Cotton
RESEARCH & DEVELOPMENT

Annual, Progress and Final Reports

REPORTS

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number: **CSP115C**

Annual Report: ☐ Due 30-September

Progress Report: ☐ Due 31-January

Final Report: ☒ Due 30-September (or within 3 months of completion of project)

Project Title: Targeted expression of genes for manipulation of the systemic acquired resistance responses of cotton for improved tolerance to fungal pathogens.

Project Commencement Date: July 2000 **Project Completion Date:** July 2003

Research Program: 5 Breeding and Biotechnology

Part 2 – Contact Details

Administrator: Graham Brill
Organisation: CSIRO Plant Industry
Postal Address: GPO Box 1600 Canberra ACT 2601
Ph: (02) 6246 5431 **Fax:** (02) 6246 5000 **E-mail:** Graham.Brill@CSIRO.au

Principal Researcher: Helen McFadden
Organisation: CSIRO Plant Industry
Postal Address: GPO Box 1600 Canberra ACT 2601
Ph: (02) 6246 5377 **Fax:** (02) 6246 5000 **E-mail:** Helen.McFadden@csiro.au

Supervisor: Dr Tony Pryor / Dr Jeff Ellis
Organisation: CSIRO Plant Industry
Postal Address: GPO Box 1600 Canberra ACT 2601
Ph: (02) 6246 5421 **Fax:** (02) 6246 5000 **E-mail:** Jeff.Ellis@CSIRO.au

Researcher 2 (Name & position of additional researcher or supervisor).
Organisation: CSIRO Plant Industry
Postal Address: GPO Box 1600 Canberra ACT 2601
Ph: **Fax:** (02) 6246 5000 **E-mail:** name.name@csiro.au

Signature of Research Provider Representative: ______________________________

Part 3.3 – Final Reports

(The points below are to be used as a guideline when completing your final report. Postgraduates please note the instructions outlined at the end of this Section.)

1. Outline the background to the project.

The overall aim of this project was to develop systems for targeting transgene expression in plants to enhance their defence responses.

We were investigating two methods for the regulation of the expression of transgenes in transgenic cotton. One method involved limiting gene function by insertion of the transposable element Ac. Transposition of the Ac element (where the Ac element "jumps out" of the gene) can cause localised re-activation of the target gene. This could be used to give small areas of localised gene expression in instances where constitutive expression of the gene in all cells would have a detrimental effect. This could be useful in the case of avirulence genes, for example. The use of transposable elements has other potential uses for gene tagging and we wished to demonstrate its feasibility in cotton. The second method involved an investigation of the behaviour of some potentially pathogen-inducible promoters as drivers of transgene expression. In this study our focus was on the PR10 gene family. Members of this gene family are highly induced by Fusarium infection of cotton – in fact they constitute a significant component of cotton's responses to Fusarium.

2. List the project objectives and the extent to which these have been achieved.

1. Determine the number of members of the PR10 gene family in cotton and determine the origin (A or D genome) of these family members.

2. Determine if there are any particular members of the PR10 family that are induced in cotton tissue specifically in response to Fusarium infection.

3. Assess the performance of potential pathogen-inducible promoters using promoter:GUS constructs in transgenic cotton. (eg. PR1:GUS, pGST:GUS and PR10:GUS).

4. Assess the behaviour of the transposable element Ac in transgenic cotton (by looking for expression of the GUS reporter gene where Ac has excised).

3. Detail the methodology and justify the methodology used.

1. The number of members of the PR10 gene family in cotton and the origin (A or D genome) of these family members was determined using Southern analysis of genomic DNA from *G. hirsutum* and native A and D genome cottons. This is a standard methodology for examination of gene copy number. In order to demonstrate that the complex banding pattern observed was not due to incomplete restriction digestion of the genomic DNA, a control hybridisation with a probe corresponding to a gene known to be present as a single copy was performed and a single band observed.

2. In order to determine if there are any particular members of the PR10 family that are induced in cotton tissue in response to Fusarium infection, we used nested PCR and real-time reverse transcription PCR with primers designed to specific PR10 genes. We also obtained information on PR10 gene expression patterns from microarray analysis of gene expression in cotton plants challenged with Fusarium.

