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REPORT FORMAT

REPORT TYPE:

Annual Progress Report: ☐

Final Report: ☑

Part 1 - Project Details

Project Title: (< 15 words) **Genetic manipulation of the systemic acquired resistance responses of cotton for improved tolerance to fungal pathogens.**

CRDC Project Number: **CSP 84C**

Part 2 - Contact Details

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Part 3 - Final Report

Expand to a maximum of 4 pages only

What were your major project objectives for the past year? (Please list)

1. Xcm avirulence (avr) genes as SAR triggers

   Characterise F1 progeny in terms of expression levels of SAR markers and tolerance to fungal infection using Verticillium and Alternaria

2. Promoters

   Screen fis-gus progeny for gus expression induced by fungal infection to establish the sensitivity of fis promoter to fungal induction. Establish gus expression after treatment with chemical SAR inducers e.g. BTH.

3. Screen other transformants for transgene presence.

Which of these objectives have been achieved?

1. Xcm avirulence (avr) genes as SAR triggers

Six different avirulence (*avr*) gene constructs (for 3 *avr* genes, *avrbin*, *avrb4* and *avrb102,* with and without signal peptide for apoplastic expression of the avirulence protein) were made and used to for cotton transformation. We obtained 27 transgenic cotton lines. The presence of the avirulence gene has been confirmed in 9 of these lines by using PCR to detect the DNA sequence. Progeny of these lines were crossed with resistant cultivars and the resulting phenotype observed. In two of these 9 lines, the expressed avirulence gene and the corresponding resistance gene in a blight-resistant cultivar interacted to give the expected phenotype – namely, generalised cell death soon after germination. The frequency with which we obtained transformants expressing avirulence genes is low, presumably because of deleterious effects of the avirulence gene product in tissue culture.

The results of these experiments demonstrate proof of the concept that we were testing, namely that bacterial blight avirulence genes can be expressed constitutively in transgenic cotton, and that they interact with existing resistance genes with the expected gene-for-gene specificity. When this interaction occurs, a generalised hypersensitive response is initiated. Thus the next step in the research, which is currently being undertaken in a new project, is to determine how to express avirulence genes in a controlled manner. We are planning to do this by the use of transposon tagging, so that only a few cells express the avirulence gene and initiate defence responses. We are also investigating potential pathogen-inducible promoters.

Interestingly, progeny of the two *avr*-gene-expressing transgenic cotton lines demonstrated an abnormal phenotype even when neither parent contained any resistance genes. Progeny of both *avr*-gene-expressing lines showed some degree of stunting (a characteristic of constitutive, non-lethal expression of systemic acquired resistance observed in other plants). We found elevated chitinase levels in the most stunted line and evidence of protection against the severity of watersoaking after inoculation with a virulent strain of bacterial blight. However, this was not accompanied by a reduction in the proliferation of bacteria in the stunted line. No protection against infection or severity of symptoms was observed after inoculation Verticillium wilt. Due to difficulties with establishing a glasshouse – based test for Alternaria infection, testing with Alternaria was not undertaken.

We attempted to use RT PCR to quantify transgene expression levels in these two *avr*-gene-expressing lines. Although we were able to detect an amplified product, it was not possible to distinguish between amplification of DNA (the genome) and RNA (the expressed gene). We therefore concluded that expression levels of the avr transgenes were very low. We were unable to detect any evidence for increased levels of expression of mRNA for genes usually associated with systemic acquired resistance responses such as acidic and basic chitinase, glucanase, and PAL in these lines. This is surprising, given the clear result showing increased levels of chitinase enzyme activity in the more stunted *avr*-gene-expressing line.

