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

Report: final

INSTITUTION: University of Georgia

Project Title: Examining *Tomato spotted wilt virus* (TSWV) resistance in GA-06G and other cultivars: what went wrong in 2015?

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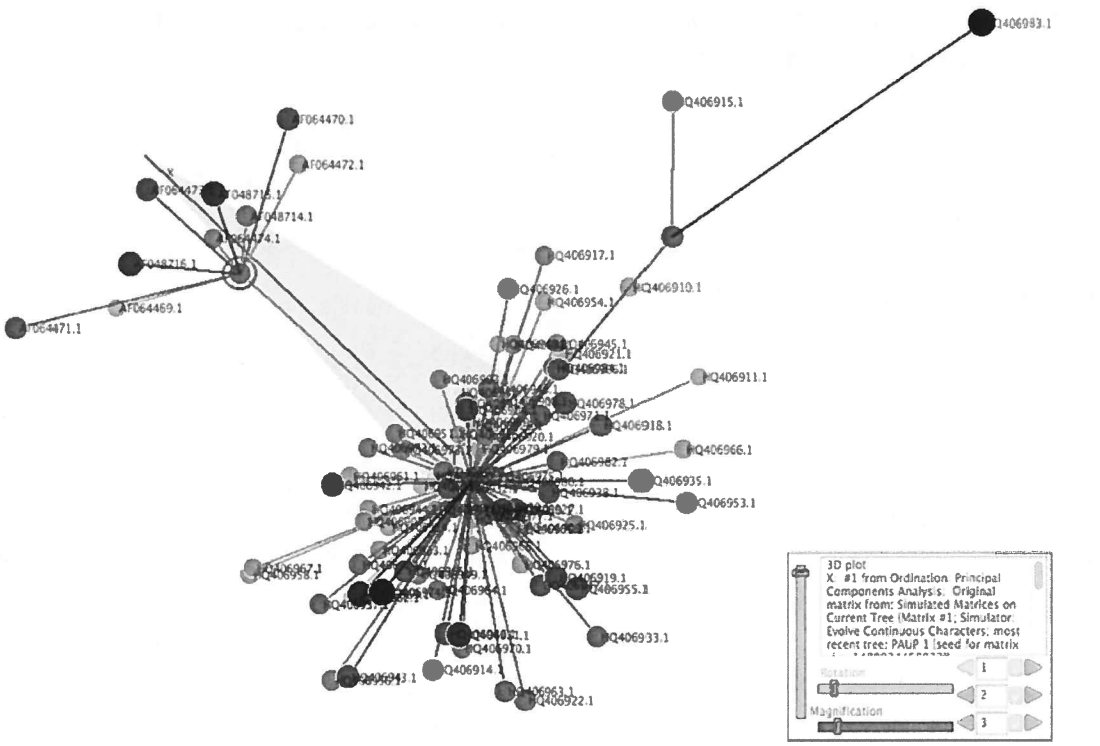
Summary

There was a spike of spotted wilt disease caused by TSWV in 2015 and 2016. Increased spotted wilt incidences were seen in what were once reliably TSWV-resistant cultivars such as GA-06G. This brought about a speculation that the virus could have evolved to become more virulent /resistance-breaking in nature. Such an event would jeopardize the usefulness of resistant-cultivars to manage spotted wilt in the long run. Our recent research has shown that partial sequences from virus isolates collected in 2010 were different than those collected in the 90s. Despite such differences there was no evidence of the presence of resistance-breaking strains of the virus then. The recent reports of increased incidences of spotted wilt in reliably resistant cultivars convinced us to reassess the situation. This project is a multi-year undertaking. My PhD student, Ms. Pin-Chu Lai is leading this effort. Ms. Lai completed her MS work at UGA doing TSWV-peanut research. We believe that sequencing the genome completely would be ideal to addressing this issue. Though the genome of TSWV is relatively small (~17KB), there have 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. We have been attempting to address the issue, and are taking numerous other approaches. In the meantime, samples from multiple plant cultivars are being collected and stored. We intend to accomplish complete sequencing this fall and in the spring of 2018.

Genotype evaluation trials conducted in the summer of 2016 and 2017, indicated that despite differences in spotted wilt incidence, almost all the cultivars exhibited symptoms of TSWV infection. Cultivars such as GA-06G had more spotted wilt incidence than others as GA-12Y and GA-14N. Foliar samples with spotted wilt symptoms were collected from multiple cultivars including some susceptible cultivars such as Georgia Green. It is not clear as to how different the virus genomes are in these cases, and if there is any evidence for selection pressure that some of the cultivars with increased field resistance would impart on the virus genome versus others.

Currently research is underway to obtain full genome sequences of TSWV. Partial genome sequencing over the years revealed that the virus has been altered over time, and that selection forces have been shaping population differences in TSWV.






Our recent research with funding from NPB has clearly shown that there have been differences in partial virus sequences from late 90s to 2010. The principal component analysis with N-gene amino acids figure above displays differences in TSWV N-gene sequences from 1998 (in small cluster) and 2010 (in big cluster). Though N-gene is important from the standpoint of plant-virus interactions, there are other genes in the virus genome that could be more influential in shaping peanut-TSWV interactions. Our goal is to identify differences in such non-structural proteins such as NSs, and assess if indeed such differences in virus genome makeup is responsible for increased TSWV incidence in recent years.

To specifically address this issue is why TSWV-whole genome sequencing was undertaken. The work is still in progress. Illumina pair-end sequencing approach (Next Generation Sequencing) was undertaken. The main challenge with this approach has been the ability to obtain high quality viral RNA from total (plant) 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 (~80%). Currently we are assessing various approaches to address this issue.

1. We are working to improve RNA quality by using an immunocapture based RNA-extraction. We are using TSWV-specific antibodies to specifically capture viral RNA and then prepare cDNA libraries subsequently. We are hoping this would improve the quality of viral RNA and that would facilitate enhancing genome coverage.

2. Numerous samples are being collected and stored during the field seasons in the meantime, once the techniques are standardized NGS sequencing will be completed.

3. In the event the coverage is still less; we intend to address this issue through traditional sequencing (Sanger sequencing). Sanger sequencing is labor intensive and may not be cost effective when labor is factored in. However, could still produce reliable results.

Work is in progress to sequence TSWV whole genomes, and we hope to achieve the goal by the end of Summer 2018. Once that is complete, we will conduct genetic analyses based on whole genomes to examine what factors are shaping TSWV populations and whether there is an indication of positive selection pressure that may jeopardize the usefulness of resistant cultivars in the near future. The protocols for conducting such analyses have all been standardized in our laboratory, and they have already been published (Sundaraj, S., **R. Srinivasan**, A. Culbreath, D. Riley, and H. Pappu. 2014. Plant resistance against *Tomato spotted wilt virus* (TSWV) in peanut (*Arachis hypogaea*) and its impact on susceptibility to the virus, virus population genetics, and vector feeding behavior and survival. Phytopathology 104: 202-210). Therefore, we do not anticipate any major issues in that regard.