

REPORTS

Part 1 - Summary Details

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

CRDC Project Number: **CSP118C**

Annual Report: Due 30-Sep-03

Progress Report: Due 29-Jan-03

DRAFT Final Report: Due 30-Sep-03

(or within 3 months of completion of project)

Project Title: Manipulating Genes to Enhance Cotton Fibre
Elongation and Cellulose Synthesis

Project Commencement Date: 9/2000 **Project Completion Date:** 9/2003

Research Program: Plant Breeding and Biotechnology

Part 2 – Contact Details

Administrator: (Name & position of officer responsible for all correspondence).

Organisation: (Organisation administering the research project).

Postal Address:

Ph: **Fx:** **E-mail:**

Principal Researcher: Yong-Ling Ruan

Organisation: CSIRO Plant Industry

Postal Address: GPO Box 1600 Canberra ACT 2601

Ph: (02)62464909 **Fx:** 62465000 **E-mail:** yong-ling.ruan@csiro.au

Supervisor: Robert T. Furbank

Organisation: CSIRO Plant Industry

Postal Address: GPO Box 1600 Canberra ACT 2601

Ph: (02)62465149 **Fx:** 62465000 **E-mail:** robert.furbank@csiro.au

Researcher 2 Danny Llewellyn.

Organisation: CSIRO Plant Industry

Postal Address: GPO Box 1600 Canberra ACT 2601

Ph: (02) 62465470 **Fx:** 62465000 **E-mail:** danny.llewellyn@csiro.au

Signature of Research Provider Representative: _____

Part 3.3 – Final Reports

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

1. Outline the background to the project.

The major fibre quality / yield problem faced by the cotton industry is the variability in fibre length, strength and uniformity. This problem is largely due to the insufficient elongation of the lint fibre to the required length, the occurrence of the fuzz fibre and the inefficient/ or uneven deposition of cellulose synthesis in the cell wall of the fibres. Understanding the mechanisms of fibre elongation and carbon partitioning to cellulose is the key to design approaches to solve this problem. To achieve this goal, we have focused on the following central issues by using multi- disciplinary approaches.

Firstly, we have studied the molecular and cellular basis of fibre elongation by focusing on the role of candidate genes involved in uptake, utilization and accumulation of osmotically active solutes, namely, sugars and K^+ in the elongating fibres. This included the role of sucrose synthase (SuSy) and transporter genes for uptake of sucrose and K^+ , i.e. SUT and KT, respectively. Secondly, the role of the opening and closing of the plasmodesmata (PD) at the fibre base in controlling fibre elongation was examined and candidate genes putatively controlling this “gating” of plasmodesmata were identified. Thirdly, we have examined the role of sucrose synthase in providing carbon for cellulose biosynthesis in cotton fibres during both primary and secondary cell wall synthesis.

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

Project Objectives:

- ❖ To clone Sut 1 and K^+ transporter cDNAs from cotton;
Both SUT and K^+ porters were cloned and expression analysis carried out.
- ❖ To analyze transgenic cotton plants transformed with constitutive-*SuSy* suppression construct;
Effects of SuSy suppression on fibre development in transgenic plants examined; fibre elongation and cellulose deposition reduced. Plant Cell paper published 2003. Full patent lodged on manipulation of SuSy in cotton.
- ❖ To transform cotton with embryo-specific *SuSy* suppression constructs;
 $\Delta 12$ desaturase promoter-*SuSy* suppression transgenics regenerated. Plants with reduced embryo SuSy had impaired embryo development.
- ❖ To investigate the opening/closing of plasmodesmata in fuzz and lint.
Lint and fuzz fibre development and associated plasmodesmatal behaviour was examined and timing of SuSy expression was also examined. Genotype comparisons were also made.
- ❖ To make constitutive fibre-specific over-expression constructs for *SuSy* and Sut1.
These constructs were made, plants have been regenerated and are under analysis in the T0 generation.

