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: UA7C

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: Genetic manipulation of fibre quality in Australian cotton

Project Commencement Date: 1/7/00 **Project Completion Date:** 30/6/04

Research Program: 5 Breeding and Biotechnology

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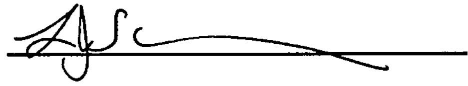
Signature of Research Provider Representative: 

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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.

We have had CRDC support for investigating the genes that control fibre morphology since 1992, with the recent realisation of one of our primary goals, namely the isolation, characterisation and *in planta* demonstration of several different fibre-specific gene promoters. The work described here aimed to capitalise on and extend this success and other exciting results obtained during CRDC projects UA4C and UA5C.

Genetic engineering to confer useful agronomic and fibre traits will lower the cost and time required for producing improved cotton varieties and will promote environmentally-friendly farm practices. The benefits of genetic engineering of cotton in Australia have been realised in the form of Bt transgenic cotton, commercialised as Ingard® and BollgardII® varieties but, despite the obvious potential, these techniques have not yet featured significantly in the improvement of fibre quality.

Genetic engineering of cotton requires both the identification and characterisation of useful genes and the capacity to insert them into the cotton genome. Research in our laboratory has aimed to address the first requirement, concentrating on genes which are expressed in fibres but not in other cotton tissues, such as leaves, stems and roots. This research is based on two main premises. Firstly, there is a strong probability that genes which are expressed at high levels only in fibre cells will have critical roles in fibre development or properties (and therefore we expect them to control fibre yield or quality). Secondly, we have identified the corresponding sequences (promoters) within the cotton genome which directly control the fibre-specificity and timing of expression of these 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, and they could therefore be vital tools in the genetic manipulation of fibre characteristics.

We have identified six different promoters within the cotton genome which directly control the fibre-specificity and timing of expression of genes. Each of the six promoters was fused to a reporter gene, β-glucuronidase (GUS), and, in transient assays, shown to direct reporter gene expression that was confined to the fibres. The first aim of this project was to test each promoter for its ability to drive fibre-specific gene expression in whole-plant transformants.

The second aim of this project was to identify which of our candidate genes have potential for alteration of fibre characteristics, by preparing gene constructs designed to change their expression and testing their effects in whole-plant transformants. Our primary candidates for fibre manipulation are the six fibre-specific genes, four of which encode structural components of the cell wall. We have also targeted some other genes, identified outside cotton, which could act to regulate cotton fibre growth. Of these, the *TTG1* and *GL2* genes, identified in the model plant *Arabidopsis*, are likely to be involved in the initiation of fibres and determination of their density on the seed coat. Other candidates are genes encoding a family of transcription factors termed MADS-box proteins, the expression of which we have detected in elongating cotton fibres.

Constructs were to be made, in which selected genes are assembled under the control of over- and under-expressing promoters and promoters that alter the time of gene expression. These promoters are available from our bank of fibre-specific isolates. Such constructs were then to be tested *in planta* by transformation of cotton. Manipulation of the genes will identify their

function and effects on cotton fibre growth and development, and transgenic cotton plants were to be screened for differences in fibre properties.

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

The original project objectives were to:

1. Identify which of our candidate genes have potential for alteration of fibre quality, by characterising them further at a molecular and functional level.
2. Prepare gene constructs designed to change the expression of the chosen gene(s) and test their effects on fibre development by transformation of whole cotton plants.
3. Evaluate fibre-specific gene promoters for their ability to drive reporter gene expression only in the fibres of transgenic cotton plants, and make them available for wider cotton research and plant breeding.

The objectives of this project were, to a large extent, achieved, and progress was significant despite the interference of a number of interruptions. The move of the Genetics Discipline to a new building in September 2000 caused longer disruption than was foreseen, particularly to plant growth facilities. Sharon Orford was on maternity leave for 12 months from 12th March 2001 and on her return to work, a large amount of time was devoted to ensuring that the project complied with new federal OGTR legislation for working with genetically modified organisms. It should also be noted that aim (2) of this project hinged on a collaboration with Dr Danny Llewellyn at CSIRO Division of Plant Industry in Canberra, where the cotton transgenesis was to be performed. Early in the project we received an indication from CSIRO that new transformation experiments would not be undertaken. Aim (2) was therefore modified to involve only those transgenics which were already underway at the commencement of the project, namely evaluating the effect of expansin misexpression on cotton fibre development.

