THE IMPACT OF INSERTING RNAI SUPPRESSOR GENE ON BACULOVIRUS PATHOGENICITY.

By
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B. Sc. Agric. Sc. (Genetics), Ain Shams University,2004.

A thesis submitted in partial fulfillment
of
the requirements for the degree of

MASTER OF SCIENCE

in
Agricultural Science
(Genetics)

Department of Genetics
Faculty of Agriculture
Ain Shams University

2012

Approval Sheet

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ABSTRACT

Shimaa Mohamed Mohamed El-Sayed El-Gamal: The Impact of Inserting RNAi Suppressor Gene on Baculovirus Pathogenicity. Unpublished M.Sc. Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2012.

Baculoviruses are natural enemies of pest insects but they have narrow host range. Recently, RNA interference (RNAi) confirmed to function as a natural defense mechanism against viruses. Too many plant and mammalian viruses encode RNAi suppressors to counteract this antiviral mechanism and enhance viral infectivity in host cells. Few studies have been done to illustrate the role of RNAi as an antiviral mechanism against DNA viruses. The influence of RNAi on baculovirus pathogenicity or replication has not been intensively studied. In the present study, the B2 gene of the flock house virus (FHV), which known as RNAi suppressor was synthesized and a recombinant baculovirus expressing it under polyhedrin promoter was produced. The mortality of this recombinant virus to *S. littoralis* larvae was compared with the mortality of a control virus. The data showed significant differences between both viruses in killing larvae, but it need three to four weeks post infection. Moreover, using the end point dilution method showed that the B2 enhanced the production of AcMNPV in *Sf9* cells. The effect of B2 on viral replication needs to be studied in details.

Key Words:

Baculovirus, RNA interference (RNAi), RNAi suppressor, B2, Flock house virus (FHV), *Spodoptera littoralis*.

ACKNOWLEDGEMENT

First of all I'd like to thank ALLAH almighty, the most merciful and compassionate, for his support, help and generosity.

I wish to express my deep sense of gratitude to **Prof. Dr. Samir Abdel-Aziz Ibrahim**, Professor Emeritus of Genetics, Genetics Department, Faculty of Agriculture, Ain Shams University, for his excellent supervision, valuable and unfailing guidance, continuous encouragement, patience, fruitful discussion during preparation of the thesis and unlimited guidance throughout writing this thesis.

I would like to express my sincere gratitude to **Dr. Sabah Mahmoud Mohamed Hassan**, Associate Professor of Genetics, Genetics Department, Faculty of Agriculture, Ain Shams University, for her supervision, keen interest, encouragement, kindness, energetic guidance and unlimited guidance throughout writing this thesis.

I would like to extend my deepest and greatest thanks to **Dr. Tamer Ibrahim Zaki Salem**, Research Associate Prof. of Microbial Molecular Biology, Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), for his supervision, kindness, encouragement, unlimited guidance throughout writing this thesis and never ending support throughout this thesis.

I would like to express my gratitude to my lab Gene Silencing and insect control (GSIC) group **Dr. Ahmed Mohamed**. Also special thanks to all my colleagues at the GSIC lab **Manal**, **Mervat** and **Rasha** for their faithful friendship and encouragement and also my gratitude to all Agricultural Genetic Engineering Research Institute (AGERI) staff.

Also, I would like to express my gratitude to the staff members of Department of Genetics, Faculty of Agriculture, Ain Shams University.

I extend my great thanks to all my friends for their love and care.

Last, but not least, my warmest thanks to all my family members specially, my father's spirit, my mother and my sister for their continuous

support and for their faithful encouragement during my entire educational career and this work could not be completed without their love, care and help.

