January, August & Final Reports

REPORTS

Part 1 - Summary Details

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

CRDC Project Number:CSP97C

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

Project Title: Cotton Biotechnology: Core Program

Project Commencement Date: July 1998 **Project Completion Date:** June 2001

Research Program: Plant Breeding and Biotechnology

Part 2 – Contact Details

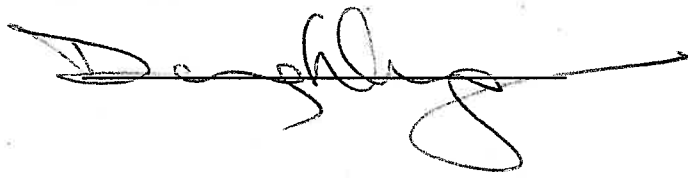
Administrator: Dianne Rosson
Organisation: CSIRO Plant Industry
Postal Address: P.O. Box 1600 Canberra City ACT 2601
Ph: 02-62465277 **Fx:** 02-62465000 **E-mail:** D.Rosson@pi.csiro.au

Principal Researcher: Dr Danny LLEWELLYN
Organisation: CSIRO Plant Industry
Postal Address: P.O. Box 1600 Canberra City ACT 2601
Ph: 02-62465470 **Fx:** 02-62465000 **E-mail:** D.Llewellyn@pi.csiro.au

Supervisor: As above
Organisation:
Postal Address:
Ph: **Fx:** **E-mail:**

Researcher 2 None
Organisation:
Postal Address:
Ph: **Fx:** **E-mail:**

Signature of Research Provider Representative:



Part 3 – Final Report Format

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

1. Outline the background to the project.

Genetic engineering techniques allow the transfer of novel genetic material from one organism to another and hence has the potential to augment classical plant breeding techniques by extending the gene pool accessible for crop improvement. We have established a program of research and development aimed at using this new technology to improve the performance of Australian cotton cultivars under our intensive production systems. The major limitations to cotton production in Australia, other than the availability of water, is competition from other organisms, be they insect pests (such as Helicoverpa larvae), weeds or fungal pathogens (such as the wilt pathogens Verticillium and Fusarium). The first generations of genetically engineered cotton plants will inevitably be aimed at minimising the impacts of these other organisms, while maintaining and improving the level and quality of Australian cotton production. Already transgenic cotton (INGARD and Roundup Ready varieties) coming through this program are making a major impact on pesticide usage and the weed control options available to the Industry. In the future, as our understanding of plant growth and development expands, other targets for genetic engineering such as improvements in quality or plant physiology will become possible.

The techniques of genetic engineering are now quite well established. A gene from a donor organism is identified with potential to improve crop performance, say through the production of a protein toxic to insect pests; the gene is isolated and characterised and if necessary restructured for correct expression in plants; the gene along with a selectable marker gene, often conferring tolerance to an antibiotic toxic to plants, is then introduced (transformed) into the recipient (cotton) using one of a variety of methods often involving the natural gene transfer organism *Agrobacterium tumefaciens* and a process of plant tissue culture and selection on the toxic antibiotic; and finally the regeneration of whole plants, all of whose cells contain the introduced gene and the selectable marker gene. Both genes (new transgenic trait and selectable marker) are incorporated into the cotton genome and are inherited in a Mendelian manner from one generation to the next. Since the cotton varities that can be transformed are often very poor agronomically, extensive back-crossing and selection is then required to incorporate the new transgenic trait into elite cotton varieties for commercial use. Cotton transformation is routine in our laboratory and we have a number of technical staff highly skilled in all the processes needed to develop transgenic cotton plants through to commercial cultivars.

