

**Annual Report National Peanut Board-Peanut Commission, 2014-2015**

# Genetic Characterization of *Cercospora arachidicola* and *Cercosporidium personatum*.

---

## Project Investigator

Renée S. Arias (Ph.D.), Research Pathologist, USDA-ARS National Peanut Research Laboratory (NPRL), 1011 Forrester Dr. S.E., Dawson, GA, USA 39842-0509, P.O. Box 509. Phone: (229) 995-7430, Fax: (229) 995-7416, E-mail: [renee.arias@ars.usda.gov](mailto:renee.arias@ars.usda.gov)

## Co-Project Investigators

Zaid Abdo (Ph.D.) Bioinformaticist, USDA-ARS, 141 Experiment Station Rd., Stoneville, MS, 38776 ([Zaid.Abdo@ars.usda.gov](mailto:Zaid.Abdo@ars.usda.gov))

Emily Cantonwine (Ph.D.) Valdosta State University, 1500 N. Patterson St., Valdosta, GA 31698 ([Egcantonwine@valdosta.edu](mailto:Egcantonwine@valdosta.edu))

## Additional Personnel

Valerie Orner, Biological Science Aid, USDA-ARS-NPRL, 1011 Forrester Dr. S.E., Dawson, GA, USA 39842-0509 ([Valerie.Orner@ars.usda.gov](mailto:Valerie.Orner@ars.usda.gov))

## Summary

Two fungal pathogens are known to cause leaf-spot disease in peanut, these are *Cercospora arachidicola* (*C.a.*) and *Cercosporidium personatum* (*C.p.*). Defoliation by leaf spots, and the multiple fungicide applications cost peanut farmers millions of dollars every year. Whereas single-spore isolation of many phytopathogenic fungi can grow in few days or even hours in laboratory, peanut leaf-spot fungi normally can take four to six months to form small colonies. This might have prevented genetic studies in the past. Progress towards finding molecular markers associated to “leaf spot” resistance in peanut, and the long term effectiveness of resistance for newly developed peanut cultivars, are both hindered by the lack of genetic information about leaf-spot pathogens. We took the task of generate genetic information about *C.a.* and *C.p.* by sequencing both genomes. We collected isolates of both species, screened six of those isolates with 96 SSR markers we had previously developed for *Cercospora soja* (unpublished), based on fingerprinting and morphology we identified one isolate of each species with consistent characteristics for the sequencing project, obtained 465 million total reads for *C.a.* and 566 million total reads for *C.p.*, and the sequences are currently being assembled in contigs. This will be a useful resource for future research on many applications: a) to distinguish these two pathogens, b) to mine the generated data for genes of interest that can be targeted in the control of leaf spot, and c) to determine the genetic diversity of both pathogens, information that is essential for peanut breeding programs.

## Hypothesis and Objectives

Having the genome sequences of *C. arachidicola* and *C. personatum* will provide a framework to understand leaf-spot diseases in peanut.

The objective of this project during the first year of work is to obtain draft genome sequences of *C.a.* and *C.p.*, and to increase the number of *C.a.* and *C.p.* isolates in our collection. Should funding become available in subsequent years, research will focus on the clarification of taxonomic differences between the two species; identification of pathogenicity genes; detection of potential genetic diversity within each of the two species; and provide peanut breeders with relevant information about the pathogens being used challenging new peanut cultivars within breeding programs.

## Results

The project was started in May 2014 when the funding was received from the National Peanut Board. A total of six isolates of *C.a.* and *C.p.* were fingerprinted using 96 simple-sequence repeat (SSR) markers originally developed for *Cercospora sojina*; these markers have not yet been published. Combining data of fingerprinting and morphology two isolates were chosen for the sequencing project, these cultures are hosted at the NPRL, were prepared for long term storage and will be submitted to an international culture collection for their long term preservation. A research agreement was signed with Valdosta State University to obtain additional fungal cultures of *C.a.* and *C.p.* and morphological assessment of cultures. We did seek collaboration from the BROAD Institute of Harvard and MIT, and Drs. J. Bochicchio, C. Nusbaum and C. Russ (from the group that participated in the Human Genome Project) to process our samples at their institute. The selected isolates were sequenced using HiSeq 2500 Sequencing System (Illumina), the current results are summarized in **Table 1**. We expect to obtain no less than 100x depth coverage for each genome, with >95% genome coverage. Sequencing results will be uploaded in NCBI GenBank database, after properly curated through bioinformatics. Sequence assembly is in progress and should be completed by approximately mid or end of February 2015. Genome annotation will be done as a separate project in the near future.

Table 1. Summary of sequencing results for *Cercospora arachidicola* and *Cercosporidium personatum* using HiSeq 2500 (illumine); ID: identification; PF: passed filter

Species	Sample ID	Total reads	PF reads	Potential coverage
<i>C. arachidicola</i>	CALF-13A	465,511,514	426,373,130	>100x depth
<i>C. personatum</i>	CPDP-13A	566,860,222	518,395,236	>100x depth

The initial expansion of our isolate collection is in progress, with 20 isolates provided by Dr. Cantonwine from Valdosta State University, and 20 isolates obtained by Valerie Orner at Arias's Lab in the NPRL. Isolates in the collection will be used in future genetic diversity studies.

### **Measurable Outcomes and Potential Impact**

Draft genome sequences of the two organisms known to cause leaf spot in peanut will be the outcome of the first year of this project. This will be a useful resource for future research on many applications: a) to distinguish these two pathogens, b) to mine the generated data for genes of interest that can be targeted in the control of leaf spot, and c) to determine the genetic diversity of both pathogens, information that is essential for peanut breeding programs.

**Financial Support:** The cost of this project (personnel, equipment, software) was covered by three USDA-ARS Units that support research on peanut (Dawson, GA; Athens, GA, and Stoneville in MS), and a grant from the National Peanut Board that covered cost of supplies, sequencing and partial salary of a faculty at Valdosta State University.