If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October. If not, please provide a written report by 30 September.

Part 1 - Summary Details

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

CRDC Project Number:

Project Title: ????

Project Commencement Date: **Project Completion Date:**

CRDC Program: - Please Select One -

Part 2 – Contact Details

Administrator: (Name & position of officer responsible for all correspondence).
Organisation: (Organisation administering the research project).
Postal Address:
Ph: **Fax:** **E-mail:**

Principal Researcher: Adrianne Machado
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Supervisor: (Name & position of senior scientist overseeing the project).
Organisation:
Postal Address:
Ph: **Fax:** **E-mail:**

Signature of Research Provider Representative: _____

Part 3 – Final Report Guide (due 31 October 2008)

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

Objectives

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

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

Outcomes

The data presented in this thesis describes the isolation and functional characterization of the cotton transcription factor GhMYB25 and demonstrates a role in cotton fibre and seed development. This gene was identified on microarray experiments as differentially expressed in ovules of two different fibreless mutants compared to wild-type. Expression analysis of Myb25 revealed that this gene is expressed in fibre initials of 0dpa ovules of DP-16. No expression of Myb25 could be detected in ovules of the mutants using RT-PCR. However a RT-PCR gel blot showed a very low expression of Myb25 in -2, 0 and 2dpa ovules of both mutants compared to the wild-type DP-16. A RT-PCR reaction using RNA from the laser captured cells revealed that Myb25 is 2 fold more expressed in the fibre initials than in epidermal cells of 0dpa ovules. The expression of Myb25 peaks at 0dpa coinciding with the fibre initiation period. The expression studies reinforced the suggestion of a role of Myb25 in cotton fibre development.

The full length Myb25 cDNA clone was isolated and cloned, and enable the promotion of an over-expression construct for the further characterization of this gene. The cDNA showed 98% homology at nucleic acid level to the GhMyb25 sequence already in the database (Genbank: AF336283) but with an unspliced intron that was not present in our clone. The Myb25 sequence belongs to a family of transcription factors with a DNA-binding domain (MYB domain) that is conserved amongst plants, yeast and animals. The Myb25 domain consists of two imperfect repeats (R2 and R3) with sequence similarity over their DNA-binding domain with other Mybs involved in plant cell shape regulation.

Myb25 is 96% identical to the *G. arboreum* fibre EST (BE054276), suggesting that Myb25 may be from the A-genome present in the tetraploid. The expression pattern study presented in Chapter 2 confirms the microarray analysis reinforcing the suggestion that Myb25 is a good candidate gene for regulating cotton fibre development. Furthermore *in situ* hybridization analysis showed a localized expression of Myb25 in the fibre initials of 0dpa DP-16 ovules sections embedded in paraffin.

The functional characterization started with the over-expression of the Myb25 in the heterologous systems, Arabidopsis and tobacco. In Arabidopsis, the over-expression of Myb25 did not alter the trichome phenotype of the transgenic lines. These results were consistent with other studies as the phylogenetic sequence analysis showed that Myb25 is more similar to AmMIXTA than to Arabidopsis GLABRA 1. AmMIXTA was reported to increase trichome density and promote the formation of novel conical cells on leaves of transgenic tobacco but not in Arabidopsis (Glover *et al*., 1998, Payne *et al*., 1999). Like the plants over-expressing MIXTA the Myb25 over-expression construct has affected the trichome development in tobacco producing an increase in leaf trichome density and branching of the transgenic lines. However, the phenotype of the transgenic tobacco plants over-expressing Myb25 differed from MIXTA as it did not promote the formation of novel conical cells on leaves. This work has contributed towards a manuscript (Expression Profiling Genes Expressed Early During Lint Fibre Initiation in Cotton) that been submitted to Plant Cell and Physiology which is currently undergoing review. The results presented in Chapter 3 indicate that cotton and tobacco trichomes may share similar developmental programs. Dang *et al*., 1996 have previously shown that cotton fibre-specific promoters have directed the transcription of reporter genes in trichomes of tobacco suggesting that the two types of trichome share some ontogenetic similarities. Besides the common N-terminal motif, homology between Myb25 and MIXTA outside of the Myb binding domain is shown to an identical positioned GIDPVTH amino acid sequence suggesting a role for this motif in cell shape regulation in tobacco.

