

**MOLECULAR GENETIC STUDIES ON HEAT SHOCK
GENES IN MAIZE (*ZEA MAYS* L.)**

By

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B.Sc. Agric. Sc. (Genetics), Zagazig University, 2004

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ABSTRACT

Fatma El-Sayed Mahmoud: Molecular Genetic Studies on Heat Shock Genes in Maize (*Zea mays* L.). Unpublished Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, University of Ain Shams, 2018.

The protein fingerprints of four Egyptian maize inbred lines (*Zea mays* L.), were performed using grain total-soluble protein electrophoretic analysis. The results showed 18 bands in a distinct pattern of K1 and K7 inbred lines, while 17 bands were present in G342 and Rg59 inbred lines as another distinct pattern indicating different genotypes. The high temperature effect on the four maize lines exposed to 45°C for 2 and 4 hours at 14-days old seedlings besides control (25°C) was studied. Four bands of heat shock proteins with molecular weights of 82, 22, 17 and 10 kDa appeared in the inbred line K1 after exposing to 45°C for 2 and 4 hours which may be indication of thermo-tolerance. One band in inbred line K1, four bands in line k7 and seven bands in line G342 were found in control appeared more concentrated after heat treatment at 45°C for 4 hours. New proteins were induced in leaves of k1 maize inbred line after exposing to heat-treatment which a high number of spots were separated by 2D electrophoresis after the treatment of 45°C for 2h and 4h. Some of these new protein spots picked off from the gel to be identified by LC-MSMS analysis. Five heat shock protein markers in the four maize lines were assessed by immunoblot with antiserum. This revealed that the antibodies against plant HSP markers were able to recognize the endogenous proteins of maize inbred lines that were absent in the control plants. These proteins were induced in the resistant maize inbred lines k1, k7 and G342 to high temperatures in contrast of the sensitive line G342,

besides, there were differences in the induction kinetics of HSP markers. The gene expression was studied by RT-PCR using three gene markers (*Zip60*, *hsp22* and *hsp16.9*). The expression of the three genes were induced strongly by heat-shock treatments in the lines k1, k7 and G342, while in Rg59 line the expression was less than the control. The *hsp22* gene was cloned and sequenced which gave a size of 657 bp cDNA sequences from the K1 line after heat treatment at 45°C for 4h. The comparison between this sequence and another gene sequences in the Gene Bank showed 100% identity in nucleotide base pairs. The gene was cloned in plant expression vector which produced a sub-cellular localization protein. Transformation of the gene, after fused with GFP and mitochondrial marker, in leaves tobacco plant by agro-infiltration was carried out to quantify the expression of the gene and its localization using an Olympus confocal microscope

Key words: Maize, Heat shock protein (*hsp*), genes, RT-PCR, Immunoblot, 2D-electrophoresis, Transformation.

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CONTENTS

LIST OF TABLES	v
LIST OF FIGURES	vii
I. INTRODUCTION	1
II. REVIEW OF LITERATURE	4
1. Heat shock proteins and high temperature tolerance in maize	4
2. Analysis of proteins by 2D electrophoresis and immunoblot	12
3. Levels of gene expression in maize inbred lines using real time PCR	22
4. Cloning and transformation of thermotolerance genes in maize	27
III. MATERIALS AND METHODS	40
1. Materials	40
1.1: Plant material	40
1.2: Strains and plasmid vectors	41
2. Methods	41
2.1. Storage protein extraction for fingerprinting	41
2.2. Heat stress condition assay by 1D-SDS PAGE	42
2.2.1. Quantification of proteins (Bio-Rad Protein Assay)	42
2.2.2. Stock solutions	43
2.2.3. Acrylamide stocks buffers (30%)	44
2.2.4. Used Buffers	44
2.2.5. Preparation of the polyacrylamide gel	45
2.2.6. Gel staining solution	45
2.2.7. Destaining solution	46
2.2.8. Application of samples	46
2.2.9. Electrophoretic conditions	46

