FINAL REPORT 2006

Part 1 - Summary Details

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

CRDC Project Number: **CSP 159C**

Project Title**:** Breeding new cotton varieties with improved yield, Fusarium resistance and fibre quality

Project Commencement Date: 01/07/2003 **Project Completion Date:** 30/06/2006

Research Program: On-Farm

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Part 3 – Final Report Guide (due 31 October 2008)

Background

Until this breeding program released cultivars, Australian growers were completely dependent on foreign-bred varieties which had shortcomings in yield, disease resistance and fibre quality. This program is dedicated to producing cottons that will increasingly provide resistance to local diseases and pests besides progressively increasing yield and improving quality. Our long term research indicates yield has been increased by 1.86% per year since CSIRO varieties were first released in 1984. We aim to continue this progress and to specifically search for better combinations of yield, disease resistance and fibre quality and to deliver those discoveries with transgenic traits sought by Australian growers.

Fusarium wilt has become one of the most serious concerns to cotton production: the disease, first identified on the Darling Downs in 1993, is very severe and has spread to new regions. As a new strain unique to Australia, there is inadequate resistance in existing varieties to make cotton production viable on heavily infested fields. Therefore, a program of research/discovery is required to identify new sources of resistance and to develop the means to incorporate these new resistances into commercial varieties quickly and efficiently. Our research has discovered some new and potentially important sources of resistance to Fusarium wilt and this project will build on those discoveries and continue to search for others. It will also incorporate the ongoing search for novel Fusarium resistance in Canberra to ensure that breeding for Fusarium wilt resistance at CSIRO is fully integrated. This component of the research will develop the breeding population and begin to search for molecular markers for Fusarium resistance genes. Such a tool would enable the breeders to more rapidly screen for Fusarium resistance and to reduce our reliance on unreliable field nurseries.

Objectives

The overall objective is to develop improved conventional and transgenic cotton varieties adapted to Australian growing conditions and producing fibre suitable for our markets. The proposal comprised five key components:

(A) Conventional breeding,
(B) Evaluation of novel germplasm,
(C) Foreign genes, and,
(D) Marker-assisted breeding,

Incorporating all components into a single project under the aegis of the CSIRO cotton breeding program integrated the delivery of new varieties to cotton growers (A) with the search for new sources of Fusarium wilt resistance (B & C), and the development of enhanced breeding tools (D). An integrated project allows for the development of tools and knowledge that can be used to maximize the potential of currently identified sources of Fusarium wilt resistance, and to continue the search for new sources of resistance in novel germplasm. As there is no certainty of success with any single approach, all avenues of discovery were employed.

Methods

This project covers conventional breeding techniques to develop new cotton varieties - both conventional as well as transgenic. Crossing between selected parents is done in the field and glasshouse then screening occurs for presence of desired traits such as disease resistance, fibre quality and transgenes if necessary. Detailed evaluation of breeding lines for yield and quality occurs in all production areas on commercial farms. These regional trials involve large numbers of plots and incur large costs in staff, travel and operating expenses.

Crossing and early-generation research of the CSIRO breeding program is aimed at new germplasm development for the yield, regional adaptation, fibre quality and disease resistance objectives. Genetic diversity is a discreet aim, as there are many dangers such as disease susceptibility with a narrow germplasm base. We have discovered some very interesting sources of better growth habit and fibre quality which are being moved through the CSIRO breeding program. Aggressive screening for bacterial blight resistance, growth habit and fibre properties is done in early generation material.

CSIRO breeding has a broad scope from innovation and discovery through to commercial variety production. We have a tradition of increasing yield and fibre quality and with innovation in areas such as host plant resistance (HPR). This approach will be continued. For example, we are raising activity with HPR for pests such as whitefly and mirids, as the new generation farming system will have these pests as constraints instead of *Helicoverpa*.

New transgenic traits such as Bollgard II and Roundup Ready Flex are being incorporated into our most advanced germplasm.

Once progeny are identified, regional trials are located at Emerald, Brookstead, Dalby, St George, Boggabilla, North Star, Moree, Merah North, Myall Vale, Breeza, Bourke, Warren and Hillston. Specific objectives are addressed at each location (such as heat tolerance at Emerald/Bourke; dryland at North Star; short season at Brookstead, Breeza, Hillston, etc). Old standard varieties are compared with existing and potential CSIRO varieties at all locations.

In eight years of CSIRO screening, there have been very few *G. hirsutum* introductions with significantly better Fusarium resistance than Sicot 189, so new approaches need to be evaluated. However, some *G. hirsutum* lines with enhanced Fusarium wilt resistance have been identified and have been incorporated into new varieties effectively. Because Fusarium wilt resistance in *G. hirsutum* is more easily incorporated into commercial varieties there is a continuing need to import diverse germplasm through quarantine and evaluate them for survival in Fusarium nurseries. Lines showing enhanced resistance will be crossed with existing elite lines to determine combining ability and additive Fusarium resistance mechanisms and the process of screening will be repeated to develop new germplasm. This area of breeding will involve large numbers of breeding lines.

Data measurements are comprehensive and involve yield, yield components, earliness, growth habit, fibre quality, disease, etc. Statistical analyses is an important component of the research, as we need to be confident of the data and having real differences between performance of new lines compared with existing cultivars.

Once elite lines are discovered, foundation seed is handed over to CSD for seed production.

Evaluation of novel germplasm. CSIRO have four separate cotton species showing exciting promise in providing enhanced resistance to Fusarium wilt. One of these species (*G. sturtianum*) is the subject of research based in Canberra and new research will continue the field evaluation and breeding the *G. hirsutum* x *G. sturtianum* lines developed under CSP120C. Other species have been discovered with very high levels of resistance and introgression with *G. hirsutum* will be initiated as part of this new proposal. This approach is difficult (but easier than *G. sturtianum* and biotechnology) and takes longer than with conventional breeding, but the robust levels of Fusarium wilt observed in preliminary trials clearly justifies persistence.

Marker assisted breeding. The use of DNA markers as surrogates for Fusarium resistance genes can increase the speed of breeding significantly and will allow selection for resistance to continue outside Fusarium nurseries. The paucity of molecular marker diversity among elite cotton varieties has hampered previous attempts to develop molecular markers for cotton breeding. However, the other *Gossypium* species in which Fusarium wilt resistance has been identified are clearly distinguishable from *G. hirsutum* at the molecular level. While breeding with these species is more difficult than *G. hirsutum*, they will be amenable to the molecular marker analysis. If successfully identified, these molecular markers will compensate, in part, for the increased difficulties these other species present. Combining the development of molecular markers with the germplasm evaluation and the development of breeding germplasm will ensure that the markers identified will be immediately applicable to the development of new commercial cultivars. Both of these evaluations will employ standard breeding populations.

Other biotechnology. CSIRO research on fungal virulence (CSP113C and CSP115C) offers the potential to develop novel approaches to genetically engineer cotton for resistance to Fusarium wilt with gene silencing. Our proposal for Fusarium breeding will cooperate with those other projects to apply any discoveries in the field and possibly to initiate breeding. This research is longer term, difficult, but promising.

Results

1 – Conventional Breeding at Narrabri

Overview

Cotton breeding has become very complex in scale and objectives. In recent years we have had to start increasing the numbers of experiments and size of breeding populations in the face of necessary targets in simultaneously improving yield, fibre quality, disease resistance and all these objectives delivered in combinations of traits such as Bollgard®II/Roundup Ready.

In addition, with the high adoption of Bollgard®II (Figure 1), the availability of grower sites with conventional cotton has substantially reduced and we are therefore concerned that our capacity to discover new germplasm will be reduced simply from the reduced number of testing sites, the reduced numbers of conventional crosses and reduced population sizes in those breeding families. Low adoption of conventional varieties also means seed companies are less inclined to provide many conventional varieties in their portfolio. In being aware of these dangers, we have consciously ensured significant activity in conventional breeding, albeit with the constraints mentioned above.

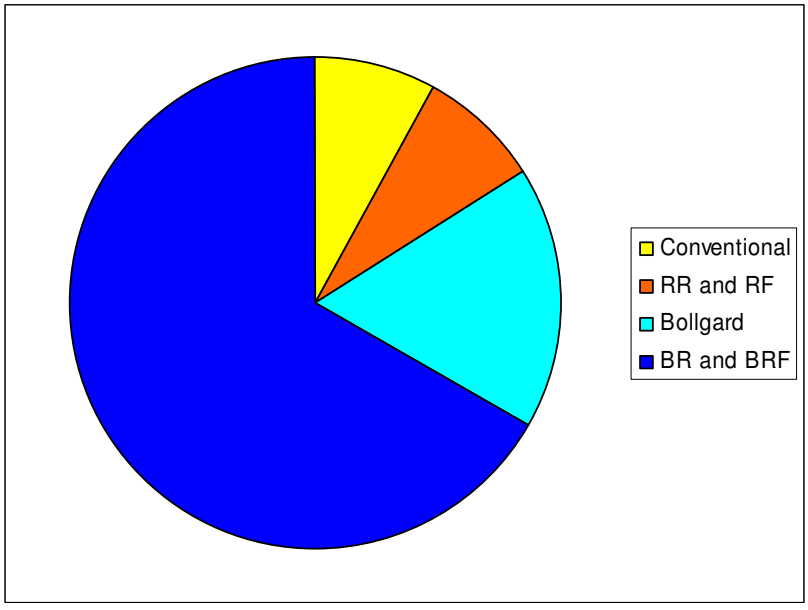

Figure 1. Trait adoption in 2006/07. The low adoption of conventional cotton presents a challenge for strategic planning in developing new conventional varieties.

