Project Title

Transcriptome-based research to identify *Tomato spotted wilt virus* (TSWV) -induced effects on newly released TSWV-resistant peanut genotypes and in tobacco thrips, *Frankliniella fusca*

Abstract /Project Summary

Our goal was to develop thrips transcriptomes for a subset of thrips that transmit *Tomato spotted wilt virus* (TSWV) and non-vectors. Our other objective was to identify variation in gene profiles in tobacco thrips with and without TSWV infection and in peanut cultivars. The objectives in this proposal are expected to be completed in the next three years.

Of the 7000 thrips species available worldwide, transcriptome for only one species of thrips, western flower thrips, is available. We proposed to directly contribute to the development of thrips database and provide more insights on how thrips interact with peanut plants and with TSWV. We developed transcriptomes for four other species. The information generated has been submitted to a public database. Identification of genes and/or their pathways in thrips that are involved in suppression of plant immune responses could be useful in breeding programs and in the development management options in the long term. Several candidate genes were idenfied last year and downstream research is being planned.

A number of TSWV resistant cultivars have been released in the recent years. However, the mechaism(s) of resistance is currently unknown. Also, our laboratory research has shown that TSWV-resistant cultivars can get infected with the virus and accumulate viral copies. Using transcriptomics we intend to identify various pathways and/or genes that may be involved in imparting resistance against TSWV. The obtained information could be extremely useful to understanding the resistance phenomenon in peanut, prolong the usefulness of resistant cultivars, and potentially assist in breeding newer cultivars. Though the proposed research does not have any immediate direct benefits to growers, it can be very valuable in developing long-term sustainable management options. More research is currently being conducted on these aspects.

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Report: Final report
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INSTITUTION: University of Georgia

Project Title: **Transcriptome-based research to identify *Tomato spotted wilt virus* (TSWV) induced effects on newly-released TSWV resistant peanut genotypes and in tobacco thrips, *Frankliniella fusca***

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EXPIRATION DATE: June 30, 2013

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The objectives of the proposal were

1. *Identify genes and their putative functions: analyze the expressed sequence tags from newly released peanut cultivars with and without TSWV infection.*

2. *Identify genes and their putative functions: analyze the expressed sequence tags of tobacco thrips (F. fusca) with and without TSWV infection.*

Despite more than twenty years of thrips and TSWV management research, thrips and TSWV still remain a problem. Though management tactics have evolved to a point where there are no significant yield reductions, this year's TSWV incidence seems to challenge the status quo. Still there is a wide gap in knowledge when it comes to understanding thrips and TSWV interactions in peanut. We attempted to use a non-traditional approach to answer some of these unknown questions. There are >7000 thrips species identified, but only <15 species are known to transmit tospoviruses (*Tospovirus*- genus of TSWV). As indicated in the objectives, our goals were to understand what makes a thrips species a vector, and what changes are brought about in thrips due to virus infection. We used transcriptomics to study these effects. Transcriptomics is used to identify transcripts in any given organism. In other words, it helps to identify genes and annotate their functions in a given organism. In accordance with our objectives we started with the thrips first, we identified two thrips species that are known to function as TSWV vectors (*F. fusca* and *Thrips tabaci*). We also selected two non-vectors viz., *Frankliniella tritici* and *Echinothrips amerciancus*. First, we developed transcriptomes for all four thrips species mentioned above. The sequences from all four thrips species have been deposited in 2012 in the NCBI (National Center for Biotechnology Information) database and are available worldwide. It should be noted that despite the availability of 7000 thrips species, prior to our study, transcriptome was available for only one species. Our efforts will be of tremendous benefit to

the thrips and *Tospovirus* community worldwide. Upon obtaining the transcriptomes we were able to compare them using various bioinformatics tools. Some details of the comparisons/analysis are described below. Details on genes involved in biological processes, immune responses, and viral reproduction are briefly explained below. The immune response genes as well as genes associated with viral reproduction were of interest, as they could be pivotal in determining the vector competence/transmission efficiency in thrips.

