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NATIONAL PEANUT BOARD/SOUTHEAST PEANUT RESEARCH INITIATIVE QUARTERLY PROGRESS REPORT FOR WORK DONE UNDER RESEARCH AGREEMENT

Report: Final report

INSTITUTION: University of Georgia

Project Title: Reemergence of TSWV in a large scale in recent years: What's going on?

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Summary

Since the early 2000s TSWV-resistant cultivars with some degree of field resistance has been the main management option for spotted wilt disease caused by thrips-transmitted *Tomato spotted wilt virus* (TSWV). This strategy has been quite successful, but in recent years, increased incidence of spotted wilt has been observed even after planting cultivars with field resistance. This prompted us to question whether TSWV isolates have evolved into a resistance-breaking strains? And, do they impact the usefulness of cultivars with field resistance? We sequenced TSWV isolates (all coding genes), and conducted phylogenetics and population genetics analyses over the last few years (2016-2018). Results are discussed below in detail. The take home points being that there is constant evolution of TSWV with respect to time, but no so much in relation to resistance identified in peanut. It could be that the temporal evolution of TSWV is favoring an adaptation to TSWV resistant cultivars, and hence the increased TSWV incidence. Work is ongoing in 2018, results presented here will concentrate on phylogenetic analysis with N-gene. Ongoing work will concentrate on others as well.

This project is a multi-year undertaking. My PhD student, Ms. Pin-Chu Lai is leading this effort. The issue that has been baffling is the increased incidence of spotted wilt caused by thrips-transmitted *Tomato spotted wilt virus* (TSWV) despite continuously planting TSWV field-resistant cultivars. The most important question being are there resistance breaking strains associated with peanut?

We believed that sequencing the genome completely would be ideal to addressing this issue. Though the genome of TSWV is relatively small (~17KB), there has been some troubleshooting regarding isolating the viral RNA from total plant RNA. Next generation sequencing did not provide the complete coverage of at least one of the RNAs. The main challenge with this

approach has been the ability to obtain high quality viral RNA from total RNA. Most samples obtained so far seem to be contaminated with plant RNA to a high degree. Specific RNA kits targeting the poly AAA tail were used to isolate viral RNA. Despite that effort, the quality of viral RNA was low and that whole genome coverage was less. This was specifically the case for the L (large) RNA. The coverage has been substantially higher for M and S RNAs (~90%). In the last quarter, we tested the following approaches to address this issue.

Since the NGS coverage was still less; we are now resorting to traditional sequencing (Sanger sequencing). Through Sanger sequencing we sequenced the five coding ORFs representing RDRP, GN/GC, Nsm, Nss, and N, respectively. Sequencing is now complete for the samples collected in 2016. For 2017 samples, 8 genomes have been partially sequenced. In total, we have 15 genomes sequenced from both TSWV-susceptible and resistant cultivars. We are still collecting TSWV-infected peanut leaf tissue samples for the 2018 field season. In some of our fields more than 30% infection was commonly observed in 2018, indicating that under high pressure the fire wall of field resistance is beginning to be crack.

In the sequencing process, primers were designed to cover all five coding genes (RDRP only partially). The gene that has been commonly studied for virus evolution is the N-gene. However, the gene that directly interacts with plant defense signaling is believed to be NSs gene. Therefore, it only makes sense to assess how much difference exists in each of these regions of the virus. Our goal is to compare resistant cultivars and susceptible cultivars to see if there are consistent substitutions that would indicate selection against host resistance.

Work flow:

Primers synthesized for each gene was evaluated for their efficiency through temperature gradients, and appropriate pairs were selected for each gene. The coding regions of the TSWV genome were amplified using gene-specific primers using RT-PCR. RT-PCR was performed using optimized parameters for each gene, then the amplicons were purified, and subsequently sequenced using a commercial facility in Huntsville Alabama. Upon receiving the sequenced products, the sequences from both directions were combined, and a consensus sequence was obtained for each sample. The sequences were then aligned using a software program that conducted both pair-wise and multiple alignments. Comparisons were made between 'old' and 'new' sequences, dating from 1998, 2008, and respectively. Sequences from 2016-2018 were also included. Comparisons were also made between resistant and susceptible cultivars. The temporal comparisons were made with N-gene sequences, as only those are available for older samples collected before 2008. The sequences were translated into their amino acid portfolios, trimmed accordingly following alignment, and then subjected to Bayesian analysis in Mr. Bayes. Upon conclusion of the analysis, the results were then subjected to Principal Component Analyses using characters from ordinations, and assuming a Brownian dispersion model. The Results from phylogenetic analyses is shown below as a Figtree output.

