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: **UA17**

Project Title: Analysis & optimisation of cotton fibre specific gene promoters

Project Commencement Date: 1/7/06 **Project Completion Date:** 30/9/09

CRDC Program: /CRDC/5. Breeding & Biotechnology

Part 2 – Contact Details

Administrator: Chelsea DuBois, Research Grants Officer
Organisation: Adelaide Research and Innovation – Research Branch
The University of Adelaide
Postal Address: Adelaide SA 5005
Ph: (08) 8303 3716 **Fax:** (08) 8303 3700 **E-mail:** chelsea.dubois@adelaide.edu.au

Principal Researcher: Dr Yinghong Liu
Organisation: School of Molecular and Biomedical Science
The University of Adelaide
Postal Address: Adelaide SA 5005
Ph: (08) 8303 3013 **Fax:** (08) 8303 4362 **E-mail:** yinghong.liu@adelaide.edu.au

Supervisor: Prof Jeremy Timmis
Organisation: School of Molecular and Biomedical Science
The University of Adelaide
Postal Address: Adelaide SA 5005
Ph: (08) 8303 4661 **Fax:** (08) 8303 4362 **E-mail:** jeremy.timmis@adelaide.edu.au

Supervisor 2 Dr Sharon Orford
Organisation: School of Molecular and Biomedical Science
The University of Adelaide
Postal Address: Adelaide SA 5005
Ph: (08) 8303 7540 **Fax:** (08) 8303 4362 **E-mail:** sharon.orford@adelaide.edu.au

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.

Our laboratory has been funded by the CRDC since 1992 to conduct research into the genetic engineering of cotton fibre traits. Using biotechnology to alter fibre traits will lower the cost and time required for producing improved cotton varieties and strengthen the international reputation of the Australian industry as a producer of high quality cotton.

Genetic engineering of cotton fibre morphology requires both useful genes and appropriate expression of the genes in cotton fibres. Our research aimed to address both these requisites, concentrating on genes which are expressed in fibres but not in other parts of the cotton plant. This research resulted in the identification of an expansin gene which is expressed only in cotton fibres. Expansins are proteins which are believed to control plant cell growth by chemical modification of cell wall components, and could therefore play a key role in determination of fibre quality and yield.

Secondly, we identified six different control regions (promoters) within the cotton genome which directly control the fibre-specificity and timing of expression of genes. Fibre-specific promoters allow the expression of any particular transgene to be targeted to the fibres only, avoiding any detrimental effects of expression on growth and morphology elsewhere within the plant. Each of the six promoters was fused to a reporter gene, GUS, and the constructs used to transform whole cotton plants, generating a total of 54 transgenic lines. Our suite of promoters was expected to drive gene expression at different times during fibre development and preliminary GUS assays carried out for one of the expansin promoter lines showed strong and very specific expression of the reporter gene during elongation of cotton fibres.

Our two areas of research came together when we used three of the fibre-specific promoters to disrupt native production of expansin in the cotton fibre. Four gene constructs were made, designed to increase, reduce or prolong expansin gene expression during fibre development, and 42 different transgenic cotton lines were recovered.

This project was primarily concerned with the evaluation of the two sets of transgenic plants: those testing the fibre-specificity of our isolated promoters, and those testing the effects of changing expansin gene expression in developing cotton fibres. In this way project UA17 was to be the culmination of 17 years of CRDC-funded research in our laboratory.

Objectives

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

Objective 1: Analysing fibre quality of cotton expansin transgenics

100% completed.

Transgenic lines derived from the four expansin transgenic cotton experiments were screened for homozygosity by PCR and 20 of the pure-breeding lines, including a non-transgenic line, were evaluated at ACRI in Narrabri for novel fibre properties.

Measurement of fibre characteristics was carried out using an HVI machine. The data was received in 2007 and includes length, uniformity, short fibre index, strength, elongation and micronaire. Statistical analysis of the data was carried out, and lines with significantly different fibre characters from those of untransformed Coker 315 were identified.

