on the stress conditions (4). The HSP 70 has a molecular weight between 68 - 73 KDa (5), composed 641 amino acid (6) and the coding region of the *hsp 70* gene in buffalo with length 1926bp (7). Despite the HSP 70 is consider as a highly conserved polypeptide (8), the polymorphisms of the *hsp 70* gene explain the variance between individuals in resistance to the different stress conditions. On one hand, the previous studies have reported the diversity of polymorphisms of the *hsp 70* gene (9-15), as well as linked some of the different production traits (16-20). On the other hand, the variations in the DNA sequences are of value because they might change the interaction of the HSP 70, thus, enhancing the animal's response to the stress conditions (21). Buffaloes have significant economic importance of the different food industry in many developing countries of Asia that regards as one of the most important sources of milk and meat production (22). Iraq has the second largest number of buffaloes after Egypt in the Middle East region, it is also ranked globally as the 12th of buffalos milk (23). In spite of the vital importance to buffaloes in Iraq, the environmental stress such as high temperatures (24), and the water salinity (25) have negatively affected on the production of buffalo. Therefore, there is an urgent need to look for mechanisms to resist the stress conditions and to select animals with a higher capacity to resist these conditions. The most important molecular mechanisms that the body possesses are the family of HSPs, especially HSP 70. It has important roles in protecting cells and keeping them alive when exposed to the different stress conditions (26), and the possibility of using its polymorphisms as an elector marker to resist stress conditions (27).

Little is known about the adaptation mechanism of local Iraqi buffaloes for surviving in stress conditions and there is a lack of studies on this subject. Therefore, the current study was designed to determine the polymorphisms of the *hsp 70* gene in the Iraqi buffaloes and study its role on the living cells in environmental stress.

Materials and methods

Animal and Sampling

This study was conducted during from November 2018 to February 2019. The number of the samples collected from buffalo females aged between 4-6 years old were 35. All the samples were selected randomly at different stages of the milk production which belonged to local farms in Basra city, Iraq. The blood samples were collected from the jugular vein using sterile tubes with size 10 ml containing 0.5 ml EDTA solution as an anticoagulant, all samples were kept frozen at - 20 °C till the DNA extraction process.

DNA extraction

The DNA was extracted from the blood samples using Genomic DNA Kit (Geneaid Biotech, Taiwan). Then it was estimated the concentration and the purity of DNA by using NanoDrop as described by Desjardins and Conklin (28).

PCR Amplification

The amplification reaction was 25 µl containing 1.0 µl DNA template (75 ng), 1.0 µl Forward primer (10 µM), 1.0 µl Revers primer (10 µM), 12.5 µl of 2 X PCR master mixes and 9.5µl water nuclease free. According to APICAL lab (formerly named First BASE Laboratories) Malaysia, the primer was used to amplify conserved coding region of the HSP 70 promoter (Table 1). PCR condition and thermal cycling protocols were summarized in Table 2. To detect PCR product was used 1.5% ethidium bromide 0.5 µg/ml stained agarose gel.

Table 1: The sequence of forward and reverse primer of *hsp 70* gene

<i>hsp70-F</i>	ATGGCGAAAAACATGGCTATCGGC
<i>hsp70-R</i>	CTAATCCACCTCCTCAATGGTGGGGCC
APICAL	(First BASE) Laboratories

(PCR product size = 1926 bp)

Table 2: Cycling protocol and temperature of PCR amplification

Cycle step	°C	Time	No. Cycles
Initial Denaturation	95	5 min	1
Denaturation	94	30 s	
Annealing	61	30 s	30
Extension	72	2 min	
Final Extension	72	10 min	1

Sequence Analysis

The sequence analysis was detected in APICAL (First BASE) Laboratories/ Malaysia. The BLAST analysis was carried out on website of NCBI. The Multiple Sequence Alignment (MAS) was done in <http://www.ebi.ac.uk/Tools/msa/clustalo/>. The result of sequence was compared with the *Bubalus bubalis hsp 70* complete gene in GeneBank (Accession number EU099315.1) as a reference sequence. Mega - x version 10.0.5 (2018) software was used to characterization expected amino acids.

Phylogenetic Tree Analysis

The sequences were compared with the top 10 hits of the *Bubalus bubalis hsp 70* gene in BLAST, represented by Accession numbers (EU099315.1, GU183098.1, GU183099.1, HM025989.2, KY912034.1, MF061305.1,

MH814759.1, MH814760.1, MH814761.1, MH814762.1), then used the MEGA - X(29) for the analysis of phylogenetic tree.

Results

The ratio of 260/280 was between 1.80 to 1.85 for all samples. PCR product size was 1926 bp (Figure 1). Based on the analysis multiple sequence alignment, two different polymorphisms were obtained according to the mutations as compared to reference sequence (Accession number EU099315.1). They were divided in to Group 1 and Group 2.

