Identification of Key Defense Genes for Resistance to Phytophthora Stem and Root Rot through Gene Silencing

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Summary of Critical Findings over the Period of Funding

The objectives of this project have been to use a newly developed gene silencing technology to examine the relative importance of a series of genes which we hypothesized may play an important role in root and stem resistance of soybean to *Phytophthora sojae.* In the period supported by this project, we examined the importance to resistance of five target genes, including two genes for isoflavone biosynthesis (chalcone reductase, CHR and isoflavone synthase, IFS) and three pathogenesis-related protein genes (PR-1a, PR-2 and MMT). These studies were carried out on a series of over twenty soybean lines which carry different Rps genes for race-specific resistance and also different genes for partial resistance to *P. sojae*.

Important results over the course of this project included the following:

- The five genes under study not only all had effects on the various forms of resistance, but also on one another's expression, allowing them to actually be placed into pathways for Rps (race-specific) or partial resistance. This information is of critical importance in that it allows us to define the overall processes involved in these two forms of resistance and, as described below, to define possible ways to enhance each form of resistance.
- Our overall results on all soybean lines show that PR-2 is a master regulatory gene of all other genes being studied and is thus essential to all forms of resistance. PR-2 is involved in releasing active elicitor (defense inducing) fragments from pathogen cell wall glucan, a major cell wall component of many oomycetic and fungal pathogens. These glucan elicitor fragments trigger all forms of soybean resistance. Since many plant pathogens contain these glucans, PR-2 plays a role not only in triggering all forms of resistance but may be effective against a wide range of pathogens.

- We demonstrated that the gene PR-1a appears to be a key gene for establishment of partial resistance, which is a more stable form of resistance than Rps-mediated race specific resistance in that it is not as easily defeated by new races of *P. sojae.*
- We demonstrated that the MMT gene appears to be a point of cross-talk between the pathways conferring race-specific and partial resistance, suggesting that it plays a regulatory role in balancing these two pathways.
- In an examination of lines carrying all available Rps genes for race-specific resistance to *P. sojae*, (Rps 1a, 1b, 1c, 1k, 2, 3a, 3b, 3c, 4, 5 and 7), we demonstrated that different Rps loci condition different hypersensitive cell death (HR) programs to pathogen attack. For instance, genes at the Rps 1 and 2 loci condition an HR involving the isoflavones, while genes at the Rps 3 and 7 loci condition an isoflavone-independent HR. This information is of great importance in allowing us to predict how best to deploy various resistance genes to complement one another.
- We fully characterized the various forms of resistance to *P. sojae* for the first time. With the exception of Rps-2, all other Rps genes were identified on the basis of providing resistance to the hypocotyls, even though root rot is probably the major source of damage in the field. We have demonstrated that three forms of resistance in roots (race-specific, partial and age-related) are expressed in different root zones. Using gene silencing, we demonstrated the relative importance of various target genes to each of these forms of resistance and that partial resistance may involve the up-regulation of age-related resistance in younger tissues.

Importance of Major Findings and Future Plans

- **PR-2.** The finding of the central, master role for PR-2 is a truly major discovery and should lead to strategies for wide spectrum control of soybean diseases. Given that this gene controls the release of elicitors from a broad spectrum of pathogens and that it controls both Rps (race specific) and partial resistance, we want to over-express this gene to see if we can enhance overall soybean resistance to pathogens. Over-expression of this gene should enhance soybean's ability to detect pathogens and trigger resistance. Current plans include developing PR-2 over-expressing soybean lines either through natural selection of lines with higher PR-2 or through genetic engineering.
- **Pr-1a.** Given that the PR-1a gene is essential to partial resistance, over-expression of it may specifically enhance expression of this more stable form of resistance. Thus, its over-expression along with PR-2 may have very complimentary effects.
- **MMT.** As a cross-talk regulatory gene between the race-specific and partial resistance pathways, the appropriate expression of MMT is critical to the balance between these two forms of resistance. While we do not yet know the optimal expression of MMT, its expression along with PR-2

and PR-1a will be important to the overall fitness of soybean against disease.

