Identification of novel plant defence genes by microarray profiling

Katherine Denby, Sally-Ann Walford, Maryke Carstens and Shane Murray

Department of Molecular and Cell Biology, University of Cape Town, Private Bag Rondebosch, 7700

Disease resistance in plants is controlled by a complex signal transduction network dependent on salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). The *Arabidopsis thaliana* mutant *cir1* exhibits constitutive expression of SA- and JA/ET-dependent genes and resistance to the bacterial pathogen *Pseudomonas syringae pv. tomato (Pst)* and the oomycete pathogen *Peronospora parasitica*. The *CIR1* gene appears to be a regulator of disease resistance and presumably, *cir1* plants express a range of genes required for resistance against both pathogens.

Double mutants were constructed between *cir1* and mutants that disrupt the SA *(NahG)*, JA *(jar1)* and ET *(ein2)* signalling pathways. These double mutants had differing resistance profiles: *cir1:NahG* lost resistance to both *Pst* and *P. parasitica; cir1:jar1* and *cir1:ein2* lost resistance to *Pst* but maintained resistance to *P. parasitica*. These double mutant phenotypes provide us with an avenue to identify defence genes required for bacterial resistance versus those required for resistance against the oomycete pathogen. *cir1:NahG* plants have lost essential defence genes, whereas *cir1:jar1* and *cir1:ein2* plants have lost genes required for resistance to *Pst* but not for resistance to *P. parasitica*.

Microarray profiling using Arabidopsis full-genome oligonucleotide slides has highlighted genes which are up-regulated in *cir1* compared to wild type. Reverse northern blots were used to verify the expression data. Interestingly, half of the genes up-regulated in *cir1* encode unknown proteins. We have obtained knockout lines for two of these *cir1*-upregulated genes, and RNA silencing is being used to generate plants specifically lacking each of the other putative defence genes. The two knockout lines show increased susceptibility to *Pst* confirming the role of the corresponding proteins in defence, and the value of these expression studies in identifying novel genes mediating disease resistance. Current work is investigating the function of these proteins during defence and extending the gene expression profiling to the double mutants.