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# Final Report
# Genetic Engineering of Cotton
## CSP71C

**Principal Investigator: Danny LLEWELLYN**
**CSIRO Plant Industry**
**P.O. Box 1600**
**Canberra City ACT 2601**
**Phone: 02-62465470**

# PLAIN ENGLISH SUMMARY

Genetic Engineering of Cotton – CSP71C

This project was to provide the basic technical support at the molecular level for CSIRO's cotton breeding program to develop new transgenic cotton varieties with improved agronomic characteristics. During the three years of the project, which was a continuation of a previous CRDC project in the same area, the three technical staff supported by the grant carried out extensive screening of transgenic breeding lines (over 500,000 samples) containing the insecticidal INGARD gene, the CryIIA gene and the herbicide resistance gene RoundupReady and various combinations of these genes in a variety of elite backgrounds. This screening work allowed the cotton breeders at Narrabri to identify those plants containing the novel genes that could be advanced in the breeding process. This is obviously an essential component of the more conventional parts of the breeding process related to the carrying out of crosses and the evaluation of the agronomic performance of the lines and directly contributed to the release of five transgenic INGARD cultivars in 1996 and a sixth cultivar in the 1998 season. On-going screening as part of a new CRDC project will have contributed to the development of new INGARD cultivars for the next decade and the two gene Bt varieties to hopefully be released in 2000 or soon after. The same technical team has also produced many hundreds of new transgenic cotton plants containing both experimental genes aimed at improving the pest and disease tolerance of cotton and potentially new commercial traits such as tolerance to the herbicide bromoxynil that are still in the evaluation phase.

## Background

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 (CSP70C) by providing access to molecular biology and laboratory skills necessary for the breeding of **transgenic** cotton varieties as opposed to conventional 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 (developed by Monsanto) that will confer protection against *Heliothis* species and herbicide tolerance genes (developed by Monsanto, Rhone Poulenc, Agrevo or as part of a CSD funded fellowship) that will confer resistance to herbicides like glyphosate, bromoxynil, Basta and 2,4-D, respectively. As the scale of the transgenic breeding program increased during the life of the project efforts have moved towards more routine (but significant) contributions towards the screening of material in the 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.

## Objectives

The project objectives were to use genetic engineering to develop new cotton cultivars with improved agronomic characters that would not be possible by traditional plant breeding techniques. This includes cultivars resistant to attack by the major insect pests and diseases of cotton and cultivars resistant to broad spectrum herbicides that could then be used in the control of problem weeds in post-emergent crops. The savings to the industry in both reductions in chemical pesticides or the use of hand chipping of weeds could be considerable. These objectives were, and continue to be, met with the release in 1996 nf the first five INGARD varieties and the release in 1998 of a sixth INGARD variety. These first generation transgenic varieties have shown enormous potential through the reduction in pesticide usage on transgenic cotton but their variable performance has indicated that they are just not quite efficacious enough for the pests we experience here in Australia. Better plants, such as the two gene varieties currently being evaluated in the breeding program, will be required and these form part of the on-going work of the project. The project has also contributed to the development of Roundup Ready cotton varieties and Roundup Ready/INGARD combinations that should he ready for release in 2000 subject to regulatory clearance. In a second stream we have been developing new transgenic germplasm for evaluation by the breeders, agronomists and weed scientists and this includes other herbicide tolerance traits such as bromoxynil and basta resistance and 2,4-D tolerance and during the course of the project a number of lines have begun to be evaluated in the field and will now become part of the mainstream transgenic breeding program. In a third aspect of the project we have been developing novel transgenes which could confer tolerance to diseases and evaluating promoters that could be useful for driving useful genes in transgenic cotton and the results of these experiments are receiving on-going evaluation in the laboratory and the field.

## Methodology

The main components of the methodology of the project are the screeniug of 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 heing 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 hiotechnology. 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-natinnal 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.