Material processed in the period 2003/04 to 2005/06 included advanced breeding lines initiated in crossing and evaluated in previous seasons. Likewise, the crosses done from 2003 to 2005 will produce breeding material for subsequent projects. Thus most new varieties take from six to ten years from crossing to commercial release. Drought has challenged establishment or success of experiments at district sites. Figure 2 shows where breeding experiments have been done in the past three seasons. We have had failed experiments at Bourke and Warren. At each of these sites, a number of experiments will be sown to include preliminary breeding lines and advanced genotypes in final assessment for possible commercial release. Breeder seed is passed to CSD when a genotype has shown excellent performance over a number of sites and seasons. CSD initiate seed production before final

decisions are made on release – this ensures early release of a variety, at the cost of occasional redundant seed production.

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Irrigated sites

Emerald, Brookstead, Goondiwindi, St George, Moree (2), Merah North, Breeza, Hillston, ACRI (Narrabri), Bourke, Warren.

Dryland sites

Dalby, Tullona, Bellata, ACRI

Fusarium nurseries

Pampas, Norwin (Darling Downs)

RRflex sites

Pampas, Moree, Bellata, Merah North, ACRI, Boggabri

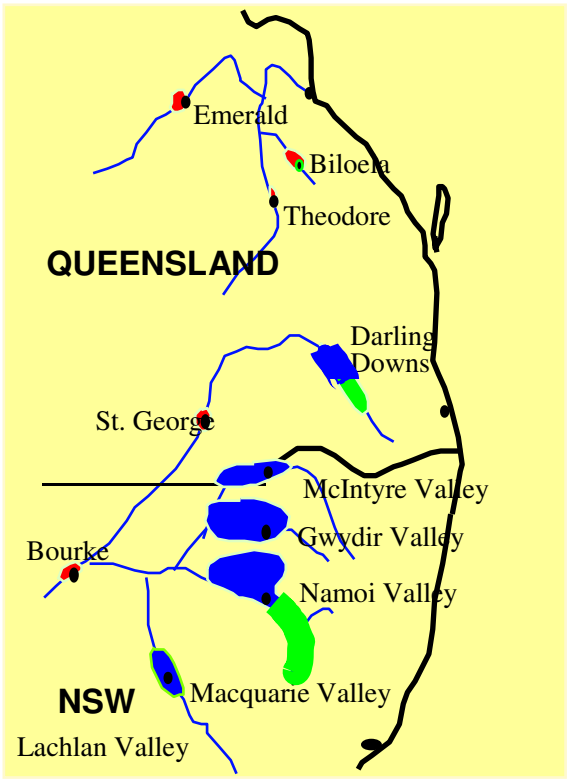

Figure 2. Sites used for breeding experiments 2003 to 2006

Conventional breeding

Tables 1, 2 and 3 show a summary of advanced central, advanced full season and advanced dryland conventional genotypes over three years of evaluation 2003/04 to 2005/06. These data are derived from the full data sets attached in the Appendices.

Table 1. Mean data for three years (covering 14 irrigated sites and six Fusarium nurseries) of breeding material for central cotton regions. 99004-434 is in advanced seed increase for potential release in 2007 as a replacement for Sicot 71 because of substantially improved fibre length and Fusarium resistance. This line has nominally been given the variety name Sicot 75 and is an important component of Bollgard II and RRFlex breeding – introgression.

Entry	YLD	LEN	STR	MIC	FIN	RFY	FRR
99004-114	2290	1.20	30.0	4.3	173	93	119
99004-434	2258	1.24	31.0	4.4	176	125	168
99035-359	2240	1.21	30.8	4.1	168	86	126
Sicot 71	2227	1.16	31.0	4.3	179	73	108
99008-207	2212	1.18	32.2	4.2	171	91	130
99004-424	2202	1.24	31.3	4.3	171	110	167
99004-826	2186	1.21	31.9	4.4	175	89	148
98034-180	2122	1.17	31.2	4.6	185	68	92
98049-2-2	2079	1.17	31.0	4.2	171	131	176
Sicot 189	2063	1.18	31.2	4.5	179	73	100
Sicot F-1	1835	1.17	31.5	4.3	171	131	193

Table 2. Mean data for three years (covering 10 irrigated sites and six Fusarium nurseries) of breeding material for full season cotton regions (Emerald, St George, Bourke). There is no plan to release any of these as a variety but some are included in Bollgard II and RRFlex introgression.

Entry	YLD	LEN	STR	MIC	FIN	RFY	FRR
Sicot 71	2595	1.15	30.4	4.4	179		
99209-284	2584	1.19	30.9	4.4	186	101	109
99216-513	2562	1.22	30.5	4.4	184	74	74
94215-442-430	2568	1.18	31.6	4.1	170	142	146
99216-415	2540	1.18	29.9	4.4	187	106	84
98236-445	2489	1.19	32.5	4.2	173	94	101
Sicot 80	2478	1.18	30.3	4.2	178		
99216-189	2507	1.18	29.2	4.3	182	103	88
Sicot 81	2492	1.22	31.6	4.2	175	118	117
99216-467	2500	1.22	29.1	4.1	180	104	90
97217-210	2478	1.19	31.4	4.4	174	98	81
99209-376	2501	1.17	30.7	4.3	182	94	94
DP16	1856	1.15	28.3	4.1	176		
Namcala	1777	1.17	33.3	3.9	161		

Table 3. Mean data for three years (covering nine dryland sites and three Fusarium nurseries) of dryland breeding material. There is no plan to release any of these as a variety but some are included in Bollgard II and RRFlex introgression.

	Relative yield	Length	Strength	Micronaire	F.rank
Siokra 24	97	1.10	31.6	4.3	102
Sicot 81	103	1.09	31.2	4.6	114
CSX225	116	1.09	30.2	4.6	75
CSX892	103	1.09	30.6	4.7	186
CSX855	94	1.15	35.3	4.6	87
CSX262	106	1.10	31.1	4.4	101

Fusarium

Table 4 shows data on advanced lines under evaluation for high Fusarium resistance. This material is the result of combination and recombination of resistant lines identified from previous screening and shows progress in Fusarium resistance as well as combining Fusarium resistance with higher yield potential in the absence of the disease.

In progress to this time with Fusarium resistance, we have noticed lower yield potential with high Fusarium resistance: Sicot F-1 has an FRank of 206, but has about 10% yield drag compared with elite material such as Sicot 71. The new lines shown in Table 4 show the negative characteristics are being removed from exotic germplasm used for Fusarium resistance and there are lines with near full yield potential with high Fusarium resistance (Figure 2).

Table 4. Examples of advanced breeding material with improved Fusarium resistance. Data from experiments in 2005/06. Yld is the average lint yield at four irrigated sites; l% is lint percentage; len, str and mic are fibre length, strength and micronaire by HVI at those four sites; Fyld and FRank are relative yield and Fusarium Resistance rank at two Fusarium nurseries.

Entry	Yld	l%	len	str	mic	Fyld	FRank
20058-319	2048	39.2	1.17	32.1	4.5	124	246
97060-69-619	1937	38.9	1.17	31.7	4.7	119	241
97060-69-536	2015	39.1	1.20	31.8	4.6	101	234
61035-315	2176	41.7	1.21	30.3	4.5	134	232
61035-137	2167	42.7	1.17	29.8	4.8	100	225
61035-260	2055	41.7	1.21	31.0	4.3	115	210
Sicot F-1	1907	38.0	1.18	31.5	4.4	118	206
61035-98	2267	43.0	1.18	30.5	4.7	134	202
61035-286	2239	41.1	1.23	30.2	4.4	114	193
98049-2-2	2167	40.4	1.18	31.6	4.3	122	193
61035-203	2136	41.5	1.21	30.7	4.5	102	192
99048-1-1	2204	39.9	1.22	31.5	4.4	116	191
61035-305	2018	40.2	1.23	31.2	4.4	99	189
20058-266	2154	40.2	1.18	31.8	4.2	116	181
97091-102-134	2220	41.6	1.22	30.4	4.3	132	178
61035-113	2146	40.6	1.28	32.1	4.4	118	177
20011-72	2034	37.9	1.21	33.0	4.4	126	177
20011-391	2024	38.9	1.22	32.4	4.4	119	171
99004-424	2265	42.7	1.23	31.0	4.4	103	171
99048-1-8	2146	39.3	1.22	31.8	4.1	116	171
20058-23	2098	38.3	1.22	32.0	4.4	120	171
97091-102-54	2209	40.8	1.20	30.2	4.4	127	168
99004-434	2307	43.1	1.25	31.2	4.5	115	168
20011-311	2056	38.1	1.24	32.4	4.5	109	161
20011-41	2189	40.9	1.19	32.9	4.3	111	155
96011-842-198	2245	40.6	1.23	31.4	4.6	112	154
97091-102-119	2193	40.8	1.21	30.6	4.2	118	150
Sicot 189	2127	39.8	1.19	31.6	4.7	63	100

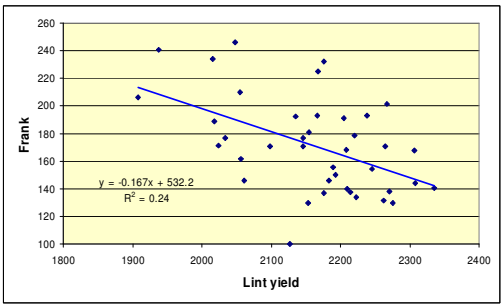

Figure 2. Association between lint yield at disease free sites and Fusarium Resistance Rank measured in a Fusarium nursery in 2005/06 advanced line data. Note the number of points with high yield and high FRank.

