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 Unpublished Ph.D. Thesis, Department of Genetics, Faculty of Agriculture, University of Ain Shams, 2018.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 45ºC 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 (*Zip*60, *hsp*22 and *hsp*16.9). 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 *hsp*22 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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