

Microarrays and identification of resistance genes; hairy roots and transformation with disease resistance genes
(Primary Investigators: Lila Vodkin, Steve Clough, Jack Widholm)

The team of Lila Vodkin, Steve Clough, and Jack Widholm bring a combination of research experience and training that enables them to apply leading-edge technologies to dissect the complex interaction of soybean with its specific pathogens and to develop biotechnology strategies to control the major diseases that affect the quality and productivity of soybeans. These research strategies include the latest approaches in structural and functional genomics and genetic transformation methodologies. This group has received funding from the National Science Foundation, the United Soybean Board, and USDA/ARS on genomics, biotechnology, and pathology research.

Goals for this project include identification of genes involved in resistance to soybean diseases using genomic approaches. Once a plant has been assaulted by a pathogen, the plant responds with a battery of defenses, many of which are the result of changes in gene expression. We will use soybean cDNA microarrays developed in the Vodkin lab to identify genes that are expressed during pathogen assaults. We are currently performing the initial microarrays with SDS and white mold infected tissue. Such genomic scale studies have yet to be undertaken by any plant group on the infection process by important agronomic pathogens in crop plants.

Initial procedures include: functional genomic analysis of the response of soybean to infection by pathogens including the major fungal soybean diseases SDS and white mold. Initial studies of white mold and SDS will be expanded to include the analysis of more specific tissues as well as analysis of genes involved in systemic acquired resistance. In addition, the number of genes to be assayed in arrays will be over 36,000 resulting from the NSF project providing a global view of expression during pathogen attack. Studies of tissue specific gene expression for both SDS and white mold will be undertaken

In addition to analyzing gene expression determined by multiple genes (quantitative traits or QTLs), defense responses that are governed by single gene resistance, known as the hypersensitive response (HR) will be analyzed. We will compare the HR induction by the bacterium *Pseudomonas glycinae* to that of a disease-causing (wild fire) strain of *P. glycinea*. All arrays will be conducted by standard approaches that have been shown to work well for soybean.

By the second year of this project, we will have sufficient data to enable us to perform a very thorough comparison of how soybean is responding (resistant or susceptible) to these three different diseases: white mold, SDS, and wild fire. The detailed bioinformatic analyses will rely on annotations made from the NSF project for the 36,000 soybean cDNAs. These cross comparisons will provide a much clearer understanding of the mechanisms that soybean uses to fight off pathogens and will have a very high probability of identifying key genes. In addition, we will cross compare the results of genes involved in defense responses to the results of microarray experiments being carried out on other projects that examine gene expression in soybean development or response to various challenges.

Experimentally, we will verify the role of key genes of interest found from the microarray experiments using the technology of quantitative PCR with the Taqman approach. These results will be used to provide the raw material for specific genes to improve plants through transformation approaches.

Transformation technologies will be employed to find and use new genes to impart disease resistance to SDS and SCN. Preliminary studies with our soybean embryogenic culture system (Finer and McMullen, 1991) show that we can insert up to four genes at once using particle bombardment. To enhance our ability to produce soybean plants by genetic engineering, we will develop root-specific promoters to focus gene expression in the pathogen's first target location. Since we have been able to greatly alter the levels and ratios of hairy root phenylpropanoids, including the phytoalexin glyceollin, we will begin inserting the identified genes into fertile soybean plants using the *rolD* promoter.