Fibre quality

In recent seasons there has been a considerable proportion of Australia's cotton crop being discounted for short staple and high micronaire. The reasons behind this problem are varied, but include:

- warm, dry, sunny seasons which cause high micronaire directly and also make irrigation scheduling difficult, so any mild moisture or heat stress can reduce fibre length directly;
- management and technology for high yield, fruit retention and earliness – all of which can cause high micronaire indirectly; and
- breeding, selection and grower choice for the highest yielding varieties – all of which can cause short staple directly and indirectly.

Spinners also regularly complain of high neps and short fibre content in Australian cotton, although there is likely an element of price negotiation in that complaint as many of our competitors have similar or worse values for these characteristics. However, it is still important to try and address these issues to ensure regular demand for our fibre. Note neps and short fibre are mainly due to aggressive ginning.

With the regular appearance of these issues by the 2000 harvest, our breeding program began to pay much more attention and priority to improved fibre quality. Prior to that time, major priorities in Fusarium resistance and transgenic trait introgression diverted attention away from fibre quality – at a time when there was also less attention from spinners. Thus our objectives were to search for a wider portfolio of variety fibre characteristics. This will ensure the Australian cotton grower will have variety options for wherever the spinning market goes in the medium term. Our targets therefore included:

- a slightly improved fibre package than available in major varieties at present – especially improved fibre length;
- a premium package at least equal to SJV Acala in all respects (length, uniformity, short fibre, strength and fineness).

These objectives need to be in high yielding varieties, with disease resistance and with popular transgenic traits such as Bollgard®II/Roundup Ready Flex. From 2000, there has been an intensive crossing and recombination program followed by screening and intensive selection among breeding lines. By 2006, we have a number of lines of great interest for further breeding.

During breeding and selection of one Bollgard®II family, an interesting line was found with excellent fibre length, increased strength and intermediate micronaire - although with a 15% yield penalty under most irrigated systems (Table 5). This variety has been released as Sicala 350B and a number of growers and merchants have been assessing this type for market interest and acceptance. It has been possible to obtain a US9c/lb premium for this fibre to make up the difference in yield. But the most important result has been the encouragement for future premium types.

Table 5. Characteristics of Sicala 350B compared with Sicot 80B. Mean of three seasons covering at least four sites each season.

	Yield (kg lint/ha)	Length (inch)	Strength (g/tex)	Micronaire
Sicot 80B	2632	1.18	29.8	4.1
Sicala 350B	2103	1.28	32.0	4.1

All current breeding of conventional and transgenic is imposing selection pressure on fibre properties to ensure future compliance with current Australian merchant discount tables. The concept new conventional release (99004-434 = Sicot 75) has excellent fibre length to overcome a potential disadvantage with Sicot 71, the most popular variety grown at present (Table 1).

Advanced premium fibre breeding lines now under multi site evaluation are demonstrating progress in combining fibre quality with better yield (Table 6). A number of lines have exceptional length, uniformity, strength and fineness. Table 6 also shows fibre properties of a SJV Acala (Maxxa GTO). Many of our elite premium fibre lines have superior length, strength and fibre maturity as well as up to 50% higher yield.

The negative association between yield-fibre quality is still of concern and we believe future research should address genetic linkages and fibre quality mechanisms as possible solutions.

Table 6. Relative yield and fibre properties of advanced premium fibre quality conventional breeding lines compared with Sicot 71 and Sicot 80. Mean of two sites in 2005/06.

Entry	RY	GIN	LEN	UNI	STR	MIC	PM	FIN
Sicot 71	122	41.5	1.17	82.9	31.2	4.5	79.1	187
Sicot 81	118	39.4	1.25	84.3	32.2	4.4	79.5	184
20228-190	118	40.2	1.27	85.0	34.0	4.1	77.2	174
20231-707	111	38.6	1.23	82.6	32.6	3.9	77.5	164
61211-372	107	39.7	1.23	84.2	33.2	4.3	80.7	175
99214-379	107	38.7	1.23	83.0	33.3	4.1	76.1	179
61220-377	106	37.8	1.23	85.2	34.8	4.5	83.1	177
61211-178	106	38.5	1.26	84.9	34.1	4.3	81.0	174
20228-64	106	37.9	1.27	83.3	36.2	3.9	75.8	163
20231-124	106	37.9	1.26	84.4	34.2	4.3	79.9	173
61219-354	105	39.3	1.25	84.2	34.3	4.1	78.9	168
61220-269	105	38.2	1.23	84.9	34.5	4.4	80.4	182
61219-79	105	38.8	1.24	84.6	34.6	4.4	80.8	177
61211-389	105	39.0	1.27	83.0	33.8	3.9	79.4	161
61211-199	105	39.5	1.25	84.4	33.7	4.2	81.7	170
61220-326	104	37.9	1.27	84.7	33.9	4.3	80.3	178
61220-350	104	37.3	1.28	84.8	34.5	4.3	80.1	174
20231-646	100	36.1	1.30	83.0	34.1	3.9	75.4	165
61211-130	100	38.4	1.26	84.2	34.1	4.1	79.8	170
61219-380	100	37.0	1.27	84.4	33.1	4.5	83.5	178
20231-457	99	36.7	1.33	84.4	33.9	4.0	78.0	164
Acala Maxxa	69	41.4	1.24	84.9	34.9	3.9	73.5	163

Roundup Ready Flex®

This trait was introduced into Australia by Monsanto about five years ago and CSIRO have a number of varieties (three Bollgard®II/RRFlex and two RRFlex) in limited release through CSD for 2006 sowing. Full transition to RRFlex from the old RR trait is expected by 2009, so we have developed a number of important breeding families in the past three years (Table 7). These breeding families occupy a major component of our field testing in 2006/07 and 2007/08.

Table 7. List of breeding families as potential varieties to be released in the next three years.

Advanced selection	Early generation breeding
• Sicot 71BRF • Sicala 45BRF • Siokra V-18BRF • CSX3607BRF (early/fusarium) • CSX3616BRF (med/fusarium)	• Sicot 75BRF • Sicot F-1BRF • Sicot 73BRF • Sicot 289BRF • Siokra 24BRF • CSX2404BRF (yield) • CSX4617BRF (yield) • CSX4620BRF (fusarium) • CSX4610BRF (fusarium) • CSX4618BRF (okra) • CSX4615BRF (dryland)

There is close collaboration and coordination with Danny Llewellyn's group in screening early generation breeding populations for transgenic traits of Bollgard®II and Roundup Ready Flex®. PCR analyses are performed in Canberra to confirm homozygosity of each of the three transgenes: Cry 1Ac, Cry 2Ab and RRFlex (where necessary, depending on the breeding population).

This screening experience and establishment of procedures and logistics is an important precedent for future use of molecular markers in the breeding program.

2 – molecular biology at Canberra

Objective 1. Make 150 backcrosses between intraspecific G. sturtianum {[(Gos-5050 x Gos-5250); (Gos-5250 x Gos-5050); (Gos-5168 x 5250)]} F_1 hybrids, polymorphic for Fov (Fusarium oxysporum var. vasinfectum), with Gos-5250 (Fov susceptible) to develop segregating BC_1 populations for Fov-QTL mapping.

