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شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم



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شبكة المعلومات الجامعية



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



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شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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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

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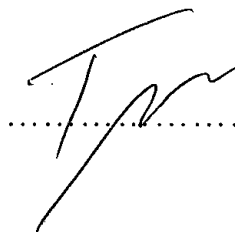
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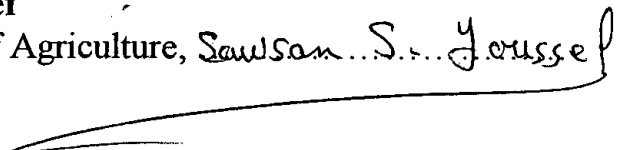
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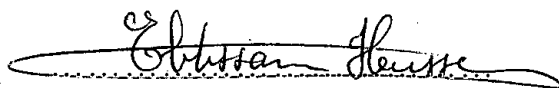
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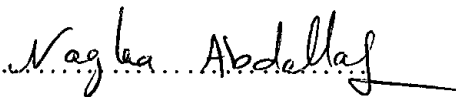
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ABSTRACT

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 AgNO₃ 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 *Ac1* or modified *Ac1/ Ac4* 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



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