Transcriptomic Analysis of the Larval Head of Egyptian Cotton Leafworm

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Figure 1. Egyptian cotton leafworm (*S. littoralis*) larvae.

Introduction

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae) is a widely-distributed and economically important pest of a wide range of crop plants (EPPO, 1997). It feeds on more than 112 plant species belonging to 44 families. It is a foliage feeder as well as a seedling cutworm. It is currently or historically a pest of cotton, maize, potatoes, forage crops, orchard crops, ornamentals, tomatoes, peppers and other vegetables in countries of Mediterranean Europe, the Middle East, North and Central Africa (Carter, 1984 and Gómez & Arroyo, 1994). In Egypt, *S. littoralis* is the most serious pest of cotton (CAPS, 2010) and damages a wide range of vegetables, ornamentals and tree crops (Belda *et al*., 1994). Over time, many leafworm populations have acquired resistance towards most conventional insecticide groups (Alford, 2000). In Egypt, the common control measures used against this insect are hand-picking of egg-masses and aerial spraying of pesticides. Both are labor intensive and little increase in their efficiency is possible. During the last two decades, research has been directed at developing novel and effective control agents against the cotton leafworm (Rashwan *et al*., 1992).

Objective

The development of an efficient insecticide to protect crops from *S. littoralis* by suppressing or silencing one or more essential genes using RNA Interference (RNAi). In this study, the transcriptome of the larval *S. littoralis* head was sequenced and analyzed for the first time to identify potential gene targets for RNAi. These data can also be used to develop a global approach for identifying genes involved in *S. littoralis* resistance to chemical insecticides.

Materials and Methods

Insects: *S. littoralis* fourth instars were obtained from a colony maintained at the insectary of the Agricultural Genetic Engineering Research Institute (AGERI), Giza, Egypt. Larvae were reared to pupation at room temperature in containers of sawdust and fed castor bean leaves (*Ricinus communis*); adults were fed 10% sugar solution and provided with leaves of *Nerium oleander* for oviposition (El-Defrawi *et al*., 1964).

Isolation of total RNA from larval heads: Fourth instars were starved for 8 h to clear their guts before total RNA isolation. Heads were separated from starved larvae and immediately transferred to a mortar containing liquid nitrogen and ground to a fine powder. Total RNA was extracted from the powdered tissue via a QIAGEN® RNeasy mini kit (QIAGEN, Valencia, CA, USA). A cDNA library was prepared from ≥ 5 µg total RNA using Superscript II Reverse Transcriptase kit (Invitrogen, Carlsbad, CA, USA).

DNA sequencing and analysis: The cDNA library was sequenced with an Illumina® Genome Analyzer IIx sequencer (Illumina, San Diego, CA) at North Carolina State University. CLC Genomics Workbench ® software (CLC bio; www.clcbio.com) was used to assemble the larval head reads into contigs (contiguous nucleotide sequences); k-mer length was 25 bp (base pairs) and the length cutoff for reads assembled into contigs was ≥ 300 bp. Blast2GO® software (Conesa *et al*., 2005) was used to align, map, and annotate the contigs. For the alignment step, the contigs were translated to proteins in all six reading frames and compared to the GenBank nr (non-redundant) protein database using the BLASTx (Basic Local Alignment Search Tool) algorithm with E-value cut-off set at 10. BLAST hits (*S. littoralis* query contigs with database-sequence matches where E-value ≤ E-3) were mapped and annotated with GO (Gene Ontology) terms. These GO terms assigned the translated query sequences to categories of putative protein function (GO level 2 functional categories) on the basis of sequence and functional conservation among organisms represented in publicly-accessible protein/gene-product sequence databases (Gene Ontology Consortium; Ashburner *et al*., 2000).

Results

Table 1. Data summary of the Egyptian cotton leafworm head cDNA library sequenced by an Illumina® Gene Analyzer IIx.