Early work in Fusarium wilt resistance done under CSP120C not only confirmed that *G. sturtianum* is a source of resistance to fusarium wilt, but that this resistance can be expressed in the *G. hirsutum* background. More importantly, the results obtained under CSP120C constituted the first report showing multilocus control of fusarium wilt of cotton in Australia. Many of the useful *G. sturtianum* genes, contained in the chromosome segments transferred to the *G. hirsutum* background, are quite probably different from these of the cultivated species and are therefore potentially useful for providing novel and effective sources of resistance to this economically significant disease. Furthermore, our results, and those reported by McFadden et al. (2004), indicate that *G. sturtianum* may constitute a good genetic model for unravelling the mode of *Fov* resistance in *G. hirsutum*. This assumption is based on the fact that *G. sturtianum* is diploid, so genetic analysis would not be complicated by the presence of duplicated homoeologs for relevant genes. Also, there are disease susceptible *G. sturtianum* accessions within our collection making this species a perfect biological model. Based on Fusarium wilt symptoms and molecular marker diversity we have identified an optimal parental combination for development of Fusarium wilt resistant segregating families. Advanced [Gos-5250 (*Fov*-resistant) X Gos-5050 (*Fov*-susceptible)] X Gos-5250 backcross progenies were generated to assess whether the basis of resistance in *G. sturtianum* could serve as a model for elucidating the resistance in *G. hirsutum*.

Approximately 256 BC_1F_1 intraspecific hybrids were transferred to a glasshouse for seed production. Currently, 218 BC_1F_1 plants have produced BC_1F_2 seeds of which 194 BC_1F_1 individuals have produced from 400 to 2700 BC_1F_2 seeds with a total of 206,000 seeds and 24 BC_1F_1 produced between 25 and 390 with a total of 5,150 seeds. Leaf tissue from this material has been collected in duplicate (approximately 6,000 leaves), freeze-dried, and stored for DNA extraction. Future work requires genotyping a substantially larger number of individuals for Fusarium wilt response bioassays. Genotyping using 34 AFLP prime combinations is required to construct a *G. sturtianum* genetic map to assist with the QTL analysis in this Fov segregating family. At the same time, we feel it is important to continue to develop the near isogenic lines (NIL) of this cross, to the extent possible, as they may also prove of great value for future work in plant pathogen interactions between *Fov* and cotton, We believe these materials merit follow-up.

Objective 2. Grow out 300 AADD genome interspecific F_2 hybrids (G. hirsutum x G. barbadense) polymorphic for Fov resistance to develop:

Segregating cotton populations for the genetic analysis of Fov resistance. Of the 266 F_2 progeny that make up our 20 cM framework cotton mapping population, 245 individuals produced F_3 seed. Production of F_3 seed (115 individuals of 61003-1 and 109 individuals of 61003-4) was done by CSIRO Myall Vale to continue the development of the 61003 recombinant inbred lines (RILs). Leaf tissue from the F_2 progeny has been collected in

duplicate, freeze-dried, and DNAs extracted to construct a 20 cM framework cotton genetic map for a QTL analysis of fusarium wilt resistance in cotton.

It is critical to have the appropriate experimental design and materials for the genetic analysis of complex traits such as Fusarium wilt of cotton. It is well known that the F_2 generation provides theoretically the most complete and most informative population for many genetic analyses. However, there are disadvantages associated with using the F_2 for genetic analysis of quantitative traits. For example, each genotype in an F_2 population is represented by only one individual, which makes it difficult to obtain replicated measurements of the same genotype. Also, the population is in a transient state, so the experiment cannot be repeated.

Generation and deployment of experimental populations is the backbone of the project and the goal is the generation of approximately 200 RIL families of 1000+ individuals each, as well as the consolidation of an immortalised mapping population for cotton genetic research in the next 3 years (Figure 3).

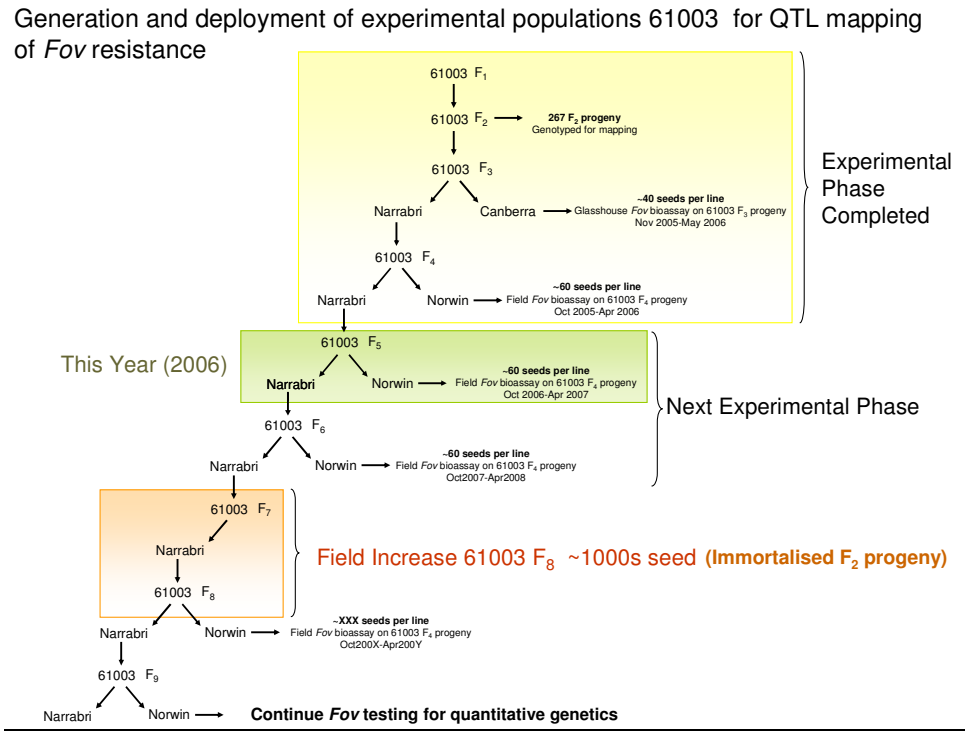

Figure 3: Experimental cotton population for QTL mapping of Fov resistance.

Fov bioassays on F_3 and F_4 progenies. To optimize the *Fov* inoculation protocol traditional pot trials were replaced by a method using single individual forestry tubes that expanded the number of individuals that could be tested at one time. Three inoculation methods (amended soil, drenching, and amended drenching) were evaluated for glasshouse screening of resistance. The inoculation method trial aimed to develop a standard glasshouse inoculation technique ideal for genetic studies designed to assess the introgression of *Fov* resistance from Australian native cotton, *G. sturtianum*, and to assess *Fov* resistance in elite cultivated cottons. The pilot trial comprised 3,600 plants were arranged in a row x column plot design including 900 Siokra 1-4 (*Fov* susceptible industry standard), 900 Sicot 189 (*Fov* resistant industry standard), 900 Sicot F-1 (best *Fov* resistant cultivar), and 900 *G. barbadense* Line

8810 (*Fov* resistant) was conducted in Canberra. The extent of fusarium wilt resistance, measured by the vascular browning index (VBI), was accessed for all individuals. The amended soil method was identified as the best *Fov* bioassay method for these trials.

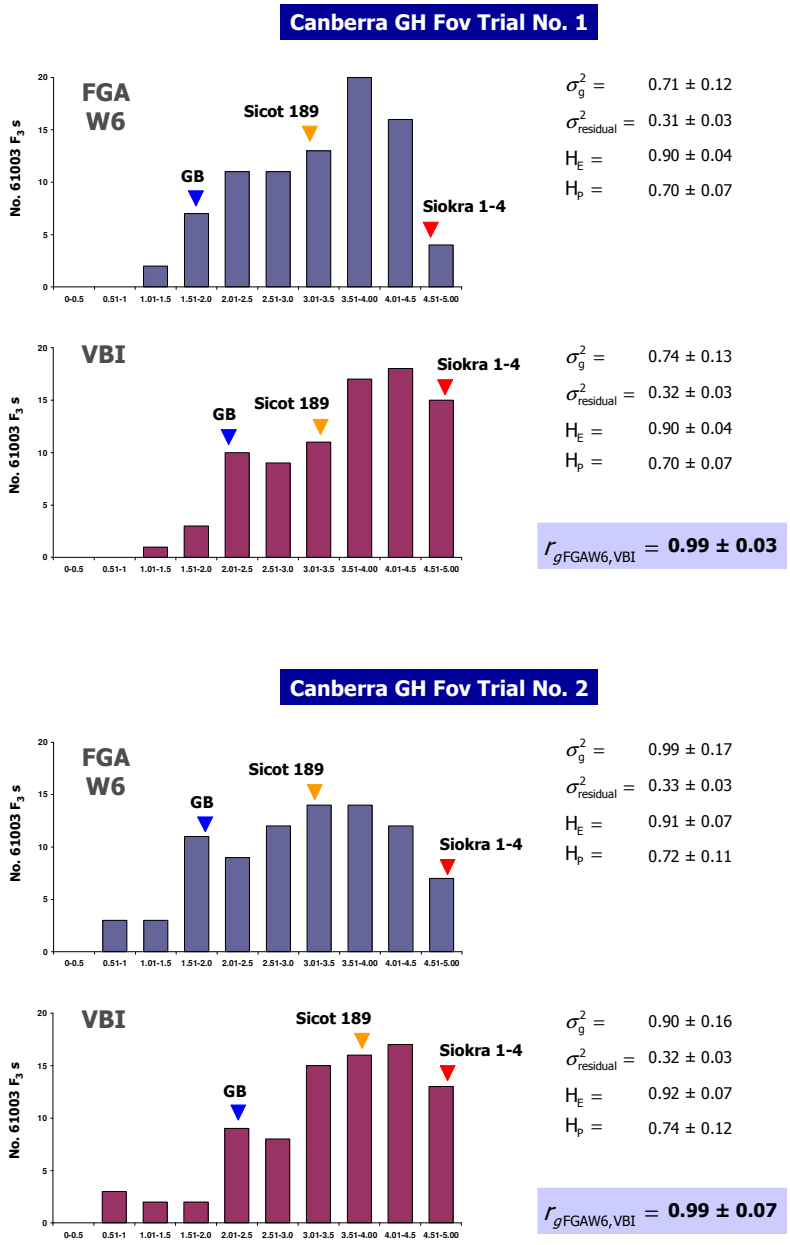

Figure 4: Frequency distribution of quantitative resistance to Fov in the F3 progeny derived from the cross GB (8810) x Siokra 1-4. Phenotypic values are shown on the x-axis. The position of the mean resistance value of the parents and the industry-standard for Fov resistance are indicated by blue, red and orange arrows, respectively.