In addition to the work described in section 4 below, Sharon Orford attended the XIX International Congress of Genetics in Melbourne in July, 2003, where she presented a poster entitled "Two WD-repeat genes from cotton can replace the function of the transparent testa glabra1 (TTG1) gene in *Matthiola incana*". In 2001 Sharon Orford was awarded an Adelaide University Small Research Grant of $10,000, an application which was supported by the CRDC. In addition to annual Discipline seminars, Sharon Orford presented results of this project in two invited seminars, the first if which was in the Department of Chemical Pathology at the Women's and Children's Hospital, Adelaide, in August 2000 and entitled "Engineering a better Australian cotton fibre". The second formed part of the Waite Precinct Plant Researchers Seminars Series in March 2004 and was entitled "The molecular genetics of cotton fibre initiation and development". In 2000 our work was the subject of an article in the "Adelaidean" newspaper and "Lab News" magazine, and Jeremy Timmis took part in a radio interview for ABC National. In 2003, Sharon Orford reviewed papers for publication in the international journals "Planta" and "Biochimica et Biophysica Acta".

A significant part of this research includes the involvement of Sharon Orford and Jeremy Timmis in the supervision of postgraduate students. Our laboratory has been particularly successful in this regard, with the awarding of two CRDC Honours scholarships (UA9C, UA10C) and three new CRDC PhD studentships (UA8C, UA11C and UA12C) during the tenure of this project. In addition, we supervised two further Honours students on cotton projects, in 2000 and 2004, and a summer student in 2003. We also supervised a PhD student (funded by ARC-SPIRT APA(I) scholarship) who was awarded her Doctorate in July 2003. These students were attracted to cotton research by results from CRDC-funded projects and contribute valuable results which underpin the more applied research which we carry out for the CRDC.

3. **Detail the methodology and justify the methodology used.**

1. Candidate genes were to be further characterised using standard molecular biological techniques, including isolation and sequencing of full-length mRNAs and corresponding genes and expression analysis by RT-PCR, Northern blotting and/or *in situ* hybridisation. Such procedures are well-established in our laboratory. In the absence of access to cotton transformation, functional analysis was to be carried out, where possible, by transformation of mutant *Arabidopsis* plants.
2. The most promising of our genes analysed to date encodes an expansin, a protein thought to control plant cell growth by chemical modification of cell wall components. Expansins could therefore play a critical role in determination of fibre quality and yield. Four constructs were made, in which the expansin gene was placed under the control of four different promoters, designed to alter native expansin expression. The gene constructs were used to transform whole cotton plants during the tenure of project UA5C, and, ideally, 8-10 independent lines from each of the four transformations were to be analysed as follows:
   - Plant transformed lines (seeds of the T1 generation) and isolate genomic DNA from 4-8 individual plants of each line.
   - Test for the presence of the transgene in each plant by PCR, using specific primers for each construct. Perform Southern blots to determine the copy number of the transgene and to confirm different genomic contexts in different transformed lines. Discard negative plants.
   - Collect T2 seed from positive plants, grow T2 plants and test for the presence of the transgene as above. Discard negative plants.
   - Collect fibres from various ages and use Northern blots or PCR techniques to show that expansin gene expression in the fibres is perturbed, as compared to that in wild-type (untransformed) plants. Evaluate fibre properties such as length, strength, micronaire, uniformity and maturity for the effects of the altered expansin expression.
3. Six different fibre-specific promoter::GUS reporter constructs were transformed into cotton as part of project UA5C, and, ideally, 8-10 independent lines from each of the six transformations were to be analysed as follows:
   - Plant transformed lines (seeds of the T1 generation) and isolate genomic DNA from 4-8 individual plants of each line.
   - Test for the presence of the transgene in each plant by PCR, using specific primers for each construct. Perform Southern blots to determine the copy number of the transgene and to confirm different genomic contexts in different transformed lines. Discard negative plants.
   - Collect T2 seed from positive plants, grow T2 plants and test for the presence of the transgene as above. Conduct a preliminary staining for GUS expression in fibres and other tissues, and discard negative plants.
   - Collect various cotton tissues at a range of time points and carry out quantitative GUS assays to confirm the fibre-specificity of each promoter and to determine the temporal expression pattern and relative strength of each promoter in cotton fibres.

