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Discovering microRNA and their targets in the red flour beetle *Tribolium castaneum* from expressed sequence tags

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Abstract

MicroRNAs (miRNAs) are endogenous, single stranded, small RNAs that are 19-24 nucleotides in length, ubiquitously found in eukaryotes and in some viruses. miRNAs majorly help in down regulation of genes by binding to the complimentary sites on 3' untranslated regions of target messenger RNA (mRNAs), and more than 30 percent of the genes in animals are believed to be under the control of miRNAs. The red flour beetle, *Tribolium castaneum* is a global pest of stored agricultural products. The use of insecticides to control beetles is challenging due to emergence of resistance against pesticide and RNA interference (RNAi) has recently been suggested as a novel and effective approach for pest control to identify potential targets in pest species. Thus, in the present study, we have developed a computational pipeline to identify miRNAs and their targets from expressed sequence tags (ESTs) of *T. castaneum*. First, 68,506 ESTs from *T. castaneum* were clustered using CD-HIT-EST to generate 35,367 ESTs that were used to search against 3,991 unique insects miRNAs dataset from miRBase release 21. As a result, 122 unique sequences were obtained which were mapped against Rfam to remove tRNA, rRNA followed by BlastX against NR database to remove the protein coding genes. A total of 89 sequences were obtained which were further validated for secondary structure predictions through Mfold and in the end, 16 miRNA sequences were obtained for target prediction using miRanda. Subsequently, 198 potential miRNA target genes were predicted for GO (Gene Ontology), metabolic pathway analysis through KEGG (Kyoto Encyclopedia of Genes & Genomes) and Protein-Protein Interaction (PPI) study through STRING annotation process. The identified 16 miRNAs with their 198 target genes in *T. castaneum* will serve as useful resource for initiating experimental validation and functional analyses of miRNA-regulated phenotypes in *T. castaneum* through gene knockdown and transgenesis.