3. Detail the methodology and justify the methodology used.

Our strategy in this work has been to study cotton fibre development and determine the key biochemical and molecular events leading to the 3 stages of fibre formation : 1. initiation, 2. elongation and 3. secondary cell wall cellulose synthesis. Once the key processes have been characterised, we set about determining which genes are likely to control these

developmental stages, focusing on the key industry traits of fibre length and cellulose content. We then used transgenic plants with reverse genetics to validate the importance of these “candidate” genes and produced transgenic plants with increased levels of key proteins to attempt to improve fibre length and cellulose content. This approach has provided us with a detailed picture of fibre development and identified several candidate genes involved in sucrose metabolism (SuSy) and transport of solutes across membranes (SUT1 and K⁺ transporters) and through plasmodesmata (callose synthesis and degradation).

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

Sucrose synthase Suppression and Overexpression in Fibres and Seeds

SuSy expression levels were reduced in transgenic cotton using both a constitutive and an embryo-specific construct. In the former case, it was determined that SuSy activity was rate limiting for early fibre elongation and that fibre cellulose content was also very dependent on SuSy activity. These data validated our approach to increase fibre SuSy for improved fibre properties. Fibre-specific and constitutive SuSy overexpression constructs have been transformed into cotton and the T0 plants are currently under investigation. Expression levels in some lines have been increased by ~ 60% in young ovules. Analysis of the effects on fibre properties will not be possible until the homozygous T2 seeds are produced. Selected high expressing lines will be examined at the three key stages of fibre development defined above. Fibre number and properties (such as length, mass, cellulose and solute content) will be measured. We would expect high level SuSy expression to increase fibre length. From these measurements the utility of this gene for fibre improvement will be determined.

Suppression of SuSy in the embryo had a strong deleterious effect on embryo development. A reduction in SuSy of around 30% caused a strong reduction in embryo growth and often abortion. It has so far not been possible to obtain plants with small reductions in embryo SuSy which had improved fibre growth.

4.2 Solute Transport and Fibre Elongation

We have shown that a coordinated expression of solute transporters (SUT & KT) and closing of plasmodesmata could be required for the rapid elongation phase of fibre development. We have shown inter-specific differences in the duration of closure of plasmodesmata which correlate with fibre length. We have developed a model for how fibre plasmodesmata are closed and then re-opened and several candidate genes controlling this process have been cloned. In a future project, these genes putatively involved in plasmodesmatal control and the sucrose transporter (SUT) will be manipulated and studied in transgenic cotton. SUT has been overexpressed in cotton and plants are growing in the glasshouse.

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

In this project we have characterised the molecular and biochemical processes controlling fibre elongation and identified several gene targets for improving this process. We have shown that 2 key processes are important in controlling fibre growth. 1. Sucrose metabolism in the fibre and 2. Solute transport into the fibre (see Fig 1). Using transgenic cotton we have shown that early in fibre growth, an enzyme of sucrose metabolism (sucrose synthase; SuSy) limits fibre elongation. Also, in fuzz fibre, SuSy activity is low and delayed. Later in elongation, the movement of solutes into the fibre control fibre elongation. We have shown that solute (sucrose and potassium) transporters may play a key role in this process and that the pores (plasmodesmata or PD) between the fibre and the seed coat must close to allow the

fibre to inflate with solutes pumped into the cell and expand to full length. Importantly, we have shown that in short fibre cultivars and in fuzz fibres, this process of PD closure is not optimal. The longer the period of PD closure, the more the fibre expands. We have identified candidate genes which could be manipulated to improve fibre length through the control of this process. We have also demonstrated the near rate limiting role of SuSy in the supply of carbon to cellulose and produced transgenic plants with increased fibre SuSy.

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

This work uses biotechnology to develop strategies for cultivar improvement in the cotton industry addressing key quality issues. The economic benefits of this are improved cultivars with more consistent and higher fibre quality. The environmental benefit may be that such cultivars would produce a higher quality product less sensitive to environmental variables such as water supply.

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

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

This work has resulted in a full patent application on the manipulation of SuSy for improved fibre elongation and cellulose biosynthesis ("Modification of sucrose synthase gene expression in plant tissue and uses therefor" : Ruan, Y-L, Llewellyn, D. and Furbank, R.T.) and recently a provisional patent application on manipulating plasmodesmatal transport of solutes to control fibre elongation.

ii.) Other information developed from research (eg discoveries in methodology, equipment design, etc.) None.

iii.) Are changes to the Intellectual Property register required? Yes. See (i).

