

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

# بسم الله الرحمن الرحيم





MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



MONA MAGHRABY



شبكة المعلومات الجامعية التوثيق الإلكترونى والميكروفيلم

# جامعة عين شمس التوثيق الإلكتروني والميكروفيلم قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



MONA MAGHRABY

## GENE EXPRESSION STUDIES OF GENES AFFECTING HEAT STRESS TOLERANCE IN SOME POULTRY LINES

By

#### ESRAA IBRAHIM SEIF EL-DEIN IBRAHIM

B.Sc. Agric. Sc., (Genetics), Fac. Agric., Ain Shams University, 2012

A Thesis submitted in Partial Fulfillment Of The Requirements for the Degree of

in
Agricultural Sciences
(Genetics)

Department of Genetics Faculty of Agriculture Ain Shams University

## **Approval sheet**

# GENE EXPRESSION STUDIES OF GENES AFFECTING HEAT STRESS TOLERANCE IN SOME POULTRY LINES

By

## ESRAA IBRAHIM SEIF EL-DEIN IBRAHIM

B.Sc. Agric. Sc., (Genetics), Fac. Agric., Ain Shams University, 2012

| This thesis for M.Sc. degree has been approved by:  Dr. Mohammed A. Rashed  Professor Emeritus of Genetics, Dean of Higher Institute for Agric Cooperation |
|--|
| <b>Dr. Eman M. Fahmy</b> Professor Emeritus of Genetics, Faculty of Agriculture, Ain Shams University  |
| Dr. Khaled A. Soliman  Professor Emeritus of Genetics, Faculty of Agriculture, Ain Shams University  |
| Dr. Alia A. M. El-Seoudy  Professor Emeritus of Genetics, Faculty of Agriculture, Ain Shams University   |
|  |

**Date of Examination:** 23/8 / 2021.

## GENE EXPRESSION STUDIES OF GENES AFFECTING HEAT STRESS TOLERANCE IN SOME POULTRY LINES

By

#### ESRAA IBRAHIM SEIF EL-DEIN IBRAHIM

B.Sc. Agric. Sc., (Genetics), Fac. Agric., Ain Shams University, 2012

#### **Under the supervision of:**

#### Dr. Alia A. M. El-Seoudy

Professor Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University (Principal supervisor)

#### Dr. Khaled A. Soliman

Professor Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University

### Dr. Aly Zean El-Abedin Abd El-Salam (Ceased or Late)

Professor Emeritus of Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University

#### **ABSTRACT**

Esraa Ibrahim Seif El-Dein Ibrahim: Gene Expression Studies of Genes Affecting Heat Stress Tolerance in Some Poultry Lines. Unpublished M.Sc., Department of Genetics, Faculty of Agriculture, Ain Shams University, 2021.

This study was conducted to determine the effect of heat stress on heat shock protein (HSP70) and heat shock factor (HSF1), through mRNA profiling in two chicken genotypes (Fayoumi and Matrouh). Fayoumi and Matrouh fully mature chickens were divided into two groups. The control group where the two genotypes were reared at room temperature (25°C) in a 55-60% humidity atmosphere. Whereas the treatment group was subjected to heat stress at 39°C for 4 hours in a 55-60% humidity atmosphere. Six blood samples were collected randomly from each genotype to tubes containing 1.0 mg/mL EDTA. Expression levels of hsp70 and hsf 1 genes were used to assess the heat tolerance of the two Egyptian local genotypes. After four hours of heat exposure, the hsp70 and hsf1 mRNA expression analysis revealed higher expression levels in Fayoumi type as opposed to the Matrouh type. These results showed that the acquired thermotolerance is positively linked to the stress memory. Within the two genotypes, Fayoumi type exhibited the highest means for hsp70 and HSF1 productions, and therefore, the heat stress is better tolerated, which indicates that Fayoumi mortality rate might be lower genetically under heat stress.

**Keywords:** Mrna; *Hsp70*; *Hsf1*; Heat Stress; Thermotolerance and Mature Chicken.

#### ACKNOWLEDMENT

I would first like to earnestly thank the **Almighty ALLAH** who help me, illuminate the right way, facilitate the difficulties, and finally crowned this work with success.