## Results

During the last three years we have continued to put much of our technical resources into supporting the transgenic cotton breeding program at Narrabri with the bulk of the seed screening work for the detection of homozygous lines occurring between May and June each year. During this period we continued screening a number of 531 INGARD lines and INGARD + CryIIA crosses for the development of commercial lines to replace the current batch of 757 INGARD lines. Screening was also carried out on INGARD + Roundup Ready crosses. The number of samples screened over the three years was in excess of 500,000 using both ELISA assays and NptII radiochemical assays. Some screening was also carried out during seed increases of commercial varieties, such as the new Sicot189i, to ensure that the seed batches were pure so that it could be sold commercially. We have had close interactions with the Quality Assurance programs within CSD.

In addition to the activities associated with the main transgenic breeding program we have continued to work with other transgenic material, mainly herbicide tolerant varieties, to make this available to the breeders and weed agronomists in time for the next seasons plantings. We have concentrated on the bromoxynil tolerant cotton we have generated in the Coker 315 variety and have been completing the molecular analysis required to identify single copy gene inserts. Currently about 16 lines have been identified and these all have tolerance to Basta at 3 L/ha equivalents and all have been crossed to Siokra V-16 and Sicala V-2 as well as selfed so that we can generate homozygous Coker lines for further field efficacy evaluation. Some of the lines were in the field last season and all four showed good field tolerance to bromoxynil. We have continued to generate some new Basta tolerant transgenic cotton lines and these are only just being evaluated now. And we have started to examine them at a molecular level. All lines that we keep are pre-screened with Basta at the equivalent of 6 L/ha (twice normal field rates). As yet we only have a few of these lines but should have more over the next few months. We have been investigating three different constructs including 35Sbar, 35Spat and S4bar. The latter construct uses the segment 4 promoter of Sub-clover stunt virus a promoter patented by CSIRO and that has good activity in cotton. We have previously used this S4bar as a selectable marker to generate some transgenic cotton containing a potential anti-fungal gene and have now shown that the S4bar in these plants gives good whole plant tolerance to Basta and may therefore be a useful construct for field tolerance and hence have commercial prospects. A number of the Basta tolerant lines should be ready for preliminary field evaluation in the coming season. We have also continued some work with our 2,4-D tolerant cotton, mainly back-crossing into elite lines and have now generated BC5 homozygous lines in L23, Sicot189, V-2 and V-16 all of which will receive preliminary agronomic evaluation in the field in 1998.

Field trial applications for all of our transgenic cotton have been prepared and granted each year and we have developed a close working relationship with all the necessary government regulatory bodies that control the testing of transgenic plants. This, for example, has included negotiations with the NRA in regard to extending the area of INGARD into Central Queensland in 1997, or the sale of lint from our trials of the two Bt gene cottons as the NRA was concerned that the CryIIA gene was as yet unregistered.

The technical team continues to generate new transgenic material in association with other researchers in our group and, for example, in the last year have concentrated on generating plants containing a number of promoter-marker gene constructs for genes promoters that may be useful in other engineering projects. This has included some potential fibre-specific promoters from Jeremy Timmis at the University of Adelaide, and some pathogen inducible promoters including a peroxidase promoter from Stylozanthes and a flax rust inducible promoter from flax. A number of transgenic lines have been generated with Rob DeFeyter containing bacterial blight avirulence genes that might induce systemic resistance to diseases and these are just starting to be analysed. New collaborations have also begun with Yong Ling Ruan and Bob Furbank for the CRDC project to manipulate carbon partitioning into the cotton seed to examine the effect on cotton

fibre development. In this respect the core technical team has a continuing and critical role to play in the development of the transgenic CSIRO cotton varieties for the future.

## Impacts

The release of the transgenic insect tolerant INGARD varieties has already had an important impact on the cotton industry in reducing chemical pesticide usage. Continuous improvements of the varieties will however be required to enhance the performance of the varieties and it is likely that the existing INGARD varieties will be replaced in the next few years with more robust two gene varieties. And similar varieties with one or more herbicide tolerance genes that are currently under development within the project. 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.

## Project Technology

The varietal development is obviously being carried out in close collaboration with Cotton Seed Distributors who sell CSIRO developed varieties and the various technology providers like Monsanto. Where appropriate we have sought patent protection, eg. The glucose oxidase genes as a potential disease tolerance gene from a project funded by CSD., ands CSIRO has sought and gained Plant Variety Rights protection for its transgenic cultivars.

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