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Comparative transcriptome analysis reveals networks of genes activated in the whitefly, *Bemisia tabaci* when fed on tomato plants infected with *Tomato yellow leaf curl virus*

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ABSTRACT

The whitefly *Bemisia tabaci* can transmit hundreds of viruses to numerous agricultural crops in the world. Five genera of viruses, including *Begomovirus* and *Crinivirus*, are transmitted by *B. tabaci*. There is little knowledge about the genes involved in virus acquisition and transmission by whiteflies. Using a comparative transcriptomics approach, we evaluated the gene expression profiles of whiteflies (*B. tabaci* MEAM1) after feeding on tomato infected by a begomovirus, *Tomato yellow leaf curl virus* (TYLCV), in comparison to a recent study, in which whiteflies were fed on tomato infected by the crinivirus, *Tomato chlorosis virus* (ToCV). The data revealed similar temporal trends in gene expression, but large differences in the number of whitefly genes when fed on TYLCV or ToCV-infected tomato. Transcription factors, cathepsins, receptors, and a hemocyanin gene, which is implicated in mediating antiviral immune responses in other insects and possibly virus transmission, were some of the genes identified.

1. Introduction

Begomoviruses (Family: *Geminiviridae*) are efficiently transmitted by the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), resulting in crop losses estimated to exceed billions of U.S. dollars annually and threatening global food security (Stansly and Naranjo, 2010; Cock, 1993). Begomoviruses affect the production of a wide range of important crops, including beans, cassava, cotton, squash, sweet potato, and tomato. Two of the most devastating diseases caused by begomoviruses include tomato yellow leaf curl disease, which is widely distributed throughout the world (Lefeuvre et al., 2010), and cassava mosaic disease, which has reached pandemic levels in African countries (Legg et al., 2014).

Begomoviruses possess single-stranded DNA genomes of ~2700 nt and are encapsidated by viral coat proteins in an incomplete icosahedral structure (Navot et al., 1991). Many begomoviruses have bipartite genomes, termed DNA-A and DNA-B, while others, including *Tomato yellow leaf curl virus* (TYLCV) are monopartite and possess a single DNA-A-like genome component, containing six genes (Basak, 2016).

Begomoviruses and *B. tabaci* have been co-evolving for millions of years (Czosnek and Ghanim, 2012; Ghanim, 2014). These plant viruses are transmitted by *B. tabaci* in a persistent, circulative manner (Czosnek and Ghanim, 2012). Once ingested by an adult whitefly, begomovirus virions pass to the midgut, where they move across the midgut membrane to the hemolymph, possibly via receptor-mediated endocytosis (Rosen et al., 2015; Kollenberg et al., 2014), and circulate back into the primary salivary glands, where they are egested with saliva during insect feeding (Czosnek and Ghanim, 2012; Hunter et al., 1998). Several proteins have been implicated in the circulation of TYLCV in the whitefly, including two heat shock proteins (Ohnesorge and Bejarano, 2009; Gotz et al., 2012), and GroEL, a protein secreted by the secondary bacterial endosymbiont *Hamiltonella,* that is thought to stabilize virion passage by interacting with the viral coat protein in the hemolymph (Morin et al., 2000; Kliot and Ghanim, 2013). Cyclophilin B was shown

Abbreviations: MEAM1, Middle East-Asia Minor 1; TYLCV, *Tomato yellow leaf curl virus*; TYLCCNV, *Tomato yellow leaf curl China virus*; AAP, acquisition access period; ANP, atrial natriuretic peptide; LDLR, low-density lipoprotein receptor; LpR, lipophorin receptor; ToCV, *Tomato chlorosis virus*

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to interact with the coat protein of TYLCV in the whitefly midgut, eggs, and salivary glands (Kanakala and Ghanim, 2016), and knottin-1 has been shown to act as a suppressor of TYLCV by restricting the number of virions that can be acquired and transmitted by the whitefly (Hariton Shalev et al., 2016).

Although the majority of plant viruses transmitted by *B. tabaci* are begomoviruses, viruses in several other genera, including *Carlavirus, Crinivirus, Ipomovirus and Torradovirus* are also transmitted by whiteflies (Navas-Castillo et al., 2011). *B. tabaci* is a complex of cryptic species formerly referred to as, "biotypes" that can colonize over 1000 plant species (Abd-Rabou and Simmons, 2010), and exhibits a range of biological diversity, including host preference, reproductive incompatibility, insecticide resistance, secondary bacterial endosymbiont composition, and virus transmission potential (Zang et al., 2006; Houndété et al., 2010; Bedford et al., 1994). One of the most broadly distributed and damaging *B. tabaci* populations is the Middle-East Asia Minor 1 population (MEAM1; formerly known as the B biotype and *B. argentifolii*) (Rosen et al., 2015; Bellows et al., 1994).

TYLCV can affect the fitness of *B. tabaci* depending on the cryptic species (Jiu et al., 2007; Rubinstein and Czosnek, 1997), and even though evidence of TYLCV replication in the whitefly host was shown (Sinisterra et al., 2005; Pakkianathan et al., 2015), conflicting reports continue to emerge (Sanchez-Campos et al., 2016). Transovarial transmission of TYLCV was recently shown to occur in *B. tabaci*, with virus entry into the whitefly ovary mediated by interactions between vitellogenin and the viral coat protein (Wei et al., 2017). TYLCV is also capable of manipulating the settling, probing, and feeding behavior of the whitefly in a way that would facilitate virus transmission (Moreno-Delafuente et al., 2013; Liu et al., 2013; He et al., 2015; Jahan et al., 2014).