Over the past few years our research team has played a pivotal role in the introduction of genetic engineering technologies to the Australian cotton industry and this is an on going process as both existing transgenic traits and new traits need to be incorporated into newly developed cultivars. Through our research collaborations with the Monsanto Company we have been able to access insect and herbicide tolerance genes (eg. INGARD and Roundup Ready) that are now having a major impact on cotton production in Australia. The aim of this project has been to develop and maintain the basic technology and expertise to produce new cotton cultivars using genetic engineering. In particular, to use the currently available molecular and tissue culture skills to produce herbicide tolerant, insect tolerant and disease tolerant cotton plants by the introduction of novel genes from other organisms. It complements and extends the more traditional cotton breeding program (CSP96C) by providing access to molecular biology and laboratory skills necessary for the breeding of transgenic cotton varieties.

This project has involved the support of three highly skilled technical staff who carry out the basic gene transfer and molecular screening techniques being used to introduce new genes into Australian cultivars of cotton. The two most prominent projects are the incorporation of the two BT-toxin genes (INGARD and Bollgard II, developed by Monsanto) that will confer protection against *Heliothis* species and herbicide tolerance genes (developed by Monsanto and Aventis) that will confer resistance to herbicides like glyphosate, bromoxynil, and Basta, respectively. As the scale of the transgenic breeding program has increased during the life of the project, a large proportion of the time of the staff is involved in routine screening and quality control work for the transgenic breeding program using molecular techniques, however, the genetic engineering technical team continues to make significant contributions to the development of new transgenic germplasm, such as disease tolerant cotton varieties.

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

In each year of the project:

- Screen leaves from at least 10000 F1 progeny of Bt cotton lines (both single and double Bt lines) in October-Dec from field plantings at Narrabri.
- Screen over 100,000 F2 seeds in April-July to identify homozygous lines for later progeny assessment by the breeders.

- Screen at least 10,000 leaf samples from material undergoing seed increase at Kununurra or in Eastern Australia to ensure high purity of the transgenic traits.
- Organise regulatory approvals (IBC,GMAC, NRA) for all summer and winter trials to be carried out by breeding program and CSD.
- Carryout purity testing of all CSIRO varieties being seed increased by CSIRO breeders and CSD as required.
- Generate at least 100 new transgenic cotton lines for cotton projects on disease tolerance, waterlogging tolerance, insect tolerance or altered fibre.
- Analyse transgenics by Southern blotting, Northern blotting and Western blotting where appropriate and analyse the segregation and copy number in T1 progeny plants.
- Carryout or participate in the appropriate bioassays to assess efficacy of the introduced transgenes. Transfer relevant material to breeders, entomologists and weed ecologists in Narrabri for appropriate field evaluation.
- Assist with the cloning and characterisation of new genes from cotton involved in waterlogging tolerance, the synthesis of terpenoid defence chemicals and genes involved in fibre development.

All of these objectives have been met during the course of the project as outlined in the detailed results below. Each year the amount of screening for the breeding program has increased but we have been able to complete the work through hiring casual staff to assist with the more routine aspects of the work. Quality control issues have become increasingly important as the size of the breeding program increases there is more potenial for mixing of lines or even different transgenic traits and through testing is critical before any of the material is transferred to the seed company or goes into commercial production. We expect this type of Quality Control work to increase as there is always the potential for transgenic and non-transgenic lines to become contaminated either through cross-pollination in the field or more likely by physical contamination and it is essential that CSIRO maintain a high degree of purity in both it transgenic and conventional breeding lines.

3. How has your research addressed the Corporations three outputs: Sustainability, profitability and international competitiveness, and/or people and community?

This project addresses the issues of sustainability of the cotton industry through the production of transgenic cotton that give the grower enhanced insect and weed control options that are environmentally sustainable. It has already resulted in the release of INGARD varieties and Roundup Ready varieties that are proving themselves in the industry to have good environmental benefits. By the end of the grant nine different transgenic varieties were released by Cotton Seed Distributors – these were Sicala V3i, Siokra V16i, Sicot 289i, Sicot 51i, Siokra 201i, Siokra S-101i, Sicala V3RRi, Sicot189RR and Sicala V-2RR. All of these had gone through the screening program in Canberra early in the project and were tested for purity any any unintended events to allow their commercial sale. Much of the work during the grant has been for the screening of the new two gene (Bollgard II) varieties that should significantly increase the level of pest control provided by transgenic cotton and allow substantial reductions in pesticide usage throughout the Industry.