Following this experiment, a series of three glasshouse trials aiming to measure *Fov* disease responses in the 61003 family were conducted between October 2005 and May 2006. A total of 8,100 61003 F_3 individuals have been tested for "foliar & general appearance" (FGA) and "vascular browning index" (VBI) in the glasshouse. All the data for this bioassay has been collected and collated and the results for the distribution of *Fov* disease symptoms for trial#1

and trial#2 are shown in Figure 4. The resistance levels of the parentals (GB: resistant & Siokra 1-4: susceptible) and the industry standard Sicot 189 are also indicated on Figure 4. On these distributions, the resistant parent (GB) was positioned at the left hand end of the distribution, whereas the susceptible parent (Siokra1-4) was positioned at the right hand end of the distribution indicating a good phenotypic separation of the two parental cultivars. High genetic correlations were observed between FGA and VBI in trial#1 ($r_{g\mathrm{FGAW6,VBI}} = 0.99 \pm 0.03$) and trial#2 ($r_{g\mathrm{FGAW6,VBI}} = 0.99 \pm 0.07$). In turn, those lines showing severe leaf symptoms and stunting did also show a high vascular browning index. Broad-sense heritability was estimated for FGA and VBI. Heritabilities were high for both traits in all trials, indicating a small genotype x environment interactions ($H > 0.90$).

Genotypic variance components were large and significantly different from zero ($P \leq 0.05$) for FGA ($\sigma_{g-Trial\#1} = 0.71 \pm 0.12$; $\sigma_{g-Trial\#2} = 0.99 \pm 0.17$) and VBI ($\sigma_{g-Trial\#1} = 0.74 \pm 0.13$; $\sigma_{g-Trial\#2} = 0.90 \pm 0.16$). In contrast, the genotype x environment interaction variance were typically smaller than the genotypic variance for FGA ($\sigma_{gxe-Trial\#1} = 0.31 \pm 0.03$; $\sigma_{gxe-Trial\#2} = 0.33 \pm 0.03$) and VBI ($\sigma_{gxe-Trial\#1\&Trial\#2} = 0.32 \pm 0.03$). The large broad-sense heritabilities for FGA and VBI and the high genetic correlations between environments indicates that a large component of the genotype x environment interaction for FGA and VBI reflected changes in the magnitude of the genetic variance in each environment with only small changes in genotype ranking. Hence, genotypes effects on Fusarium wilt resistance were large and robust owing to weak interactions with environment.

Similarly, for Fusarium wilt resistance testing under field conditions, a total of 23,000 61003 F_4 individuals were arrange in an unbalanced plot design at the *Fov* nursery at Norwin (Queensland). The data (VBI) from this field trial have been collected and a preliminary Fusarium wilt disease analysis revealed that of the 179 lines that compose the 61003 F_4 approximately 22% have exhibited the resistant levels observed in the *G. barbadense* parent (Figure 4).

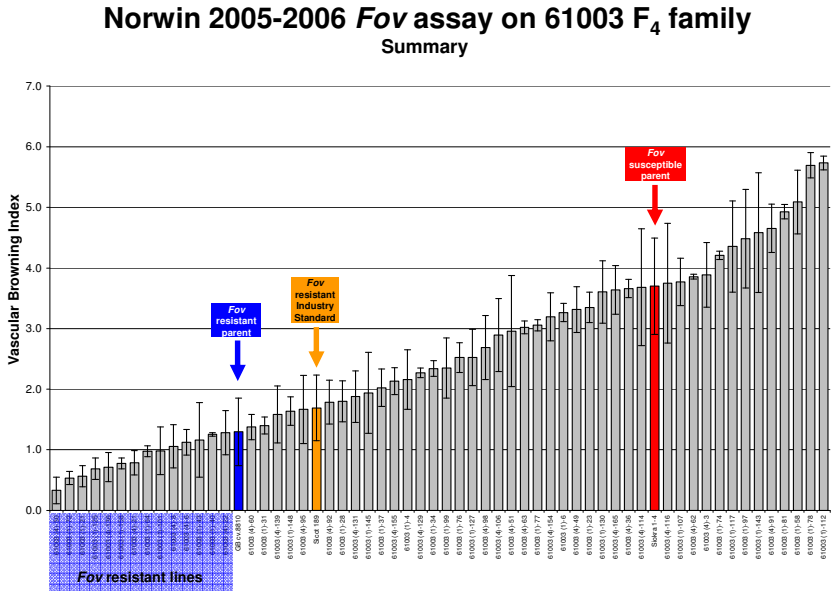

Figure 4: Vascular browning index (VBI)of 61004 F4 lines at Norwin in 2005/06 .

These data will be used to detect any significant association between vascular browning index (VBI) and marker loci in the 61003 F_2 mapping population using an appropriate QTL software analysis package.

Objective 3. Develop a STS-like co-dominant PCR marker system to construct the 20 cM framework cotton genetic-map using the 3347-Locus genetic recombination map of Rong et al. (2004).

From the 3347 loci map by Rong et al (2004), 245 cotton-STS specific loci were chosen based on their positions on the linkage map to create a "framework" map for quantitative trait locus (QTL) analysis in cotton. These markers were selected to cover the entire cotton genome with an average inter-marker distance of ~20 cM. Table 8 shows the distribution of map markers selected from chromosome 3.

Table 8: *List of marker-loci for chromosome-three based on two international cotton maps. Marker-loci in red boxes are the ones selected to develop the 20 cM Australian cotton framework map for the genetic study of Fusarium wilt resistance.*

SSR developed by Nguyen and Lacape **CIRs**
BNLs only used by Nguyen
BNLs only used by Rong
BNLs used by Rong and Nguyen
STSs used by Rong and Nguyen
Locus selected for 20cM framework map construction