Objective 2: Determination of expansin gene expression profiles in transgenic cottons

100% completed.
In order to show that expansin gene expression had been altered in our transgenic plants, and to perhaps correlate any such changes with altered fibre characters, we used quantitative PCR (Real-Time PCR) to determine expression profiles of the expansin gene family in transgenic lines. As a priority we analysed those lines with statistically significant change(s) in fibre characteristics, and 11 lines were studied in detail.

Objective 3: Electron microscopic visualisation of fibre development of transgenic cottons

100% completed.
Early fibre development in five transgenic lines was compared with that in non-transformed cotton via Scanning Electron Microscopy to determine whether a change in expansin gene expression perturbed fibre initiation.

Objective 4: Discussion of a strategy for the development of molecular markers for use in cotton breeding programs

40% completed.
Our research has resulted in a large amount of information about the expansin gene family in cotton, which could be used to track cotton fibre traits in breeding programs. Discussions were held between ourselves and Dallas Gibb in October and December 2006, regarding the development of molecular markers for cotton fibre traits. It was decided that an integrated approach with CSIRO would be best, as per feedback from the CRDC Review of Fibre Quality Projects (February 2006) but a meeting between the three parties did not eventuate.

Objective 5: Analysis of transgenic cottons in which a fibre-specific gene promoter is controlling GUS

100% completed.
A clear step in the analysis of tissue-specific gene promoters is to test those promoters in whole plants. To do this we made gene constructs in which each of the six promoters was fused to a reporter gene, GUS, and the constructs used to transform cotton plants. A number of independent lines were obtained for each experiment. Lines from four of these experiments were examined in detail. Specifically, we determined the strength and temporal control of the fibre-specific promoters *via* histochemical staining and a semi-quantitative MUG assay for GUS activity.

Methods

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

The main part of this project was to analyse the gene expression and/or fibre characteristics of eight different transgenic cotton transformation experiments, four in which the expansin gene is being misexpressed in cotton fibre cells, and four in which a fibre-specific gene promoter is controlling expression of a reporter gene. Due to position effects (differences in gene expression which result from the genomic location of the transgene), it is normally necessary to analyse 15-20 independent lines from each transformation experiment. The scope of this project allowed analysis of 4-5 lines per experiment and ideally, homozygous or pure-breeding lines should be studied. In general, well-established molecular and assay techniques were to be used, together with typical methods for the analysis of transgenic plants. Specifically:

3.1. Analysing fibre quality of cotton expansin transgenics

- Screen for presence of the transgene in cotton plants by polymerase chain reaction (PCR)
- Confirm homozygousity and copy number of transgene by Southern hybridisation
- Identify homozygous transgenic lines by PCR analysis of 12-15 seedlings (if all are positive for the transgene then the line is deemed to be homozygous (with 95% probability).
- Send homozygous transgenic seeds to ACRI Narrabri to test for modifications in fibre morphology and quality, as measured by HVI.

3.2. Determination of expansin gene expression profiles in transgenic cottons

- Determine expansin gene expression profiles by Real-Time PCR, including overall expansin gene expression (multiple gene family members) as well as expression of the transgene only.

3.3. Electron microscopic visualisation of fibre development of transgenic cottons

- Cotton tissues (ovules aged 0-2 DPA) were collected fresh and prepared for microscopic visualisation using established protocols of the Adelaide Microscopy Centre.

3.4. Discussion of a strategy for the development of molecular markers for use in cotton breeding programs

- Screen available breeding material for differences in expression of fibre-specific genes, including expansin
- Develop potential markers by screening available breeding material for polymorphisms in the gene

3.5. Analysis of the transgenic cottons in which a fibre-specific gene promoter is controlling expression of a reporter gene, GUS.

- Screen for presence of transgene by PCR analysis and grow those that are containing the transgene for GUS expression tests.
- Histochemical staining of GUS activity using X-Gluc as a substrate.
- Semi-quantitative assay of GUS activity using 4-MUG as a substrate and measurement of enzymatic conversion using a fluorometer.