An earlier transcriptome study analyzed the whitefly's response to acquisition of another begomovirus, *Tomato yellow leaf curl China virus* (TYLCCNV), where 1606 genes were differentially expressed and involved in regulating cell cycle, primary metabolism, and cellular and humoral immunity (Luan et al., 2011). Although the TYLCCNV study and earlier expressed sequence tag (EST) libraries (Leshkowitz et al., 2006; Li et al., 2011) have contributed to understanding whitefly-begomovirus relationships, technology for generating gene expression data has significantly improved and two whitefly reference genomes are now available (Chen et al., 2016; Xie et al., 2017), which provide greatly improved annotation of gene expression data.

In this study, we were interested in understanding how the whitefly responds to feeding on tomato that is infected with TYLCV, arguably one of the ten most economically important plant viruses in the world (Scholthof et al., 2011). Using RNA-Seq analysis and three biological replicates per treatment, whitefly gene expression was analyzed after feeding on TYLCV-infected or uninfected tomato for acquisition access periods of 24 h, 48 h, and 72 h. The data were also compared to a published study that analyzed the response of *B. tabaci* MEAM1 to a semi-persistently transmitted crinivirus, *Tomato chlorosis virus* (ToCV), using the same experimental procedures (Kaur et al., 2017). Together, these data provide: 1) insight into how the whitefly responds to feeding on TYLCV-infected tomato plants during acquisition and early transmission periods, and 2) a comparison of similarities and differences between whiteflies fed on tomato plants infected by either of two distinct types of viruses (TYLCV and ToCV) with different modes of transmission. These data contribute to broader knowledge on the whitefly's molecular response to feeding on a virus-infected host and vector-virus relationships, which can serve as a basis for devising new strategies to control whiteflies and whitefly-transmitted viruses.

2. Materials and methods

2.1. Insect rearing, feeding assays, and RNA isolation

An isogenic whitefly colony was established from a single female *B. tabaci* MEAM1 and reared as previously described (Chen et al., 2015). A subset of the population was transferred to broccoli (*Brassica oleracea* L. var. botrytis), a non-host for TYLCV, and maintained in a greenhouse (26 ± 5 °C). The MEAM1 population was confirmed by PCR using established primers against the mitochondrial cytochrome oxidase 1 gene (Shatters et al., 2009).

The feeding conditions for whiteflies have been previously described in (Chen et al., 2016; Kaur et al., 2017). Briefly, ~1500 adult whiteflies of mixed ages per treatment were collected from the isogenic colony reared on broccoli plants, transferred to either TYLCV-infected or uninfected tomato (cv. Moneymaker) plants (cuttings) in two separate cages, and allowed to feed. The TYLCV-infected tomato plants were generated through whitefly transmission and the virus-infection status confirmed by PCR. Test plants were maintained in a greenhouse (25–30 °C and 14 h natural sunlight). Small cuttings (3–4 leaves) were taken from test plants that were at the 10–12 leaf stage and placed in a flask of water that was sealed with a sheet of parafilm to prevent whiteflies from entering. At the end of each pre-determined acquisition access period (AAP) of 24, 48, or 72 h, 200–500 live whiteflies were collected and immediately stored at −80 °C until processing. To confirm virus acquisition, RNA was isolated from 50 whiteflies fed on either healthy or TYLCV-infected tomato from each AAP prior to RNA isolation. To determine the percent of whiteflies that had acquired TYLCV, PCR analysis was conducted using DNA preparations from each of 10 individual whiteflies per treatment feeding on TYLCV-infected plants for each AAP. Results showed that 60% (6 of 10) of whiteflies were positive for TYLCV at 24 h, and 100% (10/10) were positive for either 48 h or 72 h. as expected, individual whiteflies fed on uninfected tomato plants tested negative for TYLCV (Supplementary Fig. S1). Therefore, the genes identified as differentially expressed in whiteflies feeding on TYLCV-infected versus uninfected tomato plants were most likely exhibiting differential expression due to their responses to the acquired TYLCV or the physiological changes in the TYLCV-infected plants.

Whitefly DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, USA) and used for PCR reactions containing 2X GoTaq Green Master Mix (Promega, USA) and primers specific to the V1 coat protein of a TYLCV, South Carolina, USA isolate (GenBank: DQ139329) (Supplementary Table S1). Three biological replicates were performed per treatment at each AAP. Total RNA was processed as previously described (Chen et al., 2016). For each sample, an aliquot of the RNA was used for validation by reverse transcription quantitative PCR (RT-qPCR) (see below) and the remainder was stored using RNAstable (Sigma-Aldrich, USA).

2.2. Transcriptome sequencing and analysis

RNA-Seq libraries were constructed, sequenced, and processed as previously described (Chen et al., 2016). Briefly, 18 strand-specific barcoded RNA-Seq libraries were pooled and sequenced using an Illumina HiSeq. 2500 system with the paired-end mode. Adaptor and low quality sequences were removed from the RNA-Seq reads. The cleaned reads were aligned to the ribosomal RNA database (Gurevich et al., 2013), the assembled *B. tabaci* mitochondrion genome, and the three bacterial endosymbiont genomes of *Candidatus* Portiera aleyrodidarum, *Hamiltonella*, and *Rickettsia* (Chen et al., 2016, http://www.whiteflygenomics.org). The aligned reads were filtered. The remaining high-quality cleaned paired-end reads were aligned to the reference *B. tabaci* genome (Chen et al., 2016). Raw counts for each predicted gene were normalized to fragments per kilobase of exon model per million mapped fragments (FPKM). Differentially expressed genes between the whiteflies fed on TYLCV and uninfected tomato plants were identified using edgeR (Robinson et al., 2010). The resulting raw *p* values were adjusted for multiple testing using the false discovery rate (FDR) (Benjamini and Hochberg, 1995). For each comparison, genes with an adjusted *p* value (FDR) less than 0.05 and fold