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

1. Characterisation of candidate genes for genetic manipulation of cotton fibres

We had previously identified a number of cotton genes which could be involved in the determination of fibre morphology and these will be addressed in turn.

- **The expansin gene family in cotton**

  Expansins are a family of plant proteins thought to allow cell growth by modification of plant cell wall components The expansin work, including detailed characterisation of the gene family in cotton, analysis and testing of fibre-specific expansin gene promoters and assembly of constructs designed to misexpress the expansin gene in transgenic plants, was carried out by a PhD student (funded by ARC-SPIRT APA(I) scholarship), Sarah Harmer, who was awarded her Doctorate in July 2003 under the joint supervision of Jeremy Timmis and Sharon Orford. The research on expansin genes which was carried out as part of this project is detailed in part 2 below.

- **Putative cotton homologues of *Arabidopsis* trichome regulators**

  Arabidopsis trichome (leaf hair) development has been extensively studied as a simple example of plant cell differentiation, and the pivotal genes have been cloned. We and others have proposed that development of *Arabidopsis* trichomes is a suitable model for cotton fibre initiation because of the morphological and apparent molecular similarities between the two systems. One *Arabidopsis* gene, *TTG1*, encodes a WD-repeat protein which is absolutely required for trichome initiation, and we have sought *TTG1* homologues in cotton under the premise that similar genes could be involved in fibre initiation.

  Five *TTG1*-like genes were obtained previously from an Australian cotton cultivar, either as genomic DNA fragments (by library screening) or as genomic PCR products. Where necessary, the sequencing of these genes was completed, with at least three independent genomic PCR clones sequenced in the cases of *GhTTG2* and *GhTTG3*. The λGhTTG(B) genomic clone was subcloned into a plasmid vector, and sequencing of the coding regions showed that λGhTTG(B) overlaps with λGhTTG(C) such that they encode the same gene. The remaining three sequences were unique, resulting in the identification of four distinct *TTG1*-like sequences, a summary of which appears in Table 1.

TABLE 1: Summary of TTG1-like isolates from cotton

Cotton TTG1-like sequence	PCR clone	cDNA clone	Genomic clone
GhTTG1	TTG⑤ 699 bp	TTG(I) 1253 bp (partial)	λGhTTG(A) (pTTG(A)E5 and pTTG(A)EX2.5 subclones), 1584 bp
GhTTG2	-	TTG(II) 1694 bp	GW PCR, then genomic (three clones), 1924 bp
GhTTG3	TTG① 694bp	-	GW PCR, then genomic (three clones), 2008 bp
GhTTG4	-	-	λGhTTG(C) (pTTG(C)E2 subclone), 1837 bp λGhTTG(B) (pTTG(B)B1 subclone), 905 bp, overlaps with λGhTTG(C)

A comprehensive sequence analysis of the four cotton *TTG1*-like genes showed that they are very similar to each other and to *Arabidopsis TTG1*, as well as to a number of other genes encoding WD-40 proteins. The nucleotide sequences were deposited in the GenBank database with accession numbers AF530907-AF530912. The four sequences form two pairs, with *GhTTG1* and *GhTTG3* being similar to each other, and *GhTTG2* and *GhTTG4* grouping together. Of these, *GhTTG1* and *GhTTG3* showed the greatest similarity to *Arabidopsis TTG1*. It is possible that a single gene in *Arabidopsis* corresponds to a number of redundant genes in tetraploid cotton, which may be expressed differentially or have diverse developmental roles which are not necessarily concerned with fibre development.

Since Northern blot analysis produced only weak or undetectable signals, we investigated the expression patterns of the four genes using the more sensitive technique of reverse transcriptase PCR (RT-PCR). An alignment between the four cotton sequences allowed the design of gene-specific primers, which were tested on a bank of cotton genomic and clone DNAs. Interestingly, all four genes are derived from the diploid ancestor most closely related to *G. raimondii*, or the D subgenome.

RT-PCR was carried out on total RNA templates from fibres of various ages as well as from ovule (0 DPA), leaf, flower, stem and root tissue. As for *Arabidopsis TTG1*, transcription of the four *GhTTG* genes was detected in all organs tested, with the overall level of *GhTTG3* transcripts being lower than those of the other three genes. All the genes were expressed throughout the expansion and elongation stage of fibre development (-1 to 21 DPA) and transcripts persisted into secondary cell wall synthesis (15 to 40 DPA).