Results

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

Objective 1: Analysing fibre quality of cotton expansin transgenics

We have conducted a series of transgenic experiments which aim to perturb the expression of the expansin gene in cotton fibres. There are four experiments in which the expansin gene is being altered. The experiments are T334 (antisense expansin to decrease expression), T338 (increased expansin expression), T337 (prolonged expansin expression) and T336 (prolonged expansin expression). We completed homozygousity screening by PCR and obtained four to five pure-breeding lines per transgenic experiment, plus one null (non-transgenic) control line. Seeds from these pure-breeding lines were sent to ACRI, Narrabri in two batches in 2006. They were sown and grown by Dr Greg Constable's cotton breeding team. Mature fibres were sampled from five plants per line for testing of fibre characteristics.

Fibre characteristics were measured using an HVI machine and include length, uniformity, short fibre index, strength, elongation and micronaire. The GraphPad Prism program was used to carry out a one-way analysis of variance (ANOVA) of the data. It showed that a number of different lines have significantly different fibre traits from untransformed control cotton, Coker 315. Table 1 below shows fibre data, with the statistically significant traits in

orange. Colour shading indicates data collected from two independent batches, pink from batch 1 and blue from batch 2.

Table 1

Plant line	length	uniformity	sfi	strength	elongation	micronaire	No of plants
Coker 315	1.26	86.4	7.0	34.7	3.7	4.3	10
T334-127-3	1.27	87.5	6.3	34.5	4.4	4.8	5
T334-19-1	1.21	86.9	6.5	34.0	4.4	4.2	5
T334-19	1.18	85.6	7.1	33.3	5.4	4.1	5
T334-6-1	1.20	86.3	6.9	32.7	4.3	4.6	4
T334-6	1.21	86.2	6.9	33.5	4.6	3.9	5
T337-134	1.24	86.5	6.8	32.8	3.2	4.5	5
T337-71-1	1.25	87.3	6.3	35.0	4.8	4.1	5
T337-11	1.07	83.1	8.9	33.6	7.4	4.8	5
T337-39	1.14	83.3	8.8	33.2	7.7	4.3	5
T337-45	1.15	82.2	9.5	33.5	6.2	4.6	5
T338-137-4	1.20	84.6	7.7	30.8	4.4	4.1	5
T338-25	1.20	86.9	6.6	32.8	3.9	5.1	5
T338-52-1	1.19	85.6	6.8	34.9	5.2	4.2	5
T338-112	1.24	85.3	7.4	33.5	6.7	4.2	5
T338-137-1	1.14	83.9	7.3	32.3	6.0	4.5	3
T336-28	1.16	84.5	6.9	32.7	6.6	4.2	5
T336-31	1.20	85.1	7.3	33.4	6.4	4.2	3
T336-48-1	1.21	84.9	7.8	32.8	6.3	4.2	5
T336-63	1.17	83.9	7.7	33.9	6.1	4.4	5
Coker 315	1.18	81.8	9.9	35.3	6.0	4.0	5
T336-69-7NULL	1.19	85.0	7.0	34.4	7.1	3.9	5

A general observation is that most of the significant differences occurred in the fibre length and/or elongation traits, a result consistent with a role for expansin in fibre elongation. In addition, one line in which expansin is being overexpressed (T338-112) showed an increase in fibre length compared to untransformed Coker. Although the difference is not statistically significant according to our analysis, the result is an interesting one. It should be noted that the small numbers of plants and observations, and their intrinsic high variability, make it difficult to reach levels of statistical significance. However, small differences are potentially of commercial interest. For this reason some statistically non-significant changes were further investigated.

Objective 2: Determination of expansin gene expression profiles in transgenic cottons

In parallel with the analysis of fibre characteristics, we determined the expression profile of the expansin gene(s) in our transgenic cotton lines. In this way we could verify that we had indeed changed expansin gene expression in the cotton fibres, and potentially relate that to any modifications in the fibre traits listed in Table 1. The method used in this experiment was quantitative PCR (Real Time PCR), which requires only a small amount of RNA as starting material. RT-PCR primers were designed and tested, and three possible reference genes

(required to normalise the experimental data), were tested before *actin3* (ACT3) was chosen as the most suitable for our experiments. We grew a large number of transgenic cotton plants (off campus at the Waite Research Precinct), collected tissues, and prepared RNA from fibres aged 6, 12, 18, 24 and 30 DPA as well as leaf, stem, root and whole flower. Material was collected from all 19 transgenic lines and from non-transformed control (Coker 315) plants.

