CAN BACTERIAL BLIGHT AVIRULENCE GENES BE USED AS TRIGGERS OF COTTON DEFENCE RESPONSES?

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Introduction

Fungal diseases such as *Verticillium* wilt, *Fusarium* wilt and black root rot cause appreciable losses to the Australian cotton industry, particularly in years when environmental conditions favour disease. Research in our group is focussed on genetic engineering approaches to increase the resistance of cotton to fungal attack.

When pathogens attack a resistant plant, one often sees a localised lesion of dead cells around the site of infection, resulting in containment of the pathogen and survival of the plant. This reaction, referred to as a hypersensitive response (HR), is usually accompanied by production of many factors including proteins and phytoalexins that inhibit further pathogen growth (reviewed in Greenberg, 1997). Such a response requires the presence of one or more resistance gene(s) in the plant and specific avirulence genes in the pathogen. The localised resistance response may also be accompanied by a more widespread induction of broad spectrum resistance, termed systemic acquired resistance (SAR), to protect it against further attack (reviewed in Ryals et al 1994). SAR is usually associated with increased amounts of pathogen response (PR) proteins throughout the plant and has been well characterised in plants such as tobacco and *Arabidopsis*. In contrast to the resistant reaction, susceptible plants are either unable to mount such responses or are delayed in their induction.

As a step toward engineering cotton for improved resistance against fungal pathogens, we want to improve our understanding of its ability to mount defences against any pathogen. Bacterial blight disease of cotton, caused by *Xanthomonas campestris* pv. *malvacearum* (*Xcm*), is currently well controlled in Australia by using resistant varieties. At least 16 resistance genes have been identified in cotton against bacterial blight (Brinkerhoff, 1970); cotton varieties having one or more of these react to most strains of *Xcm* with a localised hypersensive response. Several corresponding avirulence (*avr*) genes have been isolated from strains of *Xcm* (De Feyter et al, 1993) and shown to be needed in the pathogen for induction of the HR. Recently, avirulence gene products from several other plant/pathogen systems have been shown to be secreted by the pathogens into plant cells where they function to trigger the plant defence reponses (reviewed in Mudgett and Staskawicz, 1998). We have carried out studies to investigate whether the *Xcm* avirulence gene products work in the same way in cotton, and whether they might be used as triggers of cotton's defences against other pathogens, including *Verticillium* wilt.

Results

We have tested five *Xcm* avirulence genes for the ability to induce an HR after transient expression in cotton leaves. The *avr* genes were modified for expression in plant cells by joining them to the cauliflower mosaic virus 35S promoter and nopaline synthase terminator sequences. The modified genes were inserted into an binary plasmid vector so they could be transferred into cotton cells using *Agrobacterium tumefaciens*. *Agrobacterium* cultures containing these genes were infiltrated into cotton leaves in the greenhouse. Three to six days later, necrosis of cotton tissue was observed at inoculation sites when the cotton plants contained resistance gene(s) corresponding to the particular avirulence gene in *Agrobacterium*. In contrast, necrosis was not observed in plants that did not contain the appropriate resistance gene, or when the *Agrobacterium* did not have the corresponding *avr* gene. This shows that the reactions were genotype-specific; ie. dependent on matching resistance and avirulence genes. The reactions were stronger when the *avr* proteins lacked a signal peptide sequnce, resulting in retention of the proteins inside the cotton cells and showing that the proteins act intracellularly. Control reactions in which the *avr* genes could not be transferred from *Agrobacterium* into cotton showed that transfer was required for HR induction. These experiments show that the *Xcm avr* genes behave in a similar fashion to *avr* genes from other bacterial pathogens.

We have used the same gene constructs to transform cotton variety Coker. This cultivar does not contain any known resistance genes against bacterial blight. We have obtained at least six independently transformed lines containing an *avr* gene and are currently analysing progeny for gene copy number and expression. These plants have been crossed with several cotton cultivars, containing a variety of resistance genes. Preliminary results from the first set of crosses indicate that cotton tissue necrosis occurs when the appropriate *avr* gene is expressed in the presence of a corresponding resistance gene, confirming the results of the transient expression analysis. Generalised necrosis was expected as the 35S promoter used to drive expression of the *avr* genes is constitutive and occurs throughout the plant. Plants from some of these crosses will be analysed to determine which cotton defence genes are induced and whether there might be increased fungal disease resistance. Further constructs will be made where *avr* gene expression is under the control of fungal or disease inducible promoters.

Discussion

We have shown that the *Xcm* avirulence genes can be used to trigger a genotype-specific HR in cotton in the absence of *Xcm*. This means that these genes could potentially be used as triggers of cotton's defences against a broad spectrum of pathogens, including fungal pathogens such as *Verticillium* and *Fusarium*. Ideally, expression of the *avr* triggers would only occur in the presence of a pathogen (as it does in bacterial blight disease)- this will be the next challenge for us. Such a system will require tightly controlled pathogen-inducible promoters. The triggering of cotton defence responses by *avr* genes also provides us with a tool to identify pathogen induced promoters from cotton.

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