3. In order to assess the performance of potential pathogen-inducible promoters we generated transgenic cotton transformed with various promoter:GUS constructs. (eg. PR1:GUS, pGST:GUS and PR10:GUS).

4. We assessed the behaviour of the transposable element Ac in transgenic cotton tissue by generating transgenic cotton transformed with two S4:promoter:Ac:GUS constructs, with the Ac in inverse orientations. We then looked for the expression of the GUS reporter gene that occurs where Ac has excised.

4. Detail and discuss the results including the statistical analysis of results.

1. Southern analysis of commercial cotton (A and D genome) and species with A or D genomes revealed the presence of multiple copies of PR10 genes in all species tested, with over 20 bands present in commercial cotton cultivars. Thus PR10 genes comprise a large, multigene family. No relationship between the bands present in the diploid A and D genomes and the commercial tetraploid genome was immediately evident from the patterns of bands in the various samples. No polymorphism was detected between the moderately Fusarium-resistant DeltaEMERALD and the Fusarium-susceptible Siokra 1-4. In addition, microarray analysis of expression levels of PR10 genes in Siokra 1-4 and DeltaEMERALD after Fusarium inoculation also showed no differences in the intensity, timing or identity of PR10 genes in the two cultivars after Fusarium challenge. Thus the difference in susceptibility between these two cultivars is unlikely to be due to differences in the number of copies of PR10 gene family members or differential expression of particular family members.

2. Using PR10 cDNA sequences derived from PR10 clones identified by library screening and from the microarray experiments, PCR primers specific to a selected PR10 cDNA clone (corresponding to the genomic clone from which the PR10 promoter used in subsequent experiments was derived) were designed. Induction of this PR10 gene (and possibly closely related homologues) in Fusarium-infected tissue was confirmed. It was possible to distinguish between a ribonuclease-like PR10 and other PR10 genes using specific primers in real-time reverse transcription PCR reactions. The most powerful tool used to investigate the expression of PR10 genes was microarray analysis of gene expression. In this analysis, increased expression of the ribonuclease-like PR10 in Fusarium-infected hypocotyl tissue was observed later than expression of other PR10 genes. Therefore the ribonuclease-like PR10 promoter is likely to be less suitable for driving pathogen-inducible defence responses than other PR10 gene promoters.

3. Transformation of cotton with pGST6-GUS and PR1-GUS has given 12 and 4-6 T0 plants, respectively. T1 PR1-GUS transformed cotton plants have been shown to be transgenic (NPTII) and some have shown GUS expression, demonstrating that the gene constructs are probably functional. Homozygous lines were identified for experiments with Fusarium. Transgenic plants were assessed and although GUS expression was detected, there was substantial expression in the absence of fungal infection. Thus no promoter suitable for driving pathogen-inducible gene expression in cotton roots was identified.

Cotton transformation with a PR10:GUS construct was initiated several times and putative primary transformants obtained. Other plant species were also transformed with this construct (Arabidopsis and flax) but experiments with these plants and with transformed cotton callus did not yielded any evidence of GUS expression. The sequence of the construct was checked. Lack of GUS expression in transformed plants may have

been either because the native promoter is not functional or because of an unknown flaw in the construct.

4. Cotton was transformed with two 35S-promoter:Ac:GUS constructs containing Ac in inverse orientations. Putative primary transformants were obtained. GUS expression was assessed in transformed callus, and was observed for one orientation of Ac but not for the inverse orientation. This we have preliminary evidence that the transposable element Ac is able to function in cotton tissue.

5. Provide a conclusion as to research outcomes compared with objectives. What are the "take home messages"?

The PR10 gene family in commercial cotton and in representative A and D genome cottons is large and complex. Expression of many members of the gene family is induced in response to Fusarium infection. Induction of PR10 gene expression is a major component of cotton's responses to Fusarium infection, but is not associated with the differences in Fusarium susceptibility of cultivars Siokra 1-4 and DeltaEMERALD.

There did not appear to be any particular members of the PR10 family that are induced in cotton tissue specifically in response to Fusarium infection. However, ribonuclease-like PR10 gene(s) are induced later than other PR10 genes.