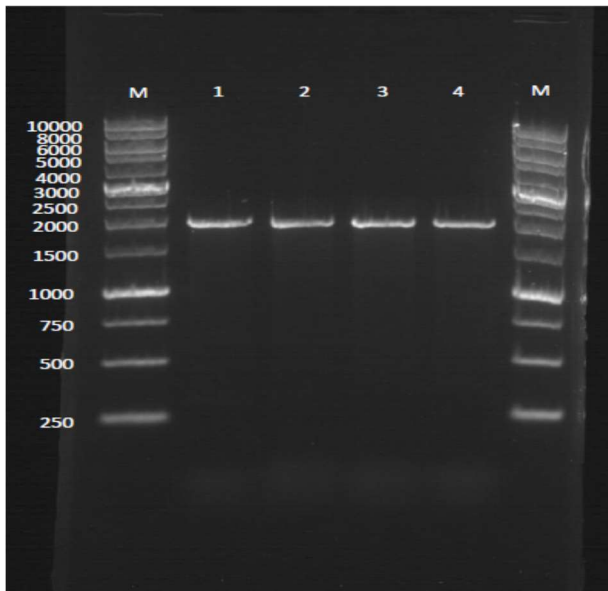


Figure 1: Gel electrophoresis of PCR products of the *hsp 70* gene. M: DNA ladder 1000bp, 1-4 DNA templates.

Group 1

The position 235 (G>C) mutation, the position 364 (A>T) a missense mutation that occurred as a result of the change of amino acid from methionine to leucine. The position 1079 (A>G), the position 1080 (C>T) both are missense mutations that occurred as a result of the change of amino acid from asparagine to serine. The position 1354 (G>A) a missense mutation that occurred as a result of the change of amino acid from aspartic acid to asparagine. The position 1638 (C>G) silent mutation and the position 1639 (T>A) a missense mutation that occurred as a result of the change of amino acid from phenylalanine to isoleucine (Record in GenBank by accession number LC496272).

Group 2

The position 35 (G >C) a missense mutation that occurred as a result of the change of amino acid from

glycine to alanine. The position 156 (C>T) a missense mutation that occurred as a result of the change of amino acid from glycine to arginine. The position 235 (G>C) silent mutation, the position 364 (A>T) a missense mutation that occurred as a result of the change of amino acid from methionine to leucine, position 562 (A>T) silent mutation, the position 563 (C>T) a missense mutation that occurred as a result of the change of amino acid from threonine to leucine. The position 1079 (A>G) and the position 1080 (C>T) are both missense mutations that occurred as a result of the change of amino acid from asparagine to serine. The position 1354 (G>A) is a missense mutation that occurred as a result of the change of amino acid from aspartic acid to asparagine. The position 1638 (C>G) is silent mutation and the position 1639 (T>A) is a missense mutation that are occurred as a result of the change of amino acid from phenylalanine to isoleucine. All the mutations were clarified in figure 2, and the changes of amino acids were clarified in figure 3. The phylogenetic tree analysis (Figure 4) showed very close affinity with all sequences of the *Bubalus bubalis hsp 70* gene. However, it did not match another sequence, but they were closer to complete the *hsp 70* gene in *Bubalus bubalis* of water buffalo (Accession Number EU099315.1)



Figure 2: Mutations in the *hsp 70* gene in Iraqi buffalo CLUSTAL O (1.2.4). EU099315.1: reference gene in GenBank. Group 1 and group 2: Sequences of the *hsp 70* gene to Iraqi buffalo(Record in GenBank by accession number LC496273).

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EU099315.1  MAKNMAIGIDLGTTCYCVGVFQHGKVEIANDQGRRITTPSYVAFTDTERLIGDAAKNQVA 60
GROUP2     MAKNMAIGIDLATTTCYCVGVFQHGKVEIANDQGRRITTPSYVAFTDTERLIGDAAKNQVA 60
GROUP1     MAKNMAIGIDLGTTCYCVGVFQHGKVEIANDQGRRITTPSYVAFTDTERLIGDAAKNQVA 60
*****
EU099315.1  LNPQNTVFDKRLLRGKFRDPVQSDMKEMFFRVINDGDKPKVQVRYGETKAFYPEETS 120
GROUP2     LNPQNTVFDKRLLRGKFRDPVQSDMKEMFFRVINDGDKPKVQVRYGETKAFYPEETS 120
GROUP1     LNPQNTVFDKRLLRGKFRDPVQSDMKEMFFRVINDGDKPKVQVRYGETKAFYPEETS 120
*****
EU099315.1  SNLVLPKMHEIAEAYLGHPTNAVITVPAYFNDSQRQATKDAQVIAGLYLGIINEPTAAA 180
GROUP2     SNLVLPKMHEIAEAYLGHPTNAVITVPAYFNDSQRQATKDAQVIAGLYLGIINEPTAAA 180
GROUP1     SNLVLPKMHEIAEAYLGHPTNAVITVPAYFNDSQRQATKDAQVIAGLYLGIINEPTAAA 180
*****
EU099315.1  IAYGLDRGTGKERNLVIFDLGGGTFDVSILTIIDOGIFEVQATAGDTHLGEEDFDSRLVDH 240
GROUP2     IAYGLDRGLGKERNLVIFDLGGGTFDVSILTIIDOGIFEVQATAGDTHLGEEDFDSRLVDH 240
GROUP1     IAYGLDRGTGKERNLVIFDLGGGTFDVSILTIIDOGIFEVQATAGDTHLGEEDFDSRLVDH 240
*****
EU099315.1  RFEEELCSDFRSTLEPVEKALADAKLDAQIRDLVVLVGGSTRIPKVKQLLQDFLNGRDLN 360
GROUP2     RFEEELCSDFRSTLEPVEKALADAKLDAQIRDLVVLVGGSTRIPKVKQLLQDFLNGRDLN 360
GROUP1     RFEEELCSDFRSTLEPVEKALADAKLDAQIRDLVVLVGGSTRIPKVKQLLQDFLNGRDLN 360
*****
EU099315.1  PTKQTQIFFTTYSNQPGLVQVYEGERAMTRNNLLGRFELSGIPPARGVPOIEVTFDI 480
GROUP2     PTKQTQIFFTTYSNQPGLVQVYEGERAMTRNNLLGRFELSGIPPARGVPOIEVTFDI 480
GROUP1     PTKQTQIFFTTYSNQPGLVQVYEGERAMTRNNLLGRFELSGIPPARGVPOIEVTFDI 480
*****
EU099315.1  ALESYAINMKSAVEDEGLKGIKISEADKKKVLKDKQEVISMLDANTLAEKDEFHKKRKELE 600
GROUP2     ALESYAINMKSAVEDEGLKGIKISEADKKKVLKDKQEVISMLDANTLAEKDEFHKKRKELE 600
GROUP1     ALESYAINMKSAVEDEGLKGIKISEADKKKVLKDKQEVISMLDANTLAEKDEFHKKRKELE 600
*****
    
