سامية محمد مصطفى

شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم

سامية محمد مصطفى

شبكة المعلومات الجامعية

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التوثيق الالكتروني والميكروفيلم

سامية محمد مصطفى

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

سامية محمد مصطفى

شبكة المعلومات الجامعية

بعض الوثائق الأصلية تالفة

سامية محمد مصطفى

بالرسالة صفحات لم ترد بالأصل

Cairo University
Faculty of Agriculture
Department of Genetics

Development of Viruses Resistance in some Plants *via* Genetic Engineering Approaches

A Thesis

Submitted in Partial Fulfillment of
Requirements for the Degree of
Ph.D. of Science
in
Genetics

By

Gihan M. H. Hussein
B.Sc. Agric. (Horticulture) 1989
M.Sc. Agric. (Vegetable Crops) Cairo Univ., 1996

2003

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2003

APPROVAL SHEET

Development of Viruses Resistance in some Plants *via* Genetic Engineering Approaches

By
Gihan M. H. Hussein

Approved

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AB STRACT

Melon and watermelon are among the most important vegetable crops of the *Cucurbitaceae*. Members of this family are susceptible to different kinds of viral diseases. This investigation was attempting to establish an efficient regeneration system for melon and watermelon and to produce transgenic plants resistant to ZYMV and WmCSV. Melon regeneration system of the melon cultivar Shahd El-Dokki was improved by adding 5 mg/l $AgNO_3$ to shoot formation medium (MR medium). This enhanced the shoot regeneration frequency from 25% to 86%. A percentage of 75% of the regenerated shoots produced roots on MS medium containing 20 µg/l NAA.

An effective plant regeneration and a gene transfer systems *via Agrobacterium tumefaciens* were developed in watermelon local cultivars (Giza 1 and Giza 21) using cotyledons as explants. The shoot regeneration frequency was 49% and 47% for *cvs*. Giza 1 and Giza 21, respectively. The regenerated shoots were rooted on MS medium containing 40 µg/l NAA.

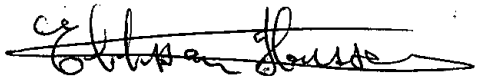
A mutation by silencing the two possible initiator AUG codons was performed in ORF AC4 of WmCSV. The mutated *Ac* 1 gene with the modified ORF AC4 was subcloned in binary vector pBin19 creating pBin/WA-11.

Melon and watermelon cotyledons from 5-days-old seedlings were inoculated with *Agrobacterium* strain LBA4404 harboring one of the binary vectors pZYMV-Eg-*cp*, pBin/WA-12 or pBin/WA-11 containing ZYMV-*cp*, modified *Ac*1 or modified *Ac*1/ *Ac*4 genes. Selection on regeneration medium supplemented with 125 mg/l kanamycin, revealed a number of kanamycin-resistant plantlets.

Integration of the transgenes in the genome of putatively transgenic plants and expression of these genes was verified by PCR and western blot analysis. The PCR results revealed that the number of positive melon plants transformed with plasmids pZYMV-Eg-*cp*, pBin/WA-12 and pBin/WA-11, were 12, 11 and 2 plants, respectively. Moreover, PCR results of putatively watermelon plants showed that three and one plants in *cv*. Giza 1 and Giza 21, respectively were positive to ZYMV-*cp* gene. Whereas, 28 and only 2 plants were positive to the *Rep* gene in the watermelon plants transformed with pBin/WA-12 and pBin/WA-11, respectively.

Western blotting analysis of the transgenic plants confirmed the expression of CP in the three PCR positive watermelon plants *cv*. Giza 1. Whereas, the tested 5 *Rep*-PCR positive melon plants showed the Rep gene expression. While, in the watermelon 48 plants exhibited a weak Rep expression. Subsequently, the resistance to WmCSV was detected using the agroinoculation assay on the T0 and T1 plants. A number of 29 watermelon plants out of 105 showed viral symptoms delay in the new shoots compared to the non-transformed plants.

Keywords: *Agrobacterium*-mediated transformation, organogenesis, Watermelon chlorotic stunt geminivirus (WmCSV), Zucchini yellow mosaic potyvirus (ZYMV), Agroinoculation, melon and watermelon



Acknowledgements

I would like to express my gratitude to

Prof. Dr. Ebtissam H.A. Hussein, Head of Genetics Department, Faculty of Agriculture, Cairo University, for her supervision and her continuous encouragement, keen interest and honest and endless help during this work and writing the thesis.

Prof. Dr. Naglaa Abdallah, Professor of Genetics, Faculty of Agriculture, Cairo University, for her critical helps, valuable advice and continuous encouragement during this work and writing the thesis.

Dr. Atef Shokry, Associate Professor of Virology, Faculty of Agriculture, Ain Shams University, for his efforts and endless helps during this study.

Prof. Dr. Bruno Gronenborn, Institut des Sciences Vegetales, for giving me the chance to work some of the experiments in his lab during the channel system mission, his efforts and sincere guidance. Also, *all the members of his lab,* for their support, helps during the period I was in France especially *Dr. Ahmed Kheyr-Pour.*

Prof. Dr. Magdy Madkour, Director of the Agriculture Research Center, for his continuous support and giving the opportunity to benefit from the channel system mission.

Prof. Dr. Hanaiya A. El-Itriby, Director General of Agricultural Genetic Engineering Research Institute, for providing all the facilities, encouragement and advices.

The members of the GTL lab, for their help and also, Mr. Khaled Hashem, at MPVL Agricultural Genetic Engineering research Institute, for his endless helps.

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