Pathosystem Development and Transcriptome Analysis of Pathogen Interactions with *Medicago truncatula*

D. A. Samac[1,2], S. Peñuela[2], D. Danesh[2], N. D. Young[2], and K. VandenBosch[3]
[1]USDA-ARS-Plant Science Research, St. Paul, MN
[2]Department of Plant Pathology, Univ. Minnesota, St. Paul, MN
[3]Department of Plant Biology, Univ. Minnesota, St. Paul, MN

Medicago truncatula is being developed as a model plant for functional genomics studies of plant-microbe interactions. A set of 30,000 non-redundant genes is being established for microarray–based gene expression studies. At present, glass slide microarrays containing 1152 cDNAs including positive and negative controls are being used for hybridization with labeled targets derived from pathogen infected and uninoculated leaf and root tissues. To fully utilize microarray analysis of plant-pathogen interactions, a spectrum of host responses, from fully resistant (incompatible) to fully susceptible (compatible), needs to be identified. Plants from a collection of 119 accessions of *M. truncatula*, including 10 cultivars, were screened for reaction to *Phytophthora medicaginis* (Phytophthora root rot), *Phoma medicaginis* var. *medicaginis* (spring black stem and leaf spot), and *Colletotrichum trifolii* (anthracnose). In seedling assays, 92% of the entries were scored as susceptible to Phytophthora root rot with brown necrotic lesions on hypocotyls and upper roots. Susceptible plants were stunted but not killed. In a detached leaf assay, 94% of the entries were scored as susceptible to *Phoma* in which more than 25% of the leaf was yellowed. Reactions of seedlings to *Colletotrichum trifolii* race 1 and 2 were similar, showing moderate to complete cotyledon yellowing. Infections only occasionally spread to stems and leaves. An alfalfa isolate of powdery mildew identified as *Erysiphe pis*i was used to inoculate 20 accessions of *M. truncatula* in the greenhouse. Two entries were resistant, three showed a hypersensitive response, and two were partially resistant while the rest were susceptible.

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 using glass slide microarrays. In the compatible *C. trifolii* interaction 54 genes were up-regulated, 35 genes were up-regulated in the compatible interaction with *P. medicaginis,* and 19 genes were up-regulated by infection with *E. pisi*. Expression profiles of plants challenged 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. Of 48 clones representing disease defense response genes, 19 were up-regulated across 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.