PRODUCTION OF CUCURBIT PLANTS EXPRESSING AN ANTIFUNGAL GENE

By

HOSSAM MOHAMED ZAKARIA
B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ. Egypt, ٢٠٠٠

THESIS
Submitted in Partial Fulfillment of the Requirements for the Degree of

MASTER OF SCIENCE

In

AGRICULTURAL SCIENCES
(Genetics)

Department of Genetics
Faculty of Agriculture
Cairo University
EGYPT

٢٠٠٧

APPROVAL SHEET

PRODUCTION OF CUCURBIT PLANTS EXPRESSING AN ANTIFUNGAL GENE

M.sc. Thesis

By

HOSSAM MOHAMED ZAKARIA

B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ. Egypt, ٢٠٠٠

Approved by**:**

Dr. MAHMOUD M. SAKER ……………………………………

Professor of Plant Biotechnology, National Research Center.

Dr. MONA H. HESSIN ……………………................

Professor of Genetics, Fac. Agric., Cairo University.

Dr. NAGLAA A. ABDALLAH ....

Professor of Genetics, Fac. Agric., Cairo University.

Date: / /

SUPERVISION SHEET

PRODUCTION OF CUCURBIT PLANTS EXPRESSING AN ANTIFUNGAL GENE

M.sc. Thesis
By

HOSSAM MOHAMED ZAKARIA
B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ. Egypt, ٢٠٠٠

SUPERVISION COMMITTEE

Dr. NAGLAA A. ABDALLAH
Professor of Genetics, Fac. Agric., Cairo University

Dr. ATEF S. SADIK
Professor of Microbiology, Fac. Agric., Ain Shams University

CONTENTS

LIST OF FIGURES

LISTT OF TABLES

Name of Candidate: Hossam Mohamed Zakaria **Degree:** MS.C.
Title of Thesis: Production of cucurbit plants expressing an antifungal gene.
Supervisors: Prof. Dr. Naglaa A. Abdelallah and Prof. Dr. Atef S. Sadik,
Department: Genetics
Branch: Genetics **Approval:** / /

ABSTRACT

Watermelon is among the most important vegetable crops of the *Cucurbitaceae*. Members of this family are susceptible to different kinds of diseases such as fungal infection. In this investigation, an attempt to establish an efficient regeneration and transformation systems of the Egyptian watermelon cvs Giza١, Giza ٢١ to produce transgenic plants resistant for fungal infection have been established. The proximal zone of hypocotyls was used as explants with four shoot formation media containing different concentrations of BAP, AgNO٣ and ABA. The regeneration system of the watermelon cultivars Giza ١ and Giza ٢١ was improved by adding ١ mg/l BA, ٠,٢٥ mg/l ABA and ٥ mg/l AgNO٣ (Media WM٤) as it gave the highest respond of hypocotyl explants. The enhanced shoots were evaluated for elongation stage on four different elongation media.EWM٢ medium, containing ٥٠ µg/l BAP, was considered as the best medium (٨٣٪ of shoots were elongated with only ٨٪ vitrification). The elongated shoots produced roots on MS supplemented with NAA at concentration of ٤٠ µg/l for both Giza ١ (٨٨,٨٪) and Giza ٢١ (٧٩٪). Transformation of explants was established using DNA bombardment with the plasmid pAB٦ carrying *gus* and *bar* genes as well as with *Agrobacterium*-mediated transformation method with the binary vector pISV٢٦٢٨ harboring the *gus*-intron and *bar* genes. Integration of the transgenes in the genome of putative transgenic plants and expression of transgenes was verified via histochemical assay, PCR and leaf painting tests. Furthermore, the plasmid pMON٢٢٦٥٣ containing the defensin gene, isolated from the leaves of alfalfa seedlings, under the control of the ٣٥S promoter was introduced into the cultivar Giza ١ *via* biolistic gene transfer to develop resistance against the fungal infection. Putitive transformants plants were screened using PCR and Southern blot analysis. Total genomic DNA of transformed plants were digested with *Hin*dIII and hybridized with labeled *Msdef*I gene. Positive labeled bands were obtained at different size fragments indicating that the gene allocated in different position in their genomes.

Keywords: Proximal zone of hypocotyls, *Agrobacterium*-mediated transformation, DNA bombardment, vitrification, defensin gene

ACKNOWLEDGMENT

I would like to express my sincere gratitude to **Prof. Dr. Naglaa A. Abdallah,** Prof. of Genetics, Genetics Department, Faculty of Agriculture, Cairo University for her supervision, continous guidance, and fruitful discussion.

Thanks are also due to **Prof. Dr. Atef S. Sadik,** Prof. of Agric. Microbiol., Department of Agric. Microbiol., Faculty of Agriculture, Ain Shams University and **Dr. Gihan M. Hosny**, Senior Scientist of PGTL, AGERI, ARC, Giza for their sincere help and supervision during the accomplishment of this manuscript.

