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Final Report

Part 1 - Summary Project Details **REPORTS**

Please use your TAB key to complete parts1,2,4 & 5

CRDC Project Number: **CSP87C**

January Report:	☐	Due 29-Jan-01
August Report:	☐	Due 03-Aug-01
Final Report:	☒	Due within 3 months of project completion

Project Title: Molecular Control of Photoassimilate Import into Developing Cotton Fibre

Part 2 – Project Contact Details

Administrative contact: Ms Di Rosson
Organisation: CSIRO Plant Industry
Postal Address: GPO Box 1600, CANBERRA ACT 2601
Ph: 02 6246 5277 **Fx:** 02 6246 5000 **E-mail:** d.rosson@pi.csiro.au

Principal Researcher: Dr Yong-Ling Ruan
Organisation: **CSIRO Plant Industry**
Postal Address: GPO Box 1600, CANBERRA ACT 2601
Ph: 02 6246 4909 **Fx:** 02 6246 5000 **E-mail:** ruan@pi.csiro.au

Supervisor: Dr Bob Furbank
Organisation: **CSIRO Plant Industry**
Postal Address: GPO Box 1600, CANBERRA ACT 2601
Ph: 02 6246 5149 **Fx:** 02 6246 5000 **E-mail:** furbank@pi.csiro.au

Part 3 – Final Report Format

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

Background to the project

The fibre length and cellulose content are the two determinates of fibre yield and quality. The rapid elongation process and massive cellulose biosynthesis in fibre cells must depend on sufficient supply of photoassimilate. Sucrose is the predominant form of photoassimilate imported to fibres for cellulose synthesis and for generating turgor pressure to drive the fibre elongation. Sucrose and other solutes may move into fibres either symplastically through plasmodesmata or apoplastically via the plasma membrane of the fibre cells. The cleavage of sucrose by sucrose synthase (SuSy) in the fibre generates UDPglucose, the immediate substrate for fibre cellulose biosynthesis. In developing cotton seeds, unloaded sucrose is utilized in diverse pathways for cellulose, starch and lipid biosynthesis by the fibre, seed coat and cotyledons, respectively. The strong competition by the latter two sink tissues could limit the availability of photoassimilate for cellulose biosynthesis in the fibre, and hence reduce the fibre yield. Previous studies sugget that SuSy expression in fibres is important for mobilising sucrose into this tissue. However, conclusive evidence is lacking regarding the role of SuSy in fibre development. Little is known on the cellular pathway of import of sucrose and other solutes into the developing cotton fibre. A clear understanding of the pathway and regulation of sucrose import into fibres is essential for designing appropriate molecular approaches to enhance photoassimilate mobilization to this biosyntheticly active sink tissue for elongation and cellulose synthesis.

The objectives and the extent to which these have been achieved

Objective 1: Understanding the cellular pathway of carbon import into fibres
We have throughly analysed the the cellular pathway of sucrose as well as K+ import into fibres.
We have also cloned two partial cDNAs for sucrose and K+ transporters and analysed their expression pattern. In addition, we observed a structural change in fibre plasmodesmata which could have important implications for controlling fibre elongation. This work is now in press in THE PLANT CELL.

Objectives 2 and 3: Control of C partitioning in cotton seed and genetic manipulation. We have provided cellular and biochemical evidence that SuSy is a key enzyme to mobilise sucrose into initiating fibres. Moreover, by using reverse genetic approaches, we demonstrated that expression of SuSy is essential for fibre cell initiation and further development. To enhance sucrose flow to fibres, we have also conducted experiments to reduce C partitioning to embryos by transforming cotton with SuSy suppression constructs driven by an embryo-specific promoter. Regeneration of these transgenetic plants is under way. Two papers have been published and one provisional patent has been lodged.

The methodology and a justification for the methodology used
A combination of molecular, cellular and biochemical methodology has been used in this study.
Using diverse but integrated approaches is essential for exploring complex issues such as the the basis of fibre elongation and the control of C partitioning to cellulose synthesis (see following for more details).