4. Detail the methodology and justify the methodology used.

The main components of the methodology of the project are the screening ofor the presence of genes in breeding material using ELISA assays and other biochemical means, the generation of transgenic cotton plants with novel traits and the backcrossing of transgenic plants to elite Australian cotton cultivars. The methodology being used is dependent on the availability of genes which in many cases must be licensed from external partners such as Monsanto and our freedom to operate in the context of international patents on biotechnology. These patents mean that it is difficult for CSIRO to act independently in this area and where appropriate we have made alliances with various multi-national companies to access core technologies that will allow Australian seed companies like CSD to use the products of our research under commercial licenses with those patent holders and to assist CSIRO in protecting any new inventions. Backcross breeding is necessary because few of the Australian cultivars can be transformed directly with novel transgenes.

Increasingly we are using DNA technologies to analyse breeding material and this is primarily using the technique of Polmerase Chain Reaction (or PCR) that allows specific sequences to be amplified out of genomic cotton DNA. We are using this technique to screen seed increase lines for the presence of so-called unintended events – particular transformed lines that have been discontinued or replaced in the breeding program eg. the 757 INGARD event is being replaced with the

more effective 531 event, but because of the large amount of the old INGARD line being grown throughout the cotton Industry there is always the potential for contamination of the 531 INGARD lines with pollen or seed from 757INGARD. Similarly CSIRO had progressed the double Bt lines containing the Cry2Aa (P2) to a very advanced stage when the P2 gene was withdrawn by Monsanto. All material that was in the breeding pipeline at that time must now be tested to ensure that it is not contaminated with the P2 gene is this is a product that will never be registered for commercial use. Similarly a number of different Roundup Ready events were in the breeding program before a single event was chosen for commecialisation by Monsanto – all RR lines must be checked for contamination with this unregistered event. PCR testing can also be used to determine the zygosity of a particular plant ie. A plant with a transgenic trait could either be homozygous or hemizygous for a particular transgene and PCR testing can help us identify which are homozygous and hence true breeding for a trait rather than testing in the next generation for absence of segregation of the trait. Monsanto has developed all of these PCR tests but we are adapting them to the high-throughput procedures need to screen large populations in our breeding programs.

5. Detail results including the statistical analysis of results.

This Core Biotechnology project is a complementary component of CSIRO's Cotton Breeding Program (funded through CSP96C) and has two main objectives, both of which contribute towards the development of new cultivars for the Australian Cotton Industry. In the first, we provide the direct molecular biology support for the transgenic component of the cotton breeding program in Narrabri that allows them to identify and track transgenes in their breeding populations, and in the second we develop new transgenic germplasm that may have novel traits that could enhance our conventional breeding program. Both of these objectives have been met over the past three years.

The first objective involves screening plants and seeds for the presence of introduced genes using various laboratory techniques, but particularly ELISA assays and more recently a histochemical assay for the GUS gene which is a marker gene linked to the new Cry2Ab gene in the Bollgard II cotton (Bollgard II is the 531 INGARD cotton retransformed with a 35Scry2Ab and 35SGUS gene using particle bombardment). This occurs primarily at two stages with the screening of F1 plants in the field during October to January to identify plants containing insecticidal genes like the INGARD or the Cry2Ab genes. Plants containing the Roundup Ready herbicide tolerance are detected by spraying the plants in the field with Roundup. The positive F2 plants are then re-screened at the seed stage in March to July to identify plants homozygous for one or more genes by assaying F3 seeds from those plants. In the last year, for example, 32,000 individual F2 plants segregating mostly for the two traits INGARD and Cry2Ab (and some for RR) were screened (64,000 ELISA tests) for the presence of both of the insecticidal genes to recover 20,000 individuals that were taken on to generate F3 seed. From these 20,000 plants seeds were again tested for both genes (480,000 ELISA tests) to detect 3,500 homozygous lines from about 18 different families which in the end will potentially result in one new cultivar per backcross family. Other screening of glasshouse samples occurs throughout the year as the breeding program generates new families using existing transgenic varieties and new elite conventional material. This has been an on-going process since the introduction of Monsanto transgenes into the CSIRO breeding program in the early 1990s. We are currently investigating using PCR techniques to identify homozygous lines and this may replace some of the F3 seed testing that we are currently doing.

