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**Southeastern Peanut Research Initiative 2017
FINAL REPORT**

UF Project Number: P0047361

Project Title: Identifying molecular tools for assessing *Sclerotium rolfsii* populations and taxonomic classification of the white mold pathogen on peanut.

Reporting Period: 1/01/17-6/30/18

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1. Abstract

Further understanding of a pathogen's population diversity is important for disease management through improved breeding programs and a better understanding of population responses to varying environments (e.g. temperature and fungicides). The goals of this project were to clarify the genetic diversity of *S. rolfsii* in the Southeastern U.S., and assess this diversity with isolates from around the world. A total of 26 *S. rolfsii* isolates were previously collected from Florida, Georgia, Alabama, and South Carolina with 18 new isolates being collected from Florida, Georgia, Alabama, Mississippi and Arkansas in this study. Type sequence data had also been previously collected from Spain, Italy, Portugal, Brazil and Chile. DNA was extracted from these isolates and amplified by PCR using ITS, RPB₁, RPB₂, and MS204 loci. The resulting sequences were aligned and analyzed using Geneious and BioEdit. Phylogenies were constructed with MEGA7 and a TCS (Templeton, Crandall and Sing, 1992) population network created in R. Using the 4 loci *S. rolfsii* isolates were separated into 2 groups with no differentiation related to host or geography. The network analysis using RPB₂ indicated that isolates from different Florida counties had different haplotypes, and that the other isolates from Georgia and South Carolina, Europe and South America grouped in different haplotypes as well. This data shows that variation does exist in the *S. rolfsii* populations, which means pathogen populations should be considered when assessing disease management strategies (e.g. cultivar resistance).

A majority of the new isolates were collected during the 2018 growing season for this grant due to low levels of white mold in 2017. Novel genes (EF1 α and β -tublin) were also examined in this study, however, data collected from these genes was not clean. Currently, tests are being done to re-run these genes and add the 18 new isolates to the data set.

2. Introduction

To effectively manage white mold caused by *Sclerotium rolfsii* in infested fields, it is critical that we understand the genetic diversity of the pathogen. This information is essential for breeding programs, assessing host specialization of *S. rolfsii*, and the efficacy of integrated management techniques. Preliminary research on 15 isolates of *S. rolfsii* collected throughout Florida's major peanut producing regions indicates that a significant amount of diversity exists within this pathogen. Another study (Xie et. al., Plant Disease) also observed that a considerable amount of variation is present for *S. rolfsii* in the Southeastern U.S., and that isolates from peanut may be specialized or adapted to optimal growth on peanuts. Further

characterization of this diversity is needed to better understand the pathogen's as it relates to pathogenicity, virulence, and other epidemiological factors (e.g. climate). We hypothesized that a high level of diversity is present within the *S. rolfisii* populations of Florida and the Southeastern U.S., and that this diversity will be present at the regional scale. Our objectives are to: 1.) Continue collecting isolates of *S. rolfisii* from production fields in Florida, Alabama and Georgia; and 2.) Examine the diversity of *S. rolfisii* isolates collected during growing seasons from 2012 to 2018 using multilocus sequencing typing – MLST with previously used loci and novel loci EF1- α and β -tublin.

3. Methods

Isolate collection and DNA extraction

Isolates were collected from Florida, Georgia, Alabama, Arkansas, Mississippi and South Carolina to represent *S. rolfisii* population in the Southeastern US. Pure cultures of *S. rolfisii* were isolated from infected peanut by surface sterilization. Actively growing mycelia were then harvested from cellophane-covered acidified potato dextrose agar plates. Total DNA was extracted using the standard protocol of DNeasy Plant Mini Kit (Qiagen, Valencia, CA). Sequences from Mediterranean *S. rolfisii* isolates were downloaded from GenBank (Accession Nos. JF267840, JF267859, JF267856, JF267855, JF267860, JF267861, JF267832, JF267848).