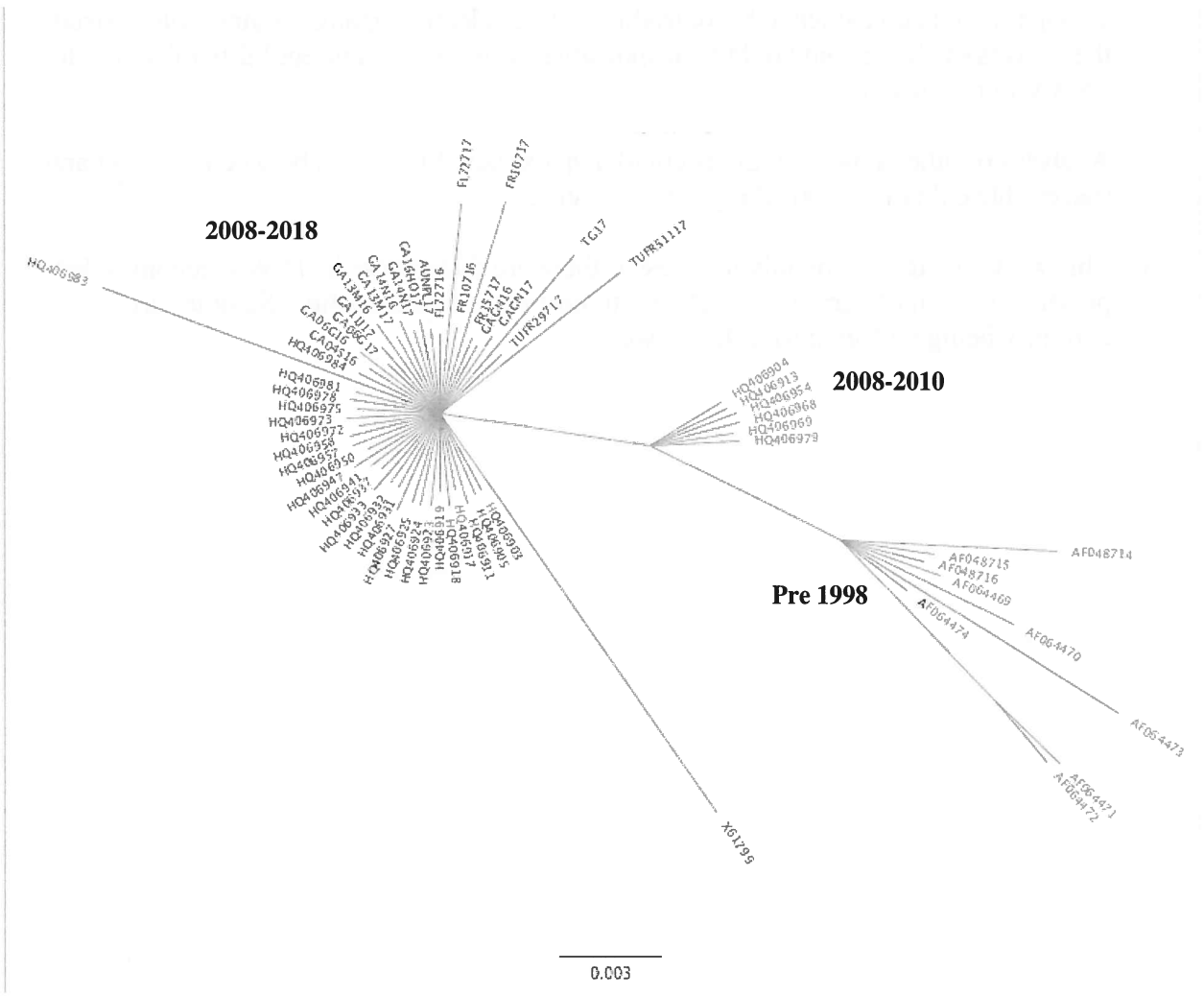

Fig. 1. A phylogenetic tree representing N-gene amino acid sequences from 1998 to 2017. The output was based on Bayesian analyses conducted for 300,0000 iterations in Mr. Bayes. The sequences with a prefix of 'AF' and 'HQ' were collected in 1998, and 2008, respectively. The remaining were collected in 2016 and 2017, and are indicated in each isolate at the end of their identification.

Interpretations

1. Like any RNA virus, TSWV seems to be evolving constantly, and this evolution seems to be influenced by population expansion or purifying selection, which is very common for a RNA virus such as TSWV.

2. What this implies is that virus is evolving constantly, and some changes seem to have occurred from 2008, and such changes are being retained in the genome.

3. Though there is no evidence for outright positive selection against peanut field resistance, the virus is evolving, and could be responsible for increased susceptibility of peanut to TSWV in recent years.

4. Analyses of other genome regions could help explain differences between resistant and susceptible cultivars, particularly the NSs gene.

5. This work in ongoing, mainly to assess if there are differences in TSWV genomes that present a pattern of variation in relation to host plant susceptibility. Samples are currently being collected for 2018 as well.