PHYSIOLOGICAL STUDIES ON IMPROVEMENT OF FABA BEAN Tolerance TO ABIOTIC STRESS BY *Agrobacterium* MEDIATED GENETIC TRANSFORMATION

By

ABEER FARAG DESOUKY MOHAMED
B.Sc. Agric. Sci. (Biotechniology), Fac. Agric., Cairo Univ., 2009

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences
(Plant Physiology)

Department of Agricultural Botany
(Plant Physiology Section)
Faculty of Agriculture
Cairo University
EGYPT

2020

Format Reviewer **Vice Dean of Graduate Studies**

APPROVAL SHEET

PHYSIOLOGICAL STUDIES ON IMPROVEMENT OF FABA BEAN Tolerance TO ABIOTIC STRESS BY *Agrobacterium* MEDIATED GENETIC TRANSFORMATION

M.Sc. Thesis
In
Agric. Sci. (Plant Physiology)

By

ABEER FARAG DESOUKY MOHAMED

B.Sc. Agric. Sci. (Biotechniology), Fac. Agric., Cairo Univ., 2009

APPROVAL COMMITTEE

Dr. SAID AWAD MOHAMED SHEHATA……..
Professor of Plant Physiology, Fac. Agric., Ain Shams University.

Dr . FARGAL ABDEL HAFEZ ZEID…….
Professor of Plant Physiology, Fac. Agric., Cairo University.

Dr. MOEMEN SAYED HANAFY……..
Researcher Professor of Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Division, National Research Center.

Dr. AHMED HUSSEIN HANAFY AHMED ……..
Professor of Plant Physiology, Fac.Agric., Cairo University.

Date: / /2020

SUPERVISION SHEET

PHYSIOLOGICAL STUDIES ON IMPROVEMENT OF FABA BEAN Tolerance TO ABIOTIC STRESS BY *Agrobacterium* MEDIATED GENETIC TRANSFORMATION

M.Sc. Thesis
In
Agric. Sci. (Plant Physiology)

By

ABEER FARAG DESOUKY MOHAMED

B.Sc. Agric. Sci. (Biotechniology), Fac. Agric., Cairo Univ., 2009

SUPERVISION COMMITTEE

Dr. AHMED HUSSEIN HANAFY AHMED
Professor of Plant Physiology, Fac. Agric., Cairo University

Dr. HEBA MOHAMED IBRAHIM
Associated professor of Agricultural Genetics, Fac. Agric., Cairo University

Dr. MOEMEN SAYED HANAFY
Researcher Professor of Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Division, National Research Center.

Name of Candidate: Abeer Farag Desouky **Degree:** M.Sc.

Title of Thesis: Physiological Studies on Improvement of Faba Bean Tolerance to Abiotic Stress by *Agrobacterium* Mediated Genetic Transformation

Supervisors: Dr. Ahmed Hussein Hanafy Ahmed
Dr. Heba Mohamed Ibrahim
Dr .Moemen Sayed Hanafy

