6.9 Asian Citrus Psyllid, Genetic Basis of Immunity

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A gene expression library was made from the alimentary tract of adult Asian citrus psyllids, AsCP. Analysis of the expressed sequence tags produced a gene dataset of 7,800 EST’s. In these ESTs, important immunity genes were identified. These transcripts with significant homology (E-value ≤10⁻²⁰ or better) were identified through homology searches to other known insect genomes. Use of genomics approaches has enabled the identification of some of the genetic basis of psyllid immunity and pathogen interactions. Further genomic analyses of AsCP, *Diaphorina citri*, will advance our understanding of the psyllid/phloem/bacterium interactions which may be linked to the acquisition and transmission of the pathogenic bacterium *Candidatus Liberibacter asiaticus*, associated with Huanglongbing. However, a much greater understanding of psyllid genomics is still needed. Continued development of these genetic products will set the foundation for further functional genomic studies to isolate AsCP specific genes to be targeted to reduce the spread of HLB and to reduce psyllid populations in an environmentally, highly specific management strategy. In this study, we focused on the genetic response of cytochrome P450 and heat shock protein 70, to treatment with imidacloprid and other stress factors, as temperature to advance the understanding of insecticide resistance development and heat tolerance in *D. citri*.

Material and Methods

Immune challenge of psyllid and isolation of RNA. Four leaf branches were treated with water, or 1 mL/3.7L admire for 3 days. Psyllids were transferred and incubated for 1 h. In order to induce heat shock responses, psyllids were incubated 1 h at 20°C or 50°C. RNA was extracted using RNA aqueous®-Micro (Ambion, Austin, TX, USA)

Quantitative real-time RT-PCR: Quantitative real-time RT-PCR was performed with the RotorGene™ 6000 Real-time rotary analyzer (Corbett Life Science, Sydney, Australia) using SuperScript™ III Platinum® SYBR® Green One Step qRT-PCR kit (Invitrogen, USA). Each reaction was carried out in 25 µL volume containing 5 pmol of forward and reverse primers and 200 ng of RNA template. Amplification cycling conditions were 50°C for 30 min, 95°C for 15 min, 30 times of 95°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec. We examined the expression of alpha tubulin (accession number is DQ675542) as an internal control.

Results and Discussion

Sequence analysis of Cyp450, Hsp70: Previously, we created and analyzed two expressed sequence tag, EST, libraries made from adult *D. citri* (Hunter et al., 2005-2008, GenBank, NCBI). Partial Cyp 450 gene transcript which was 681 bp (accession number is DQ675542) was determined. The deduced amino acid sequence of the Cyp450 displayed homology with
Antheraea yamamai cytochrome P450 CYP4G25 (76%), Drosophila melanogaster cytochrome P450 CYP4G15 (71%) and Tribolium castaneum cytochrome P450 monoxygenase Cyp4g14 (64%). The partial hsp70 gene transcript was 549 bp. The deduced amino acid sequence of hsp70 displayed homology with Chironomus tentans hs70 (62%) and Mamestra brassicae hs70 (56%). The partial hsc70 gene is 550 bp (accession number is DQ675540). The deduced amino acid sequence of hsc70 displayed homology with Bemisia tabaci hs70 (87%) and Mamestra brassicae hs70 (86%).

Quantitative real time RT-PCR analysis of immune induced genes in psyllid. Adult D. citri were exposed to imidacloprid via plant sap. Neither the gene expression level of Cyp 450 nor hsp70 was changed by this treatment. This is similar to D. melanogaster Cyp4G15 expression pattern which showed the same expression between resistant and susceptible strains to the insecticide DDT and pyrethroid (Maibeche-Coisne et al, 2000). Drosophila melanogaster, the Cytochrome P450 gene Cyp6g1 was shown to be capable of metabolizing imidacloprid. However, the expression of Cyp4 family cyp4G33 from Chironomus tentas was induced by Atrazine (Londono et al., 2007). Atrazine is a herbicide used to stop pre- and post-emergence broadleaf and grassy weeds in major crops by binding to the plastoquinone-binding protein in photosystem II, inhibiting electron transport. Cytochrome P450 comprise a super family of enzymes that are involved in the biosynthesis of many biologically important compounds and metabolism of a variety of chemicals. The D. citri Cyp 450 may play a role in endogenous compound metabolism rather than in detoxification.

Heat shock treatment (Figure) revealed that hsp70 gene was strongly induced. Heat shock genes are known to respond to a variety of stresses, such as exposure to xenobiotics, heavy metals, metabolic poisons and temperature extremes. While the D. citri hs70 was not induced by imidacloprid, it was involved in heat stress. Similarly, cyp450 transcript expression in D. citri was not changed by heat shock. Understanding the interactions between D. citri immune physiology under hot Florida summers and insecticides may lead to more efficacious insecticide applications and reduced costs to growers when managing these important economical pests in citrus groves.

In summary, only a few insecticides are being used to manage the Asian citrus psyllid, AsCP, Diaphorina citri, (Hemiptera: Psyllidae) to reduce the spread of the phloem-inhabiting bacterium Ca. Liberibacter asiaticus, associated with Huanglongbing, (Citrus greening disease). Imidacloprid is the most important systemic insecticide currently being used to control plant pests including psyllid, as either soil, seed or foliar treatments. Imidacloprid acts as an agonist at the nicotinic acetylcholine receptor and interferes with the transmission of stimuli in the insect nervous system. This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in the insect’s paralysis, and eventually death. In Drosophila melanogaster, the cytochrome P450 gene Cyp6g1 was shown to be capable of metabolizing Imidacloprid. Cytochrome P450 comprise a super family of enzymes that are involved in the biosynthesis of many biologically important compounds and metabolism of a variety of chemicals. To understand the genetic basis of how the AsCP responds against imidacloprid and other environmental stresses is important to effective management of AsCP and citrus greening. In this study, we show psyllid immune gene responses against imidacloprid and other stress factors such as temperature to advance the understanding of insecticide resistance development.
and heat tolerance. Heat shock protein 70 gene was induced by heat treatment demonstrating a response to stress within the psyllids. Further experiments are under way, to identify more genes which respond to biological and environmental stresses. These insights provide genetic targets to effectively reduce psyllids by increasing susceptibility to low dosage insecticides and to Florida hot summer temperatures.

References


Hunter WB, Dang, P, McKenzie, CL. 2005 Diaphorina citri (Hemiptera) DN465721-DN470410, NCBI.


Hunter, WB, Hunnicutt, LE, Hall, DG. 2006. Ribosomal proteins of the Asian citrus psyllid, Diaphorina citri. 88 Proteins, DQ675535-DQ673441. NCBI.


Figure 1. Quantitative real time RT-PCR analysis of transcriptional levels of psyllid genes, by heat shock treatment.