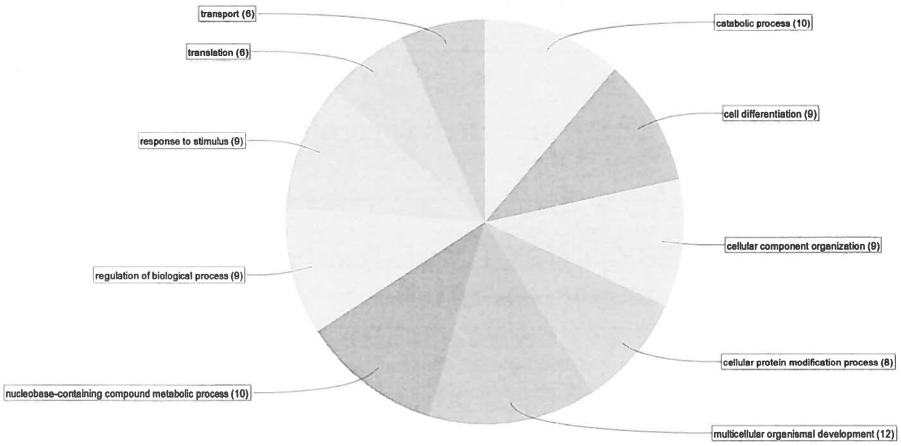

Fig. 1. Sequence distribution by biological process in vector species

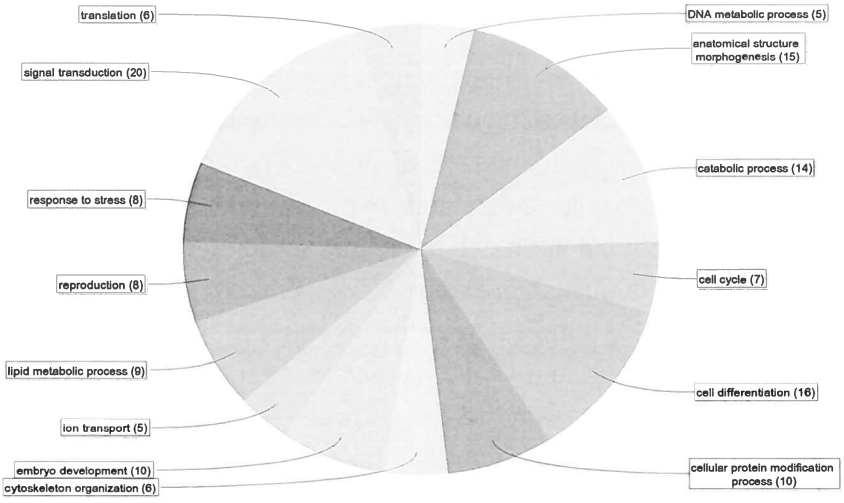

Fig. 2. Sequence distribution by biological process in non-vector species

Figs. 1 and 2 clearly indicate that there are differences in gene identity and function. My graduate student, Ms. Anita Shrestha, further characterized the differences between the two. For instance, the immune response genes, genes involved in viral reproduction, as well as putative viral receptor sites in vectors and non-vectors were identified and annotated. This in thrips world is equivalent to a 'treasure trove.' Below, I will attempt to explain how the genes in vectors and non-vectors are different.

