

Transcriptome Analysis of Pathogenic Interactions with *Medicago truncatula*

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The forage legume, *Medicago truncatula*, is an excellent model for investigating functional genomics of plant-microbe interactions. As a basis for functional genomic analysis, 17 cDNA libraries were constructed, 11 from plants at different developmental states, 3 from pathogen-infected plants, 2 from elicitor-treated plants, and one from mycorrhizal roots. Partial sequencing of clones from these libraries has contributed to the 164,441 ESTs from *M. truncatula* that have been deposited in GenBank as of May 2002. Database mining shows that a number of genes are more prevalent in pathogen-infected or elicitor-treated plants, but no clones are specific to only pathogen interactions.

At present, glass slide microarrays containing 1152 cDNAs ('kiloclone set') printed in triplicate are being used for hybridization with labeled targets derived from pathogen-infected and noninoculated leaf and root tissues. The kiloclone set contains positive and negative control and clones of known tissue-specific patterns. It is also rich in clones encoding proteins with putative functions in signal transduction, transcriptional regulation, control of cell division and cell death, pathogen response, secondary metabolism, and a number of genes of unknown function. Approximately 17% of the cDNAs are involved in plant-microbe interactions. Comparisons of gene expression profiles of *M. truncatula* "Jemalong A17" interactions with two foliar pathogens (*Colletotrichum trifolii* and *Erysiphe pisi*) and one root pathogen (*Phytophthora medicaginis*) were performed. Cluster analysis revealed subsets of genes that are differentially expressed across tissues and pathogens. In the *C. trifolii* interaction, 54 genes were up-regulated, 19 genes were up-regulated by infection with *E. pisi* and 35 genes were up-regulated by *P. medicaginis* infection. Hierarchical clustering of pathogen-challenged tissues showed that expression profiles of pathogen-challenged Jemalong A17 with *P. medicaginis* and *C. trifolii* were more closely related than to the profile observed with *E. pisi*. This may reflect the strong hypersensitive reaction observed in the *E. pisi*-Jemalong A17 interaction. Two-dimensional clustering analysis showed that out of 48 clones representing disease defense response genes, 19 were up-regulated across all experiments. These genes included the classical pathogenesis response proteins, chitinases, glucanases, chalcone reductase, chalcone isomerase and peroxidases, among others. Only 5 clones showed down-regulation in all experiments. Out of 127 genes with unknown function, 14 were down-regulated in all experiments and only one clone was up-regulated in all cases. Several subgroups of unknowns were associated with defense-response genes using self-organizing maps partitioning. These novel genes will be studied further to assign putative functions related to disease response. A time course experiment of *M. truncatula* roots challenged with *P. medicaginis* showed a number of differentially expressed genes at each time point. At 2 days after inoculation, with the development of the first symptoms, 52 genes were up-regulated, including most of the defense-response genes, and 26 genes were down-regulated, including many known nodulin genes.

To test the utility of *M. truncatula* microarrays for investigating gene expression in alfalfa, RNA was extracted from healthy 21-day-old roots of alfalfa and *M. truncatula*. Comparable signal intensities and expression patterns were seen for the two *Medicago* species indicating the potential of these microarrays for application to other legume crops.