Detailed results including the statistical analysis of results

Objectives 1 : Carbon import to fibre

To explore the pathway and mechanism controlling solute import into and elongation of the cotton fibre, we have studied the gating of plasmodesmata (PD) with possible co-ordinated expression of sucrose and K+ transporter genes in fibres. Confocal imaging of a membrane-impermeable fluorescent molecule, 5(6)-carboxyfluorescein together with osmotic potential measurement and plasmolysis analysis demonstrates that fibre PD are open for solute import at the initiation / early phase of elongation, ie. 0 - 6 days after anthesis (DAA) and also during the secondary cell wall cellulose biosynthesis period (16 to 30 DAA). However, a transient closure of PD is evident at the later phase of elongation (~10 DAA). A developmental switch from simple to branched plasmodesmata was also observed in fibres at 10 DAA and onwards. Interestingly, the closure of fibre plasmodesmata (~ 10 DAA) precedes the elevation of fibre turgor, suggesting the former causes, at least partially, rather than responds to, the turgor increment.

The closure of fibre PD at ~10 DAA (see above) would necessitate solute import across the plasma membrane of fibres interconnecting the underling seed coat cells. Using a partial sucrose transporter and a K+ transporter cDNA probes we isolated from cotton, we have shown that both sucrose and K+ transporter genes are expressed at their highest level at ~ 10 DAA, coinciding with the closure of plasmodesmata. The cell wall loosening gene, expansin, however, is expressed highly at the early phase of elongation (~6 to 12 DAA). Together, the results suggest that (i) the initial fibre elongation is largely achieved by cell wall loosening; (ii) the closure of PD and the coordinated expression of the sucrose and K+ transporter at ~ 10 DAA cause the establishment of higher turgor which drives the rapid fibre elongation; (iii) the re-opening of PD and diminished expression of the transporters at ~ 16 DAA lead to the loss of high turgor in the fibres, thus terminating the elongation process and allowing secondary cell wall thickening to occur.

To the best of our knowledge, this is the first demonstration that the gating of PD in a given cell is developmentally reversible and highly coordinated with the expression of membrane transporters and cell wall expansion. The novelty and quality of this work has been evaluated very highly by peer scientists. The work has been accepted for publication by THE PLANT CELL, a leading journal in plant science. The discovery has also established a basis for further studies to ultimately genetically engineer fibre length, a key determinant of fibre yield and quality.

Objectives 2 and 3: C partitioning in cotton seed and genetic manipulation

Our previous studies has identified sucrose Synthase (SuSy) to be the key enzyme to mobilize sucrose into cotton fibres. This notion is now further strengthened by our recent results that (i) sucrose transporter protein was immunologically undetectable in fibre cell initials, but SuSy was highly expressed and (ii) sucrose moves into fibre symplastically at the initiation and early phase of elongation as well as during the secondary cell wall cellulose synthesis period. Down-regulation of SuSy in the fibre using antisense or co-suppression approaches could yield unequivocal evidence regarding the role of SuSy in fibre development. To achieve this, we have made sense and antisense gene constructs using the 3' region of the cotton SuSy cDNA driven by the subclover stunt virus segment 7 promoter, which is expressed throughout the plant but very highly in developing fibres. Our recent analysis of T1 seeds from 12 transgenic lines demonstrates that SuSy indeed plays a crucial role in fibre development as the level of SuSy suppression in cotton seeds correlates well with the degree of fibre inhibition in those lines.

In developing cotton seed, the phloem-unloaded sucrose is not only partitioned to fibres for cellulose biosynthesis but also mobilised to embryos for protein and lipid biosynthesis by SuSy expressed in cotyledons. Fibre competes poorly with the embryo for C, particularly under sub-optimum conditions when plants are stressed. To enhance sucrose flow to fibres for cellulose synthesis, we are attempting to reduce C partitioning to embryos through reducing SuSy expression in this tissue. To achieve this, we have evaluated several seed/ embryo specific promoters and concluded that the cotton seed delta-12 desaturase promoter is the suitable one as it activates specifically in embryo during the period of fibre elongation and cellulose synthesis. Using this promoter we have now made inverted repeat and sense repeat SuSy suppression constructs to reduce SuSy expression specifically in the embryo. These constructs have been transformed into cotton and regeneration of the transgenic cotton is under way. These plants will be analysed as part of our new CRDC project.