Rong's 2004 cotton Map							Nguyen's 2004 cotton Map						
Sort ID	SeqName	Chr	Locat	GenBank Accession.	SSR	User_ID	Sort ID	SeqName	Chr	Locat	GenBank Accession.	SSR	User_ID
Chr.03						Chr.03							Chr.03
143	Gate4DG02	Chr.03	0				78	pGH639b	Chr.03	0			
144	PAR0476	Chr.03	0	CA993033		PAR0476	79	BNL3408b	Chr.03	0		Yes	
145	Gate4DC11	Chr.03	1	BE053871	Yes	GA__Ea0004F22							
146	Unig25G07	Chr.03	3.7	CD777495		Unig25G07							
							80	E5M1_400	Chr.03	7			
							81	**CIR030**	Chr.03	9		Yes	
150	Gate2AA08	Chr.03	12.9	BE054377		GA__Ea0002A15							
151	A1748	Chr.03	23.4	CA994178		A1748	82	E3M2_110	Chr.03	23			
							84	BNL2443b	Chr.03	25		Yes	
							86	E3M7_82	Chr.03	31			
152	Coau4H06	Chr.03	36.5	AI055600		Coau0004H06	87	pAR172a	Chr.03	36			
							88	E2M6_148	Chr.03	38			
							90	**CIR058**	Chr.03	39		Yes	
							92	BNL3441	Chr.03	39		Yes	
							93	BNL3627b	Chr.03	41		Yes	
							94	BNL1059b	Chr.03	41		Yes	
153	Gate1BD11	Chr.03	41.8	BE055048		GA__Ea0001G22	97	E1M2 450	Chr.03	42			
155	Gate2AC01	Chr.03	44.2	BE055489		GA__Ea0002E01							
156	pGH639	Chr.03	44.2	CA993936		pGH639							
157	Gate4BE01	Chr.03	46.2	BE054679		GA__Ea0004I02							
159	PAR0185	Chr.03	52.2				99	E5M8-143	Chr.03	52			
161	A1182	Chr.03	52.3	CA994024		A1182							
							100	E5M8 87	Chr.03	53			
162	Unig06D07	Chr.03	54.7	CB066369		GA__Ec0006D07	102	E7M4_185	Chr.03	55			
164	Gate4CD12	Chr.03	54.7	BE054441		GA__Ea0004H23							
166	PAR0149	Chr.03	57.1	CA992747	Yes	PAR0149							
							104	E8M5_155	Chr.03	61			
167	Coau4A11	Chr.03	62.9	AI055448		coau0004A11							
168	Unig24B10	Chr.03	67.2	CB066446		GA__Ec0024B10	105	A1145	Chr.03	67			
170	Gate2CD07	Chr.03	69.6	BE052278		GA__Ea0002H13							
171	Unig22B04	Chr.03	70.5	CB066384		GA__Ec0022B04							
172	Gafb15O14	Chr.03	70.5	BM359051	Yes	GA__Ea0015O14							
173	Coau3B05	Chr.03	71.7	AI055112		coau0003B05							
179	pAR0050	Chr.03	72.1	CA992680	Yes	pAR0050							
180	PAR0879	Chr.03	72.6	CA993492		PAR0879							
182	Unig26F10	Chr.03	75.1	CB066515		GA__Ec0026F10							
183	Gate2BC05	Chr.03	76	BE055498		GA__Ea0002E10							
185	Unig25A01	Chr.03	77.7	CB066464		GA__Ec0025A01							
186	Unig23D03	Chr.03	78.5	CB066423		GA__Ec0023D03							
187	Unig28F06	Chr.03	79.3	CB066560	Yes	GA__Ec0028F06							
190	pAR4-14	Chr.03	80.2	CA992986	Yes	PAR04-14							
193	pGH551	Chr.03	81.9	CA993917		pGH551	106	BNL226b	Chr.03	82		Yes	
196	A1474	Chr.03	82.8	CA994110		A1474							
197	Unig22G01	Chr.03	85.5										
204	PAR0110	Chr.03	88.2	CA992729		PAR0110							
205	A1788	Chr.03	89.2										
							107	BNL3989	Chr.03	90		Yes	
206	A1145	Chr.03	91	CA994004		A1145							
208	A1449	Chr.03	93.7	CA994100	Yes	A1449	110	**CIR245**	Chr.03	94		Yes	
209	pGH358	Chr.03	94.6	CA993871		pGH358	111	**CIR332**	Chr.03	95		Yes	
211	BNL0226	Chr.03	96.9										
213	PGH740	Chr.03	98.1	CD777473		pGH740							
214	pGH550	Chr.03	99.3	CA993916		pGH550							
215	P11-28	Chr.03	101.1	CD777504		P11-28							
220	Coau4A15	Chr.03	102	AI055453		coau0004A15							
222	Gate4AE05	Chr.03	103.1	BE054689		GA__Ea0004I09	113	**CIR212a**	Chr.03	103		Yes	
							117	pVNC163c	Chr.03	105			
224	Gate3BE09	Chr.03	108.3	BE053668		GA__Ea0003I18							
							118	BNL3259a	Chr.03	109		Yes	
							119	E7M1_172	Chr.03	112			
225	Gate4AB05	Chr.03	112.8	BE053073		GA__Ea0004C09							
226	A1418	Chr.03	114.6	CA994090	Yes	A1418							
227	Gate2CB02	Chr.03	121.1	BE055187		GA__Ea0002D03							
228	Unig27E01	Chr.03	124.4										
230	Gate3BG09	Chr.03	128	BE054805		GA__Ea0003M18							
							120	**CIR084b**	Chr.03	129		Yes	
232	G1129	Chr.03	129.5	CA994233		G1129							
235	pXP3-89	Chr.03	132.4	CA992640		pXP3-89							
							121	**CIR228a**	Chr.03	138		Yes	
236	G1164	Chr.03	143.8	CA994242	Yes	G1164	122	**CIR133**	Chr.03	144		Yes	
237	Gate1BF05	Chr.03	150.8	BE052111		GA__Ea0001K10							
							123	**CIR202**	Chr.03	153		Yes	

The 245 cotton-STS specific primers were screened against the two parental lines of the 61003 family (*G. hirsutum* Siokra 1-4 and *G. barbadense*). A total of 239 co-dominant PCR markers had a positive PCR amplification from the parental lines. The results of this screening revealed that of the 239 markers 68 (28%) were polymorphic between *G. hirsutum* cv. Siokra 1-4 and *G. barbadense*. These polymorphic loci were selected for genotyping to generate mapping data from the 61003 interspecific families (Figure 6).

As an adjunct to the Rong & Paterson AD genetic linkage map, a second published cotton map has been investigated. Nguyen *et al.*, [TAG (2004) 109:167-175] developed a cotton genetic map composed of approximately 1200 loci. This information will assist in increasing the coverage of our proposed 20cM cotton framework map (Table 8). Approximately, 330 cotton SSRs have been selected and screened from the Nguyen's map. Of the 330 SSRs, approximately 230 (70%) were polymorphic between *G. hirsutum* cv. Siokra 1-4 and

G. barbadense. These polymorphic loci were selected for generating mapping data on cotton interspecific family 61003 (Figure 7).

In order to analyse this cotton map markers on 266 F_2 progenies a fluorescent-labelled PCR product and a laser detection system has been proposed. One of these primers has to carry a fluorescent dye label, which may be NED, FAM, VIC and PET. These fluorescent dyes are expensive and in a large genetic mapping project costly. In order to overcome this financial burden, we have adopted the universal fluorescent-labelled M13 nested PCR approach (Schuelke, 2000), which can reduce the primer costs by an order of magnitude.

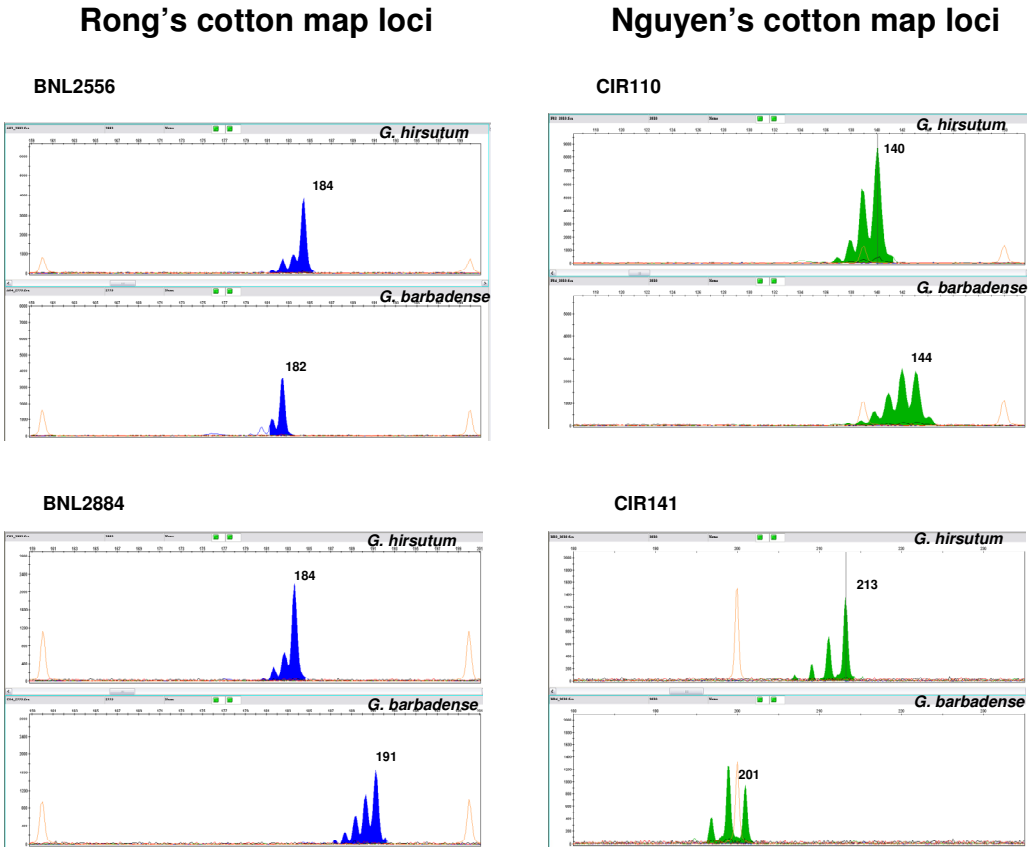

Figure 6: GeneMapper gel image showing co-dominant markers between G. hirsutum cv. Siokra 1-4 and G. barbadense.

Objective 4. Direct mapping of cotton STS- and SSR-mapped makers.