I would like to express my special appreciation and thanks to my **professor. Dr. Alia A. M. El-Seoudy**, Emeritus Professor of Genetics, for her valuable supervision and who suggest the problem. I would like to deeply thank her for all efforts made, the given advice, continuous monitoring, and criticizing the thesis. Without her support, this work would not have appeared in this form, so for her I must be grateful forever.

I would like to offer my sincere thanks and gratitude to my supervisor **Prof. Dr. Khaled A. Soliman**, Professor of Genetics for his successful guidance, assistance, and his patience through the work period. He always monitored and evaluated the practical performance to achieve better results.

I would like to extend my thanks to **Dr. Lamiaa M. Radwan**, Associate Professor of Poultry Production for her contribution to the scientific ideas to successful completion of this work.

I would also like to offer grate thanks for the late **Prof. Dr. Ali Zean El- Abedin**, Emeritus Professor of Genetics for his guidance and scientific ideas contributed significantly to the successful completion of this work.

There are no words of gratitude and appreciation enough to thank **Dr. Mahmoud Magdy El-Mosallamy**, Associate Professor of Genetics for his many scientific assistance and distinctive design of the experiment that led to the success of this work. He was always offering guidance to walk on the right path, in addition to continuous follow-up and effective performance in the writing and completion of this thesis.

I would also like to deeply thank **Mrs**. **Hager El-Hefnawy**, Assistant Lecturer of Genetics for helping me through the practical work steps.

Last, but not least I am grateful to all my friends and colleagues who have helped me through my way in the completion of this thesis.

## **CONTENTS**

| LIST OF TABLES  | III |
|---|-----|
| LIST OF FIGURES   | IV  |
| LIST OF ABBREVIATIONS                                   | V   |
| 1. INTRODUCTION   | 1   |
| 2. REVIEW OF LITERATURE                                 | 4   |
| 2.1. The effect of heat on the performance of chicken   | 4   |
| 2.2. The effect of heat on the physiology of chicken    | 6   |
| 2.3. Heat shock proteins                                | 8   |
| 2.4. Heat chock factors                                 | 8   |
| 3. MATERIALS AND METHODS                                | 13  |
| 3.1. Materials  | 13  |
| 3.1.1. Sample collections                               | 13  |
| 3.2. Methods  | 14  |
| 3.2.1.DNAsequencingforheatshockgenes17                  | 14  |
| 3.2.1.1 DNA extraction                                  | 14  |
| 3.2.1.2 Agarose gel electrophoresis protocol            | 15  |
| 3.2.1.3 PCR amplification                               | 17  |
| 3.2.1.4 DNA sequencing                                  | 18  |
| 3.2.2 Gene expression studies                           | 19  |
| 3.2.2.1 RNA extraction                                  | 19  |
| 3.2.2.2 cDNA synthesis                                  | 20  |
| 3.2.2.3 Real-time quantitative PCR (RT-PCR)             | 21  |
| 4. RESULTS AND DISCUSSION                               | 23  |
| 4.1 DNA sequencing for heat shock genes                 | 23  |
| 4.1.1 DNA isolation                                     | 23  |
| 4.1.2 PCR amplification                                 | 23  |
| 4.1.3. DNA sequencing                                   | 25  |
| 4.1.4. BLAST results                                    | 25  |
| 4.1.5. Multiple sequence alignment and DNA polymorphism | 27  |
| 4.2 RNA isolation                                       | 28  |
| 4.2.1. Real-time PCR                                    | 29  |

| 5. SUMMARY     | 34 |
|----------------|----|
| 7. REFERENCES  | 36 |
| ARABIC SUMMARY |    |

# LIST OF TABLES

| <b>Fables</b> |   | Pages |
|---------------|---|-------|
| No.           |   |       |
| 1             | BLAST results for the two Egyptian chicken genotype   | 26    |
|               | including genotype name, pairwise %, %GC and          |       |
|               | GenBank accession numbers.                            |       |
| 2             | The CT values, delta-CT, ratio of relative expression | 30    |
|               | of hsp70 for each experimental genotype (G).          |       |
| 3             | The CT values, delta-CT, ratio of relative expression | 32    |
|               | of hsf1 for each experimental genotype (G).           |       |

