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| **Reporting Period** | Final Report   |
| **Proposal** | [2259 R-gene Clusters for Phytophthora Sojae Resistance: Cloning Rps Genes](http://moss.unitedsoybean.org/Lists/Proposals/DispForm.aspx?ID=2709&RootFolder=*)   |
| **Committee** | Production   |
| **Target Area** | Supply   |
| **Project Start Date** | 1/1/2012   |
| **Project End Date** | 12/31/2012   |
| **Project Number** | 2259   |
| **Project Status**Summary:* **Summary of what was accomplished or learned during the project**

*Phytophthora sojae* wiped out several fields in the past two years, even in drought, when varieties with *Rps1c* and *Rps1k* were placed in genetic backgrounds with little to no field resistance.  The need to diversify the available *Rps* genes in cultivars is great.  We have re-sequenced the regions spanning *Rps2* and *Rps8* from the original sources, and are rapidly completing key regions that span *Rps3a*.  Based on our preliminary analysis, there are numerous, novel R-gene like sequences in each of the lines.  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.  ***Rps2***:  The region encompassing *Rps2* has been sequenced and characterized.  Sequence from six individual BACs spanning 370,000 basepairs identified 26 candidate resistance genes.  In addition to *Rps2*, this region also carries the powdery mildew resistance gene (*Rmd*) and the ineffective nodulation gene *Rj2.* Three VIGS constructs are under development to break the candidate genes into subgroups.***Rps8/Rps3a***:  Rps8 and Rps3a are not alleles, but fall in the same region on soybean chromosome 13.  This is good news in that these two Rps genes can be stacked to confer resistance to a broader range of *P. sojae* pathotypes.  This finding was confirmed using allelism tests, and linkage analysis was used to determine the genetic distance and positions of *Rps*3a, *Rps*3c and *Rps*8. The challenging part of the study has been to identify isolates of *P. sojae* with different virulence patterns that can be used to distinguish between all three genes.  A total of 75 *P. sojae* isolates were tested to identify the correct virulence pattern. With the correct isolates, we could evaluate disease resistance within F2:3 and F3:4 mapping populations derived from crosses of soybean genotypes with *Rps*3a and *Rps*8, as well as *Rps*3c and *Rps*8*.* To accomplish this task, 167 SSR and PAMSA markers were used on three populations (326 lines from L83-570xPI399073 F3:4, 554 lines from L92-7857xPI399073 F3:4, 22 lines from Williams x PI 399073 BC4F5:6).  Linkage analysis studies indicate that *Rps*3a and *Rps*8 are linked at a genetic distance of ≥15.5 cM on soybean chromosome 13, while *Rps*3c and *Rps*8 are not linked, suggesting *Rps3c* is located on a different chromosome. Genetic linkage maps of the long arm of chromosome 13 have been successfully developed from L83-570 (*Rps*3a*Rps*3a) PI399073 (*Rps*N*Rps*N*Rps*8*Rps*8) and L92-7857 (*Rps*3c*Rps*3c). In this study, *Rps*8 was mapped to a previously reported location on chromosome 13, north of the simple sequence repeat marker Satt114, and flanked by markers Sat\_103 and Sat\_234. Highly significant association was identified between *Rps*3c and single nucleotide polymorphism marker on chromosome 18 (BARC-032785-09037, P < 0.005).  Four BAC clones from L83-570 the source of *Rps3a* and 22 BAC clones PI 399073 the source of Rps8 were added to the sequence contigs which span this region.   In addition, one 10kb region was amplified directly and sequenced.  With this piece, our preliminary results indicate that we have covered the region that corresponds to *Rps8*. The combined results of these genetic and physical mapping efforts also highlight the complex genetic landscape of this resistance gene-rich region of soybean chromosome 13 and 16. **Functional Analysis:** The final step will be to identify which R-gene like sequence is the actual gene that triggers the rapid resistance response.  To accomplish this, the vascular puncture inoculation method was adapted to expedite gene silencing strategies for functional analysis of these potential R-genes.  The final proof will be the silencing of the gene in the plant and detection of the change from resistant to susceptible following inoculation.  This method is very nicely outlined in the attached poster. * Assessment of progress achieved toward each project performance measure, and if a performance measure was not achieved, an explanation

BACs from the Rps3a region are limited and we are slow in identifying those.  This is in part to due one student completing his M.S. thesis and training a new person to pick up this research.  Two M.S. students have completed their degrees on this project, one now works with Wayne Parrot and the second is in John Finer’s lab.Sequence analysis and assembly has also been slower than we would have liked as hiring of additional qualified employees has been difficult, especially when the positions have been vacated midyear. However, we have had a couple of joint bioinformatic training sessions for the Dorrance Lab so that we can analyze data independently and enhance data analysis capabilities* 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

More markers and sequence is now available for the soybean community to utilize (industry and public).**Plans:** Publications/Presentations 2012Publications:Frasch, R.M., Lincoln, L.M., Carroll, J., Whitham, S.A., Dorrance, A.E., and Graham, M.A. 2012.  Using vascular puncture inoculation and virus-induced gene silencing to identify the *Rps2 Phytophthora sojae* resistance gene.  Soybean Molecular Meetings, August, 2012, Des Moines, IA.Thesis:  Guadi, A. 2012. Characterization of *Rps8* and *Rps3* Resistance Genes to *Phytophthora sojae* through Genetic Fine Mapping and Physical Mapping of Soybean Chromosome 13. M.S. Thesis, The Ohio State University  |
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| **Attachments**  |

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| [Rps2poster\_soybeanmolecularmeeting\_2012.pdf](http://moss.unitedsoybean.org/Lists/ProjectStatusReports/Attachments/6646/Rps2poster_soybeanmolecularmeeting_2012.pdf)     |

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