Department: Agricultural Botany **Branch**: Plant Physiology

Approval: / /**2020**

ABSTRACT

Legume crops have been the primary targets for improvement by genetic transformation due to their importance for human and animal consumption worldwide. Many of these important legume crops were difficult to genetically engineer especially faba bean crop, mainly due to high phenolics content and their recalcitrance to *in vitro* regeneration. Therefore, a series of experiments were performed in order to evaluate the growth, morphological changes and production of phenolics in the *in vitro* plantlets of five Egyptian faba bean cultivars (Giza 843, Sakha 1, Sakha 3, Nubaria 2 and Nubaria 3). The obtained results revealed that cultivars Nubaria 2 and Sakha 3 had the highest regeneration frequency (85.3% and 78.6%), respectively, and low in total phenols concentration. The effect of different levels of chitosan (0, 2, 4, 8, 15, 30, 60, and 120 mg /l) on shoot regeneration from mature embryo axes of cv. Nubaria 2 were studied The obtained results indicated that high level of chitosan have lethal effect on the development of embryo axes. However, low levels of chitosan (2 mg /l and 4mg /l) in combination with 4.5 mg/l BAP had a positive effect on shoot regeneration. HPLC analysis showed changes in the polyphenols concentrations in faba bean plantlets that exposed to low levels of chitosan (2, 4 and 8 mg /l). The obtained results indicated also that the concentration of GA_3 and ABA were increased in the faba bean plantlets that exposed to the low levels of chitosan. The effect of salt stress imposed by the addition of sea salt at concentrations of 1000, 3000, 5000 and 7000 ppm to the culture media on the selected cultivars was studied .The results revealed that decreasing in plant growth parameters, sugars and phenols concentrations with increasing sea salts concentrations.This was highly pronounced in Sakha 3 cultivar followed by Nubaria2. the effect of antioxidants (DTT , Cystein and Sodium thiosulfate) on the transformation efficiency was studied. The results obtained indicated that the addition of DTT, cysteine and sodium thiosulfate to the culture media had positive effects on increasing the transformation efficiency of faba bean. Overexpression of PR10a gene from potato were studied in transgenic faba bean lines (T2 and T3) cv.Tattoo in comparison with wild type plants using physiological measurements (net photosynthesis rate, stomatal conductance, transpiration rate, chlorophyll fluorescence Fv'/Fm' and electron transport rate ETR).This measurement were recorded using Li-Cor Li6400XT portable photosynthesis system (Licor ,Inc.,Lincoln, NE 68504) at 1200 PAR under drought and salt stress conditions using hydroponic culture system containing 150mM sodium chloride.

Key words: Embryo axes, regeneration frequency, phenols, GA_3, ABA, sugar, DTT, cysteine, sodium thiosulfate, PR10a, LI6400XT, PAR, net photosynthesis rate, stomatal conductance, transpiration rate, chlorophyll fluorescence parameter Fv'/Fm', ETR.

ACKNOWLEDGEMENT

First of all, I would like to express my deepest thanks to "ALLAH" who gave me the power, knowledge and helped me to carry on and finish this work.

I'd like to express my sincere appreciation and gratitude to ***Dr. Ahmed Hussien Hanafy Ahmed*** *Professor of Plant Physiology, Faculty of Agriculture, Cairo University, for his sincere supervision, continuous and valuable advice. Special thanks and deep appreciation are also to* ***Dr. Moemen Sayed Hanafy*** *Professor and Head of Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Division, National Research Center for his sincere supervision and especially, i would like to thank him for his detailed and constructive comments and for his important support throughout this work. My thanks also are directed to* ***Dr. Heba Ibrahim*** *Associated professor of Agricultural Genetics,Faculty of Agriculture, Cairo University, and* ***Dr.Abdel Salam Reda*** *Emeritus Professor of Plant Biotechnology, Biotechnology and Genetic Division ,National Research Center for continuous advice. Grateful thanks Directed to Professor* ***Hartmut Stützel,*** *Institute of Biological Production Systems, Leibniz University Hannover, Germany for his continuous advice and assistance in my research .Deep and Grateful thanks also extended to* ***Ms. Yichen pao*** *Ph.D. student at Institute of Biological Production Systems, Leibniz University Hannover, Germany for her continuous advice and assistance in my research. Special thanks and deep appreciation are also to* ***Ms.Illona Napp*** *Institute of Biological Production Systems, Leibniz University Hannover Germany for her technical assistance in my search . I'd like to give a delicate dedication to my family who surrounded me with all love and care ,thanks are also extended to my sister* ***Sally*** *for her support . Grateful thanks are also extended to the staff members of Plant Biotechnology Department, Genetic Engineering and Biotechnology Research Division ,National Research Center . My thanks also are directed to Alexander von Humboldt (AvH) foundation, Germany for funding .*

CONTENTS

CONTENTS (Cont.)

LIST OF TABLES