Recent incidents in Europe and the US have highlighted the importance of Quality Control in the commercialisation of transgenic crops. In the US transgenic corn containing the Cry9C gene (StarLink corn commercialised by Aventis) was released for animal food uses only but was subsequently detected in corn chips and other products used for human consumption and this has resulted in the voluntary withdrawal of this product from the market and a massive and expecsive cleanup operation to remove it completely from the human food chain. Conventional cotton varieties destined for Greece have been found to be contaminated with transgenic seed and this has halted all foreign sales of cottonseed into Greece and other European countries. Conventional canola seed planted in England and Europe has also been found to be contaminated with transgenic seed. Contamination can occur at any stage during the breeding or seed increase of a variety and CSIRO and CSD have instituted a number of programs to test transgenic seed at various stages for both purity of the trait and for the presence of unintended events (obsolete events that have been superceded or discarded from the breeding program). This type of testing started in 2000 when Monsanto made its event-specific PCR tests for INGARD and RR unintended events available to CSIRO. The tests involved making DNA preparations from about 30,000 plants representing all the different CSIRO 531 INGARD (and RR) varieties that had been undergoing seed increase for commercial release in 2000 or 2001 and carrying out a PCR amplification and analysis on an agarose gel for each of the different possible unintended events. Each of the backcross family still had a number of potential lines that could form a new variety so testing had to be carried out on more than one line per family. About 60 lines were tested and four were found to have low level contamination with another event of the same transgenic trait – further testing indicated that most, but not all of this contamination occurred during seed increase by physical contamination with the old 757 INGARD cotton. Contamination by cross pollination has so far not been observed. One RR line was found to have been contaminated with seed of an abandoned RR event and this must have occurred during handling of seed lots during the breeding process. Contaminated

lines were either discarded or an earlier non-contaminated seed increase or a clean single plant selection used for subsequent seed increase. This first batch of testing highlighted that contamination can occur and that testing will have to be done for all future transgenic varieties. The second year of testing has just been completed and no contamination detected in 51 lines representing five different backcross families that are due for commercial release in 2001. Hightened awareness of the potential for contamination and changes to the management of trias and the physical handling of transgenic lines appears to have been effective in reducing the level of contamination of breeding lines and will ensure that CSIRO's transgenic cotton varieites will continue to have a high degree of purity and performance. The potential for contamination of conventional varieties with transgenic varieites will also have to be addressed as part of the breeding program and the seed production program. The requirement for this testing has considerably increased the workload of the team in Canberra and required an additional staff member to co-ordinate and carryout much of the QC testing.

At a regulatory level we have continued to maintain close contacts with the various Australian regulatory bodies such as GMAC and the NRA and in all cases the appropriate approvals have been gained for all of our breeding and other experimental trials in the Eastern States and in Northern Australia. CSD have gained their own relevant approvals and permits for seed increase trials in Kununurra and elsewhere. We are currently switching over to the new system of regulation through the Office of Gene Technology Regulation (OGTR) that was recently legislated and the first applications to this new body are being submitted. Already it is evident that gaining approvals is going to be more difficult and time-consuming but hopefully this will settle down as the new Office gains some experience in the process of assessing and granting approvals for field testing of transgenic plants. It is hoped that Monsanto will submit applications for General Release of Bollgard II soon so that individual application for field trials of this transgenic trait will not be required after 2003.