Southern blots of cotton genomic DNA, using the four *TTG1*-like genes as probes, indicated the presence of three-five WD-repeat family members related to each of the two groups, even when a gene-specific probe was used. This is in agreeance with all other cotton genes isolated in our laboratory, which appear to be members of gene families.

Although molecular analysis of the cotton *TTG1*-like genes as part of this project yielded new and valuable information, it is difficult to tell from the results described here which of the four genes, if any, is a functional homologue of *Arabidopsis TTG1*. A functional analysis of the genes was approached by attempting to complement *ttg1* mutations in heterologous plant systems, namely *Matthiola incana* and *Arabidopsis thaliana*. These experiments, carried out by a PhD student in the laboratory under the joint supervision of Jeremy Timmis and Sharon Orford, are detailed in reports for project UA9C, UA12C and in our publication of this work (see section 9).

A second trichome gene, *GLABRA2* (*GL2*), is a member of the homeodomain (HD) class of transcription factors and is involved in the control of trichome cell expansion. We isolated an excellent candidate (as a cDNA clone) for the cotton homologue of GL2 as part of project UA5C, and work was to be continued on a collaborative basis with CSIRO Division of Plant Industry. We had isolated one genomic clone in a library screen with the *GL2*-like cDNA, and a 4 kb subclone had been constructed by Jeremy Timmis as part of project UA6C. During the tenure of this project, we sequenced the subclone to obtain approximately 2 kb of non-contiguous sequence. Sequence analysis showed that the gene corresponded to our cDNA and that the coding region was interrupted by a large number of introns. The genomic subclone contained approximately 1.5 kb of promoter sequence and the initiation codon but appeared to be partial, missing about one third of the coding sequence at the 3'end. The information was shared with our collaborators, who undertook a number of other experiments to be included in a joint publication (see section 9).

- **The MADS-box family of transcription factors**

  Previous work in our laboratory by Sharon Orford (UA5C) and Damien Lightfoot (UA10C) had shown that at least four genes encoding MADS-box transcription factors are expressed in developing cotton fibres, and that one of these genes, 24.11, is alternatively spliced. Alternative splicing is a phenomenon by which several different proteins can be produced from transcription of one gene and is an important source of variation in higher organisms. A large number of 24.11 cDNA clones were sequenced, and four sequence variants were observed in addition to the splicing variants. Genomic sequence was obtained for only one of the variant groups. In this project we aimed to complete the work begun in UA5C and UA10C, by cloning additional gene(s) which gives rise to the alternatively-spliced MADS-box transcripts.

  Two genomic clones containing MADS-box genes had been obtained from a library screen. The one which hybridised most strongly to a cDNA probe was subcloned into a plasmid vector, but our set of four MADS-box primers could not amplify a product from the subclone and no further analysis had been carried out. As part of this project, fresh glycerol and plasmid DNA stocks of the subclones were prepared, their identity checked by Southern blotting and the previous PCR results verified. A PCR-based experiment was therefore used in an effort to clone the 24.11 gene(s). Gene-specific primers for 24.11 yielded a 1.1 kb PCR product from tetraploid cotton (cv. Siokra 1-4), and direct sequencing showed it to be a mixture of amplification products from more than one template gene. The PCR product was cloned and 11 individual clones sequenced in full. The clones formed three groups, one consisting of seven members which matched the existing isolate, one containing three clones which matched the other main cDNA sequence variant, and one gene which was clearly 24.11-like but did not correspond in sequence to any 24.11 cDNA clones. Gene-specific primers were designed in an attempt to isolate the two remaining 24.11-like genes, and the PCR reaction conditions adjusted until specific products were obtained from a bank of RT-PCR and genomic clone DNAs. PCRs on cotton genomic DNA with the same sets of primers yielded products which were cloned, but sequencing results of two independent clones from each experiment were inconclusive. The sequences were clearly 24-11 genes but, as the only sequence differences between these genes and the existing isolates are built into the primers, it cannot be determined whether or not they correspond to new variants. Results from the MADS-box experiments were written into a manuscript for publication (see section 9).

