STUDIES ON GENE REGULATION OF SOME GENES THAT RELATED TO BT. TOXINS RESISTANCE IN THE COTTON WORM,

Spodoptera littoralis

By

HAGER MOHSEN HASSAN ALI KHALIL

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2013

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

2021

Format Reviewer

Vice Dean of Graduate Studies

Name of Candidate: Hager Mohsen Hassan Ali Khalil Degree: M.Sc. Title of Thesis: Studies on Gene Regulation of Some Genes that Related to

Bt. Toxins Resistance in the Cotton Worm, Spodoptera littoralis.

Supervisors: Dr. Nagwa Ibrahim Abdelfattah Elarabi

Dr. Dalia Sayed Ahmed Hasaneen

Dr. Saad Mohamed Moussa

Department: Genetics **Branch:**

Approval: 27/3/2021

ABSTRACT

A better understanding of the processing of *Bacillus thuringiensis* (Bt) Cry1C toxin in the midgut of *Spodoptera littoralis* larvae is very important to characterize the main regulatory elements of Bt tolerance. The current study aimed to evaluate the mRNA level of trypsin (Try), aminopeptidase N (APN), alkaline phosphatase (ALP), cadherin (Cad), and cytochrome P450 (CYP) in both susceptible and Cry1C- tolerant strains of S. littoralis. Total RNA was extracted from susceptible and tolerant strains to construct cDNA. Quantitative real-time PCR (qPCR) showed a significant upregulation of CYP gene in tolerant strain. In contrast, the levels of expression of Try, ALP and Cad were significantly downregulated in tolerant strain. APN relative mRNA expression did not show significant differences between susceptible and tolerant strains. Histologically, the midgut of late third-instar larvae of tolerant population S. littoralis showed vacuolization of the epithelium and disruption of both the peritrophic membrane and the striated boarder compared to the susceptible strain. Our data indicate the important roles of CYP, Try, ALP and Cad in the resistance development and toxicity to Bt Cry1C. The obtained results are useful for further illustrating of Bt Cry1C processing and S. littoralis tolerance.

Key words: *Spodoptera littoralis, Bacillus thuringiensis, Bt* Cry1C toxin, Tolerance, qPCR

CONTENTS

INTRODUCTION	
REVIEW OF LITERATURE	
1. Egyptian cotton leafworm "Spodoptera littoralis"	
2. Pest control	
3. Control of the cotton leafworm	
4. Bacillus thuringiensis (Bt) as a biological control agent	
5. Mode of action of <i>B. thuringiensis</i>	
6. Resistance and tolerance against <i>Bt</i> toxins	
7. Genes influence in <i>Bt</i> resistance evolution	
a. Cadherin as a midgut receptor	
b. Aminopeptidases N as a midgut receptor	
c. Alkaline phosphatase as a midgut receptor	
d. Gut proteases as a Bt protoxin activation enzyme	
e. Cytochrome P450 as a detoxification enzyme	
f. ATP-binding cassette as a transporter	• • • •
g. β-1,3-Galactosyltransferase enzyme	
h. Esterase enzyme	
MATERIALS AND METHODS	· • • • •
RESULTS	
1. Tolerance level of S. littoralis larvae to Bt Cry1C toxin	
2. Histopathological studies	
3. Primers design for gene expression quantification	
4. Determination of gene expression level using qRT-PCR	
DISCUSSION	
SUMMARY	
REFERENCES	
ARARIC SUMMARY	

LIST OF TABLES

No.	Title	Page
1.	qPCR primers sequences for gene expression measurement	37
2.	Mortality of susceptible and <i>Bt Cry1C</i> - tolerant strains of <i>S. littoralis</i> larvae	51
3.	Susceptibility of susceptible and <i>Bt Cry1C</i> - tolerant strains of <i>S. littoralis</i>	52
4.	Genes name, Species, and GenBank accession number. Cad, APN, ALP, Try, CYP, and 28S rRNA.	56
5.	qPCR analysis: Relative fold change expression of Cad, ALP, APN, Try and CYP in midgut of susceptible (Sus.) and <i>Bt Cry1C</i> - tolerant (Tol.) strain of <i>S. littoralis</i>	62
	vivo i wing	52

LIST OF FIGURES

No.	Title	Page
1.	Spores and protein crystals of <i>B. thuringiensis</i>	12
2.	Schematic representation of the three-domain Cry toxin structure	13
3.	Mode of action of the <i>Bt</i> Cry toxin according to the sequential binding model	15
4.	Schematic representation of the "ping-pong" binding mechanism.	16
5.	Mode of action of the <i>Bt</i> Cry toxin according to the signal transduction model	17
6.	Chromatin prestained protein ladder map	34
7.	1Kb DNA ladder map	36
8.	SDS PAGE gel (10%) showing partially-purified <i>Bt</i> Cry1C toxin	50
9.	Midgut of late third-instar larvae of <i>S. littoralis</i>	54
10.	Integument of late third-instar larvae of <i>S. littoralis</i>	55
11.	Total RNA isolation from midgut of 3 rd instars larvae of susceptible (A) and <i>Cry1C</i> - tolerant (B) strains of <i>S</i> .	
	littoralis	58
12.	Relative fold change expression of Cad gene in midgut of susceptible (Sus.) and tolerant (Tol.) <i>S. littoralis</i>	59

13.	Relative fold change expression of APN gene in midgut of susceptible (Sus.) and tolerant (Tol.) S. littoralis	59
14.	Relative fold change expression of ALP gene in midgut of susceptible (Sus.) and tolerant (Tol.) <i>S. littoralis</i>	60
15.	Relative fold change expression of Try gene in midgut of susceptible (Sus.) and tolerant (Tol.) <i>S. littoralis</i>	60
16.	Relative fold change expression of CYP gene in midgut of susceptible (Sus.) and tolerant (Tol.) <i>S. littoralis</i>	61