Transcript Profiling in *Medicago truncatula* Responding to Infection by *Erysiphe pisi* and *Colletotrichum trifolii*

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Medicago truncatula is a model for legume genomics, exhibiting simple genetics and a genome highly conserved with alfalfa and pea. The large collection of molecular tools make *M. truncatula* an excellent system to identify genes involved in biotic and abiotic stress tolerance in legumes. We are using *M. truncatula* to isolate disease resistance genes and to identify and characterize the function of genes expressed during resistant interactions with pathogens.

A collection of 119 accessions of *M. truncatula*, including 10 cultivars, was screened for reaction to *Colletotrichum trifolii*, the causal agent of anthracnose of alfalfa. A majority of the entries showed moderate to complete cotyledon yellowing, classified as a resistant response. In only about 4% of the entries did infection result in necrosis or spread of the pathogen to stems and leaves, a reaction classified as susceptible. Approximately 18% of the entries showed little to no response, classified as a potential non-host reaction. Histochemical staining showed that the reduction or absence of symptoms on a least one accession was correlated with reduction in spore germination compared with germination on the resistant genotype. Microscopic evaluation revealed that under certain conditions, spore germination in *M. truncatula* accessions was reduced compared with germination on Saranac and Saranac AR alfalfa. In the resistant interaction, production of hydrogen peroxide, epidermal cell browning and callose production was observed 48 hours post inoculation. Little to no mycelial growth was observed on resistant accessions compared with a highly susceptible accession.

An alfalfa isolate of powdery mildew identified as *Erysiphe pisi* 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. At 48 hours post inoculation the resistant accession exhibited auto-fluorescence at the site of fungal penetration, indicating the presence of phenolic compounds, and the germinating spore had collapsed. To map the location of genes controlling resistance to powdery mildew, plants from 90 recombinant inbred lines from a cross between Jemalong 6 (resistant) and DZA315.16 (susceptible) were scored for powdery mildew development. Major genes for resistance are located on linkage group 5 near a cluster of potential resistance genes with NBS-LRR motifs.

Glass slide microarrays with 6,384 M. truncatula ESTs were used to examine gene expression in *M. truncatula* responding to *E. pisi* or *C. trifolii*. Cotyledons of Jemalong A17 (resistant) were inoculated with spores of *C. trifolii* and harvested 24 h after inoculation. Mature leaves of *M. truncatula* Jemalong 6 (resistant), Jemalong A20 (moderately resistant) and DZA315.16 (susceptible) were inoculated with spores of *E. pisi* and harvested 12 hours after inoculation. For each interaction, RNA was extracted and pooled from three replicate experiments, labeled and hybridized with six replicate slides. In response to *C. trifolii*, 84 genes were up-regulated at least 2-fold and 2 genes were down-regulated, compared with mock-inoculated plants. In the resistant response to *E. pisi* 361 genes were up-regulated, 297 genes were up-regulated in leaves of the moderately resistant accession and 192 genes were up-regulated in leaves of the susceptible accession. Among the three interactions, 75 gene were up-regulated in common. A total of 50 genes were up-regulated by both *E. pisi* and *C. trifolii* in resistant interactions indicating that the response pathways to both pathogens overlap significantly. A majority of the characterized genes are involved in flavonoid, stilbene, lignin, and phytoalexin production and cell-death regulation, consistent with the phenotypes observed. Several genes of unknown function were also differentially regulated, highlighting this approach as a tool for gene discovery.