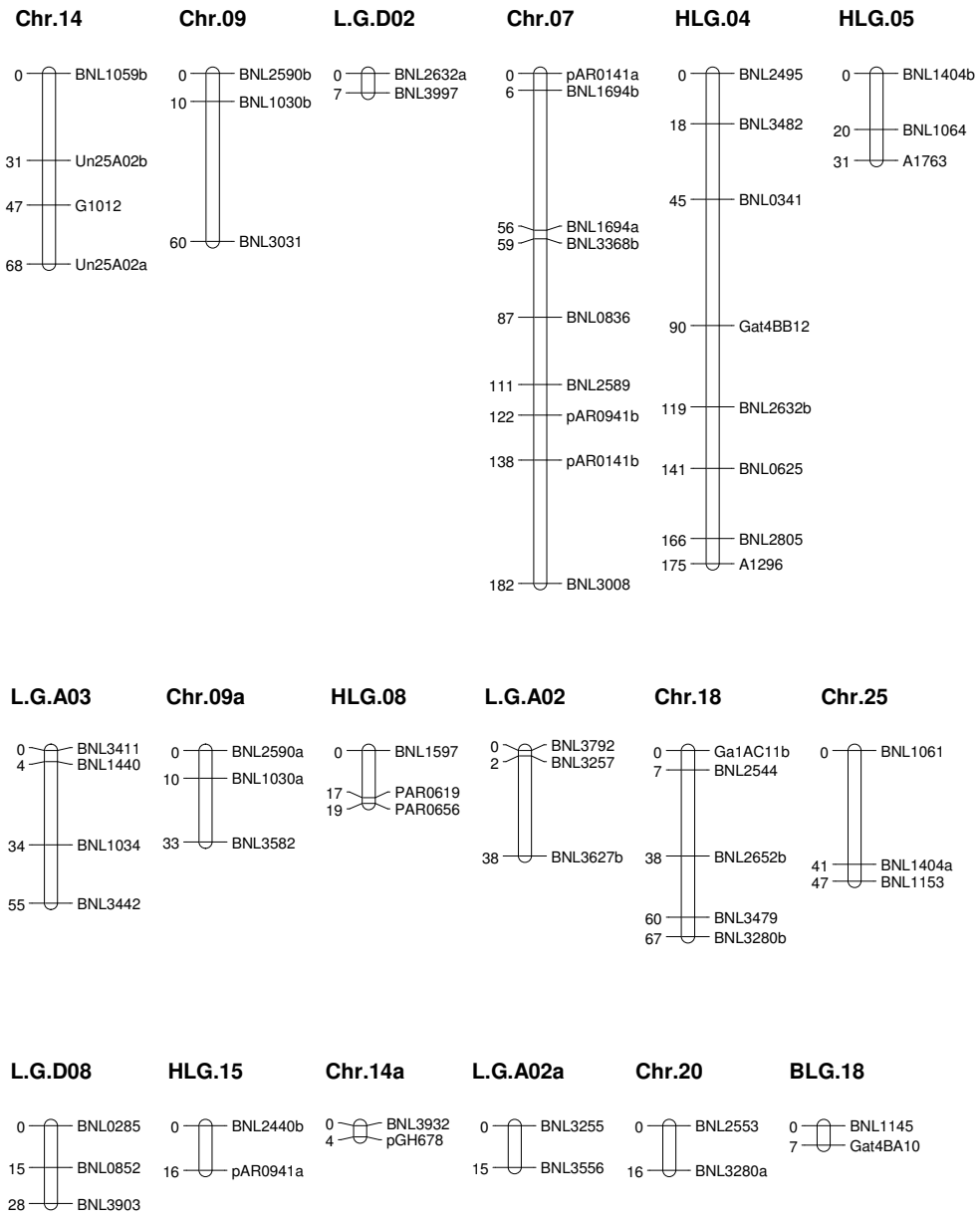

Figure 5: Australian cotton framework map showing 18 genetic linkage groups resolved at a LOD > 5 and θ = 0.25.

An STS/SSR framework map using 61003 F_2 populations is being constructed. So far, 298 markers have been selected for genotyping and mapping. Of the 298, 126 have been mapped in the population. The data gathered using an ABI 96 (3730 xl- AGRF) and 16 (3130 xl-in-house) capillary systems have been scored and analysed using GeneMapper ABI prism software v3.7 and v4.0. The integrated map covered approximately 830 cM with an average interval size of approximately 35 cM on the framework map (Figure 4). The current cotton framework map will be critical for analysing quantitative traits loci (QTLs) of Fusarium wilt

resistant but also it will be of great value to assess in the future other traits such as agronomic characteristics, and yield and fibre quality components.

Objective 5. Identification of quantitative trait loci (QTL)

A preliminary analysis to identify QTLs linked with Fusarium wilt resistance in cultivated cotton was undertaken. Overall, the correlation of phenotypic and genotypic variances in 61003 F_3 and F_4 allowed the identification of 8 QTLs associated with Fusarium wilt resistance (Figure 8). In general, the proportion of the observed phenotypic variation explained by significant QTLs was quite high. For the FGA and VBI, the chromosomal location was consistent between the glasshouse and field trials. For example, a QTL with large effect in FGA (35%) was detected on LG.15 (Chr.1 or Chr.15) (Figure 8A). A second QTL, which explain an additional 22% of the phenotypic variation in FGA, was identified on LG.D08 (Figure 8B). These two loci together explain 57% of the FGA phenotypic variation among lines. For VBI, a QTL with a large effect (29%) was also found in the same location on chromosome LG.15 (Figure 8C). A second QTL, which explain an additional 19% of the phenotypic variation in VBI, was identified on LG.D08 (Figure 8D). These two loci together explain 48% of the VBI phenotypic variation among lines.

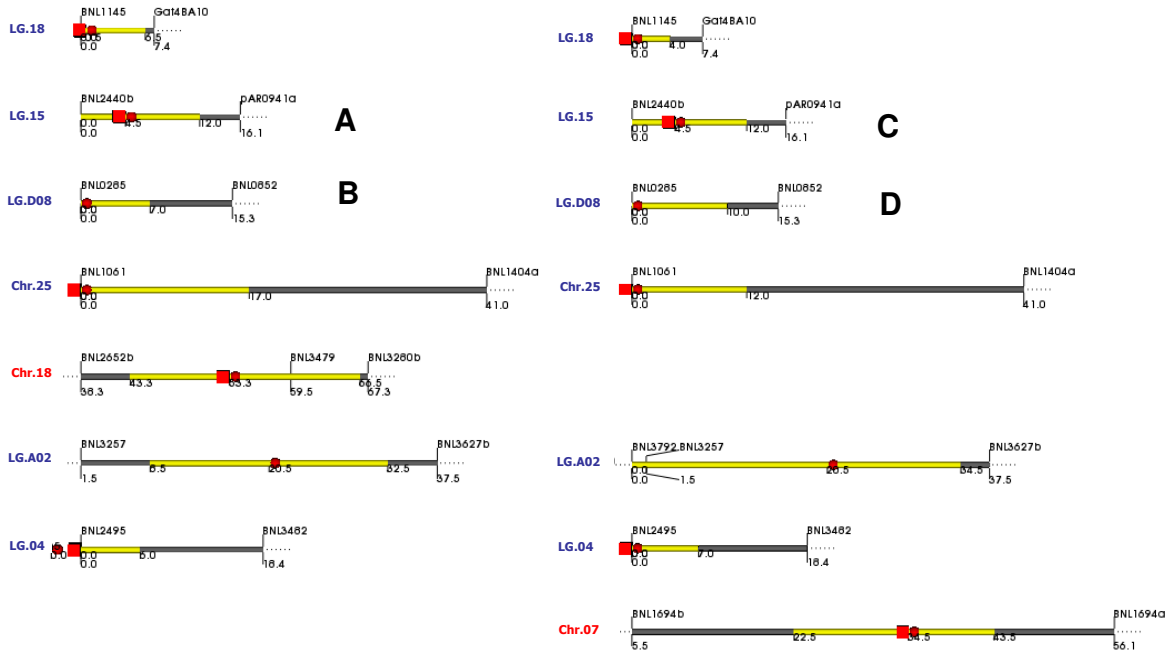

Figure 8: The location of QTL for FGA and VBI on chromosome LG.15 and LG.D08. The yellow area is significant (95%) by permutation test.

5. Conclusion

The CSIRO cotton breeding program has been careful with strategic planning to ensure we have options for future industry requirements. We have ambitious targets for yield, fibre quality and disease resistance - innovation and ideas are being applied in conventional breeding and biotechnology to address these targets.

We have made good progress with molecular markers in CSIRO germplasm to address Australian issues: Eight Quantitative Trait Loci have been identified on two specific chromosomes for Fusarium resistance. We will exploit these discoveries in the breeding program. Of importance is that these discoveries can be applied to many more traits than Fusarium resistance.

With variety releases in the past three years (36 in total), we have introduced high Fusarium resistance (Sicot F-1, Sicala 45, Sicot 14B); Bollgard®II (a large suite including Sicot 71B and Sicot 71BR); a fuller suite of Roundup Ready (Sicot 71RR, Sicot 80RR); premium fibre (Sicala 350B); and limited RRFlex release (Sicot 80BRF, Sicala 60BRF, Sicot 43BRF).