On the Research and Development side, the same group of technicians funded by this grant have continued to produce and analyse transgenic cotton plants for our other commercial interactions and in-house projects and have achieved all the desired objectives. Over a hundred new transgenic lines have been generated with a variety of gene constructs for both commercial and research projects. These include new insecticidal genes from Novartis, various promoters for expressing transgenes both constitutively and in response to diseases of cotton, waterlogging tolerance genes, fibre –specific promoters and genes from our own fibre biology projects and the University of Adelaide group. Although we continue to collaborate with the Adelaide group of Sharon Orford and Jeremy Timmis and are currently introducing nine different constructs into cotton, the workload to do this has expanded to the point where we have to seriously consider how we interact with Adelaide and whether we can continue generating and analysing transgenic plants for them given that our first priority must be towards the breeding program and CSIRO's own biotechnology projects. We have generated many more reporter gene containing cotton plants with various combinations of the sub-clover stunt virus promoters driving GUS and these are currently growing in the field to fully evaluate their potential in cotton to drive useful genes such as Bt genes. We have been working closely with Dr Yong-Ling Ruan to introduce some of his carbon partitioning constructs into cotton and the first trasnsgenic plants have shown interesting phenotypes and highlight the important role of the enzyme sucrose synthase in channelling photosynthetic sucrose into the fibre cell for elongation.

Continuing discussions with Aventis have indicated that they are rationalising their transgene portfolio and it now seems unlikely that the bromoxynil tolerant cotton we generated during the course of this project will have any commercial future (either here or overseas) despite their excellent performance in the field. While this is disappointing for us, we have to face the commercial realities of working with transgenic plants and genes owned by large biotechnology companies. Aventis is still pushing ahead with Basta tolerant cotton (as Liberty-Link cotton), at least in the US and possibly Australia, using transgenic events of their own, but are also considering some of the events generated by CSIRO. The work carried out with these two herbicide tolerance traits has increased our experience with the production and testing of elite events of transgenic cotton and will be shortly written up for publication.

Our transgenic cotton team continues to work with Belinda Townsend (who was awarded her Doctorate from the ANU resulting from her research as funded by CRDC (ANU3C)). New constructs introduced into cotton will be described in her separate Annual Report for her PostDoctoral Fellowship (CSP105C). We are also still working closely with Dr Helen McFadden and Dr David Jones (ANU) in the development of transgenic cotton material that may have enhanced tolerance to diseases such as Verticillium and fusarium wilts.

In conclusion, the contributions of this project to the breeding effort for transgenic cultivars continues to occupy a considerable proportion of the time of both the technical team and the Principal Investigator, but the rewards are the development of new cultivars for the Industry to use. RR cultivars were released for the first time in 2000 along with the first of the new 531 INGARD lines and many more are due for release next season. The Bollgard II varieties are on track for release in 2003 and should considerably enhance the performance of CSIRO's transgenic cotton in insect control.

