# MOLECULAR GENETIC STUDIES ON A MIDGUT RELATED GENE IN PINK BOLLWORM (*PECTINOPHORA GOSSYPIELLA*)

**By**


## MERVAT RAGAB MANSOUR ALI DIAB

**B. Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2005**


## THESIS

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# APPROVAL SHEET

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SUPERVISION SHEET

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**Title of Thesis:** Molecular Genetic Studies on a Midgut Related Gene in Pink Bollworm (*Pectinophora gossypiella*).
**Supervisors:** Dr. Ebtissam H.A. Hussein
Dr. Samir M. M. Abd-alla
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## ABSTRACT

Vacuolar - $H^+$ ATPase (V-ATPase) is a multi subunit enzyme. It consists of two domains ($V_1$ and $V_O$). $V_1$ domain has eight subunits (A-H) while $V_O$ domain has six subunits (a, c, c', c", d and e). The V-ATPase is an ATP-driven proton pump and is essential to the function of eukaryotic cells. In insects, V-ATPase is located in apical membrane of goblet cells in the midgut. It acts as an energizer of the plasma membrane, driving nutrient uptake, fluid secretion and in some cases alkalizing the gut lumen. The aim of the present study was knocking down the V-ATPase gene(s) using RNAi in an attempt to disturb the food process within the pink bollworm (PBW) midgut and eventually causing the insect death. Based on the conserved regions of the V-ATPase subunits A and D genes in different insects available in the GenBank, degenerate primers were synthesized and used to amplify the two genes. The amplified genes encoding the V-ATPase subunits A and D transcripts were sequenced. The sequencing results confirmed the isolation of the full length genes encoding the V-ATPase subunit A was 2602 bp in length, encoding 618 amino acids. While, the gene encoding V-ATPase subunit D was 1467 bp, encoding 249 amino acids. Three dsRNA fragments were designed, two of them (VATPA756-1155 bp and VATPA347-753 bp) were targeting subunit A and the third (VATPD221-703 bp) to knockdown subunit D. Three bioassay methods were assayed on the larvae of the pink bollworm (PBW), i.e., droplet feeding, mixing the dsRNA with the diet and dsRNA injection. Injection of 200 ng of the three dsRNA fragments (VATPA756-1155 bp, VATPA347-753 bp and VATPD221-703 bp) into the thorax of the third instar caused larval mortality of 46.3, 43.9 and 25%, respectively. Furthermore, treating the larvae with the two dsRNA fragments targeting the V-ATPase subunit A caused shrinkage of the bodies and slower development indicating the starvation effect induced by the treatment with the dsRNA.
**Key words:** Pink bollworm, *Pectinophora gossypiella*, RNA interference, Subunit A, Subunit D and Vacuolar ATPase.

# *DEDICATION*

*I dedicate this work to my heartfelt dearness, love and thank. To my late father, my mother, my husband (Ahmed), my kids (Hisham, Aya and Mohammed), my brothers (Ali, Sayed, Ahmed and Mustafa) and my sisters (Mariam, Hanaa and Rabab). I would like to express my deepest thanking for all their kindness, patience, support, helping and encouragement.*

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| *A. albopictus* | *Aedes albopictus* |
| *A. aegypti* | *Aedes aegypti* |
| AGERI | Agriculture Genetic Engineering Research Institute |
| ARC | Agriculture research center |
| *Bt* | *Bacillus thuringiensis* |
| BLAST | Basic local alignment research tool |
| *B. mori* or (Bm) | *Bombyx mori* |
| Cry | Crystalline |
| dsRNA | Double-stranded RNA |
| *D. melanogaster* or (Dm) | *Drosophila melanogaster* |
| E value | Expectation value. |
| *H. armigera* | *Helicoverpa armigera* |
| h. | hours |
| *M. sexta* or (Ms) | *Manduca sexta* |
| mRNA | Messenger RNA |
| miRNAs | MicroRNAs |
| min | minutes |
| *N. lugens* | *Nilaparvata lugens* |
| no. | number |
| ORF | Open reading frame |
| *O. furnacalis* or (Of) | *Ostirina furnacalis*, |
| *P. gossypiella* or (Pg) | *Pectinophora gossypiella* |
| PBW | Pink bollworm |

| | |
|---|---|
| RACE | Rapid amplification of cDNA ends |
| RISC | RNA-induced silencing complex |
| RNAi | RNA interference |
| rpm | Round per minute |
| sec | seconds |
| siRNA | Short interfering RNA |
| SIT | Sterile insect technique |
| V-ATPase | Vacuolar ATPase |

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