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

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

A proposal was submitted to CRDC in 2003 to extend this work and exploit the transgenic lines produced in the current project. This proposal was not funded. CSIRO has secured support from our other commercial partners to continue the project.

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

The current work is being written up in the form of two papers which will be submitted in the next 3 months. Dr Furbank has presented the work at two international conferences in the past 12 months as has Dr Ruan.

(c) for future research.

Another CRDC proposal will be submitted in 2004 to further exploit this research.

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

Ruan, Y-L., Llewellyn, D.J. and Furbank R.T. (2000) Pathway and control of sucrose import into initiating cotton fibre cells. *Australian Journal of Plant Physiology* 27: 795-800.

Ruan, Y-L., Llewellyn, D.J. and Furbank, R.T. (2001) The control of cotton fibre elongation by developmentally reversible gating of plasmodesmata and co-ordinated expression of sucrose and K⁺ transporters and expansin. *Plant Cell* 13: 47-60.

Ruan, Y-L, Llewellyn, D.J. and Furbank, R.T. (2003) Suppression of sucrose synthase gene expression represses cotton fibre initiation, elongation and development. *Plant Cell* 15: 952-964.

Ruan, Y-L, Llewellyn, D.J., White, R. and Furbank, R.T. (2003) Plasma-membrane association of sucrose synthase is essential for secondary cell wall cellulose synthesis. (in preparation).

Ruan, Y-L, White, R. Xu, S.-M. and Furbank, R.T. (2003) The role of plasmodesmatal gating in cotton fibre elongation. (in preparation).

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.

The results from this project provide a mechanistic view of fibre development in cotton from initiation to cellulose synthesis. This developmental characterisation has to date not been available, making the elucidation of genes controlling cotton fibre development difficult to achieve. The benefits of this research will be primarily through further research on the roles of the candidate genes identified here in controlling fibre quality in cotton. This will become more apparent when characterisation of the transgenic plants produced in this project is complete and the genes cloned can be used as markers or transgenes in cotton breeding. As this work is not “near market” it is difficult to produce a cost / benefit analysis on the CRDC investment in this project.

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.

Cotton fibres are the fastest growing and among the longest single cells in the plant kingdom. In the space of about 16 days, these single cells can expand from a few micrometers to 3 cm in length. When the fibres stop growing they then thicken with cellulose, becoming over 90% cellulose by the end of maturation. As long fibres are important for cotton fibre quality, we set out to study cotton fibre elongation using biochemistry and molecular genetics. We identified several potential gene targets for improving this process of elongation. We have shown that 2 key processes are important in controlling fibre growth. 1. Sucrose metabolism in the fibre and 2. Solute transport into the fibre. Using transgenic cotton we have shown that

early in fibre growth, an enzyme of sucrose metabolism (sucrose synthase; SuSy) limits fibre elongation. Also, in fuzz fibre, SuSy activity is low and delayed. Later in elongation, the movement of solutes into the fibre control fibre elongation. The fibre must inflate analogous to a filling balloon. We have shown that solute (sucrose and potassium) transporters play a key role in this process and that the pores (plasmodesmata or PD) between the fibre and the seed coat must close to allow the fibre to inflate with solutes pumped into the cell and expand to full length. Importantly, we have shown that in short fibre cultivars and in fuzz fibres, the timing of this process of PD closure is not optimal. The longer the period of PD closure, the more the fibre expands. We have identified candidate genes which could be manipulated to improve fibre length through the control of this process. We have also demonstrated that during cell wall thickening the enzyme SuSy also plays a key role in supplying sugars for cellulose biosynthesis and that a small decrease in the level of this enzyme has a large negative effect on fibre cellulose content. We have produced transgenic cotton plants with increased seed SuSy and will analyse fibre properties in these plants. We hope to use the genes we have identified to produce cultivars with longer fibres and more consistent cellulose content.