Further characterization studies were carried out by over expressing and silencing the Myb25 gene in transgenic cotton. The Myb25 RNAi transformants showed several phenotypes, fibre production was severely affected in some primary transformants which had developed distorted fruits with several seeds aborted. SEM images from young cotton ovules revealed that the suppression of Myb25 have affected the fibre at early developmental stage. The reduced fibre initials of the transgenic ovules showed an unsynchronized and delayed growth compared to the uniform and advanced fibre development of the wild-type. Myb25 appears also to be crucial for a normal embryogenesis as the seeds were aborted in the primary transformants. Myb25 expression was detected in 20dpa embryos indicating that this gene may play a role in the embryo development.

The reduced fibre phenotype of the Myb25 suppression lines may indicate segregation of the transgene on the seed coat repressing only the fibre development without any impact on seed development. On other hand the shrunken aborted seeds seem to be derived from the suppression of the Myb25 in developing embryo.

Another important observation in this study is the impact of Myb25 suppression on trichome development of leaves and stem. The severe fibre reduction phenotype correlates with a repression in trichome development of leaves and stem. It is well known that different trichome types may occur on the same plant with type and spacing determined by the plant

organ from which the trichome extends, and the developmental phase of the plant at the time of organ initiation (Payne et al., 1999). Although the cotton leaf and seed trichomes are morphologically distinct structures presumably the Myb25 acts regulating the activity of a common gene controlling the initiation of both types of trichome.

In Arabidopsis both GL1 and TTG 1 are necessary for normal trichome initiation (Marks and Feldmann, 1989). Mutations at either of two loci, TTG 1 and GL1, result in the virtual absence of leaf trichomes from Arabidopsis. In addition the homozygous mutants failed to produce anthocyanin pigments and seed coat mucilage (Koorneef, 1981). In cotton the molecular basis of trichome differentiation remains unclear. Herein is shown that Myb25 suppression disturbs the trichome development of cotton leaves and petiole as well as embryogenesis and fibre development.

A Myb25 promoter-GUS fusion construct will be useful to verify if the Myb25 promoter can direct the specific expression of the GUS reporter gene in trichomes. The expression of Myb25 under strong fibre specific promoters could help to improve the fibre traits

The work presented in this thesis represents a characterization of the cotton transcription factor GhMyb25. The analysis of cotton genes through a study of fibreless mutant versus wild-type using microarray selected a candidate gene for a role in cotton fibre development. Functional characterization of GhMyb25 revealed that this gene play an important role in fibre and seed development. It also provides a starting point for future research using biotechnological approaches to enhance cotton fibre quality.

Conclusion

5. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

Extension Opportunities

6. 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.

8. A. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

   B. Have you developed any online resources and what is the website address?

Part 4 – Final Report Executive Summary

Despite the long history of cotton production and research, little is known about the genes that control the initiation and growth of the cells on the outer surface of the seed that become fibres. The work described in this thesis uses cDNA microarray technology to study global gene expression differences between normal linted cotton and two lintless mutants to uncover the genetic control of lint production. By comparing differences in gene expression between

the mutants and the wild-type, a small number of genes that might be important for fibre development were identified, including a transcription factor GhMyb25, which was assumed to be the best candidate for a master fibre control gene. During normal lint production this gene is predominantly expressed in the cells on the surface of the seed at the same time that fibre cells start to grow, but also in the trichomes on the leaf and stem of developing plants.

Over-expression of GhMYB25 in transgenic tobacco led to an increase in the number and branching of multicellular trichomes on the adaxial leaf surface. By contrast, ectopic expression of GhMyb25 in Arabidopsis had no effect in trichome development suggesting that Arabidopsis and tobacco trichomes are regulated differently.

Over-expression and silencing of Myb25 in transgenic cotton was also performed to validate its role in fibre development. Analysis of primary transformants revealed that the RNA interference (RNAi) of GhMyb25 caused a significant reduction in fibre development, as well as, the production of distorted fruits with aborted seeds. Apparently the suppression of GhMyb25 in the embryo has inhibited the development of the seed entirely. Trichome development on the petiole and leaves of the transgenic lines was also affected by the suppression of the GhMyb25. The inhibition of the trichome development correlated inversely with the over-expression phenotype, where an increase in trichome production on leaves and stems was observed from a preliminary analysis of a number of primary transformants.

The outcomes of this project will provide a better understanding of the genes controlling fibre development and should enable genetic engineers to design new strategies using biotechnological approaches to genetically improve cotton fibre yield and quality.