Expression analysis of the Rpp1-mediated immune reaction to Phakopsora pachyrhizi using the Affymetrix Soybean Genome Array

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Introduction

Soybean rust is a devastating foliar disease of legumes, caused by the fungus Phakopsora pachyrhizi. Field infections can sometimes lead to 100% yield loss (Fig 1.), and since the U.S. produces over 70 million tons of soybeans per year, soybean rust is an important concern for U.S. agriculture. As of early November 2006, soybean rust had been identified in 15 U.S. states, as far north as Illinois and Indiana. Soybean rust is currently managed through the use of fungicides, and the development of rust tolerant or resistant varieties of soybeans is a top priority. There are currently four independent rust resistance genes (Rpp1-1 to Rpp4-4) that have been identified in non-commercial cultivars. Several isolates of soybean rust have been identified that overcome the resistance provided by Rpp1-Rpp4. Therefore, new sources of rust resistance are needed.

Microarray technology allows the expression of thousands of genes to be simultaneously measured without any prior knowledge of the genes. Using this technology, we will be able to identify soybean genes that change expression in a rust resistant reaction. The cultivar Komata (PI200492) carries the Rpp1 rust resistance gene. When inoculated with specific isolates of P. pachyrhizi, Komata displays resistance to rust that has been termed the "Immune" reaction and is characterized by a lack of visible symptoms. In this study we compare the gene expression in the Immune reaction (Fig 2, below left) to gene expression in the fully susceptible "Tan" reaction (Fig 2, below right) using the Affymetrix Soybean Genome Array. Genes that show significantly different expression between Immune and Tan reactions are good candidates to be involved in rust resistance.

Methods

• Soybean plants carrying the Rpp1 resistance gene (PI200492, Komata) were inoculated with P. pachyrhizi isolates Hawaii 94-1 and Taiwan 72-1 that result in an Immune or Tan reaction, respectively.
• After inoculation the plants were incubated at 20° C with constant dew for approximately 24 hours.
• Whole leaves were harvested and flash-frozen in liquid nitrogen at 6, 12, 24, and 48 hours post inoculation.
• Leaflets were pooled from 4 to 6 plants at each time point, from three independent experiments, and RNA was extracted using Trizol/GITC (Fig. 3).
• RNA samples from various time points were labeled with biotin using the Affymetrix One-Cycle kit and hybridized to arrays for 16 hours.
• RNA samples were washed and stained with R-phycocerythrin Streptavidin using the Affymetrix Fluidics Station 450, and scanned using the Affymetrix Gene Chip Scanner 3000.
• Raw data were subjected to quality control procedures and background correction, normalization, and summarization were applied.
• Summarized data was analyzed using Bioconductor software and the statistical language "R" to produce a set of differentially expressed genes.
• Differentially expressed genes were identified by BLAST X similarity search to proteins in GenBank and assigned to GO functional categories using the UniProt and PFAM databases.

Results

Fig 5. The distribution of transcripts identified as statistically significantly different (p < 0.05, with Benjamini False Discovery Rate control) between the Immune and susceptible reactions. A total of 1133 genes were differentially expressed in the Immune reaction.

Discussion

• Soybean genes have been identified that are induced or suppressed in the Immune reaction following infection with soybean rust.
• In the comparison of the Immune reaction to the susceptible reaction, the host genotype (cultivar Komata, Rpp1) is identical between samples. Therefore, differential expression of genes should result from the difference between the two treatments, the rust isolate giving an Immune or Tan reaction.
• Many of the genes induced in the Immune reaction are genes that have been shown in other plant-microbe interaction studies to be involved in plant defense and stress responses, such as pathogenesis-related (PR) proteins and isoflavone reductase.
• The induced genes can be grouped into categories by similar functions. Upregulation of genes such as nitrite reductase, glutaredoxin, catechol reductase and isoflavone reductase suggests that redox reactions may play role in the Immune reaction, possibly by regulating cellular metabolism.
• Induction of lipases and lipid/membrane metabolism genes and a group of carbohydrate metabolism and cell wall-related genes such as expansins suggests that remodeling of the cell wall and cellular membranes is a component of the immune reaction, even though no visible lesions are present on the inoculated plants.
• Heat-shock proteins, heat-shock transcription factors, and abiotic stress response genes are upregulated in the Immune reaction. The heat-shock 70 family of genes has been implicated in oxidative stress in Arabidopsis, and heat-shock transcription factors have been shown to regulate transcription of defense genes.

References:


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Fig. 4. An example of the raw data that is produced by the Affymetrix GCOS software.

Fig. 3. A schematic illustrating the workflow of the experiment.

Fig. 2. Photographs illustrating the Immune reaction and the Tan reaction on Komata plants.

Fig. 1. A list of genes that are differentially expressed in the Immune vs. Tan reactions. The genes are grouped by time point, with induced genes in the top colored areas, and suppressed genes in the bottom grey areas. Gene names in Blue = transcripts related to reduction/oxidation reactions, Brown = cell wall synthesis and cell biogenesis, Orange = heat shock and abiotic stress, and Green = lipid metabolism.