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Figure 3: changes in amino acids to the *hsp 70* gene to Iraqi buffalo as a compare with same gene in GenBank. EU099315.1: reference gene in GenBank. Group 1 and group 2: sequences: Sequences of the *hsp 70* gene to Iraqi buffalo.

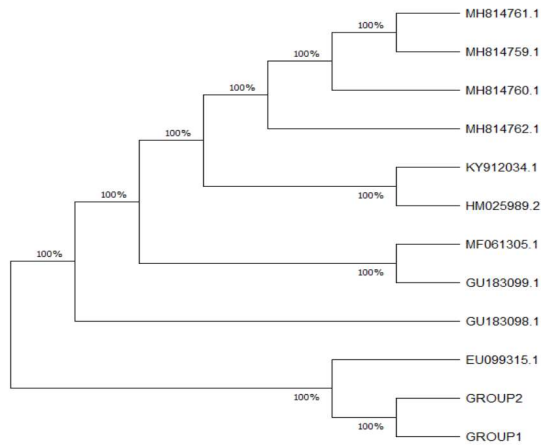


Figure 4: The Phylogenetic tree analysis of the *hsp 70* gene in Iraqi buffalo. Group 1, 2: sequences of the *hsp 70* gene in Iraqi buffalo EU099315.1, GU183098.1, GU183099.1, HM025989.2, KY912034.1, MF061305.1, MH814759.1, MH814760.1, MH814761.1, and MH814762.1: Accession numbers of top 10 hits *Bubalus bubalis* the *hsp 70* gene sequences in GenBank

Discussion

The ratio of 260/280 was the best ratio for the purity of the DNA as mentioned (28). The results are in line with what (7,19), noting the possibility of polymorphism for the

hsp 70 gene in Buffalo, but the results did not match 100% with any pre-recorded GeneBank sequence, including the complete gene of the *hsp 70* gene to *Bubalus bubalis* (Accession number EU099315.1). The corresponding ratio in the first group and the second group were 99.64 and 99.43% (30) respectively. This finding suggests that the Iraqi Buffaloes have different polymorphisms of the *hsp 70* gene. The silent mutations can alter the capacity of an mRNA to code for protein via impact the average of the translation, by the change in codon usage through the production of budding protein (31). As for the missense mutations can be molecular markers to resist the heat stress (32, 33). This Genetic diversity may give Iraqi buffalo the ability to withstand various stress conditions (34). Moreover, the polymorphism in the coding region of the *hsp 70* gene is associated with an advantage of longevity and survival, as well as the polymorphisms are correlated with increased heat tolerance (35). Nevertheless, the result of the Phylogenetic tree analysis is supporting the hypothesis that the Iraqi buffalo have adapted to environmental conditions, because these genetic changes can determine the ability of animals to withstand different stress conditions (7).

Conclusion

In conclusion, the current study showed two different groups of the *hsp 70* gene, these differences may refer to the diversity of animals' ability to tolerate different stress conditions. Therefore, more studies to investigate the role of the polymorphism of the *hsp 70* gene and the different production traits in the Iraqi buffalo are needed.

Acknowledgments

We extend our appreciation to the Associate Professor Dr. Wessam Monther Mohammed Saleh, college of Veterinary Medicine, university of Basra for his supporting, aiding, and direction in sampling process. Many gratitude and thanks to the farmers who cooperated with us to complete this work.

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