Based on the statistical analysis of the fibre data (see Table 1), we selected the antisense (T334), overexpression (T338) and prolonged expression (T337) lines for further study, since it was in these lines that most of the significant differences in the fibre length and/or elongation were found. Real-Time PCR was carried out on four T338 lines (including T338-112), three T337 lines and two T334 lines, and in each case we measured expression of the transgene as well as overall expansin gene expression in fibres at 6, 12, 18, and 24 DPA. In some cases, 30 DPA samples were also examined. Table 2 below provides a summary of the transcript accumulation of transgenic expansin and overall expansin genes detected in these lines. Fibre traits significantly different from the untransformed control (WT), taken from Table 1, are also shown with arrows indicating increase (↑) and decrease (↓) in value.

Table 2

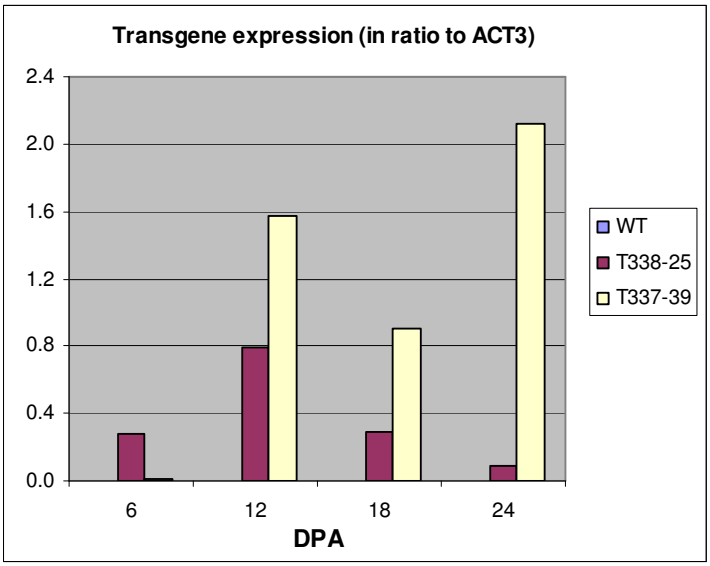
Plant line	Promoter::transgene	Expression of transgene in fibre	Fold increase in total expansin expression in fibre c.f. WT	Fibre traits significantly different from WT
T338-112	FS6::sense-expansin	undetectable	Up to 7.5	**uniformity ↑**
T338-52-1	FS6::sense-expansin	undetectable	Up to 3.5	**length ↓, elongation ↑**
T338-137-4	FS6::sense-expansin	very low	Up to 1.5	**length ↓, uniformity ↓, strength ↓**
T338-25	FS6::sense-expansin	**high**	Up to 4.0	**length ↓, micronaire ↑**
T337-11	FS18::sense-expansin	very low	Up to 12.7	**length ↓, elongation ↑, micronaire ↑**
T337-39	FS18::sense-expansin	**very high**	Up to 10.8	**elongation ↑**
T337-71-1	FS18::sense-expansin	very low	Up to 9.3	**elongation ↑**
T334-19	FS6::antisense-expansin	undetectable	Up to 2.3	**length ↓, elongation ↑**
T334-6	FS6::antisense-expansin	very low	Up to 6.3	**elongation ↑**

In most cases, expression of the transgene was low or undetectable. However, surprisingly, fibres from all eight lines (Table 2) showed overall expansin gene expression which was higher than that in the untransformed control and was independent of whether the transgene was expressed. This unexpected observation could be the result of complex interactions between members of the expansin gene family such that the overall expression of the gene family is altered. Silencing of the transgene is common in transgenic experiments. Also, as the T334 and T338 experiments both used the same promoter (FS6), it could be that the FS6 promoter is not functioning as expected, and that there are interactions occurring between it

and native members of the expansin gene family. It appears therefore that the transgene was not being expressed as expected in most of our transgenic plants, but large changes were observed in overall expansin transcript accumulation. The high variability between lines is typical for a transgenic experiment, where results are dependent on position effects (i.e. the genomic site of insertion of the transgene). Normally analysis of 15-20 transgenic lines from any one experiment is required to counter position effects.

