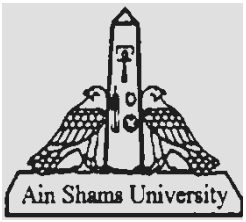

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Thesis

Submitted for Partial Fulfillment of

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B.Sc. in Science (Botany)

(2010)

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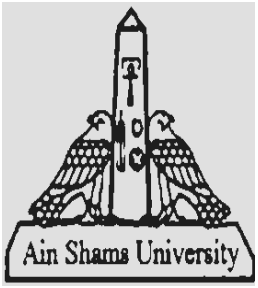

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(وَقُلْ اعْمَلُوا فَسَيَرَى اللَّهُ عَمَلَكُمْ وَرَسُولُهُ وَالْمُؤْمِنُونَ وَسَتُرَدُّونَ
إِلَى عَالِمِ الْغَيْبِ وَالشَّهَادَةِ فَيُنَبِّئُكُمْ بِمَا كُنتُمْ تَعْمَلُونَ (105)
وَآخَرُونَ مُرْجَوْنَ لِأَمْرِ اللَّهِ إِمَّا يُعَذِّبُهُمْ وَإِمَّا يَتُوبُ عَلَيْهِمْ وَاللَّهُ
عَلِيمٌ حَكِيمٌ (106))
سورة التوبة (104-105)

This thesis has not been previously submitted for any degree at this or any other university.

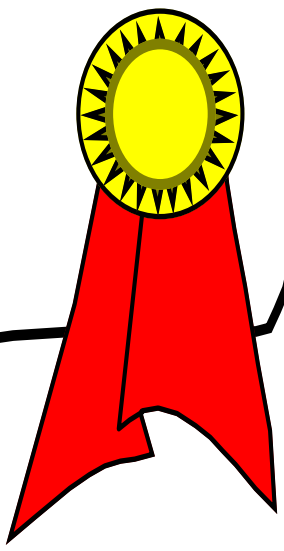
The references in the text will show specifically the extent to which I have availed myself of the work of other authors

Nehal Talaat Mohamed

Dedication

To my mother,
my little daughter,
my brother and my husband.

To the soul of my beloved father and uncle Tarek Agamy.



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I am also greatly indebted to my ***parents****,* ***my husband, my lovely little daughter****,* ***my family****,my colleagues and all the staff in Botany Department for their kind support throughout my work.*

LIST OF ABBREVIATIONS

ABB.	COMPLETE NAME
2,4-D	2, 4-Dichlorophenoxyacetic acid
ABA	Abscisic acid
APX	Ascorbate peroxidase
ASA	Ascorbic acid
BAP	Benzyl-aminopurine
BLAST	Basic Local Alignment searching tool
CAT	Catalase
CTAB	Hexadeyl trimethyl-ammonium bromide
DW	Dry weight
EC	Electrical conductivity
EDTA	Ethylene diamine tetra acetate
EL	Electrolyte leakage
FRAP	Ferric reducing antioxidant power assay
FW	Fresh weight
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Total reduced glutathione
GUS	ß-glucuronidase reporter gene
H_2O_2	Hydrogen peroxide
HPLC	High-performance liquid chromatography

IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
INDELs	Missence, insertion and deletion mutation
Kn	Kinetin
LD80	Sub-lethal concentration
MDA	Malondialdehyde
MS	Murashige and Skoog medium
MSI	Membrane stability index
NAA	Naphthalene-acetic acid
NCBI	National center for biotechnology
OD	Optical density
ORF	Open reading frame
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
POX	Peroxidase
PPO	Polyphenol oxidases
PSI	Pressure per square inch
PVP40	Polyvinyl pyrrolidone
RAPD	Random amplification of polymorphic DNA
RGR	Relative growth rate
ROS	Reactive oxygen specie
RWC	Relative water content
SNPS	Single Nucleotide Polymorphisms
SOD	Superoxide dismutase
TAC	Total antioxidant capacity

TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TW	Turgid weight

ABSTRACT

Callus was induced from seeds of tomato plant (*Lycopersicon esculentum* cv. Super strain B) on MS medium supplemented with 0.5 mg/L Naphthalene-acetic acid (NAA), 0.5 mg/L Indole-3-acetic acid (IAA), 2 mg/L Benzyl-aminopurine (BAP) and 2 mg/L Kinetin (Kn). This callus was subjected to osmotic stress using elevated concentrations of mannitol (0.0, 50, 100, 150, 200, 250 and 300 mM). The sub-lethal concentration which killed about 80% of callus cells (ID_{80}) was determined to be 250 mM mannitol. The remaining 20% of cells which survived the sub-lethal concentration was selected visually and multiplied on the same medium composition and are considered somaclonal variant cells and designated as "selected callus line". Experiments were conducted to compare different metabolic activities of the selected callus line with the non-selected one (control callus, 0.0 mannitol). Results showed reduction in fresh weight of the selected line and non-significant reduction in relative water content (RWC) of both callus lines. No significant difference was recorded in electrolyte leakage percentage (EL%) or malondialdehyde content (MDA) between both lines. An enhanced total antioxidant capacity (TAC), peroxidase (POX) and ascorbate oxidase (ASO) activity and reduced activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX) was found in the selected callus line. No change in the activity of catalase (CAT) between both lines was noticed, however hydrogen peroxide content (H_2O_2) increased in the selected line. The selected callus line accumulated more ascorbic acid (AsA), GSH and abscisic acid (ABA), indicating that the selected callus line tends to rely on the non-enzymatic antioxidant mechanisms rather than enzymatic ones in spite of remarkable decrease in total phenols content and lycopene concentration in the selected line.

Effect of osmotic stress, induced by mannitol, on stress-related genes such as TAS14 gene and TSW12 gene was studied. The genomic DNA of both the selected and non-selected callus line was extracted according to CTAB protocol. The extracted genomic DNA was used for Ploymerase Chain Reaction (PCR) using specific 1 set of primers covering the entire coding sequence of TAS14 gene and TSW12 gene through the 2 exons then, the amplified product was sequencing and analyzed. The analysis revealed the presence of novel and published mutations in TAS14 and TSW12 genes. Some of these mutations lead to changes in amino acid sequence and consequently in protein structure.

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