	Head
Total # of read sequences	51,252,862
Read length (base pairs)	101
Total number of bases read	5,176,539,062
GC%	40%
Number of contigs	17,318
Shortest contig (base pairs)	500
Median contig (base pairs)	1,122
Longest contig (base pairs)	16,676
Average contig (base pairs)	1,514

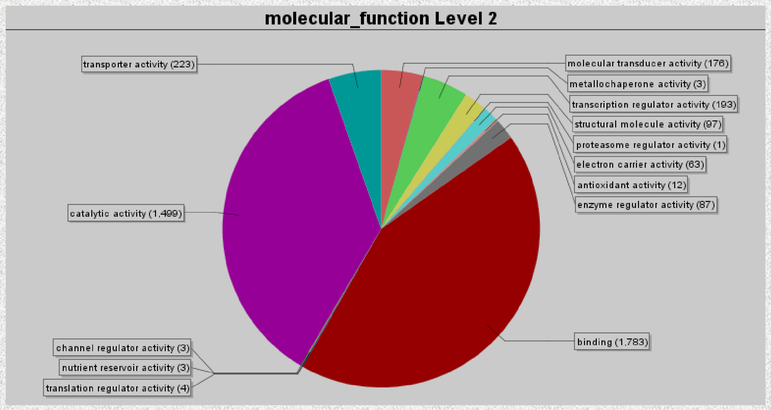

Figure 2. GO analysis results for the larval Egyptian cotton leafworm head contigs.

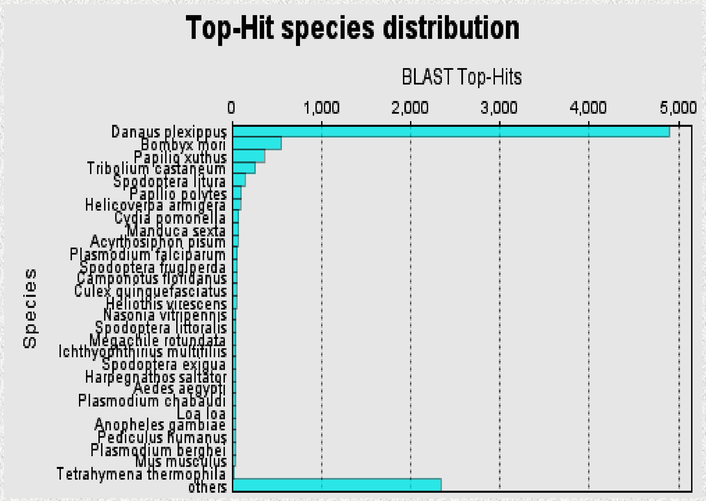

Figure 3. Species distribution of blast hits for contigs of Egyptian cotton leafworm.

Table 2. Examples of putative hormones found in the larval head transcriptome of the Egyptian cotton leafworm.

Message	No. bp	E-value
Allatostatin	1158	2.03E-151
Eclosion hormone	763	1.67E-50

Table 3. Example of hormone receptors found in the larval head transcriptome of Egyptian cotton leafworm.

Message	No. bp	E-value
Diuretic hormone receptor	1762	0
Adipokinetic hormone receptor	2598	0

Table 4. Examples of putative proteins involved in juvenile hormone (JH) biosynthesis found in the larval head transcriptome of Egyptian cotton leafworm.

Message	No. bp	E-value
Farnesyl pyrophosphate synthetase	958	3.88E-55
JH acid methyltransferase	1281	2.44E-117

Table 5. Example of enzymes associated with juvenile hormone (JH) found in the larval head transcriptome of Egyptian cotton leafworm.

Message	No. bp	E-value
JH epoxide hydrolase	1555	0
JH esterase	2962	0
ecdysone 20-monooxygenase	3163	0

Table 6. Putative insecticide receptors and xenobiotic enzymes found in the larval head transcriptome of Egyptian cotton leafworm.

Message	No. Bp	E-value
Acetylcholine receptor alpha-like	2014	0
Nicotinic acetylcholine receptor beta-1	1148	0
Aminopeptidase N	4189	0
Cadherin	4680	0
Glutathione S-transferase	2117	4.65E-152
Cytochrome p450	3443	0
Carboxylesterase	2388	0

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