TRANCSCRIPTOMIC ANALYSIS OF THE LARVAL HEAD OF EGYPTIAN COTTON LEAFWORM

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Abstract

Egyptian cotton leafworm, *Spodoptera littoralis*, is the most important pest of cotton in Egypt. Current technologies offer limited protection of cotton. Transcriptomes for the larval head and abdomen of this pest were sequenced for the first time using Illumina® technology. Here we present current results of on-going analysis of the larval head transcriptomic cDNA library. A total of 51,252,862 larval head read sequences were assembled into 17,318 contigs which are being analyzed to identify their function. Bioinformatic analysis has thus far identified sequences associated with functions critical to the insect's growth and development. Among these are sequences associated with JH regulation and synthesis, moulting, metabolism, water balance, insecticide activity, and olfaction. Genes identified have the potential to be targeted with RNAi transgenes. Our sequencing results can also be used to develop a global approach for identifying genes involved in *S. littoralis* resistance to chemical insecticides.

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; CAPS, 2010). It feeds on more than 112 plants belonging to 44 families (Moussa *et al*., 1960; Salama *et al*., 1970; Brown & Dewhurst, 1975). It is a foliage feeder as well as a seedling cutworm. It is currently and/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 Africa, and Central Africa (Carter, 1984; Inserra & Calabretta, 1985; Gómez & Arroyo, 1994).

In Egypt, *S. littoralis* is the most serious pest of cotton (Bishara & El Zoheiry, 1940 ; CAPS, 2010) and damages a wide range of vegetables, ornamentals and tree crops (Belda *et al*., 1994; Domínguez 1993). Many populations have acquired resistance towards most conventional insecticide groups (Alford, 2000). The main control measures now used against this insect are hand-picking of egg-masses and aerial spraying of pesticides. Both are intensive, and little increase in their efficiency is possible. During the last two decades, research has been directed at developing new control agents effective against this pest (Rashwan et al., 1992).

The prospect of developing an insecticidal transgene to protect crops from *S. littoralis* may lie in manipulating this pest's RNAi (RNA interference) mechanism to suppress or silence its critical-functioning genes (Kennerdell & Carthew, 2000). The objective of our work was to sequence and analyze - for the first time - the transcriptomes of the larval *S. littoralis* head and abdomen in order to identify potential targets for RNAi. Our sequencing results can also be used to develop a global approach for identifying genes involved in *S. littoralis* resistance to chemical insecticides. We report here results for the head transcriptome.

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; Elbarky et al., 2008).

Isolation of total RNA from larval heads

Fourth instars were starved for 6 hours in order to clear their guts before isolation of total RNA. Heads were separated from starved larvae and immediately transferred to a mortar containing liquid nitrogen. A pestle was used to grind head tissue to a fine powder while the tissue was submerged in liquid nitrogen. 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 via an Invitrogen® 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 E10 (10 +1). BLAST hits (*S. littoralis* query contigs with database-sequence matches where E-value ≤ E+10) 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 and Discussion

Sequencing and assembly results

Sequencing and assembly were completed in July 2012. Results are presented in Table 1. Total read count was 51,252,862 for the head cDNA library. Read length was 101 nucleotide bp. The head reads were assembled into 17,318 contigs. Contig sequence length ranged from 500 – 16,676 nucleotides, with an average of 1,514.

Table 1. Sequencing and assembly summary for *S. littoralis* larval head cDNA library sequenced with an Illumina® Gene Analyzer IIx.

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 assembled	17,318
Smallest (nucleotides)	500
Median (nucleotides)	1,122
Largest (nucleotides)	16,676
Average (nucleotides)	1,514

Contig analysis: Homology with database insect sequences

The assembled contigs were batch BLASTed, mapped and annotated with Blast2GO software (Conesa et al., 2005). Contigs with BLAST hits numbered 14,251. Figure 1 shows the species distribution for these hits.