6. Discuss the results, and include an analysis of research outcomes compared with objectives.

The research outcomes from this project have been considerable: 10 different transgenic cotton cultivars will be available for commercial release in 2001 including, Sicala V3i, Siokra V16i, Sicot 289i, Sicot 51i, Siokra 201i, Sicot 42i, Siokra S-101i, Sicala V3RRi, Sicot189RR and Sicala V-2RR, and many more will be available in the coming year. The double Bt lines (Bollgard II) are all on track for commercial release in 2003 and these will have a major impact on pesticide usage in the Australian cotton industry. The Quality Control programs we have instituted will ensure that all released varieties will conform to International standards for purity and will comply with the Gene Technology Act 2000 for purity of both trait and event. CSIRO has established a close working relationship with all of the relevant regulatory bodies in Australia and is now relied upon for its expertise in the area of release of transgenic crops and risk and safety assessment of transgenic plants. Our team of technicians is highly regarded for their skills in the production of transgenic cotton plants and this has allowed us to gain access to transgenic traits (often at an early experimental stage) from International biotechnology companies that might not have previously been available to Australia. Many transgenic plants are being produced and analysed in the field making Plant Industry the most prolific organisation in Australia to field test transgenic plants and gaining us considerable experience in the evaluation of transgenic plants. As yet we have not produced a new trait with clear commercial potential but the experiences have sharpened our abilities in genetic engineering and this knowledge can be put to good use with future projects and genes.

7. 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 and future research needs.

The release of the transgenic insect tolerant INGARD varieties has already had an important impact on the cotton industry in reducing chemical pesticide usage by over half on the currently legislated area allowed for transgenic cotton (30% of plantings). Continuous improvements of the varieties will however be required to enhance the performance of these initial varieties and it is likely that the existing INGARD varieties will be replaced over the next couple of years first with improved single gene varieties like Sicot 289i, and then by about 2003 with the first two gene or Bollgard II varieties. Bollgard II varieties are already proving to have considerably better insect control and are therefore likely to increase pesticide reductions over and above that now seen for INGARD. These two gene varieites will offer a more robust product in terms of management options for controlling the development of resistance to Bt-toxins and will allow the expansion of the total area planted to transgenic insect protected varieties. This could result in over a 70 % reduction in pesticide usage for the whole of the Industry and should significantly enhance the environmental impact of the Industry. The first herbicide tolerant varieties were released in 2000 (Sicala V-2RR and Sicot 189RR along with the first of the stacked traits Sicala V-3RRi) and these are going to have a siognificant impact on weed control in cotton. More of these stacked varieties are in the pipeline and will be released over the coming years. Transgenic varietal improvement needs to run parallel with conventional varietal improvement and we expect that this will be an on-going project that will have on-going benefits to the cotton industry.

The work in developing new transgenic germplasm with a variety of traits from both technology providers and CSIRO's own research is continuing to maintain our expertise in this area and developing new experimental lines that require on-going testing and evaluation. None of these has yet produced a commercially valuable trait, but trait development is a long term process and we are continuing to explore new areas of disease tolerance, waterlogging tolerance, and fibre quality improvement.

8. Describe the project technology (eg. commercially significant developments, patents applied for or granted licenses etc).

The transgenic varieites developed through this project and CSP96C are being protected by Plant Variety Rights both here and overseas.

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

None

10. Detail a plan for the activities or other steps that may be taken;

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

The project is obviously on-going and we were successful in attracting a new round of funding from the CRDC (CSP136C) to continue the molecular work surrounding CSIROs transgenic cotton breeding program. All aspects of the project are still being pursued and we are refining and honing our techniques to allow us to handle the very large numbers of samples needed to produce effective commercial transgenic cultivars. Work is continuing to produce and analyse new transgenic plants with altered fibre properties (eg., Manipulating Genes to Enhance Cotton Fibre Elongation and Cellulose Synthesis), gossypol levels (CSP105C), and disease tolerance.

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

The work carried out as part of this project has resulted in the release of new cultivars and these are presented to cotton growers through the CSD variety tour and in publications by CSD (including their new web site). The research and development findings of the project are being published in the cotton literature (eg. ACGRA Cotton Conferences) and in peer-reviewed Journals (see reference list below). Some of the information and techniques generated in the project are subject to CSIRO's confidentiality arrangements with Monsanto and must remain in-house.

11. List the publications arising from the research project.

1998

G. Charles, M. Hickman, D. Llewellyn and G. Constable (1998) Field evaluation of transgenic 2,4-D tolerant cotton. Proc. 9th Australian Cotton Conf. (Broadbeach 12-14 August, 1998) 193-201.