2.3. 2D Electrophoresis and analysis for k1 line	47
2.3.1. Protein extraction	47
2.3.2. The two dimensional gel electrophoresis	47
2.3.2.1. The first dimensionel	47
2.3.2.2 The second dimensione	48
2.3.3. Protein identification by MS and database search	49
2.3.3.1. In-gel digestion	49
2.3.3.2. Acquisition of MS/MS spectra by LC-ESI-MSMS	49
2.3.4. Data analysis	50
2.4. Immunoblot analysis (Western blot analysis)	50
2.4.1. Western transfer	50
2.4.2. Western blot	51
2.5. RNA analysis	53
2.5.1. Extraction and purification of plant total RNA	53
2.5.2. cDNA synthesis	546
2.5.3. PCR primers	56
2.5.4. Polymerase Chain Reaction (PCR)	57
2.5.4.1. Sample preparation	57
2.5.4.2. PCR program	58
2.5.4.3. Agarose gel electrophoresis	58
2.5.5. Gene sequencing	59
2.6. Quantitative RT-PCR analysis	59
2.7. Isolation and plasmid construction of <i>hsp22</i> gene	60
2.7.1. Purification of PCR product band from agarose gel	60
2.7.2. Ligation of cDNA extracted in PCR2.1 Topo vector (3.9kb)	61

2.7.3. Transformation of <i>E.coli</i> competent cells	61
2.7.4. Purification of plasmid DNA from <i>E.coli</i>	62
2.8. <i>Agrobacterium</i>-mediated transformation of tobacco	63
2.8.1. Transformation of <i>Agrobacterium</i> competent cells	63
2.8.2. Transformation of tobacco by <i>Agrobacterium</i>	64
2.8.3. Agroinfiltration and localization of <i>ZmHSP22</i> protein	65
2.8.4. Transient expression in <i>N. benthamiana</i> leaves and laser-scanning confocal microscopy	66
IV. RESULTS AND DISCUSSION	68
1. Protein purification and electrophoresis	68
2. Effect of high temperature on protein synthesis	71
3. Two dimensional electrophoresis (2D) analysis in the line K1 leaves under heat stress	77
4. Two experimental approaches were carried out in this study to investigate if the resistance to high temperatures of maize inbred lines correlates with expression levels of HSP markers	88
4.1. A comparative analysis of induction levels of five protein markers by immunoblot (western blot)	88
4.1.1. Induction of BiP antibody in response to heat- shock	88
4.1.2. Induction of HSP 70 in response to heat-shock	90
4.1.3. Induction of mitochondrial HSP22 in response to heat- shock	91
4.1.4. Induction of cytosolic HSP17.9 in response to heat-shock	92
4.1.5. Induction of cytosolic HSP17.6 in response to heat-shock	93

4.2. Expression levels of three heat-induced gene markers by RT PCR	96
4.2.1. ZIP 60 transcription factor involved in thermal stress and drought	96
4.2.2. hsp 22 gene encoding for a maize mitochondrial hsp 22kD	96
4.2.3. hsp 16.9 gene encoding for a cytoplasmic hsp 16.9 kD	96
5. Isolation and cloning of heat shock protein gene 22kDa	105
6. Transformation and localization of 22 heat shock protein gene	113
6.1. Cloning in PCAMBIA vector	113
6.2. Transformation in Agrobacterium agro-infiltration in tobacco leaves	114
V. SUMMARY	118
VI. REFERENCES	122
VII. الملخص العربي	

LIST OF TABLES

Tables		Page
Table (1)	The four maize inbred lines used in the study and their pedigrees.....	40
Table (2)	Composition of 13% resolving gel and 3 % staking gel. (Mini gel).....	45
Table (3)	Genomic DNA elimination reaction components are.....	55
Table (4)	Reverse-Transcription reaction components.....	55
Table (5)	List of specific primers and their nucleotide sequences.....	56
Table (6)	SDS-PAGE profile of embryos total proteins of four maize inbred lines, representing band number and molecular weight (MW), where (+) means presence and (-) means absence of band.....	70
Table (7)	SDS-PAGE profile for two maize inbred lines after exposing to heat shock. C (control): 25°C, T1 (treatment 1): 45°C for 2 hours and T2 (treatment 2): 45°C for 4 hours. (+) means presence and (-) means absence of band.....	74