Other Important Outcomes of the Project

The funding for this project helped us to refine our gene silencing strategies and publish two papers (with others to come). These are noted below. A very important outcome of these publications is that we are the world leaders in gene silencing for soybean. The four other major soybean defense laboratories (two in the US, one in Canada and one in Germany) have adopted our soybean gene silencing protocols and additional collaborative efforts with a laboratory in Japan have begun.

Based on the work funded through IMBA, we have successfully received funding from the Ohio Soybean Council for three consecutive years, for a total of approximately $160,000.

Bibliography of manuscripts, abstracts and presentations that have resulted (to any degree) from IMBA funding.

Book Chapters:

Graham TL, Graham MY and Yu O. 2007. Genomics of Soybean Secondary Product Metabolism, an invited chapter for a book on Soybean Genomics, Gary Stacey, ed., in press.

Day B, Graham TL. 2007. The Plant Host-Pathogen Interface: Cell Wall and Membrane Dynamics of Pathogen Induced Responses. Ann. N.Y. Acad. Sci. 1113: 123–134.

Peer-reviewed journal articles:

S. Subramanian, M.Y. Graham, O Yu., and T. L. Graham 2005. RNA Interference of Soybean Isoflavone Synthase Genes Leads to Silencing in Non-transformed Tissue and to Enhanced Susceptibility to Phytophthora sojae, Plant Physiol. 63:141-149

Graham TL, Graham MY, Subramanian S, Yu O. 2007. RNAi silencing of genes for elicitation or biosynthesis of 5-deoxyisoflavonoids suppresses race specific resistance and hypersensitive cell death in *Phytophthora sojae* infected tissues. Plant Physiol. 144: 728-740.

Presentations:

T.L. Graham, 2005. RNAi-based Defense Gene Silencing in Soybean, Department of Plant Pathology, University of Wisconsin, September 27, 2005, invited by the Plant Pathology graduate students.

T. L. Graham, 2005. A Tale of Two Projects: Soybean Defense Gene Silencing and Novel Defense Activators from Plant, OPBC Annual Meeting, Mohican Resort, Perrysville Ohio, November 4-5, 2005.

T. L. Graham, 2005. RNAi Silencing of Soybean Defense Genes Leads to Altered Responses to Elicitors and Infection in both Transformed and Distal Tissues, 2005 PMBB Research Symposium, April 15-16, 2005. **Note: Our labs work was the cover story for the symposium**.

T.L. Graham, 2006. "Use of Gene Silencing to Dissect Resistance Responses to *P. sojae* in Soybean", an Invited presentation at the Joint Annual Meeting of the Plant Molecular Biology and Biotechnology (PMBB) and Molecular Plant Microbe Interactions (MPMI)@Ohio Annual Meeting., Shisler Conference Center, Wooster, Ohio, March 31, 2006.

T. L. Graham, 2006. "Functional dissection of soybean defense pathways and their regulation using metabolomics and RNAi gene silencing", an invited presentation at the Symposium, "Signals, Pools and Pathways to Host Resistance", Joint Annual Meeting of the American Phytopathological Society (APS), Canadian Phytopathological Society (CPS) and Mycological Society of America (MSA), Quebec City, Quebec, July 2006. This was an invited presentation at an international symposium sponsored by the APS/CPS Biochemistry and Molecular Biology Committees. Attendance was excellent, estimated to be over 150 scientists.

Graham TL, Graham MY, Subramanian S, Yu O. 2007. Use of Gene Silencing and Metabolomics to Characterize Interactive Stress and Defense Pathways in Soybean. Stress 2007, Budapest, Hungary, August 23-26, 2007. (I was also the Organizing Chairman for this Symposium at the Meeting…see Major Accomplishments and Service for details)

Graham TL. 2007. Gene Silencing as a Tool for Dissection of Soybean Defense Responses. Third Symposium on The Sao Paulo-Rutgers-Ohio State Tripartite Graduate Program, Rutgers University, April 9, 2007.

Graham MY, Sinden MR, St. Martin S, Graham TL. 2007. The Effects of Silencing Homologs of the Arabidopsis Defense Regulator NPR in Soybean. Ohio Plant Biotechnology Consortium Annual Meeting, Columbus, Ohio October 27, 2007.