G. Consatble, D. Llewellyn and P. Reid Biotechnology risks and benefits: the INGARD cotton example (1998) Proc. Australian Society of Agronomists (Wagga 1998)

R. DeFeyter, H. McFadden, D. Llewellyn and E.Dennis (1998) Can bacterial blight avirulence genes be used as triggers of cotton defense responses. Proc. 9th Australian Cotton Conf. (Broadbeach 12-14 August, 1998) 595-597.

E. Finnegan, D. Llewellyn and G. Fitt (1998) What's happening to the expression of the insect protection in field grown INGARD plants? Proc. 9th Australian Cotton Conf. (Broadbeach 12-14 August, 1998) 291-297.

E. Finnegan, D.Llewellyn and G. Fitt (1998) Expression of Bt transgene in field grown cotton in Australia. Proc. 4th Asia-Pacific Conference on Agricultural Biotechnology. (Darwin 13-16 july, 1998) (P. Larkin Ed.) 225-227.

K Kazan, F. Murray, K. Goulter, D. Llewellyn and J. Manners (1998) Induction of cell death in transgenic plants expressing a fungal glucose oxidase. Molec Plant Microbe Interactions, 11: 555-562.

Llewellyn, D and Higgins, T.J. (1998) Biotechnological approaches to crop protection: novel sources of insect tolerance genes other than Bt-toxins. Proc. Applied Entomol. Research Conference (Brisbane 29 Sept-3 October 1998)

McFadden, H. Grover, A. DeFeyter, R. and Llewellyn, D. (1998) Transgenic cotton expressing a gene for chitinase shows improved tolerance to verticillium wilt in glasshouse trials. Proc. 9th Australian Cotton Conf. (Broadbeach 12-14 August, 1998) 591-594.

Y-L Ruan, R. Furbank and D. Llewellyn (1998) Towards genetic engineering of sucrose synthase to enhance fibre cell initiation and cellulose biosynthesis in cotton. Proc. 9th Australian Cotton Conf. (Broadbeach 12-14 August, 1998) 751-155.

D. Rungis, D. Llewellyn, E.Dennis and B.R. Lyon (1998) DNA markers: A new tool for improving the breeding of Australian cotton cultivars. Proc. 9th Australian Cotton Conf. (Broadbeach 12-14 August, 1998) 757-761.

B. Surin, P. Boevink, P. Keese, P. Chu, P. Larkin, D. Llewellyn, R. Kahn, G. Ellacot and P. Waterhouse. (1998) A suite of promoters and terminators for plant biotechnology. Proc. 4th Asia-Pacific Conference on Agricultural Biotechnology. (Darwin 13-16 july, 1998) (P. Larkin Ed.) 121-122.

1999

Last,D.I. and Llewellyn,D.J. (1999) A detoxification gene in transgenic *Nicotiana tabacum* confers 2,4-D tolerance. Weed Science 47: 401-404.

F. Murray, D. Llewellyn, H. McFadden, D. Last, E. Dennis, and J. Peacock (1999) Expression of the *Talaromyces flavus* glucose oxidase gene in cotton and tobacco gives some protection against fungal infection but exhibits a phytotoxic phenotype. Molecular Breeding 5: 219-232.

2000

Ellis, M, Millar, A, Llewellyn, D, Peacock, W.J. and E.S. Dennis (2000) Transgenic cotton over-expressing alcohol dehydrogenase shows increased ethanol fermentation but no increase in tolerance of oxygen deficiency. Aust. J. Plant Physiol, 27: 1041-1050.

Hughes, P, Dennis, E, Whitecross, M, Llewellyn D and Gage P (2000) The Cytotoxic Plant Protein, βPurothionin, Forms Ion Channels In Lipid Membranes. J. Biol. Chem. 275: 823-827

Llewellyn, D (2000) Genetically Modified Crops: from humble origins to World domination? Microbiology Australia 10-12.