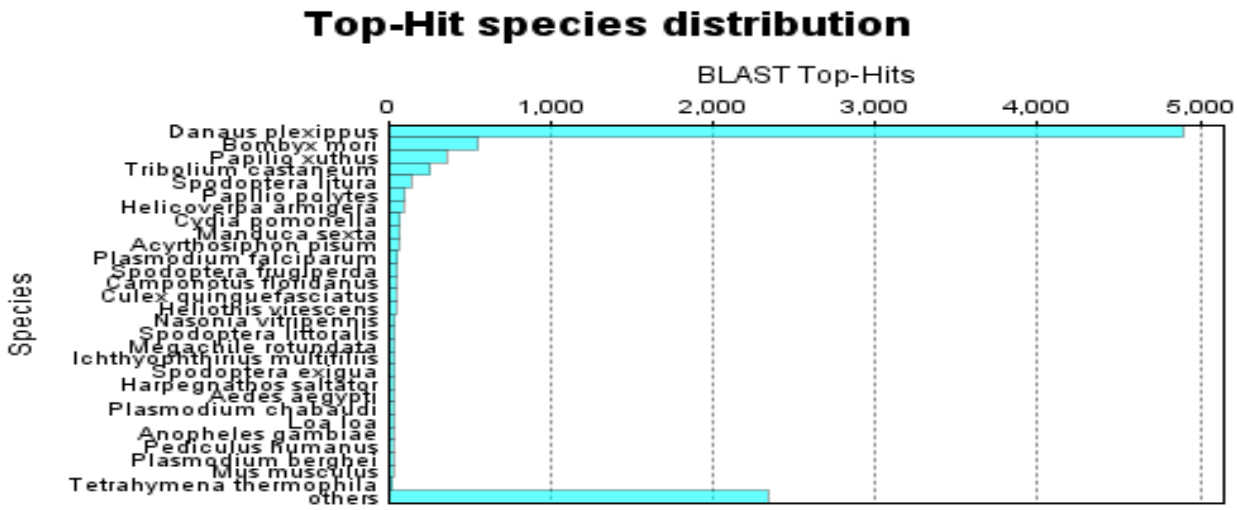

Figure 1. Species distribution of top BLAST hits for the contigs of *S. littoralis* larval head.

Results of BLAST analysis showed many *S. littoralis* sequences were most related to lepidopteran database sequences (Table 2.) The Egyptain cotton leafworm head larval transcriptome had 2,244 BLAST hits identical to *Danaus plexippus* and 287 hits identical to *Bombyx mori.*

Table 2. Number of *S. littoralis* larval head contig BLAST hits [identical] (E-value = 0) to database Lepidoptera sequences.

Lepidoptera insects	Number of matching *S. littoralis* contigs
Danaus plexippus	2244
Bombyx mori	287
Helicoverpa armigera	63
Helicoverpa assulta	2
Heliothis virescens	16
Spodoptera Spp.	143
Spodoptera littoralis	35

Biological function

Blast2GO® mapping and annotation steps resulted in 14,251 contigs (sequences) being assigned to GO (Gene Ontology) categories of putative protein function (Table 3.). Translated contig BLAST hits (E-value 10) assigned to GO level 2 functional categories were associated with catalysis, binding, transport, signal transduction, and structural molecule activity. Contigs were also associated with enzyme regulation, electron carrier activity, antioxidant activity, and other GO functional categories (Table 3.).

Table 3. Numbers of *S. littoralis* larval head translated contig BLAST hits assigned to GO level 2 functional categories.

GO Term	Number of assigned BLAST hits
Binding	3343
Catalytic activity	2870
Receptor activity	249
Structural molecule activity	166
Nucleic acid binding transcription factor activity	180
Transporter activity	390
Electron carrier activity	90
Molecular transducer activity	323
Enzyme regulator activity	168
Antioxidant activity	20
Protein binding transcription factor activity	52
Nutrient reservoir activity	3
Translation regulator activity	5
Morphogen activity	2
Metallochaperone activity	3
Channel regulator activity	3
Protein tag	1
Receptor regulator activity	2

Gene identification and functional annotation

Analysis of *S. littoralis* larval head BLAST hits is still in progress. Functional annotation has thus far identified several messages associated with neuropeptides, hormones, and receptors that regulate insect growth and development. Contigs with potential as candidates for insecticide research are presented in Tables 4–8. These include sequences associated with JH (juvenile hormone) regulation and synthesis (Tables 4, 6, and 7), moulting (Tables 4 and 7), as well as with diglyceride utilization (adipokinetic hormone) and insect water balance (diuretic hormone) (Table 5.). Receptors and enzymes associated with insecticide activity are presented in Table 8. Additionally, several sequences have been identified as olfactory binding proteins, including several categorized as pheromone-binding proteins, general odorant-binding proteins, and chemosensory proteins.

Table 4. Examples of putative neuropeptides found in the head larval transcriptome of *S. littoralis*.

Message	Length (base pairs)	E-value
Allatostatin	1158	2.03E-151
Eclosion hormone	763	1.67E-50

Table 5. Examples of hormone receptors found in the head larval transcriptome of *S. littoralis*.

Message	Length (base pairs)	E-value
Diuretic hormone receptor	1762	0
Adipokinetic hormone receptor	2598	0

Table 6. Examples of putative enzymes involved in juvenile hormone (JH) biosynthesis found in the head larval transcriptome of *S. littoralis*.

Message	Length (base pairs)	E-value
Farnesyl pyrophosphate synthetase	958	3.88E-55
JH acid methyltransferase	1281	2.44E-117

Table 7. Putative enzymes involved in juvenile hormone (JH) degradation and ecdysteroid synthesis found in the larval head transcriptome of *S. littoralis*.

Message	Length (base pairs)	E-value
JH epoxide hydrolase	1555	0
JH esterase	2962	0
Ecdysone 20-monooxygenase	3163	0

Table 8. Putative insecticide receptors and xenobiotic enzymes found in the larval head transcriptome of *S. littoralis*.

Message	Length (base pairs)	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

Summary

A larval head transcriptome for the Egyptian cotton leafworm, *Spodoptera littoralis* was recently sequenced using Illumina® technology. On-going bioinformatic analysis has thus far identified sequences associated with functions critical to the insect's growth and development. Among these are sequences associated with JH regulation and synthesis, moulting, metabolism, water balance, insecticide activity, and olfaction.

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