However, in the T337-39 line, in which we were hoping to extend expansin expression beyond its normal range, the FS18 promoter appears to conform more closely to expectation. Comparison between the T338 and T337 lines which have detectable transgene expression showed that the T337 lines which used the FS18 promoter did have a prolonged expression pattern. Figure 1 below shows the transgenic expansin expression profile of T338-25 and T337-39 in which levels of transcript accumulation from the transgene were high to very high.

Figure 1

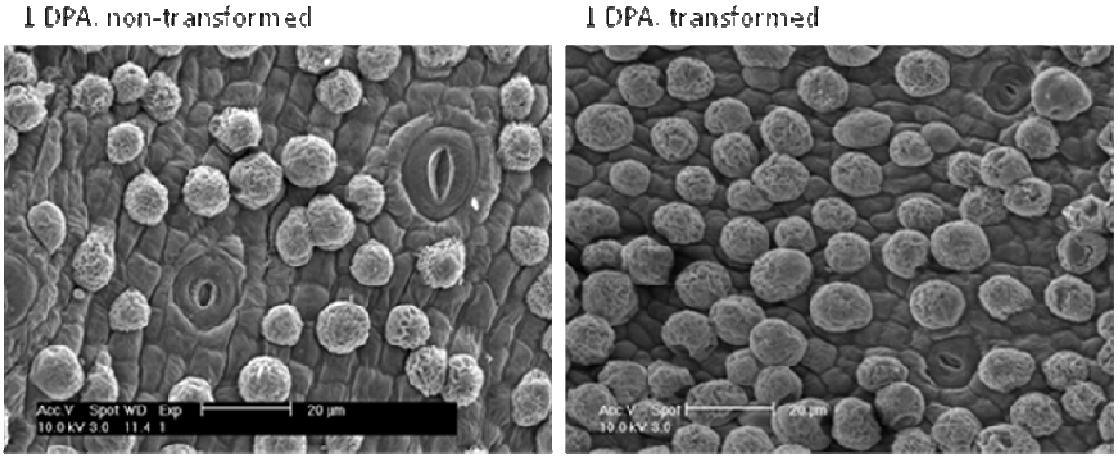


The transgene transcript level in T337-39 remained high at 18 and 24 DPA, while the level decreased in T338-25 at the same ages. This was also the case for two other independent lines, T337-11 and T337-71-1, though the detectable transcript level was lower than that in T337-39. All three T337 lines showed a significant increase in fibre elongation compared to the untransformed Coker in the statistical analysis of fibre characteristics (Table 1). These results suggest that the promoter FS18 is functioning as expected and therefore, serious consideration should be given to the possible commercial utilisation of this promoter in breeding new cotton varieties.

Objective 3: Electron microscopic visualisation of fibre development of transgenic cottons

In addition to measuring fibre traits, we wished to determine whether fibre initiation in our transgenic plants was altered. To this end, ovules aged 0-2 DPA from one or two lines per experiment and from a non-transgenic line were observed via Scanning Electron Microscopy (SEM), using equipment in the Adelaide Microscopy Centre. An example of the image generated is shown in Figure 2. No obvious differences in fibre initiation (morphology or density) were observed between transgenic and non-transgenic cotton, even in the lines where we have altered expansin expression at 6 DPA.

Figure 2



Objective 4: Discussion of a strategy for the development of molecular markers for use in cotton breeding programs

Discussions were held between ourselves and Dallas Gibb in October and December 2006, regarding the development of molecular markers for cotton fibre traits. It was decided that an integrated approach with CSIRO would be best, as per feedback from the CRDC Review of Fibre Quality Projects (February 2006) but a meeting between the three parties did not eventuate. Molecular markers have been assigned by other cotton researchers to the expansin genes identified in our laboratory and these markers could be used to track our genes through genetic crosses designed to introgress our genes into elite commercial cultivars.