McFadden, H, de Feyter R and Llewellyn D (2000) Molecular biology approaches to understanding and controlling fusarium wilt in cotton. Proceedings of the Australian Cotton Conference Brisbane. Pp463-464.

McFadden, H., de Feyter, R., Murray, F., Grover, A., Llewellyn, D., Dennis, E., and Peacock, W.J. (2000) Genetic Engineering Approaches to the Improvement of Cotton's Tolerance to Verticillium Wilt. In: Advances in Verticillium Research and Disease Management. Tjamos, E.C., Rowe, R.C., Heale, J.B. and Fravel, D.R. (Eds.) The American Phytopathological Society, St. Paul. pp 187-191.

McFadden H., de Feyter R. and Llewellyn, D. (2000) Expression of bacterial avirulence genes from *Xanthomonas campestris* pv *malvacearum* in cotton. Poster presented at the First International Symposium on Induced Resistance to Plant Diseases, Corfu, May 2000.

Ruan Y-L, Llewellyn D and Furbank RT (2000) Pathway and control of sucrose import into initiating cotton fibre cells. Aust. J. Plant Physiol., 27: 795-800.

Rungis, D, Llewellyn, D, Dennis ES and Lyon BR. (2000) Characterisation and mapping of the xcm resistance locus in australian cotton cultivars by the use of molecular marker techniques. Proceeding of the Australian Cotton Conference, Brisbane. pp 465-469.

2001

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: 47-60.

Townsend B and Llewellyn D. (2001) 'Potential for the Genetic Manipulation of Gossypol in Cotton – a Defense Chemical with Negative Impacts on Cottonseed Products' Abstract and poster submission at the Keystone Symposium on Plant Foods for Human Health: Manipulating Plant Metabolism to Enhance Nutritional Quality, held in Colorado, USA, April 6 - April 11 2001.

12. Are changes to the Intellectual Property register required?

No.

Part 4 – Final Report Plain English Summary

Provide a half to one page Plain English Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web.

The process of developing and commercialising a novel trait in cotton generated by genetic engineering involves four steps:

1. Isolating the genes that confer the novel trait and engineering them for expression in plants.
2. Introducing the novel genes into transgenic cotton and evaluating the plants in the laboratory or glasshouse.
3. Breeding the plants into elite germplasm that will have all the desired attributes of conventional cultivars and producing pure breeding stocks for commercial seed increase.
4. Extensive field evaluation and regulatory assessment before commercial release to growers.

CSIRO has developed the research and technical capacity to handle all of these elements of transgenic cultivar development. Over the last three years this project, which is an integral part of the overall CSIRO cotton breeding program, has contributed to the development of 10 new transgenic cultivars that will be available through Cotton Seed Distributors in 2001. This includes INGARD and Roundup Ready (RR) cotton cultivars and the first stacked variety with both traits, Sicala V-3RRi. Improvements to these initial varieties are on-going, with the the first double Bt (Bollagrd II and stacks with RR) varieties, which are still in the breeding pipeline, due for commercial release perhaps by 2003. Quality Control with transgenic plants has become a significant national and international issue and we have been developing the testing regimes for ensuring that all CSIRO developed varieties are not contaminated with different traits or transgenic lines.

CSIRO also has the capacity to develop and test new transgenic germplasm which is often at a much more basic level that the commercial traits being accessed from international biotech companies such as Monsanto. This project is generating new transgenic plants with novel genes that might have the potential to improve the waterlogging tolerance of cotton, the level of gossypol in cotton seeds, modifying the characteristics of the cotton fibre and enhancing the tolerance of cotton to debilitating diseases like fusarium wilt. Some of this material is undergoing field testing at Narrabri, but as yet none of these experimental lines looks promising. Biotechnology is a high risk research area but the rewards for the successful development of a new transgenic trait can be considerable.