Thanks also due to **Prof. Dr. Hanaiya A. El-Itriby**, former President of ARC for her endless help, encouragement during this work.

I would like to express my thanks to **Dr. Roba M. Ismail** and my colleagues; **El-Dessoky, Attia, Ismail, Tarek** and **Khaled Hashem** at AGERI, ARC, for their encouragement during this study.

INTRODUCTION

Cucurbitaceae is an economically important family which includes ١١٨ genera with ٨٢٥ species, and is widely distributed throughout tropical and subtropical regions (Whitaker and Davis, ١٩٦٢). In Egypt, the cultivated area of melon is ٧٥,٠٠٠ hectares, providing an export value that exceeds ٣٠٠,٠٠٠ dollars annually (FAOSTAT, ٢٠٠٥). Plant diseases caused by fungi are often one of the most destructive agent and difficult to control. Watermelon is infected with a large number of fungal diseases including *Fusarium,* downy mildew, powdery mildew, etc. Serious diseases caused by fungi are usually controlled by chemical fungicides that the farmer applies to crops. Two different types of fusarium infection affect watermelon cultivation all over the world. Fusarium crown and foot rot is caused by *Fusarium solani* f. sp. *Cucurbitae* which exhibits host specificity for all cucurbits. Also, Fusarium wilt is caused by a seed- and soilborne fungus that is specific to melon and watermelon (Bruton *et al*. ٢٠٠٧).

Genetic engineering has opened new avenues to modify crops, and provided new solutions to solve specific needs. The powerful combination of genetic engineering and conventional breeding programs allows useful traits to be introduced into commercial crop varieties within an economically appealing time frame (Hansen and Wright, ١٩٩٩).

Plants are amazingly adaptive at meeting environmental stresses and attacks from insects, fungi and viruses. Over the millennia and throughout millions of generations, plants have developed innovative ways to survive and prosper under harsh conditions. Several molecules in plants have been reported to contribute to plant defense activity: proteins or peptides, such as lectins, ribosomal inactivating proteins, chitinases, proteases, defensins, peroxidases, ubiquitin-like peptides, ribonucleases, arginine- and glutamate-rich peptides and some unclassified proteins, organic compounds, classified into phytoanticipins and phytoalexins, which include phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins and dienes and active nitrogen and oxygen species, such as reactive nitrogen oxide species and hydrogen peroxide (Wang and Ng, ٢٠٠٣).

Higher plants have developed a range of systems by which to protect themselves from damage associated with biotic and abiotic stress (Tregear *et al.*, ٢٠٠٢).

Plants have developed a first line of defense toward off attacking fungi, called defensins. Plant defensins facilitate genetic modification of crops for enhanced resistance to fungal attacks. In general, plant defensin blocks calcium channels, which are used as a communication network by the fungus to direct its spread by disrupting the calcium pathway that will inhibit fungi growth. A number of studies has been performed to unravel the mode of action of plant defensins. It has been shown that plant defensins induce an array of relatively rapid responses in fungal membranes, including increased $Ca^{٢+}$ uptake, K^{+} efflux, increased uptake of fluorescent dyes, and membrane potential changes. Furthermore, the existence of high-affinity binding sites for plant defensins on fungal cells and plasma membrane fractions has been demonstrated (Thevissen *et al.*, ٢٠٠٠). The first step in the path leading to fungal growth inhibition would be the binding of plant defensins to specific sites on the plasma membrane of fungal hyphae. Interaction with these binding sites would subsequently enable plant defensins to insert into the plasma membrane, thus affecting membrane structure and permeability to certain solutes, such as $Ca^{٢+}$ and K^{+}, some of which play an important role in fungal growth and development.

In vitro regeneration of adventitious shoots is a critical stage of genetic transformation as they increase the frequency of transformation. Recently, a novel direct shoot regeneration using the proximal hypocotyl was efficiently applied in melon by Curuk *et al.* (٢٠٠٢), flax by Dedicova *et al.* (٢٠٠٠), cotton by Ouma *et al.* (٢٠٠٤) and canola by Cardoza and Stewart (٢٠٠٣).

Fungal diseases are the one of the most important problems that restrict the production of watermelon. Genetic engineering approach is a valuable biotechnology for breeding purposes and can be used to introduce resistant gene not found in the gene pool of particular species.

The main aim of this work was to produce transgenic watermelon plants resistant to fungal infection, especially fusarium using the plant defensin gene. Achieving this goal required the following:

١- To improve the previously developed regeneration system of the two Egyptian watermelon cultivars Giza١ and Giza٢١ using the proximal zone of hypocotyls.

٢- To compare between *Agrobacterium*-mediated transformation and microprojectile transformation systems in watermelon for establishing the best conditions for transformation system in watermelon.

٣- To introduce the plant defensin gene into the Egyptian watermelon cultivars for acquiring resistance for fungal infection.

٤- To confirm the integration and expression of the transgenes in the putative transgenic plants by molecular analysis.