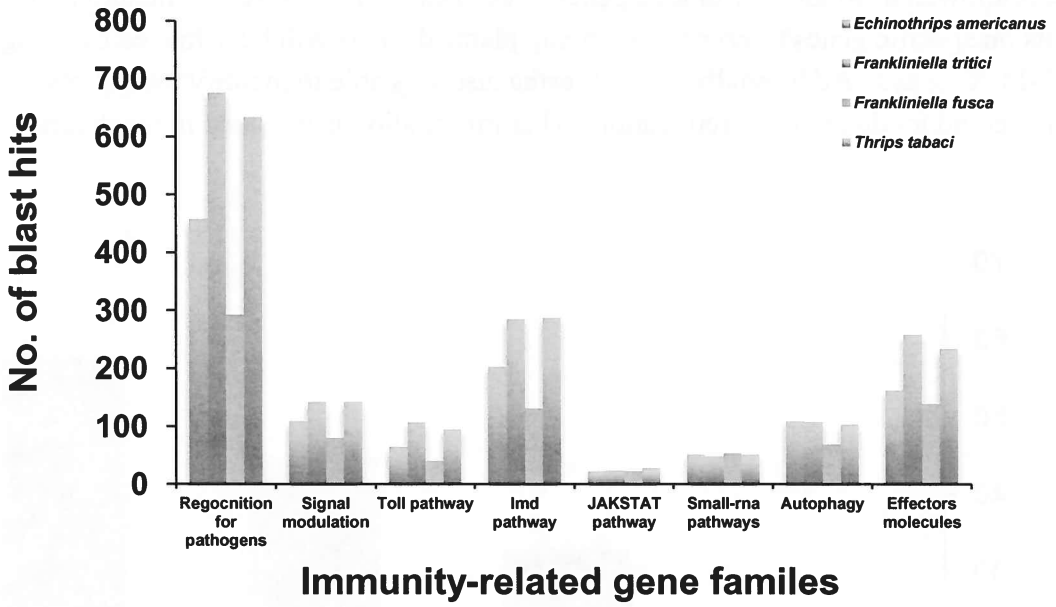

Fig. 3. Immunity related genes, their potential functions, and the various pathways associated with immune response.

This information clearly indicated that there exist some differences between vectors and non-vectors. The number of orthologs and non-orthologs in each category is explained below by a Venn diagram.

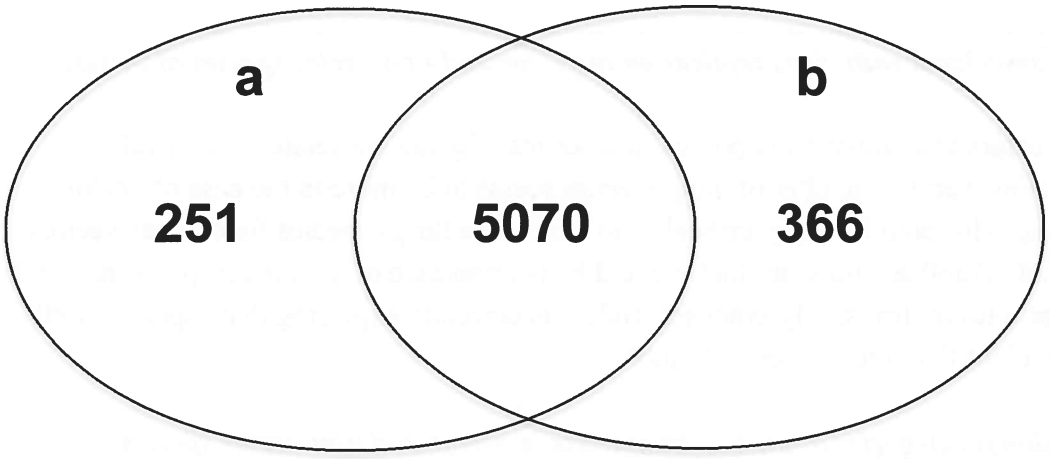

Fig. 4. Assembled transcripts from thrips species were blasted against each other and orthologous groups were clustered based on 50% sequences similarity and e-vale of 1e -5. The overlapping region represents the number of transcripts shared among vector and non-vector species. The circle "a" and "b" represent transcripts that clustered among vector and non-vector species respectively.

This process allowed us to identify unique genes in each category. Currently, quantitative assessments on specific genes/transcripts are being planned. This will be achieved by using routine RT-PCR assays. Additionally, Ms. Shrestha also was able to identify transcripts of genes in thrips that could modulate virus replication. That information is included in the diagram below.

