MOLECULAR BIOLOGY APPROACHES TO UNDERSTANDING AND CONTROLLING FUSARIUM WILT IN COTTON

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Expression of avirulence genes from bacterial blight in transgenic cotton plants

The resistance of commercial cultivars of cotton to bacterial blight, caused by *Xanthomonas campestris* pv. *malvacearum*, is due to the interaction of resistance gene products in cotton and avirulence gene products in the pathogen. This interaction triggers a hypersensitive response at the site of infection that leads to localised cell death and hence containment of the pathogen. Associated with the localised hypersensitive response is a more generalised induced resistance that can be detected elsewhere in the plant. This induced resistance can be effective against a range of other pathogens. We are conducting experiments to determine if it is possible to utilise the interaction of bacterial avirulence genes expressed in transgenic plants already containing blight resistance genes to trigger defence responses including generalised induced resistance.

We have demonstrated, using a transient transformation assay, that bacterial avirulence genes can function in plant cells and that the products generated interact with existing resistance genes with the expected gene–for–gene specificity. We have also obtained transgenic plants that express the avirulence genes in all their cells. When the parent transgenic lines (the Coker cultivar that does not contain blight resistance genes) are crossed with cultivars that contain corresponding resistance genes, the progeny either fail to germinate, or die shortly after germinating. This demonstrates that the avirulence gene and resistance gene products present are interacting successfully to initiate an artificial hypersensitive response. Thus we have demonstrated the potential for the system to trigger plant defence responses, although we have not been able to demonstrate the subsequent activation of induced resistance. We were interested to observe that, in the absence of a resistance gene, ie: in the parent Coker background, two lines that express an avirulence gene showed some aspects of induced resistance responses. They were initially stunted, a phenomenon associated with constitutive activation of resistance in some *Arabidopsis* mutants, and showed elevated levels of chitinase, a pathogenesis-related protein, in leaves. Extracts of leaves of plants from one line had 10-fold more chitinase activity than an extracts from an untransformed control line, while another line showed a 2-fold increase in chitinase activity. We have been unable to demonstrate improved resistance to pathogens in these lines. However, it would appear that some aspects of induced defence pathways can be triggered by the expression of the avirulence gene in the absence of a known resistance gene. This may be because cotton contains homologues of blight resistance genes that are not effective in generating resistance to bacterial blight infection, but which can still interact in some way with the avirulence gene products.

The challenge now is to control the expression of the avirulence genes in transgenic plants in such a way that the deleterious effects of the hypersensitive response are minimised. We are therefore looking for pathogen-inducible promoters for use in this system. We have demonstrated that a peroxidase promoter from *Stylosanthes humulis* (provided by John Manners, CSIRO Tropical Agriculture) linked to the GUS marker gene in transgenic cotton is activated by *Alternaria macrospora* infection of leaves and by *Verticillium dahliae* infection of roots. We have also isolated cDNA and genomic clones for genes of homologues of the pathogenesis-related PR10 proteins. We have shown that expression of these genes is induced in cotton stems in response to Verticillium inoculation. Generation of constructs containing a PR10 homologue-promoter sequence linked to the GUS marker gene for evaluation in transgenic cotton is in progress.

We are also planning to investigate the use of a transposon-inactivation system for control of avirulence gene expression in transgenic cotton. In this case, the avirulence gene is inactivated by the insertion of a transposon. Thus in almost all the cells of the plant the avirulence gene will not be expressed. However, in a small number of cells, the transposon will excise from the avirulence gene which will be reactivated. The hypersensitive response will then be expressed in these cells and, hopefully, induced systemic resistance will be activated in the rest of the plant. Although this system has been shown to work in tomato (David Jones, Australian National University, unpublished results), it has not been demonstrated in cotton. Therefore initial work will aim to determine if this control mechanism is effective in cotton.

Discovery of genes involved in the interaction of cotton and Fusarium wilt by the application of microarray technology

We are about to start a new project utilising the exciting new microarray technology. This approach should allow us to identify genes that are important in the interaction of cotton and Fusarium wilt. This may allow us to identify the pathways that are important for the establishment of tolerance in less susceptible cotton cultivars. It may also be possible to utilise the interaction of more resistant native cotton species with Fusarium wilt to give insight into the genes important for the selection of resistance traits.

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