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: **CSP148C**

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: Pilot studies to determine the feasibility of potential new directions for work with Fusarium oxysporum f.sp. vasinfectum

Project Commencement Date: 1/7/2002 **Project Completion Date:** 30/6/2003

Research Program: 5 Breeding and Biotechnology

Part 2 – Contact Details

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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.

Fov is the causal organism of Fusarium wilt disease in cotton. This disease is of serious concern to the cotton industry in terms of potential yield loss.

Existing CRDC projects were in progress to identify genes associated with cotton's response to Fov infection (CSP114C) including those genes associated with the expression of moderate resistance. Once potential genes have been identified as being associated with resistance responses, it is necessary to confirm that these genes are playing an active role in the expression of this resistance. One approach that may be used to verify the role of any particular gene in resistance is to generate transgenic plants in which the expression of this gene has been altered and to look for a change in disease resistance. This is not feasible in cotton but would be feasible in the model plant *Arabidopsis thaliana*, where suitable genomics tools (e.g. rapid and simple transformation, complete genome sequence, diploid plant) are available. One of the aims of this project was therefore to establish a model *Arabidopsis/Fusarium oxysporum* system to complement studies with cotton and Fov. We wished to know if the importance of genes detected in the cotton microarray work (CSP114C) could be assessed rapidly in *Arabidopsis* and if it would have been feasible to perform gene discovery work in the system for application to the cotton system. In order to achieve this we needed to set up and develop expertise in the *Arabidopsis/Fusarium* infection system.

The second part of the pilot project dealt with developing the tools required to undertake a study of the molecular basis of pathogenicity of Fov. This may have lead in the long term to the development of novel disease resistance strategies. In order to undertake molecular analysis of the pathogen, a transformation system for Fov is required. We wished to apply an Agrobacterium-based transformation method developed for *Fusarium oxysporum* pathogens of *Arabidopsis* to the cotton pathogen Fov. Initially we planned to introduce reporter genes into the fungus to generate transgenic strains with immediate practical use in studying Fov infection dynamics in experimental systems and in the glasshouse.

It was hoped that development of these technologies would form the basis for a further project aimed at verification of the role of genes already discovered, the discovery of novel plant genes and the elucidation of pathogenesis mechanisms in Fov.

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

1. To develop transformation procedures for Fov and generate strains expressing a marker protein such as green fluorescent protein (GFP) or beta-glucuronidase (GUS).

Successful transformation procedures were developed and applied. GUS-expressing Fov strains were generated. Despite several attempts using several different constructs, we were unable to generate detectable GFP expression in transgenic Fov. Further work with Fusarium transformation also demonstrated the feasibility of techniques for disrupting gene function. Loss of pathogenicity as a result of gene disruption was demonstrated. This technique would have provided the basis for systematic mutagenesis of the pathogen to generate non-

pathogenic mutants. Characterisation of the mutants was anticipated to provide information regarding the genes required during Fov infection.

2. To establish a procedure for the infection of *Arabidopsis thaliana* with a strain of *Fusarium oxysporum,* and a method for the assessment of disease severity.

The required strains of *Arabidopsis* and Fusarium were imported and a quarantine facility for their use established. We were successful in setting up a protocol for *Arabidopsis* infection and implemented a system for the assessment of disease severity.

3. Detail the methodology and justify the methodology used.

The main portion of the financial support given in this project funded a visit to our laboratory by Dr Andrew Diener from Massachusetts General Hospital. Dr Diener has extensive experience with *Arabidopsis* and its Fusarium pathogens. He provided the protocols that we used and assisted us with establishment of this technology. The fact that we were able to achieve all we hoped with his assistance demonstrates the effectiveness of this strategy for technology transfer.

Fusarium transformation was achieved using an Agrobacterium-based protocol. This was effective and simple. *Arabidopsis* infection was achieved using drenching of seedlings grown on peat pellets. Although large quantities of inoculum were required, this protocol gave reproducible infection and was preferable to any technique requiring transplantation of the very small *Arabidopsis* plants.

