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Differential Expression Analysis of Busseola fusca Larval Transcriptome in Response to Cry1Ab Toxin Challenge

Authors : Bianca Peterson, Tomasz J. Sańko, Carlos C. Bezuidenhout, Johnnie Van Den Berg

Abstract : Busseola fusca (Fuller) (Lepidoptera: Noctuidae), the maize stem borer, is a major pest in sub-Saharan Africa. It causes economic damage to maize and sorghum crops and has evolved non-recessive resistance to genetically modified (GM) maize expressing the Cry1Ab insecticidal toxin. Since B. fusca is a non-model organism, very little genomic information is publicly available, and is limited to some cytochrome c oxidase I, cytochrome b, and microsatellite data. The biology of B. fusca is well-described, but still poorly understood. This, in combination with its larval-specific behavior, may pose problems for limiting the spread of current resistant B. fusca populations or preventing resistance evolution in other susceptible populations. As part of on-going research into resistance evolution, B. fusca larvae were collected from Bt and non-Bt maize in South Africa, followed by RNA isolation (15 specimens) and sequencing on the Illumina HiSeq 2500 platform. Quality of reads was assessed with FastQC, after which Trimmomatic was used to trim adapters and remove low quality, short reads. Trinity was used for the de novo assembly, whereas TransRate was used for assembly quality assessment. Transcript identification employed BLAST (BLASTn, BLASTp, and tBLASTx comparisons), for which two libraries (nucleotide and protein) were created from 3.27 million lepidopteran sequences. Several transcripts that have previously been implicated in Cry toxin resistance was identified for B. fusca. These included aminopeptidase N, cadherin, alkaline phosphatase, ATP-binding cassette transporter proteins, and mitogen-activated protein kinase. MEGA7 was used to align these transcripts to reference sequences from Lepidoptera to detect mutations that might potentially be contributing to Cry toxin resistance in this pest. RSEM and Bioconductor were used to perform differential gene expression analysis on groups of B. fusca larvae challenged and unchallenged with the Cry1Ab toxin. Pairwise expression comparisons of transcripts that were at least 16-fold expressed at a false-discovery corrected statistical significance (p) ≤ 0.001 were extracted and visualized in a hierarchically clustered heatmap using R. A total of 329,194 transcripts with an N50 of 1,019 bp were generated from the over 167.5 million high-quality paired-end reads. Furthermore, 110 transcripts were over 10 kbp long, of which the largest one was 29,395 bp. BLAST comparisons resulted in identification of 157,099 (47.72%) transcripts, among which only 3,718 (2.37%) were identified as Cry toxin receptors from lepidopteran insects. According to transcript expression profiles, transcripts were grouped into three subclusters according to the similarity of their expression patterns. Several immune-related transcripts (pathogen recognition receptors, antimicrobial peptides, and inhibitors) were up-regulated in the larvae feeding on Bt maize, indicating an enhanced immune status in response to toxin exposure. Above all, extremely up-regulated arylphorin genes suggest that enhanced epithelial healing is one of the resistance mechanisms employed by B. fusca larvae against the Cry1Ab toxin. This study is the first to provide a resource base and some insights into a potential mechanism of Cry1Ab toxin resistance in B. fusca. Transcriptomic data generated in this study allows identification of genes that can be targeted by biotechnological improvements of GM crops.

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