Amplification and Sequencing

To amplify each locus polymerase chain reactions (PCR) were conducted using a Mastercycler thermocycler (Eppendorf, Hamburg, Germany). Reaction mixtures included USB Taq PCR Master Mix 2X (Affymetrix, Santa Clara, CA) and appropriate primers at 5 μ M concentration (Table 1). For each primer pair, the PCR cycling parameters described in corresponding citations from Table 1 were used. Amplification products were checked on a 1.2% agarose gel stained with ethidium bromide. PCR products were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (ThermoFisher, Waltham, MA) following the standard protocol. Cleaned PCR products were sent to Eurofin Genomics for sequencing with 2X coverage (forward and reverse).

Table 1.

Locus / gene	Primers	Length (base pairs)	Description
Previously used loci			
ITS	ITS1 – ITS4 (White et al 1990)	~680 bp	Internal transcribed spacer region
RPB1	Af – Cr (Matheny et al, 2002)	~1300 bp	RNA polymerase subunit 1
RPB2	bSr_1F – b11R1 (Matheny et al, 2002 and Remesal et al, 2013)	~1000 bp	RNA polymerase subunit 2
MS204	E1F1 – E4R1 (Walker et al, 2012)	~1000 bp	guanine nucleotide-binding protein subunit beta-like protein
Further Loci			
EF1- α	EF1-526F – EF1-567R (Rehner, 2001)	~1500 bp	Eukaryotic translation elongation factor 1 alpha
β -tubulin	F- β tub2r – A- β tub2r (Elmax et al, 2002)	~400 bp	Beta tubulin

Population analysis

DNA sequences were aligned using MUSCLE (Edgar, 2004) and edited manually using Geneious (Kearse et al, 2012) and BioEdit (Hall et al, 1999). Phylogenies were constructed using maximum likelihood approach with 1000 bootstrap replicates using MEGA 7 software (Kumar, 2016). Haplotype networks were constructed using the TCS algorithm (Templeton et al, 1992) and visualized using PopART (<http://popart.otago.ac.nz>). A haplotype is group of genes within an organism that inherited from the same parent, indicating genetic similarity. To detect whether there is significant population structure, an Analysis of Molecular Variance (AMOVA) was conducted using Arelquin 3.5 software (Excoffier et al, 2010).

4. Results

Analysis of molecular variance had a p-value of 0.125. The AMOVA indicated that ~14% of the variation occurs among the populations. It also indicated that most (~86%) of the variation occurred within the populations.

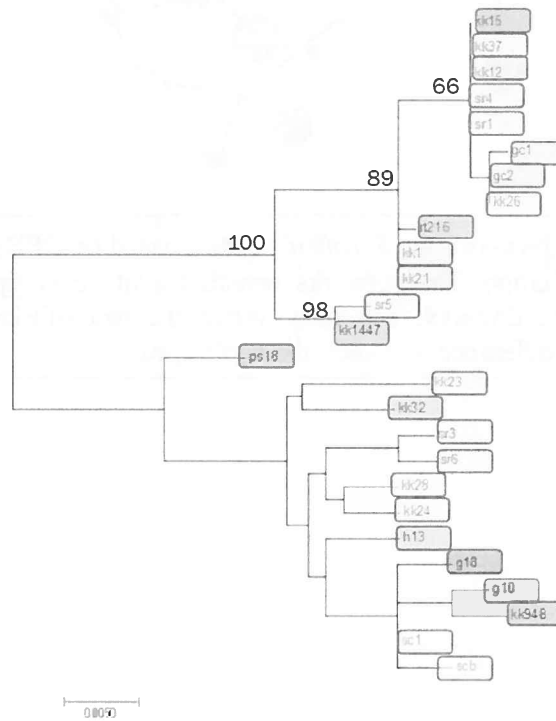


Figure 1: Multilocus sequence typing (MLST) based on a neighbor-joining phylogeny using concatenated nucleotide sequences of ITS, RPB1, RPB2, MS204. Different colors represent isolates from different counties in Florida and the states of Georgia (g), South Carolina (sc), and Alabama (h). The multilocus phylogeny shows that the isolates are separated into two major groups, however no trends are apparent for the geographic regions based on county or state.

