PRODUCTION OF TRANSGENIC CANOLA PLANTS RESISTANT TO BLACK CUTWORM *"Agrotis ipsilon"*

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2006
M.Sc. Agric. Sci. (Genetics), Fac. Agric., Cairo Univ., 2010

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APPROVAL SHEET

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SUPERVISION SHEET

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Title of Thesis: Production of transgenic canola plants resistant to black cutworm "*Agrotis ipsilon"*
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ABSTRACT

In order to improve insect resistance in canola,transgenic canola plants expressing the *Cry1Ia5* gene were developed. The cultivars tested differ genetically for callus induction and somatic embryo formation. The cultivar Serw-4 exhibited high regeneration efficiency (97%) followed by Serw-3 (88%). The genetic differences in regeneration ability among the four canola cultivars were resolved by RAPD and AFLP analyses. The genotype-specific markers were determined which can be verified as useful genetic markers for the regeneration capacity of the different canola cultivar used. The efficiency of sonication assisted *Agrobacterium* and particle bombardment mediated gene delivery systems was compared. The data indicate that 5 sec. sonication followed by *Agrobacterium* infection improves the transformation efficiency to 55% and 33% compared with 25% and 16% that resulted from biolistic gun for the cultivars Serw-4 and Serw-3, respectively. The number of transgenic plants recovered from the Agro-sonication method was higher in comparison to biolistic mediated transformation. The Agro-sonication method was employed to develop transgenic canola plants expressing the *Cry1Ia5* gene. Stable integration of the *Cry1Ia5* gene into plant genome was confirmed by PCR and Southern blot analyses. Northern blot analysis of the T_2 plants confirmed the expression of the *Cry1Ia5* gene. The insecticidal activity of five transgenic canola lines against the 2^{nd} larval instars of black cutworm was tested. The transgenic line 5 was more toxic, it causes 100% mortality after four days of feeding. The transgenic lines L2, L3, L4 cause 60, 20, 80 mortality % respectively after six days of feeding. Significant decreases in total protein, protease, lipase, alpha esterase and AChE activities were detected in the body of larvae fed on transgenic material compared with the control. The data show a significant increase in GST and chitinase activities in the body of larvae fed on *bt* compared with the control canola plants. Transgenic plants showed high resistance to black cutworm and can serve as a novel genetic resource in future breeding programs. Transgenic plants expressing BT protein were normal in phenotype with as good seed setting as the non-transgenic control plants.

Key words: *Cry1Ia5* gene, Insect resistance, Canola, Transformation.

DEDICATION

I dedicate this work to whom my heartfelt thanks: to my father, mother, sister and brother for all the support and encouragement they continually offered along the period of my post-graduate studies.

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