2. Promoters

Lines transformed with the flax fis promoter-gus construct and the Stylosanthes inducible peroxidase promoter-gus construct were generated (by Danny Llewellyn and members of his team). We have selected several independent homozygous lines for each construct and performed experiments to determine the patterns of gus expression in these plants. We found that in plants transformed with the Stylosanthes inducible peroxidase promoter-GUS construct there was increased GUS expression in response to Alternaria, Verticillium and Xanthomonas inoculation. Neither promoter is activated by methyl jasmonate or BTH (chemical inducers of different types of defence response pathways) under the conditions used. Unfortunately, we also observed background levels of gus expression in vascular and floral tissues. The work on these promoters is complete and a publication is being prepared. We have isolated several genomic and cDNA clones of genes for cotton PR10 proteins. We demonstrated that expression of these genes is increased in stems in response to Verticillium inoculation. The genomic clone should therefore yield another potential pathogen-inducible promoter. We have also obtained a promoter for the Arabidopsis GST6 gene from Karam Singh. This promoter is activated by salicylic acid and hydrogen peroxide in Arabidopsis roots. We plan to test the activity and inducibIity of this promoter in cotton. This highlights the need for a system whereby we can generate transgenic cotton roots in tissue culture without going through whole plant transformation. Hopefully, we will be able to develop a suitable system in this laboratory in the near future.

3. Screening other lines.

Although we have only tested 9 of the 27 transgenic lines obtained, we have demonstrated what we needed to demonstrate, so testing of the remaining lines will not be given a high priority in future work. Instead, we will concentrate on generating new lines where the expression of the avirulence gene is controlled.

Which objectives were not achieved and why not? (please detail any problems you have had during the year)

Other work relating to this project

We are still pursuing lines of tomatoes that have homozygous expression of all three components of David Jones' Cladosporium avr/R GEAR (genetically engineered acquired resistance) gene system. In these plants the resistance gene expression is controlled by transposon tagging so that the plants have minute necrotic lesions of an artificial hypersensitive response. These plants have been shown to have elevated systemic acquired resistance. We have established a Verticillium infection system and plan to test the transgenic plants with Verticillium to see if this system is able to give protection against Verticillium wilt.

What are your project objectives for the coming year?

Under the new project, we have commenced work on the synthesis of constructs containing a transposon to give limited expression of the avirulence gene. Avirulence gene expression should be limited to small necrotic flecks. We are anticipating that this will prime the natural defence responses of the plant so that they have improved resistance to pathogen attack.

To what extent have your research results to date been disseminated to other researchers, growers, and or industry ?

Publications arising from this project.

1998 de Feyter, R., McFadden, H. and Dennis, L.
Five avirulence genes from *Xanthomonas campestris* pv. *malvacearum* cause genotype-specific cell death when expressed transiently in cotton.
Molecular Plant Microbe Interactions 11: 698-701.

1998 de Feyter, R., McFadden, H., Llewellyn, D., and Dennis, L.
Can bacterial blight avirulence genes be used as triggers of cotton defence responses?
In: Proceedings of the 9th Australian Cotton Conference (Broadbeach, Queensland: August 12-14, 1998) pp.595-597.

2000 McFadden[1] H., de Feyter[1] R. and Llewellyn, D.
Molecular biology approaches to understanding and controlling Fusarium wilt in cotton.
In: Proceedings of the 10th Australian Cotton Conference (Brisbane, Queensland: August 16-18, 2000) pp. .

McFadden, H.G., Chapple, R.M., de Feyter, R. and Dennis E.S. Expression of pathogenesis-related genes in cotton stems in response to infection by *Verticillium dahliae*.
Physiological and Molecular Plant Pathology
submitted.

McFadden, H.G. The fis promoter from flax, *Linum usitatissimum,* and the Shpx6 peroxidase promoter from *Stylosanthes humulis* drive increased levels of expression of the *gus* gene in transgenic cotton plants treated with pathogens.
Physiological and Molecular Plant Pathology
In preparation.

Will your research results be useful to other researchers, growers, and or industry in the next year, and if so how do you intend to communicate these results or findings?

The generation of transgenic lines of cotton with constitutive expression of avirulence genes is not of immediate benefit to growers. We still need to find a mechanism for the control of this system for switching on plant defence responses, and test if this does give increased host tolerance to the important cotton pathogens. We plan to continue work towards this goal.