We have continued to discover better combinations of yield, fibre quality and disease resistance in conventional germplasm. Sicot 75 is one example and this will provide a new base for future conventional and transgenic varieties. CSIRO cotton varieties have increased yield by an average of 1.96% per year in the past 25 years (Figure 9). Recent releases have maintained that progress and our objective is to maintain or even increase that rate of increase.

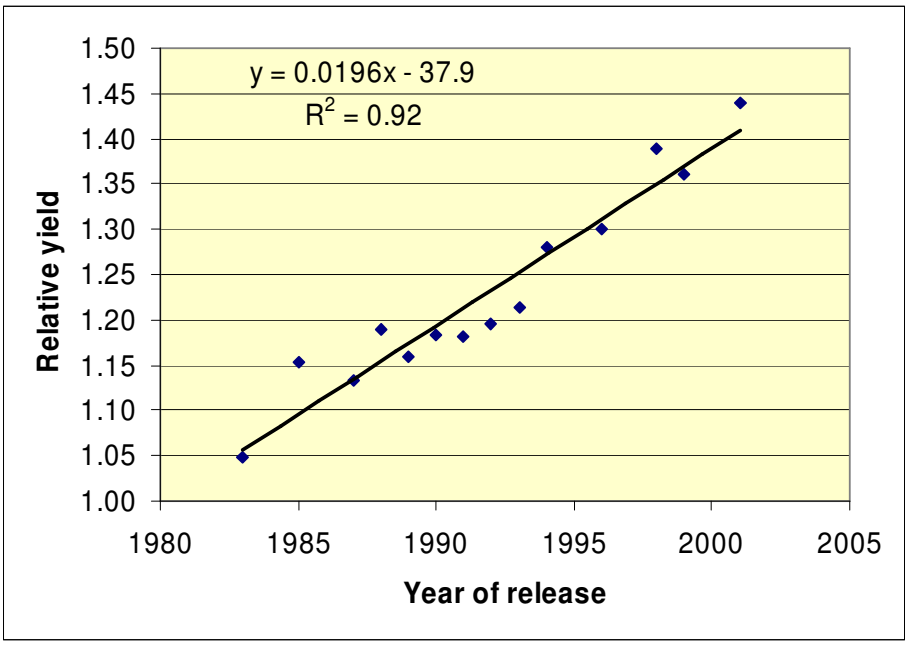

Figure 9. Yield progress with release of CSIRO cotton varieties. Each point is a new variety and is the mean yield from ten sites in each of three years compared with DP16 and Namcala in the same experiments. Conventional varieties only; the last point in 2002 is Sicot 71.

Staff
Narrabri: Greg Constable, Peter Reid, Warwick Stiller, Lindsay Heal, Chris Tyson, Kellie Cooper, Max Barnes, David Shann, Marilyn Smith, Terry Murphy, Tricia Hadley, Kay Smith, Chris Allen, Sandra Magann.
Canberra: Augusto Becerra, Curt Brubaker, Bronwyn Matheson, Walter Tate.

Extension opportunities
Cotton breeders participate in many extension activities, especially in promotion of new varieties and their management. Grower adoption of our research is in adoption of our varieties. CSIRO currently has a 91% market share in Australia.

Publications
Reid, P., Allen, S., Kochman, J., Stiller, W., McNamara, G., Lehane, J. and Constable, G. (2004). Sicot F-1: a variety with increased resistance to Fusarium wilt. In: Quality Cotton - A Living Product, Not Just Another Yarn, Proceedings 12th Australian Cotton Conference (Gold Coast, 10-12 August, 2004) 2 pp
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8b. Variety releases during CSP 159C.

2003	2004	2005
Sicala 45 a normal leaf variety with medium maturity and a Fusarium resistance rank better than Sicot 189. **Siokra V-18** a medium maturity okra leaf, compact growing variety with large bolls and improved Fusarium resistance rank. **Sicot 11B** (Bollgard® II) a relatively early maturing compact plant type with larger bolls than other Bollgard II varieties available in 2003. **Sicot 12B** (Bollgard® II) an early to medium maturing variety with intermediate growth habit and small to medium sized bolls. **Sicot 13B** (Bollgard® II) a medium maturing taller plant type with average boll size. It has good yield potential but low Fusarium resistance ranking and should therefore be grown only in regions free of Fusarium Wilt such as Tandou, lower Namoi and Emerald. **Sicot 14B** (Bollgard® II) a medium maturing compact plant type with average boll size. Very good Fusarium resistance. In longer season locations, agronomic management should ensure full plant size is obtained.	**Sicot F-1** a full season variety with very high Fusarium resistance. It has vigorous growth habit and is targeted at fields with high incidence of Fusarium. **Sicot 73** a broadly adapted full season type with high yield potential. It has longer staple than Sicot 71 and more vigorous growth habit. **Siokra 24** a full season okra leaf variety with vigorous growth habit, with long and fine fibre. It is best adapted to early dryland sowings and for central Qld irrigated areas. It has much improved Fusarium resistance compared with Siokra V-16. The okra leaf trait provides some pest resistance, particularly mites. **Sicot 289RR** a full season variety with vigorous growth habit, adapted to central, western and northern production areas. It has slightly improved yield and Fusarium resistance to Sicot 189RR. **Sicot 60RR** a medium season cultivar, best suited to central and southern production areas. It has improved Fusarium resistance compared with Sicala V-2RR. **Sicot 289BR** a full season variety with vigorous growth habit best suited to central, western and northern production areas. It has good yield potential and a good disease resistance package. **Sicot 71BR** a full season variety with compact growth habit suited to most production areas. It has high yield potential and a good disease resistance package **Sicala 60BR** a medium season variety adapted to central, southern and eastern production areas. It has good disease resistance and good fibre quality. **Sicala V-3BR** a medium season variety with compact growth habit best suited to southern and eastern production areas. **Siokra V-16BR** a full season okra leaf variety for dryland production systems free of Fusarium wilt. The okra leaf trait provides some pest resistance, particularly mites. **Sicala 40BR** a short season variety with compact growth habit for southern and eastern production areas. It has good fibre quality. **Sicot 289B** a full season variety with vigorous growth habit best suited to central, western and northern production areas. It has good yield potential and a good disease resistance package. **Sicot 80B** a full season variety with	**Sicot 71B** a full season variety with compact growth habit suited to most production areas. It has high yield potential and a good disease resistance package **Sicot 71RR** a full season variety with compact growth habit and good yield potential suited to most production areas. It has a good disease resistance package **Sicot 43BR** a medium maturing normal leaf variety with good fusarium tolerance. **Sicot 43B** a medium maturing normal leaf cultivar **Sicot 43RR** an early to medium maturing variety best suited to southern and eastern districts. **Sicot 80RR** a full season variety with vigorous growth habit and long fine fibres. Well suited to dryland systems.

	vigorous growth habit suited to central, northern and western production areas and for dryland systems. It has a good disease resistance package. **Siokra V-18B** a medium maturing okra leaf variety best suited to southern and eastern production areas. Disease resistance and fibre quality packages are good. The okra leaf trait provides some pest resistance, particularly mites. **Sicala 40B** a short season variety with compact growth habit for southern and eastern production areas. It has good fibre quality. **Siokra V-16B** a full season okra leaf variety for dryland production systems free of Fusarium wilt. The okra leaf trait provides some pest resistance, particularly mites.	

Part 4 – Final Report Executive Summary

The overall objective of this project was to develop improved conventional and transgenic cotton varieties adapted to Australian growing conditions and producing fibre suitable for our markets. The project comprised key components of conventional breeding and development of markers for future use in marker-assisted breeding.

Conventional breeding experiments and line evaluation are done in all major production regions in Australia; our mobile sowing and harvesting equipment is used at 15 sites and comprises more than 20,000 yield plots each year. New elite lines have been identified with increased yield potential, improved fibre properties and increased resistance to diseases such as Fusarium wilt.

Up to eight Quantitative Trait Loci have been identified for resistance to Fusarium wilt. These results are being used to develop molecular markers for Fusarium resistance to enable quicker and more accurate breeding and development of new varieties. At present there are 9,000 plots per year in field Fusarium nurseries with variable success; markers would enable this process to be improved at cheaper cost.

There have been 36 new varieties released in the past three years. We have introduced high Fusarium resistance (Sicot F-1, Sicala 45, Sicot 14B); Bollgard®II (a large suite including Sicot 71B and Sicot 71BR); a fuller suite of Roundup Ready (Sicot 71RR, Sicot 80RR); premium fibre (Sicala 350B); and initial limited RRFlex release (Sicot 80BRF, Sicala 60BRF, Sicot 43BRF).

We have continued to discover better combinations of yield, fibre quality and disease resistance in conventional germplasm. Sicot 75 is one example and this will provide a new base for future conventional and transgenic varieties. Thus the 1.96% improvement in yield potential due to breeding from previous years will continue. Yield increases are needed to help keep cotton growers remain viable.