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LIST OF ABBREVIATION

AaIT	*Androctonus australis* insect selective toxin
a.a.	amino acid
AcMNPV	*Autographa californica* nucleopolyhedrosis virus
AGERI	Agriculture genetic engineering research institute
Ago2	Argonaute
Anti	antisense
ATP	Adenosine triphosphate
B.P.B.	Bromophenol blue
BEVS	baculovirus expression vector system
Bt	*Bacillus thuringiensis*
BV	Budded virus
C. elegance	*Caenorhabditis elegans*
CrPV	cricket paralysis virus
CTV	Citrus tristeza virus
dsRNA	double stranded RNA
DTT	Dithiothreitol
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
EGFP	enhanced green fluorescent Protein
egt	ecdsteroid UDB-glucosyltransferase gene
Fig.	Figure
FHV	Flock house virus
GP64	Glycoprotein 64
GVs	Granuloviruses
h.p.i.	Hour post infection
HaSNPV	*Helicoverpa armigera* nuclear polyhedrosis virus
Hc-pro	helper component protein
HzSNPV	*Heliothis zea* nuclear polyhedrosis virus
IE-0	immediate early - 0
IPTG	Isopropyl-beta-thio galactopyranoside
LacZ	Lactose Z gene

LB	Luria Broth media
miRNAs	microRNAs
MOI	multiplicity of infection
NoV	Nodavirus
NPVs	nucleopolyhedrosis viruses
nt	nucleotide
NTC	no template control
O.D.	Optical density
ODV	occluded derived virus
PCR	polymerase chain reaction
PD	proportionate distance
Pfu taq	proof reading Taq DNA polymerase enzyme
Pfu/ml	plaque forming unite/ml.
pIBs	polyhedral inclusion bodies
Polh	Polyhedrin
Pre-miRNAs	precursor microRNAs
PTGS	post transcriptional gene silencing
Rec.	recombinant
RISC	RNA induced silencing complex
RNAi	RNA interference
rpm	round per minute
S. littoralis	*Spodoptera littoralis*
S2	Schneider line 2
SDS	sodium dodecyl sulfate
Sf9/*Sf21*	*Spodoptera frugiperda* 9/21
siRNAs	small interfering RNAs
ss	single stranded
TAE	Tris acetate EDTA solution
$TCID_{50}$	tissue culture infectious dose
TEV	Tobacco etch virus
WhNV	Wuhan Nodavirus
X-gal	5-bromo-4-chloro-indolyl-β-D-galactopyranoside

I. INTRODUCTION

Baculoviruses consider one of the most prominent natural enemies of pest insects. They are host specific and have narrow host range. Moreover, they are safe to human, plants, animals and environment. However, the major impediments on using them as biopesticides include low infectivity in some cases and slow killing speed. Recombinant viruses were the key to solve these problems. Too many recombinant viruses are produced using insect specific toxins to speed up their killing time **(Stwart *et al.,* 1991 and Tomalski and Miller 1991).** Other genes have been inserted to or deleted from baculovirus genome to improve baculoviruses as biopesticides **(O'Reilly and Miller 1991and Inceoglu *et al.,* 2001)**.

One of the methods that has not been examined before is whether or not RNA Interference (RNAi) has effect on baculovirus replication or infectivity.

RNAi machinery **(Fire *et al.,* 1998)** occurs in most of eukaryotic organisms and has been described as Post-transcriptional gene silencing (PTGS) in plants and quelling in fungi **(Pandit and Russo, 1992 and Romano and Macino, 1992).** This mechanism can degrade viral and transgene RNAs in a sequence specific manner. RNAi machinery is triggered by the presence of double stranded RNAs (dsRNAs) in most of the eukaryotic cells. This mechanism functions as a natural defense mechanism against viruses; therefore too many plant and mammalian viruses encode RNAi suppressors to counteract this antiviral mechanism in host cells **(Delgadillo *et al.,* 2004).** The researchers found that some of these suppressors have other functions during virus infection such as B2 suppressor of Nodamura virus, which can increase viral RNA accumulation in both vertebrate and invertebrate cell lines **(Johnson *et al.,* 2004).** In addition, B2 protein of Flock house virus enables virus infection in Drosophila cells **(Li *et al.,* 2002)**. Moreover, nonstructural

protein NSs of tomato spotted wilt virus enhances the Baculovirus replication in permissive and semipermissive insect cells. **(Oliveira *et al.*, 2011).**

It is important to study the impact of RNAi suppressor protein isolated from insect virus on DNA virus pathogenicity, which may uncover the Role of RNAi machinery against DNA viruses as well as RNA viruses. In addition, more understanding for virus host interaction can be obtained from this study.

The main aim of this study is to synthesize the B2 gene of the FHV and express it under polyhedrin promoter to study if RNAi plays role in baculovirus infectivity.