2. *The effect of expansin gene misexpression on cotton fibre development*

In previous work, four genetic constructs were made, in which the expansin gene was placed under the control of different promoters designed to alter native expansin expression in cotton fibres. The gene constructs were used to transform whole cotton plants late in 1999, and the first T1 seeds from the experiments were sent to Adelaide in December 2002. We received T1 seed from a total of 42 different transgenic lines during the tenure of this project.

Between December 2002 and April 2004, 4-8 seeds from 25 of the transgenic cotton lines were planted. Initial experiments involved trialling of a number of different methods for small-scale genomic DNA preparation from leaves of the transgenic plants. Genomic DNA from each plant was tested for the presence of the transgene by PCR, using primers for the kanamycin resistance gene (*NptII*) and/or for the specific construct. Of these, 16 lines were 100% transgenic (that is, a PCR product was detected for all plants tested), and nine lines were segregating for the transgene. Negative plants were discarded and plants grown to fruiting before T2 seed was collected from between one and four plants per transgenic line. A summary of this work appears in Table 2.

TABLE 2: Summary of transgenics with expansin misexpression constructs

Construct name	Composition	Expected expansin expression	Construct sent to Canberra	Expt	First seeds obtained	No. T1 lines recovered *	T2 seed collected (no. lines)*
pPR6EXP	pFS6 promoter::EXP	increased	Dec 1999	T338	Jan 2003	9 (6)	9(6)
pFS18AEXP	FS18A promoter::EXP	prolonged	Dec 1999	T336	Jan 2003	10 (9)	5 (5)
pFS18BEXP	FS18B promoter::EXP	prolonged	Dec 1999	T337	Dec 2002	16 (14)	5 (5)
pPR6antiEXP	pFS6 promoter:: antisense EXP	decreased	Jan 2000	T334	Dec 2002	7 (4)	6 (3)
					TOTAL	**42 (33)**	**25 (19)**

*Numbers in brackets refer to the number of lines known to be independent. In some transformed lines, more than one plant was obtained from the same callus, and, whilst they should be clones of the same transformation event, they have been treated as different lines in these experiments until Southern analysis can resolve the question.

Due to the time-consuming nature of cotton transgenics, the transgenic lines were grown to T2 during this project but further analysis was not carried out. The fibres of the T1 plants did not show any startling characteristics (for example, the antisense transformants contained fibres) but fibre properties were not evaluated in any detail. Genomic Southern blots were performed in an effort to distinguish between the lines and to determine the copy number of the transgene, but these were unsuccessful due to a number of technical difficulties such as DNA loading on the agarose gel and use of a heterologous probe. A more appropriate probe was obtained but time constraints prevented a repeat of the experiment.

3. Demonstration of fibre-specific gene promoter activity in transformed cotton plants

In previous work, genetic constructs were made in which our six putative fibre-specific gene promoters were fused upstream of a GUS reporter gene. The gene constructs were used to transform whole cotton plants in 1999, and the first T1 seeds from the experiments were sent to Adelaide in February 2002, although the majority were received in 2003, such that a total of 50 different plant lines were recovered.

Between September 2002 and March 2004, 4-8 seeds from a total of 24 different lines were planted. Genomic DNA from extracted from leaf samples of each plant and tested for the presence of the transgene by PCR as for the expansin misexpression experiments described above. Of these, 12 lines were 100% transgenic, ten lines were segregating for the transgene and in two cases all plants tested negative for the transgene. Negative plants were discarded and plants grown to fruiting. Plants from two of the lines did not set fruit. T2 seed was collected from between one and four plants per transgenic line for 20 lines. A summary of this work appears in Table 3.

TABLE 3: Summary of transgenics with promoter::GUS reporter fusions

Construct name	Composition	Construct sent to Canberra	Expt	First seeds obtained	No. lines recovered	T2 seed collected (no. lines)
p3GUS1.5	pFS3 promoter::GUS	June 1999	T312, T313	Feb 2002	6 (6)	4 (4)
p6GUS1.4	pFS6 promoter::GUS	June 1999	T311	Jan 2004	1 (1)	1(1)
p18GUS1.2	pFS18 promoter::GUS	June 1999	T310	Jan 2003	10 (9)	6(5)
p17GUS1.2	pFS17 promoter::GUS	Nov 1999	T332	July 2003	24 (7)	7 (7)
pGUSEX1.2	pFS14 promoter::GUS	June 1999	T315	Feb 2002	3 (3)	2 (2)
pGUSEX1.4	Expansin promoter::GUS	June 1999	T316	July 2003	10 (4)	-
				TOTAL	**54 (30)**	**20 (19)**

*Numbers in brackets refer to the number of lines known to be independent, as in Table 1 above.