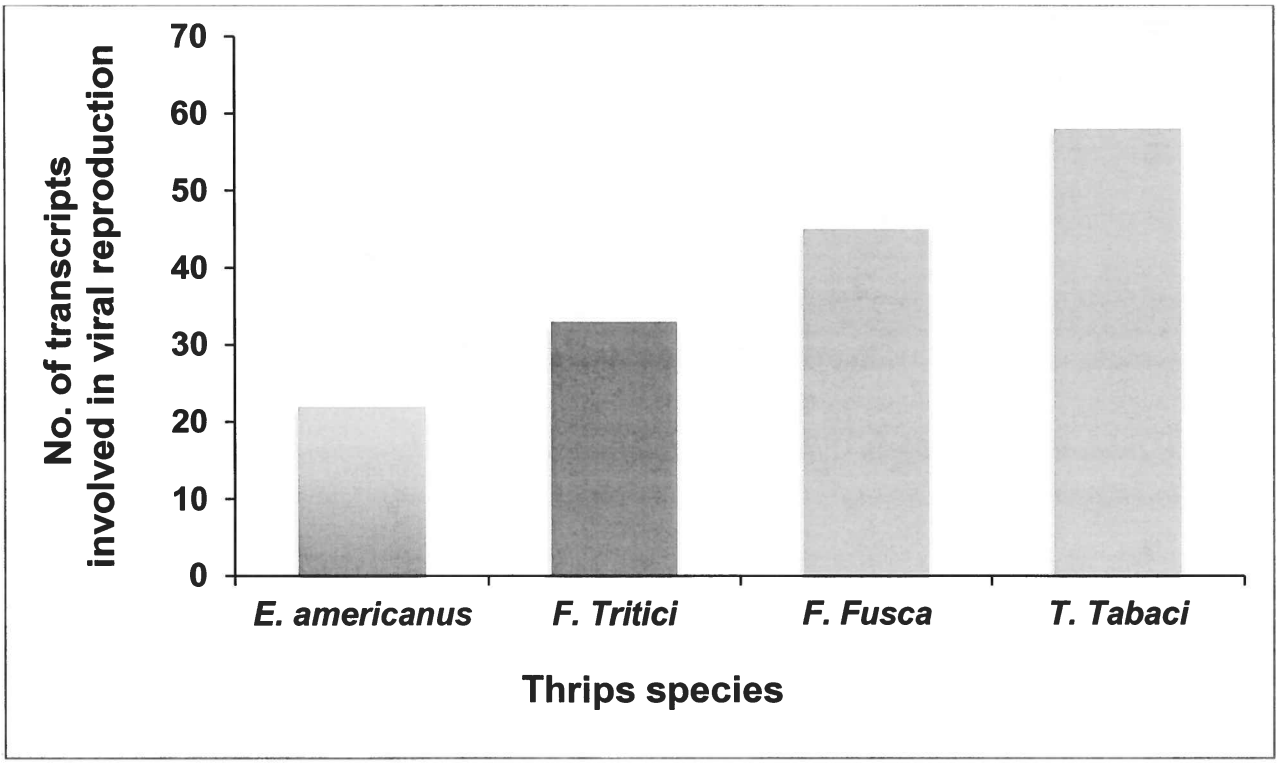

Fig. 5. Genes associated with virus replication in vector and non-vector species of thrips.

It seems both vectors and non-vectors possess transcripts of genes associated with viral reproduction. However, the number of unique genes seems to be more in the case of vectors than non-vectors. This could be very critical as to why some thrips species function as vectors and others do not. Another important factor could be the presence of unique receptors that could bind to virus particles in thrips. My graduate student is currently exploring that aspect as well and has already identified a few receptor homologs.

Table 1. Functional categories of unique transcripts in vector and non-vector species

Functional categories	No. of transcripts in vector species	No. of transcripts in non-vector species
Host molecules participating in virus life cycle	8	4
Immune response genes	3	12
Genes involved in transcription/translation/mRNA processing	16	17
Response to stress	4	5

Despite the qualitative differences that are illustrated, there could be quantitative differences that could hold the key to assessing vector competence in thrips species. Ms. Shrestha is currently attempting to understand the differential expression of genes in thrips with and without viral infection. She is using only vector species, *F. fusca*, as her model. Despite the infection status she will also be exploring the differences in all three-life stages (larvae, pupae, and adults). This is critical, as only larvae are known to acquire the virus. In other words, if adults acquire the virus for the first time they will not be able to transmit the virus successfully. This indicates that there is a barrier in the developmental stage. The differential expression of genes using transcriptomes of infected and non-infected thrips and their life stages could be extremely useful to decipher these interactions. Ms. Shrestha's research focus will now be on differential gene expression between TSWV-infected *F. fusca* and non-infected *F. fusca*.

Despite the availability of resistant genotypes, TSWV incidence has increased in 2013. Although the precise reasons for this spike are unclear. What is clear is that under substantial thrips and TSWV pressure yield losses could still occur. One of the main constraints in increased TSWV incidence could be due to the fact that the mechanisms conferring resistance in these newly released genotypes, or any peanut genotype for that matter, are unknown. Our greenhouse studies over the last two years seem to shed some light on the same. However, a robust approach such as transcriptomics could be pivotal to decoding the minute details that are key to contributing field resistance. We plan on studying these details in 2013 and 2014. We believe that this research, though not immediately applicable as a management tactic, would significantly improve our understanding on thrips-TSWV interacitons as well as thrips-TSWV-peanut interactions. Additionally, understanding the mechanisms of resistance could also significantly benefit peanut breeding and will be complementary to the ongoing peanut genomics initiative.