Discussion of results including an analysis of research outcomes compared with objectives

We have elucidated the cellular pathway for photoassimilate import into fibres and identified the closure of fibre plasmodesmata and co-ordinated expression of sucrose and K+ transporters as the controlling points for fibre elongation. We have now obtained direct evidence that SuSy gene expression is critical for fibre initiation and subsequent development.
These are all novel discoveries which are now recognised by both the international science community and industry (see publication and patent list). Overall, we have achieved the objectives in this project with several additional discoveries such as the cloning and expression of a K+ transporter cDNA.

Assessment of the likely impact of the results and conclusions of the research project for thecotton industry. Where possible include a statement of the costs and potential benefits to the Australian Cotton industry and future research needs
The results obtained from this research (see above) provide much needed information for identifying traget genes for genetic engineering to improve fibre quality and yield. More specifically, the results show that sucrose and K+ transporters are potential targets for engineering to enhance fibre elongation, while enhancing SuSy gene expression in fibres could enhance fibre cell initiation and

cellulose synthesis. Down-regulation of SuSy in embryo may have a similar effect, i.e. enhancing sucrose flow to fibres at the expense of the oil and starch produced in the embryo.

Description of the project technology (e.g. commercially significant developments, patents applied for or granted, licenses, etc).

Analyses of transgenic cotton transformed with SuSy suppression constructs driven by subclover stunt virus segment 7 promoter have shown SuSy not only plays a critial role in fibre development but also important in pollen and embryo developemt. This discovery has wide industry implications including enhancing fibre development, maintaining / or removing male sterility and producing seedless fruit etc. A provisional patent entitled "Modification of sucrose synthase gene expression in plant tissue and uses therefor" has been submitted to both Australia and USA.

A technical summary of any other information developed as a part of the research project including discoveries in methodology, equipment design, etc.

Recommendations on the activities or other steps the may be taken to further develop, disseminate, or to Exploit the Project Technology
These experiments have set the framework for targetted manipulation of developmental and physiological process that determine both quality and yield characteristics of cotton fibres and these will be investigated in the new CRDC funded project.

A list of publications arising from the research project

Publications

(1) Ruan, Y-L. Llewellyn, D.J.and Furbank, R.T . (2001) The control of single-celled cotton fiber elongation by developmentally reversible gating of plasmodesmata and coordinated expression of sucrose and K+ transporters and expansin. Plant Cell 13 (1) in press.

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

(3) Ruan, Y-L., Furbank, R.T. and Llewellyn, D.J. (1998) Towards genetic engineering of sucrose synthase to enhance fibre cell initiation and cellulose biosynthesis in cotton. In: Cotton Covering Our Future, Proceedings of 9th Australian Cotton Conference, (Gold Coast, August 1998) pp. 751-755.

Patent

Modification of sucrose synthase gene expression in plant tissue and uses therefo. This patent application has been lodged both in Australia and USA in December 2000.

Part 4 – Final Report Plain English Summary

A plain English summary not exceeding 200 words

In this project, we have used different molecular and physiological approaches (I) to understand how the cotton plant controls the flow of energy (as sugar) into developing fibres and (II) to identify the key genes that might control fibre cell elongation and the partitioning of sugar to cellulose synthesis. We found that sucrose, the major photoassimilate, moves into fibres through plasmodesmata (pores at the base of the fibre that control nutrient import), except for the period of 10 to 16 days after anthesis (DAA). During this most rapid elongation stage, the fibre plasmodesmata are closed and sucrose as well as K+ moves into fibres by their respective cell membrane transporters that actively pump these moleculaes into the expanding fibre cell. This closure of the pores and the accelerated import of the solutes by their transporters generates the higher turgor pressure seen inside the fibres, thus driving the rapid elongation process. We have also identified sucrose synthase (SuSy) to be the key enzyme to mobilize phloem-unloaded sucrose into fibers for their initiation and for cellulose synthesis. Indeed, by a "knock down" of SuSy gene expression using genetic engineering approaches, we achieved, depending on gene dosage in the embryo, a fibre-less phenotype early in development and thinner/ short fibres at maturation. This provides direct evidence that SuSy does play a crucial role in fibre development. The above discoveries established a solid basis for the newly-funded CRDC project -" Manipulating genes to enhance fibre elongation and cellulose synthesis" where we will attempt to enhance fibre properties by manipulating the expression of key genes like SuSy.