Once again, a small number of known independent transgenic lines was able to be grown to T2 during this project and further analysis was prevented due to time constraints. In addition, the transgenic testing of our most promising fibre-specific promoter did not provide seed until January 2004, and then only from one line. It is this promoter which we are most interested in characterising in detail. However, as much progress as possible was made with the material and growth facilities available.

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

The research objectives were completed as fully as allowed by the time- and labour-consuming nature of cotton transgenics. Whilst not providing the scope envisaged in the initial project application, the collaboration with CSIRO has been a fruitful one, with the receipt of seeds from 96 different transgenic cotton lines, from 10 transformation experiments. Where possible, at least five independent lines from each experiment was tested for presence of the appropriate transgene. Plants from a total of 49 (41 confirmed independent) lines were cultivated and tested and T2 seed was collected from positive plants of 45 (37 confirmed independent) lines. The T2 seed awaits planting and a full evaluation of the effects of each transgene on development of cotton fibres.

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

The primary impact of our work lies in the area of cotton biotechnology. Our ultimate aim is to produce cotton varieties with improved fibre characteristics, thereby ensuring the profitability and international competitiveness of the Australian cotton industry. Genetic manipulation of fibre properties requires an understanding of the genes which are involved in fibre development, a subject which is poorly understood at the molecular level. It is this paucity in knowledge which we are addressing in our research at Adelaide University.

In addition, genetic engineering to confer useful agronomic and fibre traits will lower the cost and time required for producing improved cotton varieties and will promote environmentally-friendly farm practices. The benefits of genetic engineering of cotton in Australia have been realised in the form of Bt transgenic cotton, commercialised as Ingard® and Bollgard®II varieties but, despite the obvious potential, these techniques have not yet featured significantly in the improvement of fibre quality.

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 aims of this project were to characterise and evaluate a number of cotton genes and promoters, obtained in Adelaide, for their eventual use in cotton genetic engineering. A number of genes were characterised using molecular biological techniques, and one of the

genes was used in misexpression studies in transgenic cotton plants. Transgenic seeds were also obtained in which a reporter gene is under the control of several different fibre-specific gene promoters. Whilst a large number of transgenic cotton lines were obtained during the tenure of this project, they were not evaluated in any detail beyond testing for presence of the transgene and collection of T2 seed. It is in the next, detailed phase of testing that we expect to generate results of industrial significance (see section 8).

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.

There is a clear path for this project in terms of future research and development of the project technology. Having completed the initial testing phase in 10 transgenic cotton experiments, it only remains to grow the T2 seed and evaluate plants from each line for the effect of the transgene on measurable fibre properties, or in the case of testing of the fibre-specific promoters, quantify reporter gene expression.

Results from the first component will contribute valuable information on the gene(s) involved in cotton fibre development, as well as providing novel germplasm for use in cotton breeding programs. The second component will provide a range of extensively characterised gene promoters which could be used to express any gene specifically in fibre cells and in a defined temporal manner. These promoters, after full evaluation, will be available for use in other cotton research, such as gene misexpression studies (like those carried out in this project) or in the production of coloured cotton fibres. We would look to the CRDC for direction as to how best to supply these resources to the cotton breeding and biotechnology community.

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

Conference presentations during the tenure of this project:

S Harmer, S Orford and J Timmis, "Characterisation of the expansin gene family in *Gossypium hirsutum* (cotton)", 23rd Lorne Genome Conference, Victoria, Australia, February 2001 (poster)

S Delaney, S Orford and J Timmis, "Functional analysis of a cotton fibre-specific gene promoter", Australian Cotton CRC Annual Scientific Meeting, Toowoomba, June 2002 (paper)

S Delaney, S Orford and J Timmis, "Development of gene promoters for cotton fibre improvement", 11th Australian Cotton Conference, Brisbane, August 2002 (poster)

S Orford, J Humphries and J Timmis, "Two WD-repeat genes from cotton can replace the function of the *TRANSPARENT TESTA GLABRA1* (*TTG1*) gene in *Matthiola incana*", the XIX International Congress of Genetics, Melbourne, Australia, July 2003 (poster)