## LIST OF FIGURES

| Fig. |   | Pages |
|------|---|-------|
| No.  |   |       |
| 1    | Developed chicken strains, including the Matrouh              |       |
|      | strain, which came through crossbreeding between the          |       |
|      | (Dam line) Dokki 4 and the (Sir line) White Leghorn.          | 13    |
| 2    | Agarose gel electrophoresis of total DNA isolated from        |       |
|      | a final set of eight collected samples from the two           |       |
|      | genotypes (El Fayoumi and Matrouh).                           | 23    |
| 3    | Gel electrophoresis of amplified DNA F (Fayoumi), M           |       |
|      | (Matrouh) using a set of heat stress protein primers;         |       |
|      | samples no. 1-2(HSF1), 3-4(HSF3), 5-6(HSP90), 7-              |       |
|      | 8(RPL5), 9-10(HSP70F), 11-12(MRPS27), 13-                     |       |
|      | 14(HSP70L)  | 24    |
| 4    | An example of forwarding direction trace file                 |       |
|      | (chromatogram) sample for the <i>hsf1</i> gene of a single    |       |
|      | sample.   | 25    |
| 5    | Comparative nucleotide alignment of the <i>hsf1</i> gene      |       |
|      | among the two chicken genotypes, with a total                 |       |
|      | alignment length of 219 bp is shown. Two sequences            |       |
|      | per breed was genotyped—samples of 4/5 Fayoumi                |       |
|      | (FAY) and sample 7/8 Matrouh (MAT).                           | 28    |
| 6    | Agarose gel electrophoresis of total RNA isolated from        |       |
| Ü    | a final set of 14 collected samples from the two              |       |
|      | genotypes (El Fayoumi and Matrouh).                           | 28    |
| 7    | Fold change histogram for expression levels of <i>hsp70</i>   | 20    |
| ,    |   | 31    |
| 0    | in the two-genotype using Q-PCR technique                     | 31    |
| 8    | Fold change histogram for expression levels of <i>hsf1</i> in | 22    |
|      | the two-genotype using Q-PCR technique                        | 33    |

#### LIST OF ABBREVIATIONS

8-OHdG 8-Hydroxydeoxyguanosine

AA Arbor Acres aa Acetic Acid

AHS Acute Heat Stress

AL Ad Libitum

BJY Beijing You chicken

BW Body Weights

CASP6 Down Regulating Caspase 6

CAT Upregulating Catalase
CB Commercial Broilers

CCK Cholecystokinin
CE Control Exposed

ChIP Chromatin Immunoprecipitation Assay

CHS Chronic Heat Stress

CK Creatine Kinase
CN Cou Nu Hubbard

CT Cloacal Temperature

DD Hsp70 genotype
CT Cycle Threshold
ED Embryonic Days

FABP1 Fatty Acid Transporter 1

Fas Factor Associated

GOT Glutamic-Oxaloacetic Transaminase

GPT Glutamic-Pyruvic Transaminase

GSH-Px Glutathione Peroxidase

HE Heat Exposed HS Heat Stress

HSE Heat Shock Element

HSF Heat Shock Factors
HSP Heat Shock Protein
HSR Heat Shock Response

IGF-1 Insulin-Like Growth Factor 1

LDH Lactate Dehydrogenase
MOS Mannan Oligosaccharides

mRNA Messenger RNA

Na Naked-Neck

NR3C1 Glucocorticoid Receptor NR3C1 Glucocorticoid Receptor

PBMCs Peripheral Blood Mononuclear Cells

RJF Red Jungle Fowl RO Ross 508 Aviagen rpl5 House-Keeping Gene

RR Respiration Rate

RT-qPCR Reverse Transcription-Quantitative Polymerase Chain

Reaction

SNPs Single Nucleotide Polymorphism

SOD Superoxide DismutaseSRBC Immune ParameterT3 Triiodothyronine

TBARS Thiobarbituric Acid Reacting Substances

TBE Tris-Boric-EDTA

THI Temperature-Humidity Index

TM Thermal Manipulation

TN Thermoneutral

TNF Tumor Necrosis Factor UCP Uncoupling Protein

VF Village Fowl