Another way forward with this work is to conduct crosses between our transgenic lines and screen the progeny. It is possible that the transgenes will interact with each other to produce further fibre variants, and we conducted ten preliminary crosses between our transgenic lines, including crosses between T337-39/T337-11 and T338/T336 (another construct of prolonged expansin expression). Seeds from these crosses were collected. T337 lines are those shown to have altered expansin expression with a significant increase in fibre elongation (see above). However, as we lack the facilities for a large-scale breeding and screening program, no further crosses were conducted or planned.

Objective 5: Analysis of GUS expression driven by fibre-specific promoters in transgenic cotton plants

Previous CRDC-funded research resulted in the isolation of several fibre-specific gene promoters. Constructs were made in which four of the promoters were driving expression of a reporter gene, GUS. These were transformed separately into cotton to generate a number of lines stably transformed with reporter constructs for each of the four promoters. The aim of this work was to continue analyse the expression of GUS in the transgenic reporter lines. Seeds were sown for 3-6 lines per construct. PCR testing was carried out to identify plants carrying the transgene, and negative plants were discarded. Positive plants were grown and fibres (6-12 DPA) collected from 14 lines for preliminary GUS staining. Fibres from only five of the lines stained blue, indicating expression of the GUS gene. High variation between transgenic lines not unexpected and is a result of position effects, as discussed above. Tissue was collected from fibres aged 6, 12, 18 and 24 DPA as well as leaf, stem and whole flower of the five positive lines plus a non-transgenic line. Protein extractions were carried out on

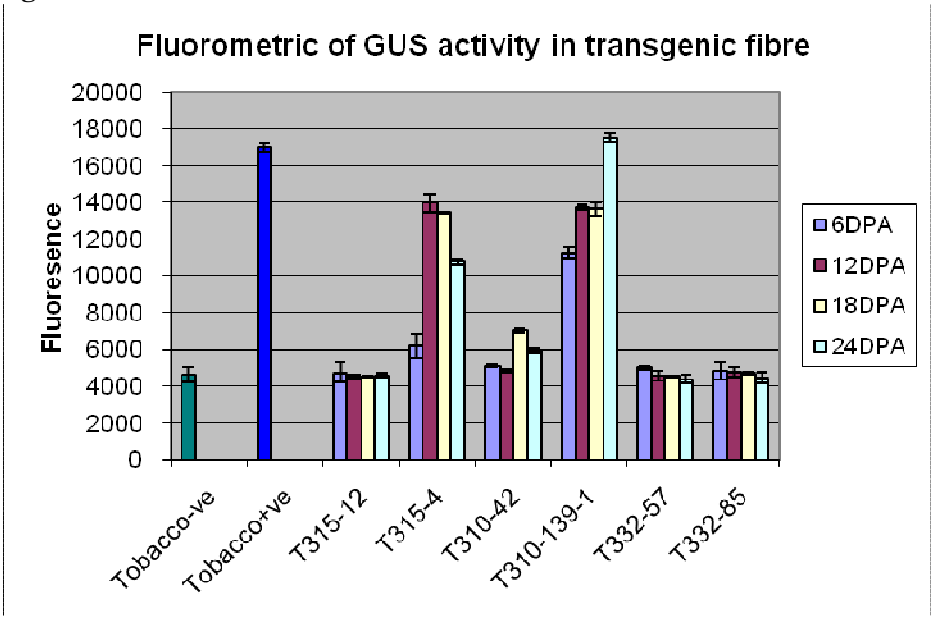
these tissues in preparation for quantitative MUG assays to determine the fibre-specificity and strength of each promoter. GUS activity (as determined by GUS staining or by MUG assay) was not detected in any tissues other than fibre. Table 3 below summarises GUS staining and MUG assays of the collected fibre samples and Figure 3 shows the fluorometric profiles of the fibre samples in the MUG assay.

Table 3

Line	Promoter	No of plants tested	GUS staining	MUG assay reading
T315-12	Non-transgenic	1	-	Baseline level*
T315-4	pFS14	2	+++	**High**
T310-42	pFS18	3	++	**Low**
T310-139-1	pFS18	3	+++	**Very high**
T332-57	pFS17	4	+	Very low to baseline level
T332-85	pFS17	4	+	Very low to baseline level

* A leaf sample of untransformed tobacco was used as a negative control for the MUG assay, and the fluorometric readings from this sample were used to set the baseline level for all other samples. A leaf sample of tobacco transformed with 35S::GUS was used as a positive control for the MUG assay. The number of "+" indicates the strength of the blue GUS staining in 6-12DPA cotton fibres.

