Molecular Analysis of the Fusiform Rust Disease Interaction

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Fusiform rust is a disease of southern pines that is characterized by the formation of woody galls on the stems and branches of infected trees. It is caused by a fungus named *Cronartium quercuum* f. sp. *fusiforme*. This organism has a number of interesting effects on the trees it infects. Three of the most profound are its ability to change the rate of xylem cell division, its ability to alter the pattern of xylem cell differentiation, and its ability to modify xylem cell size and shape. Our goal is to determine how *C. q. fusiforme* causes these changes in wood development. By studying fusiform rust from this perspective, we expect to gain insight into the molecular control of normal wood development as well as the mechanisms underlying fusiform rust gall formation. As the human population expands, there is an increasing need to produce wood more efficiently from the available land base. A more detailed understanding of wood development is likely to assist in this regard. In addition, there is a significant economic incentive to improve our understanding of the fusiform rust disease interaction; throughout the southeastern United States, this disease is estimated to cause losses ranging from $25M to $135M/year (Cubbage et al. 2000).

Our initial hypothesis was that *C. q. fusiforme* mediates at least some of its effects on wood by altering the expression of pine genes at the level of transcription. If so, we reasoned that we should be able to identify genes controlling development at the vascular cambium by looking for pine genes whose expression changes in response to rust infection. To look for such genes and to test this hypothesis, we have compared gene expression patterns in galled and healthy pine seedlings of the same age. We used two different techniques to identify and isolate transcripts that were present at different levels in these two tissues. We began with a technique called differential display (Liang and Pardee 1992), but eventually switched to suppression subtraction hybridization (Diatchenko et al. 1996) because differential display proved to be very laborious for a relatively low yield of interesting clones.

Using differential display we isolated 19 cDNAs that are differentially expressed in galled and healthy tissue. Six of these originated in pine and all six were suppressed in galls relative to healthy tissue. The differential expression of these clones was quantified by repeated northern analysis or reverse-transcriptase PCR. Their suppression in galls ranged from 5-fold to 1.4-fold. The remaining 13 clones were derived from *C. q. fusiforme*.

Using suppression subtraction hybridization, we made two libraries. The “galled library” is enriched for cDNA sequences expressed only in galls, or expressed at higher levels in galls than in healthy stems. The “healthy library” is enriched for sequences expressed only in healthy stems, or expressed at higher levels in healthy stems than in galls. We sequenced 123 clones from the galled library and among them there were 62 different sequences. Thirty-nine of these sequences were homologous to other sequences in GenBank. Most of these clones are predicted to be from *C. q. fusiforme*; based on their homologies only 4 are known or predicted to come from pine. We are currently conducting PCR experiments to determine the genome of origin of

each of these 62 clones. We sequenced 200 clones from the healthy library and among them there are 43 different sequences. Twenty-nine of them are homologous to other sequences in GenBank. All of these clones are predicted to come from pine because the healthy tissue is not infected with *C. q. fusiforme*.

In total, suppression subtraction hybridization yielded 105 new genes. Because this is too many to study individually we are collaborating with John Davis (University of Florida) on the use of clone arrays to quantify mRNA levels in galled and healthy tissues. In our first experiment, we detected significant differences (i.e. $\geq$ 3x increase or decrease in galled tissue relative to healthy tissue) for approximately 50% of our clones. Most of the remaining clones also exhibited differences in expression as expected, but these differences were of a smaller magnitude. On-going work focuses on replicating these experiments with probes from both loblolly and slash pine.

Two gall-suppressed pine clones have been chosen for further functional analyses. One of these encodes an NAC domain protein. Multiple lines of evidence suggest that NAC domain proteins are transcriptional regulators (Kikuchi et al. 2000; Ren et al. 2000; Xie et al. 2000). Those that have been characterized in herbaceous plants play roles in development or defense (Souer et al. 1996; Aida et al. 1997; Sablowski and Meyerowitz 1998; Xie et al. 1999; Ren et al. 2000; Xie et al. 2000). We plan to localize the expression of the pine NAC domain protein in stem cross sections as a first step toward determining if it is likely to play a role in development or defense. The second clone of interest, DD55, encodes a protein of unknown function. The genome of *Arabidopsis thaliana* contains a single gene ortholog of DD55. It encodes a protein that is 76% identical and 86% similar to DD55. Given the ease with which *A. thaliana* can be manipulated, we plan to determine the function of the DD55 ortholog in this model plant through a series of transgenic experiments. Once its function in *A. thaliana* is known, more specific hypotheses about the function of DD55 in pine will be designed.

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