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| **Reporting Period** | Final Report   |
| **Proposal** | [1320-532-5659 R-gene Clusters for Phytophthora sojae Resistance: Cloning Rps Genes](http://moss.unitedsoybean.org/Lists/Proposals/DispForm.aspx?ID=3148&RootFolder=*)    |
| **Committee** | Production   |
| **Target Area** | Supply   |
| **Project Start Date** | 1/1/2013   |
| **Project End Date** | 12/31/2013   |
| **Project Number** | 1320-532-5659   |
| **Project Status** | R-gene clusters for *Phytophthora sojae* Resistance Cloning *Rps* genes  ·         Summary of what was accomplished or learned during the project  Through the analysis of sequence from the source of Rps genes for Rps2, Rps3a, and Rps8 we have identified more than 40 candidate R-genes. Developed a better understanding of the challenges and arrangements of these complex loci Students that have completed degrees  Andika Gunadi, M.S. 2012, The Ohio State University, Research Assistant, Finer Lab  ·         Assessment of progress achieved toward each project performance measure, and if a performance measure was not achieved, an explanation **Strategy 1:  Identification of candidate *Phytophthora* resistance genes for *Rps2, Rps3* and *Rps8.*** **Performance Measure(s) for Strategy 1** Completion of BAC contigs for both *Rps2* (L76-1988)*,* *Rps8* (PI399073), and Rps3a (L83-570). Sequencing of core BACs andidentification of candidate R-genes for *Rps2* *Rps8 and Rps3a.****Rps2:*** Previously, we used resistance gene analog primers and bacterial artificial chromosome (BAC) fingerprinting to develop and sequence a BAC contig for the  *Rps2*  locus in the susceptible parent Williams 82. Using available genetic markers and BAC-end derived primers from the Williams 82 contig, we have now developed a complete BAC contig for the same region in the resistant parent L76-1988.  The contig, which contains six overlapping BACs, spans approximately 370,000 bases.  In addition to containing the Rps2 resistance genes, this region also carries resistance to powdery mildew (*Rmd)* and the ineffective nodulation gene *Rj2.* Bioinformatic analyses identified 26 predicted resistance genes in L76-1988 and revealed extensive colinearity between L76-1988 and Williams 82. While the genes are very similar to each other across lines, there is one gene with an obvious deletion in Williams 82, making it top candidate resistance gene. The *Rps8* BAC contig was more complicated, and from the two BAC libraries we are missing coverage.  Direct sequencing of both RNA and DNA have helped to fill the gaps.  Two DNA-Seq runs have been carried out using MiSeq for soybean line PI399073, which contains *Rps8* and other *P. sojae* resistance genes. Approximately 40 million reads have been generated of 300 bp. This sequence data will greatly aid in our reassembly of chromosome 13, which contains several R-genes but is poorly assembled. The *Rps3a* BAC contig was delayed, the one graduate student completed degree without finishing this objective.  However, additional BACs were identified in a recent screen and are being prepared for initial analysis. In total we have completed sequencing of the following:Total BACs sequenced -**29** (Rps8-27, Rps3a-2)BACs sequenced using Illumina -**26** (Rps8-24, Rps3a-2)BACs sequenced using BAC end sequencing-**5** (Rps8-4, Rps3a-1)  **Strategy 2:  Using Virus Induced Gene Silencing (VIGS) to assay the function of candidate *P. sojae* resistance genes.** **Performance Measure(s) for Strategy 2**a) Make changes to the current VIGS and *P. sojae* inoculation protocols to allow silencing of candidate genes to occur at soybean stage suitable for *P. sojae* infection.Based on available literature, we have made several modifications to the vascular puncture protocol (VPI).  In each trial we have modified a single parameter at a time to determine how each parameter affects the efficiency of VPI with Bean Pod Mottle Virus. We have checked the effect of seed soaking by changing temperature from room temperature to 10 degrees C.  We have altered soaking duration from 15 minutes, 30 minutes and 60 minutes. We have altered the ratio of virus infected tissue to buffer from 10:1, 5:1, 3:1, 1:1, 1:3, 1:5 and 1:10. We have altered the buffer from 50 mM to 100 mM. We have investigated different puncture tools including an engraving tool with 3 pins and a scalpel.  At each point we have tested the protocol modification on 20 seeds per treatment, of these 75% germinate following the treatment.  Of these conditions, a combination of a 15 minute seed soak at 10 degrees C, a buffer ratio of 3:1 with a 50 mM buffer concentration yield 25% of plants infected with Virus and confirmed by Elisa tests.  We have a also been testing *Rpp2* resistant (L76-1988) and susceptible (L82-2024) lines using a hypocotyl inoculation method with *P. sojae*. While *Rps2* mediated resistance is thought to be slow, we are able to phenotype plants within 72-96 hours.  We have tested five different  *P. sojae* isolates to determine which give the most consistent result.  Given these findings we may screen candidate gene VIGS constructs using both method methods.  Our next experiments will determine if different genotypes can have more robust infection.  These tests will focus primarily on the *Rpp2* resistant and susceptible genotypes.  We will also begin to infect these genotypes with *Phytopthora sojae* (5 fields races that will trigger *Rpp2* resistance.a) Use VIGS to silence the *Rps2, Rps3*, and *Rps8* candidate resistance genes. Infect silenced plants with corresponding isolates of *P. sojae*.Candidate gene sequences for silencing have been identified for all genes, we are currently moving forward with what we have to develop the VIGs vectors. In addition, we have identified 3 candidate defense genes that appear to be common to multiple pathogen responses.  We will begin screening these VIGS constructs in the Rps2, Rps3 and Rps8 backgrounds to determine if they are required for resistance.  If these test work, we may test other defense related genes for their involved in *P. sojae* resistance.  ·         If the findings to date for this USB funded research project have been instrumental in leveraging additional non-USB funding, please briefly list the funding source, the amount of incremental funding and how these results might have influenced that funding decision  Internal Funding was leveraged through an Ohio State University program, Center of Applied Plant Sciences.  This program has provided funds for a post-doc with bioinformatic expertise which was greatly needed for this project. The Dorrance Lab is currently evaluating germplasm for resistance towards *Phytophthora sojae* for one company that has just begun to develop varieties.   NOTE: Submitted by Katie Williams on 1/31/2014 on behalf of Anne Dorrance. |
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