We did not identify a potentially useful pathogen-inducible promoter among those tested (PR1:GUS, pGST:GUS and PR10:GUS). However, induction of many genes was identified in the microarray analysis of gene expression (CSP114C). Further investigation of these may identify potentially useful pathogen-inducible promoters.

We have obtained preliminary evidence that the transposable element Ac is able to function in cotton tissue. This could provide the basis for further work in cotton, particularly in diploid cottons, where generation of tagged loss of function mutants could be useful.

6. Detail how your research has addressed the Corporation's three Outputs - Economic, Environmental and Social?

25/9/2003 4:40 PM
Deleted:

25/9/2003 4:40 PM
Formatted: Indent: Left: 0 cm, Hanging: 0.63 cm

In this project, our goal was to develop technology to assist with finding solutions to the Fusarium wilt problem. Such solutions would contribute to the continued economic viability of the cotton industry.

7. Provide a summary of the project ensuring the following areas are addressed:

25/9/2003 4:40 PM
Deleted:

25/9/2003 4:40 PM
Formatted: Indent: Left: 0 cm, Hanging: 0.75 cm

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.)

b) other information developed from research (eg discoveries in methodology, equipment design, etc.)

c) are changes to the Intellectual Property register required?

PR10 genes were identified as a major component of cotton's responses to Fusarium wilt infection, but as association of timing, intensity or identity of PR10 genes with improved resistance to Fusarium wilt (observed in DeltaEMERALD) was not observed, we have no evidence that increased expression of PR10 genes contributes to resistance.

Potentially pathogen-inducible promoters were assessed but no useful pathogen-specific inducibility identified in transformed cotton tissue. Induction of many other genes in

response to infection was observed in microarray analysis of gene expression. Further analysis of these may yield potentially useful pathogen-inducible promoters.

We have some evidence that the transposable element Ac is able to function in cotton tissue. This could provide the basis for further work in cotton, particularly in diploid cottons, where generation of tagged loss of function mutants could be useful.

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

(b) for the future presentation and dissemination of the project outcomes.

(c) for future research.

Further analysis of genes identified by microarray analysis of gene expression these may yield other potentially useful pathogen-inducible promoters. **9. List the publications arising from the research project and/or a publication plan.**

A paper including the real time reverse transcription PCR analysis of PR10 gene expression studies has been submitted for publication.

“Gene Expression Profile Changes in Cotton Root and Hypocotyl Tissues in Response to Infection with Fusarium oxysporum f. sp. vasinfectum”

by Caitriona Dowd, Iain W. Wilson and Helen McFadden

submitted to Molecular Plant Microbe Interactions.

10. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry or the Australian community.

In this project, our goal was to develop technology to assist with finding solutions to the Fusarium wilt problem. Such solutions would have contributed to the continued economic viability of the cotton industry. However, this project did not identify any technology that is applicable to the development of improved resistance to Fusarium wilt.

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

The PR10 gene family in commercial cotton and in representative A and D genome cottons is large and complex. Expression of many members of the gene family is induced in response to Fusarium infection. Induction of PR10 gene expression is a major component of cotton’s responses to Fusarium infection, but is not associated with the differences in Fusarium susceptibility of cultivars Siokra 1-4 and DeltaEMERALD.

25/9/2003 4:41 PM
Formatted: Indent: Left: 0 cm, Hanging: 0.75 cm

25/9/2003 4:41 PM
Formatted: Indent: Left: 0 cm, Hanging: 0.75 cm

25/9/2003 4:41 PM
Deleted: ... [1]

25/9/2003 4:42 PM
Deleted:

25/9/2003 4:42 PM
Formatted: Indent: Left: 0 cm, Hanging: 0.75 cm

There did not appear to be any particular members of the PR10 family that are induced in cotton tissue specifically in response to Fusarium infection. However, ribonuclease-like PR10 gene(s) are induced later than other PR10 genes.

We did not identify a potentially useful pathogen-inducible promoter among those tested (PR1:GUS, pGST:GUS and PR10:GUS). However, induction of many genes was identified in the microarray analysis of gene expression (CSP114C). Further investigation of these may identify potentially useful pathogen-inducible promoters.

We have obtained preliminary evidence that the transposable element Ac is able to function in cotton tissue. This could provide the basis for further work in cotton, particularly in diploid cottons, where generation of tagged loss of function mutants could be useful.