

Pathosystems and resistance gene genomics in *M. truncatula*

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An underlying theme of the *Medicago truncatula* NSF genomics program is a focus on plant-microbe interactions, including both pathogenic and symbiotic microorganisms. In the course of this research, the NSF group has been developing host-pathogen systems for a diverse range of microorganisms, including Oomycetes, fungi, nematodes, bacteria, and parasitic seed plants. We are also screening ecotypes of *Medicago truncatula* to identify sources of resistant and susceptible germplasm. Ultimately these germplasm resources are expected to provide the basis for genetic analysis and map-based cloning of resistance genes. In parallel to these phenotypic screens, the cDNA microarray resource of *Medicago truncatula* (developed jointly at the University of Minnesota and the Samuel Roberts Noble Foundation) is being used to investigate differential host gene expression in response to infection by many of the above-mentioned pathogens. This topic will be covered in presentations by Kathryn VandenBosch and Deborah Samac in this same session.

As a complement to the phenotypic screens, described above, the laboratories of Doug Cook (University of California-Davis) and Nevin Young (University of Minnesota) have been characterizing the genomic distribution and phylogeny of *Medicago truncatula* resistance genes. Three approaches were used to identify sequences with homology to resistance genes: PCR with degenerate primers designed against the nucleotide binding site (NBS) domain that is highly conserved in most of the known plant resistance genes (so-called resistance gene analogs or RGAs); direct hybridization with characterized soybean resistance genes; and BLAST analysis against the *Medicago truncatula* EST database (www.tigr.org/tdb/tgi/mtgi). The combined effort yielded in excess of 150 unique RGA sequences. Phylogenetic analysis revealed two main groups of sequences, corresponding to those with homology to the toll interleukin receptor (TIR) subfamily and those with homology to the subfamily of RGAs lacking a TIR domain. Each of the subfamilies were further subdivided into several minor clades, with all clades containing examples of transcribed genes based on the TIGR database. Phylogenetic analysis using all available R gene homologous sequences as of January '02 indicates that the diversity of *Medicago truncatula* RGA sequences is highly representative of other legume RGAs. The closest homology was consistently to RGA sequences characterized from *Medicago sativa*, with many minor clades containing pairs of genes from each species (*truncatula* and *sativa*). We suggest that such gene pairs represent orthologous sequences, or sequences recently derived from an orthologous ancestor. Genetic mapping of the majority of these RGAs permits several general conclusions. First, most, but not all, of the RGAs occur in clusters, with the largest cluster containing in excess of 30 RGAs and comprising a significant portion of linkage group 6. Second, several cases of synteny were observed for the genomic position of phylogenetically conserved RGAs between *Medicago truncatula* and pea, and *Medicago truncatula* and soybean. This suggests that at least some of these RGA clusters derive from ancestral RGA clusters that predate radiation of these legume species.