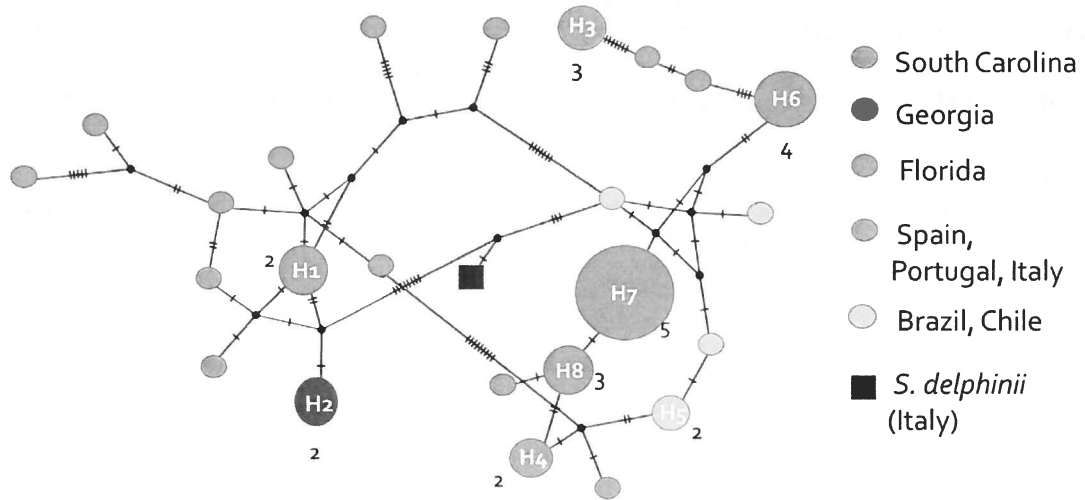


Figure 2: Haplotype Network for *S. rolfsii* isolates based on RPB₂ loci in 3 US states, South America and Europe. This networks reveals haplotype groups but also considerable haplotype diversity, especially within the state of Florida. Each tick mark indicates the relative difference between the haplotypes.

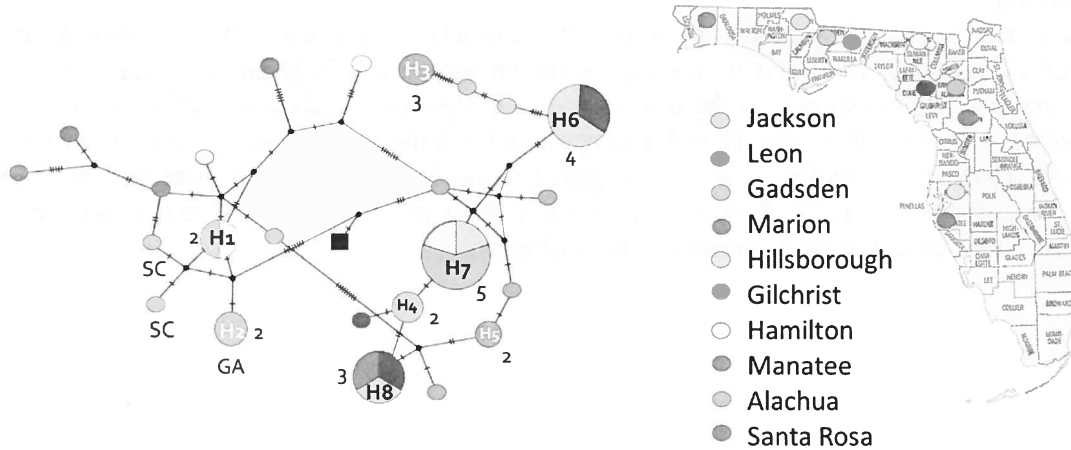


Figure 3: Haplotype Network for *S. rolf sii* isolates based on RPB₂ loci with overlay for the various counties in which isolates were collected in Florida. Data shows that isolates from different counties have different haplotypes, indicating the Florida population to be diverse.

5. Summary

Unfortunately, due to low quality from the DNA extractions, the new loci were not able to be added to the analysis. The previous analysis is shown here, and it is believed that the new loci will improve the confidence in the diversity seen in the isolates already. Further isolates were collected (18) from two new states Mississippi and Arkansas. However, a majority of these isolates (15) were collected in 2018. The goal is to finish the analysis of these new in 2018 and add the new loci in 2019. A new factor (host type) will also be added to this data set as isolates were collected from tomatoes and soybeans.