S Delaney, S Orford and J Timmis, "Functional analysis of a cotton fibre-specific gene promoter", the XIX International Congress of Genetics, Melbourne, Australia, July 2003 (poster)

D Lightfoot, S Orford and J Timmis, "Protection of cotton fibres", 25th Lorne Genome Conference, Victoria, Australia, February 2004 (poster)

J Humphries, S Orford and J Timmis, "Analysis of fibre initiation in cotton", 25th Lorne Genome Conference, Victoria, Australia, February 2004 (poster)

Publications:

Harmer SE, Orford SJ and Timmis JN (2002) Characterisation of six α-expansin genes in *Gossypium hirsutum* (upland cotton). *Mol. Genet. Genom.* 268: 1-9

Houben A, Orford SJ and Timmis JN (2004) *In situ* hybridisation to plant tissues. In Darby, IA (ed) "*In situ* Hybridisation Protocols", 3rd Edition, Humana Press, *accepted for publication*

Orford SJ, Humphries JA, Walker AR and Timmis JN (2004) Two WD-repeat genes from cotton are functional homologues of the *Arabidopsis thaliana TRANSPARENT TESTA GLABRA1* (*TTG1*) gene, *submitted to Plant Molecular Biology*

*Orford SJ, Malone KM, Lightfoot DJ and Timmis JN (2004) Evidence for alternative splicing of MADS-box transcripts in developing cotton fibre cells of *Gossypium hirsutum* (upland cotton), *in preparation*

*Orford SJ, Press L, Llewellyn DJ and Timmis JN (2004) Towards functional characterisation of members of the homeobox gene family in *Gossypium hirsutum* (upland cotton), *in preparation*

*Significant progress has been made on the papers in preparation and they will be submitted to the appropriate scientific journals as they are completed.

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

11. 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.

The aim of this project is to develop the molecular tools required for genetic improvement of cotton fibre properties. The research has two main components, namely:

1. Characterisation and evaluation of genes with significant roles in the determination of cotton fibre characteristics, and;
2. Assessment of a bank of cotton gene promoters for their ability to drive fibre-specific gene expression in transgenic cotton plants.

We therefore expect the project to provide novel, partially characterised germplasm for use in cotton breeding programs aimed at improving cotton fibre properties, and a valuable bank of molecular tools which would allow expression of any gene in a defined manner in cotton fibre cells. These resources will lower the cost and time required for producing cotton varieties with improved fibre characteristics, the commercial cultivation of which will ensure the profitability and international competitiveness of the Australian 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.

CRDC Project UA7C:
GENETIC MANIPULATION OF FIBRE QUALITY IN AUSTRALIAN COTTON

Genetic engineering to confer useful agronomic and fibre traits will lower the cost and time required for producing improved cotton varieties and will promote environmentally-friendly farm practices. Genetic improvement of cotton fibre morphology requires both useful genes and appropriate expression of the genes in cotton fibres. Previous CRDC-funded research in our laboratory has aimed to address both these requisites, concentrating on genes which are expressed in fibres but not in other cotton tissues.

We have identified six different 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, in transient assays, shown to direct reporter gene expression that was confined to the fibres. The six promoter::GUS constructs were then used to transform whole cotton plants and a large number of transgenic lines were recovered. These have been tested for presence of the transgene and T2 seed collected. Future work involves quantitative GUS assays on fibre extracts in order to confirm the fibre-specificity of each promoter and to determine the temporal expression pattern and relative strength of each promoter in cotton fibres of T2 plants.

The second aim of this project was to identify which of our candidate genes have potential for alteration of fibre characteristics, by preparing gene constructs designed to change their expression and testing their effects in whole-plant transformants. The most promising of our genes encodes an expansin, a protein thought to control plant cell growth by chemical modification of cell wall components. Expansins could therefore play a critical role in determination of fibre quality and yield. Four constructs were made, in which the expansin gene was placed under the control of four different promoters, designed to alter native expansin expression. These promoters were available from our bank of fibre-specific gene promoters. The gene constructs were used to transform whole cotton plants and a large number of transformed lines were recovered. These have been tested for presence of the transgene and T2 seed collected. T2 plants will be tested for the effects of the transgene on fibre properties such as length, strength, micronaire, uniformity and maturity.

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