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

The primary goal of this project was to set up mechanisms and protocols and to demonstrate that we had the capacity to undertake a more extensive project in this area. We generated GUS-expressing Fov lines that were shown by Southern analysis to be transgenic. These GUS-expressing Fov were shown to be as pathogenic as the non-transformed parents in glasshouse assays. We cloned an Fov homolgue of a gene for MAP kinase, a gene that is required for pathogenicity in *Fusarium oxysporum lycopersici* (tomato pathogen). Sequencing showed a high degree of homology between MAP kinase genes from the cotton, *Arabidopsis* and tomato pathogens. We obtained a construct for "knockout" of the MAP kinase gene from Dr Diener and used it to generate Fov mutant strains with a defective MAP kinase gene. Disruption of the gene was demonstrated by Southern analysis and serious reduction (but interestingly, not complete loss) of pathogenicity was demonstrated in glasshouse assays.

Fusarium infection of several *Arabidopsis* ecotypes was achieved and a moderately resistant and a susceptible ecotype identified.

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

The main conclusion from the work achieved was that we had the capacity to develop novel research based on the techniques that we set out to establish. We demonstrated the potential of the techniques to form the basis for further research. Unfortunately this will not be undertaken due to funding restrictions as a consequence of the drought.

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

The research here was not sufficiently advanced to make a contribution to output. However, our goal was to develop the groundwork for further studies aimed at 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:

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?**

The main thrust of this project was to achieve establishment and application of known methodologies, thus the main output was to achieve local technical advances.

The aims of the project were to develop transformation procedures for Fov and generate strains expressing a marker protein such as green fluorescent protein (GFP) or beta-glucuronidase (GUS) and to establish a procedure for the infection of *Arabidopsis thaliana* with a strain of *Fusarium oxysporum*.

Successful transformation procedures were developed and applied. GUS-expressing Fov strains were generated. Further work with Fusarium transformation also demonstrated the feasibility of techniques for disrupting gene function. Loss of pathogenicity as a result of gene disruption was demonstrated. This technique would have provided the basis for systematic mutagenesis of the pathogen to generate non-pathogenic mutants. Characterisation of the mutants was anticipated to provide information regarding the genes required during Fov infection.

The required strains of *Arabidopsis* and Fusarium were imported and a quarantine facility for their use established. We were successful in setting up a protocol for *Arabidopsis* infection and implemented a system for the assessment of disease severity.

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.

We submitted a project proposal that encompassed the use of the *Arabidopsis*/Fusarium model system for the evaluation of the effectiveness of potential antifungal genes for later work in cotton. We identified a potential target gene (to be provided by QDPI/CSIRO Plant Industry Queensland) that coded for a protein that had been shown in *in vitro* studies (in our laboratory and elsewhere) to be effective against Fov. We also planned to generate transgenic *Arabidopsis* plants with altered expression of some of the genes identified in other projects (CSP114C). We proposed to use Fusarium transformation technology to generate a library of mutants with disrupted genes, some of which would have had reduced pathogenicity. Subsequent analysis of these mutants could have identified factors required for Fov infection. Knowledge of the factors contributing to Fov's effectiveness as a pathogen could inform the

development of novel disease control strategies. As part of the proposal, we planned to investigate the potential for gene silencing in Fov using RNAi technology. There is a slight possibility that this strategy could be employed for the development of plants that can deliver dsRNA and disrupt the function of infecting fungal cells.

9. List the publications arising from the research project and/or a publication plan.

This research was not sufficiently advanced to yield any publications. The work undertaken was aimed at demonstrating the feasibility of certain approaches, and was not sufficiently novel to be publishable.

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.

Unfortunately, in the absence of further work, this research is likely to have minimal impact of the cotton industry.

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.

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This project was successful and all aims were achieved. Successful transformation procedures were developed and applied. GUS-expressing Fov strains were generated. Further work with Fusarium transformation also demonstrated the feasibility of techniques for disrupting gene function. Loss of pathogenicity as a result of gene disruption was demonstrated. This technique would have provided the basis for systematic mutagenesis of the pathogen to generate non-pathogenic mutants. Characterisation of the mutants was anticipated to provide information regarding the genes required during Fov infection.