Figure 3



The MUG assay confirmed the preliminary GUS staining results. Both preliminary staining and MUG assay confirmed that the non-transgenic T315-12 had no detectable GUS activity. It therefore served as a negative control for transgenic fibre samples. T315-4 and two T310 lines showed higher fluorometric readings than the negative control, indicating the expression of the reporter GUS gene in these lines. While T315-4 and T310-139-1 both showed high GUS activity, the GUS expression in T310-139-1 fibre remained high even at 24 DPA. The

GUS activity was also higher at 18 and 24 DPA fibres in T310-42, although its level was lower compared with T310-139-1. These results suggest that FS18 promoter, which is active throughout elongation and secondary cell wall synthesis in the cotton fibre, is functioning as expected in some of the GUS transgenics. This is in agreement with the findings for our transgenic expansin lines shown above. In contrast, the level of GUS activity in T332 lines were very close to that of the negative controls, which is consistent with very faint blue observed in GUS staining. In conclusion, the FS18 promoter is the most efficient and predictable promoter among all fibre-specific promoters we have tested.

Outcomes

5. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

In this project we have analysed the fibre traits from transgenic cotton plants which used three different fibre-specific promoters to alter expansin gene expression. Expansin transcript accumulation was altered in transgenic lines containing the FS18 promoter and these lines all showed a significantly increased fibre elongation compared to the untransformed cotton. We have also analysed a range of transgenic cotton plants which used fibre-specific promoters driving expression of the GUS reporter gene. Again, FS18 was shown to be the most effective in promoting fibre-specific GUS gene expression among our suite of promoters in transgenic cotton. This represents the culmination of 17 years of CRDC-funded research in our laboratory.

The findings that the FS18 promoter produced elevated expression of expansin and that the transgenic lines bearing the construct showed altered fibre elongation demonstrate that it is practically possible to manipulate gene expression in a tissue-specific manner by utilising native cotton genes. Moreover, the lines which exhibit altered fibre properties provide valuable material for the industry in the form of germplasm for cotton breeding programs aimed at fibre improvement. In addition, this research contributes significantly to our understanding of the functional aspects of cotton fibre-specific promoters and genes.

6. Please describe any:-
   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.); and
   c) required changes to the Intellectual Property register.

   a) In this project we perturbed the expression of expansin, a gene known to be involved in cell elongation and growth, in developing cotton fibres. Specifically, the FS18 promoter extended expansin expression beyond its normally confined period during fibre development. Transgenic plants containing the construct have modified fibre characteristics that could be incorporated into new crop varieties and this is potentially of commercial significance. Throughout this and our other projects, we consulted regularly with Adelaide Research and Innovation (the University of Adelaide IP arm and its lawyers), with a view to applying Provisional Patents to our findings. However, significant work is required to fully explore the commercial potential of our results.

b) A large amount of valuable resources, in the form of seeds and plant material, was generated during the course of this project. Future research in this area will require suitable long-term storage and cataloguing of this material.

c) None.

Conclusion

7. 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?

Progress in plant breeding programs aiming to improve fibre quality in cotton has reached a plateau. Most genetic modification uses ubiquitously expressing promoters that often result in yield penalty. Very little progress has been made in this field. In this project, we used several fibre-specific promoters that we identified previously to drive a native cotton expansin gene and successfully obtained transgenic lines with modified fibre characteristics. We identified the FS18 promoter as the most effective and predictable among our suite of fibre-specific promoters and the resultant transgenic plants have considerable potential in breeding new cotton varieties. More specifically, we have immediate access to plant lines in which expansin gene expression in fibres is extended well beyond its normal range. Coupled with this is the observation of significant correlation with fibre elongation. The FS18 promoter could be used to drive a variety of other genes and confine their expression specifically to the fibre. Those transgenic lines with modified fibre traits could be incorporated into new crop varieties that may provide a beneficial agronomic effect for the cotton industry.

