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| **Reporting Period** | Final Report |
| **Proposal** | [0275 R-gene Clusters for Phytophthora Sojae Resistance: Cloning Rps Genes (Year 1 of 1)](http://moss.unitedsoybean.org/Lists/Proposals/DispForm.aspx?ID=1949&RootFolder=*) |
| **Committee** | Production |
| **Target Area** | Supply |
| **Project Start Date** | 7/1/2010 |
| **Project End Date** | 12/31/2011 |
| **Project Number** | 0275 |
| **Project Status**  **Final Report - #0275**  **R-gene Clusters for *Phytophthora sojae* Resistance:  Cloning *Rps* Genes**  **Summary:**   * **Summary of what was accomplished or learned during the project**   *Phytophthora sojae* developed in several areas of the Midwest this summer, where heavy rains occurred after emergence and again later in the season.  Soybean varieties planted in two locations with a combination of an *Rps* gene and partial resistance had the highest yields compared to varieties with moderate levels of partial resistance and no *Rps* genes or low levels of partial resistance.  Within the lines that serve as sources of *Rps* genes it is important that we clone each *R-* gene, in order to determine which one is conferring resistance to *P. sojae*.  These R-gene rich regions span large areas of the genome, which are also areas for high recombination.  Thus, without a marker tied to the correct R-gene, the resistance could easily be lost through the current process of rapid cultivar development.  ***Rps*2:**  As part of this project, a BAC library was developed from soybean line L76-1988, which carries the *Rps2* resistance gene.  A BAC contig spanning the *Rps2* regionwas developed using this library. This region, which spans ~370 kb, has now been completely sequenced.  Computational analyses have identified 25 candidate resistance genes.  In order to better understand how this region confers resistance, the corresponding region from the publicly available genome sequence (from a susceptible genotype) was also analyzed. While the gene content and gene order were conserved across the two lines, the susceptible line had only 22 candidate resistance genes (see Figure). In both lines, many of the resistance gene sequences were missing core elements likely required for function, as indicated by their small size.  Interesting, L76-1988 (*Rps2*) had one intact R-gene that is truncated in the susceptible genotype (dashed box in Figure). While we will target all of the genes using virus-induced gene silencing (VIGS), this gene will be tested first.  ***Rps*8**:  Using an Illumina sequencer (short reads), we sequenced 10 BACs covering over half a million bp of the 2.23 Mbp region corresponding to *Rps8*.  These results combined with previous sequence analysis we have 2 gaps, of approximately 50,000 and 215,000 bp to fill.  Three populations were advanced (against all odds of late planting and rainy harvest) to narrow this region down even further.  ***Rps*3a**:  A new BAC library was made from the Williams *Rps*3a isoline, L83-570, two BACs from this region were also sequenced with the Illumina platform covering approximately 166,000 bp in the region.  The very preliminary bioinformatics of these sequences indicate that there is a 4 kb gap in this PI compared to the Williams82 sequence.  Further analysis and Sanger sequencing will be required to confirm if this is true.  We have placed markers on two populations (*Rps*8 x *Rps*3a and *Rps*8 x *Rps*3c). In both of these populations, there is evidence for rearrangements based on changes in marker order, similar to what we have seen in the Williams x *Rps*8 crosses.  We will use these maps to target our Rps3 BAC sequencing to facilitate rapid coverage of this region in the genome.  **New Method Developed**:  A protocol using vascular puncture inoculation (VPI) in seed with a virus to induce gene silencing (VIGS) in soybean was developed and refined.  This procedure will now allow us to screen all of the R-genes that were identified in these regions to determine which one is actually recognizing *Phytophthora sojae* and conferring resistance.   * **Assessment of progress achieved toward each project performance measure, and if a performance measure was not achieved, an explanation**   The biggest challenge we have had is identifying BACs in the PI 399073 library, which is the source of *Rps*8.  Numerous BACs were identified previously, but then aligned to the duplicated regions in the soybean genome.  A new protocol for BAC confirmation was developed this year, which adds considerable time to the process but to date we have not identified a BAC that does not go to this region.  Previously we were using molecular markers that were around repeat regions, now we take sequence from PI 399073 and amplify up a unique region that is specific to Chromosome 13.  Personnel changes and USDA hiring freeze were limitations this past year.  We fully expected to be further along.  At this point we are fully staffed for the first 6 months of 2012, when one student from the Dorrance lab will graduate and a new student will be starting.  **Plans:**  ***Rps*2**:  Complete development of constructs to be used in the Virus-induced-gene-silencing experiments.  Begin to silence genes to determine which gene is the one that encodes *Rps2*. Develop gene-specific primers for all of the genes in the *Rps2* region to determine which genes are active during *Phytophthora* infection.  In addition, screen germplasm to assess how prevalent this gene is in the soybean germplasm collection.  ***Rps*8**:  Intensively screen the BAC library to fill in the remaining gaps across this region.  Our goal is to have this completed by April, and then a round of Illumina sequencing.  The backcross populations that were advanced in 2011 are currently being evaluated with molecular markers and resistance response to 3 *P. sojae* isolates for the region of introgression from *Rps*8 to see the changes.  This should narrow the region down even more so we can focus on the R-genes that are in the BAC that corresponds with the markers from that region.  ***Rps*3/*Rps*8**:  Map these loci in the large segregating populations.  The DNA for most of these populations has been isolated and molecular markers are currently being used.  Several isolates have been selected that will give us the appropriate response in this region – this will be completed by May 2012.  ***Rps*3**:  Screen the BACs for additional clones that correspond to this region.  These will also be sequenced.  Our hope is to cover this region by the end of the summer 2012. | |
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| **Attachments** | |  | | --- | | [0275\_Rps2locus\_Jan2012.pdf](http://moss.unitedsoybean.org/Lists/ProjectStatusReports/Attachments/4763/0275_Rps2locus_Jan2012.pdf) | |

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