MOLECULAR IDENTIFICATION AND RNAI OF THE AQUAPORIN GENE(S) FROM THE EGYPTIAN COTTON LEAFWORM, *SPODOPTERA LITTORALIS*

By

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

Shimaa Mohamed Mohamed EL-Sayed EL-Gamal: Molecular Identification and RNAi of Aquaporin Gene(s) from The Egyptian Cotton Leafworm, *Spodoptera littoralis*. Unpublished Ph.D Thesis, Department of Genetics, Faculty of Agriculture, Ain Shams University, 2018.

Aquaporins (AQPs) are integral membrane proteins belong to the Major Intrinsic Proteins (MIP) superfamily identified in different tissues from all living organisms including mammals, plants, invertebrates and microorganisms. Aquaporins play crucial physiological roles in insects besides maintaining the appropriate water homeostasis. Therefore, they were suggested as good targets for insect control through RNAi. Only few AQPs were isolated and studied from terrestrial insects. In the present study, we report the cloning of partial cDNA sequences of AQP1 and AQP3 genes from *Spodoptera littoralis*. Our study revealed that AQP1 and AQP3 genes are expressed in all insect developmental stages (eggs, larval instars, pupae, and adult males and females), while AQP2 gene expression was detected in all insect stages except for eggs and adult females. Furthermore, AQP1, AQP2 and AQP3 gene expression was detected in tissues of the digestive and the excretory systems (foregut, midgut, hindgut and Malpighian tubules) of fourth, fifth and sixth larval instars. Gene expression of AQP1 and AQP3 was detected in the reproductive system of adult females and males, while AQP2 gene expression was detected in the reproductive system of adult males only. Silencing of AQP2 gene was more effective than silencing of AQP1 or AQP3 genes as it gave higher mortality ratio and more delayed adult emergence than silencing of AQP1 and AQP3 genes. The highest reduction in gene expression through RNAi was achieved for AQP2 gene compared to AQP1 and AQP3 genes. Consequently, we suggest that these AQP genes have critical role in maintaining water balance in *Spodoptera*

littoralis insects, specially the AQP2 gene. The role of aquaporins in insect development and reproduction is still in need to more investigation.

Keywords: *Spodoptera littoralis,* Lepidoptera, aquaporin (AQP), gene expression, water homeostasis, RNA interference (RNAi)

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LIST OF ABBREVIATION

ABBREVIATION	Name
a.a.	Amino acid
Amp.R	Ampicillin resistance gene
Acc. no	Accession number
AQP	Aquaporin
ANOVA	Analysis of variance
BLAST	Basic Local Alignment Search Tool
BME	beta mercapto ethanol
B. mori	*Bombyx mori*
bp	base pair
B.P.B.	Bromophenol blue
Bt	*Bacillus thuringiensis*
cDNA	complementary DNA
C. elegance	*Caenorhabditis elegans*
C. supperssalis	*chilo supperssalis*
dsRNA	double stranded RNA
DDT	dichlorodiphenyltrichloroethane
DTT	Dithiothreitol
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
Fig.	Figure
G. molesta	*Grapholita molesta*
GLPs	aquaglyceroporins
h.p.i.	Hour post injection
H. armigera	*Helicoverpa armigera*
IPM	Integrated Pest Management
IPTG	Isopropyl-beta-thio galactopyranoside
LacZ	Lactose Z gene
LB	Luria Broth media
MIPs	Major intrinsic proteins

NF	Nuclease-Free
NTC	no template control
O.D.	Optical density
PCR	polymerase chain reaction
qRT-PCR	Quantitative real time PCR
P. rapae	*Pieris rapae*
RISC	RNA induced silencing complex
RACE	Rapid amplification of cDNA ends
RNAi	RNA interference
rpm	round per minute
RT-PCR	reverse transcriptase PCR
Sp. littoralis	*Spodoptera littoralis*
Sp.li	*Spodoptera littoralis*
Spoli-DHR	*Spodoptera littoralis* Diuretic Hormone Receptor
Sp. litura	*Spodoptera litura*
Sp. lu	*Spodoptera litura*
T. castanium	*Tribolium castanium*
RH	Relative humidity
RT	room temperature
SE	standard error
siRNAs	small interfering RNAs
ss	single stranded
TAE	Tris acetate EDTA solution
UTR	un translated region
X-gal	5-bromo-4-chloro-indolyl-β-D galactopyranoside

INTRODUCTION

Water regulation is one of the critical physiological mechanisms in all living organisms as water supports all the biochemical processes within the living cells. So it is important to maintain water balance during all developmental stages. Water regulation is maintained by some hormones (*eg.* diuretic and antidiuretic hormones), their receptors and water channels (aquaporins). Aquaporin proteins are integral membrane proteins belong to the major intrinsic proteins (MIPs) superfamily. Aquaporins make the membrane 10 to 100 fold more permeable to water than membranes lacking such channels (**Krane and Kishore 2003**). The first aquaporin was identified from human blood cells by **Agre *et al.*, (1993)**, then followed by a number of different AQPs which have been identified in all kinds of tissues of mammals, invertebrates, plants, and microorganisms (**Gomes *et al.*, 2009**). In insects, aquaporins are expressed in different tissues such as Malpighian tubules, hindgut, fat bodies, ovaries, olfactory organs and others. Regarding lepidopteran insects, some AQP genes were isolated and characterized from only five species; *Bombyx mori*, *Spodoptera litura*, *Grapholita molesta*, *Ectropis oblique*, and *chilo suppressalis* (**Kataoka *et al.*, 2009b; Azuma *et al.*, 2012; Liu *et al.*, 2013; Li *et al.*, 2016; Lu *et al.*, 2018**).

Aquaporins play many important secondary physiological roles in insects besides maintaining water homeostasis such as drought and freeze tolerance **(Kikawada *et al.*, 2008; Philip *et al.*, 2011)**, the excretion process **(spring *et al.*, 2009)**, gas exchange **(Cooper and Boron 1998; Endeward *et al.*, 2006)**, ion transport through the central pore of the tetramers (**Cohly *et al.*, 2008)**, neural signal transduction and cell to cell adhesion **(Tatsumi *et al.*, 2009)** and role in maintaining the appropriate aqueous environment of olfactory organs (**Ishida *et al.*, 2012).** Therefore disrupting insect aquaporins was suggested as a good target for insect control.