Extension Opportunities

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.

(a) A significant amount of research and development would be required to exploit the technological output of this project. A clear way forward is to incorporate our important transgenic lines, such as T337-11, into existing breeding programs. A breeding experiment involving crossing our transgenic lines with current Australian elite varieties and selection for the FS18-expansin transgene would necessarily involve breeders and molecular genetic backup to provide marker assisted selection. Similarly, it would be of interest to combine the transgenes by crossing our transgenic plants as outlined in section 4.4, which could produce additional fibre variants. However, it is difficult to predict the likely outcomes of breeding experiments such as these and such project would therefore have a high risk.

(b) A paper with the results of our research is currently being prepared for publication in a scientific journal.

(c) As our research ends with this project, it is for the CRDC to determine how much further they wish to extend our findings. The CRDC Research and Development Plan for 2008-2013 reflects a shift away from biotechnology and towards an emphasis on farming systems, sustainability, improved routes to market and human resources. Options for future research are outlined above.

9. 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)

- Orford SJ, Delaney SK and Timmis JN (2007) Genetic modification of cotton. In Gordon, S. and Hsieh, Y.-L. (eds) "Cotton: Science and Technology", Woodhead Publishing Ltd, Cambridge, UK, pp 103-129
- Delaney SK, Orford SJ, Martin-Harris M and Timmis JN (2007) The fiber specificity of the cotton *FSltp4* gene promoter is regulated by an AT-rich promoter region and the AT-Hook transcription factor GhAT1. *Plant Cell Physiol*. 48: 1426-1437
- Lightfoot DJ, Malone KM, Timmis JN and Orford SJ (2008) Evidence for alternative splicing of MADS-box transcripts in developing cotton fibre cells. *Mol Genet Genom.* 279:75-85
- Lightfoot DJ, Timmis JN and Orford SJ (2009) Identification and characterisation of genes specifically expressed in the boll wall of cotton. *In preparation*
- Liu YH, Harmer SE, Orford SJ, Llewellyn DJ and Timmis JN (2009) Altering the expression of the expansin gene family in cotton fibres has an effect on fibre characteristics. *In preparation*

2

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

No.

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.

Advances in our knowledge of the genes expressed in cotton fibres make biotechnology a viable means to improve fibre characteristics. Most genetic modification uses ubiquitously expressing promoters that often result in yield penalty. We have developed a strategy which uses fibre-specific promoters to manipulate and confine gene expression to fibre cells.

We used several fibre-specific gene promoters to drive expression of an expansin gene that is native to cotton and fibre-specific as well. Expansin is a protein known to be involved in cell elongation and growth, and therefore could impact on fibre traits such as length. Normally, the fibre-specific expansin gene expresses predominantly in the first 18 days of fibre development. By assembly of the expansin gene with a promoter (FS18) that usually drives expression of a lipid transfer-like protein that is active late in fibre development, we have been able to extend expansin expression for a further 4-10 days in transgenic plants. The chimeric gene increased the total amount of expansin in fibre cells and altered the morphology of the fibre in ways that are potentially advantageous to the industry. Linked experiments in which this promoter was used to drive a GUS reporter gene in a separate set of transgenic plants showed that the FS18 promoter can drive a high level of transcription specifically in fibre cells to a much later stage of fibre development. The FS18 promoter could be used to express any gene of interest in an effective and predictable manner in cotton fibre cells, and could be an important tool in fibre biotechnology.

Transgenic plants containing the FS18-expansin construct have modified fibre characteristics that could be incorporated into existing breeding programs. In this way new fibre variants

could be generated, contributing to germplasm stocks and helping to maintain the reputation of the Australian cotton industry as a producer of premium quality cotton. Our strategy of manipulating gene expression in a tissue-specific manner by utilising native cotton genes is feasible and potentially beneficial to the cotton industry.

For more information, contact:
Professor Jeremy Timmis
Discipline of Genetics
School of Molecular and Biomedical Science
The University of Adelaide
AUSTRALIA 5005
Ph : +61 8 8303 4661
Fax : +61 8 8303 5338
e-mail: jeremy.timmis@adelaide.edu.au