

GENETIC CHARACTERISATION OF CHROMOSOME INHERITANCE IN *G. HIRSUTUM* X C GENOME ALIEN CHROMOSOME ADDITION LINES: FUSARIUM WILT RESISTANCE IN WILD AUSTRALIAN *GOSSYPIUM*

L.A. Becerra Lopez-Lavalle^{1,2}, H.G. McFadden² and C.L. Brubaker^{1,2}

Centre for Plant Biodiversity Research¹

CSIRO Division of Plant Industry, Canberra²

Introduction

In 2000/01, the Australian cotton industry produced approximately 3.4 million bales with a gross estimated value of 1.7 billion dollars. Although cotton production in Australia is efficient, diseases, such as Fusarium wilt, are causing severe production losses. Unfortunately, the incidence of Fusarium wilt in Australia has been increasing since it was identified in NSW during the 93/94 season (Kochman, 1995). The disease is now present in many commercial cotton fields in NSW and Queensland.

Although the resistance of elite Australian cotton cultivars is improving steadily, increasing the Fusarium wilt resistance of cotton cultivars remains a priority. While Australian cotton breeders are actively trying to enhance resistance to Fusarium wilt in their current breeding materials, they are also searching for novel sources of resistance genes. One possible source of new genes may be the native Australian *Gossypium* species, that are distant relatives of the cultivated cottons.

Preliminary surveys of the wild Australian *Gossypium* species suggest that some of the C and K genome species may be resistant to the two *Fusarium oxysporum* (*Fov*) pathotypes (VCG 0111 and 0112) affecting cotton cultivation in Australia (unpublished data). If this resistance proves to be robust, then the introgression of these genes from the wild species into the cultivated cottons depends on the generation of fertile hybrids and the frequency of genetic recombination (Stewart, 1995). Several studies have been made using Australian diploid species in breeding programs. Brubaker *et al.* (1999) evaluated the production of fertile hybrid germplasm with diploid species in the C, G and K genomes using tetra- and hexaploid bridging families. They concluded that the G genome species hybridise most readily with *G. arboreum* (A genome), while C and K genome species are more compatible with *G. hirsutum* (AD genome). However, all the intergenomic hybrids were sterile and colchicine treatment was needed to restore fertility before backcrossing to

G. hirsutum. The only exceptions were four *G. hirsutum* x K genome triploids, which exhibited limited female fertility when backcrossed to *G. hirsutum*. This low fertility was attributed to the occurrence of unreduced gametes. Brubaker *et al.* (1999) also obtained 18 new fertile synthetic polyploids and 23 self-fertile derivatives of 2 synthetic hexaploids. These fertile hybrids represent a possible avenue of introgression of genes from the wild Australian *Gossypium* species into cultivated cotton and justify further attempts to use hexaploid bridging families to access lines of potential utility (e.g. *Fov* resistance) for in future breeding efforts.

Materials and methods

Plant Material

The chromosomal locations of *G. sturtianum*-specific AFLP markers were determined using 13 *G. sturtianum* BC₁ multiple chromosome addition lines that were generated by crossing a synthetic *G. hirsutum* x *G. sturtianum* allohexaploid (Gos 5271) to *G. hirsutum* (CPI138969) to generate an AADDC pentaploid F₁ (Hyb 629) that contained a haploid component of the *G. sturtianum* chromosomes. This pentaploid was subsequently backcrossed to *G. hirsutum* (Sicala V2) to generate the 13 BC₁ multiple chromosome addition lines that contained the full *G. hirsutum* tetraploid genome complement accompanied by mixed subsets of the *G. sturtianum* haploid genome. These BC₁ multiple chromosome additions lines were subsequently backcrossed to *G. hirsutum* (Sicala V2) to generate 114 BC₂ multiple chromosome addition lines.

AFLP analysis

Genomic DNA was extracted from 100 mg of young leaves, preferentially taken from just below the shoot apex to reduce the level of recalcitrant compounds in the leaf tissue. The plant material was ground in a 1.5 ml microcentrifuge tube with a plastic pestle in liquid nitrogen. The powdered leaf material was resuspended in 1 ml of lysis buffer [100 mM Tris-HCl (pH 8.0); 1.4 M NaCl; 20 mM EDTA (pH 8.0); 2% (w/v) CTAB; 2% (w/v) PVP-40; 0.1% (w/v) DIECA; 0.1% (w/v) ascorbic acid; 0.2% (v/v) β -mercaptoethanol], incubated 20 min at 60°C, extracted at least twice with chloroform:isoamyl alcohol (24:1), and precipitated with isopropanol.

Amplified fragment length polymorphisms (AFLPs) were identified using 6 adapters (2 *Eco*RI, 2 *Pst*I and 2 *Mse*I) and 34 selective primers using 1 μ g cotton genomic DNA for each individual sample following Vos *et al.* (1995). The AFLPs were resolved on 6% denaturing polyacrylamide gels run at 50 watts using 1X Tris-Taurine-EDTA (TTE) buffer (10.8 g Trizma base; 3.6 g Taurine; 0.2 g Na₂EDTA2H₂O). The AFLP

autoradiograms were scored visually and data relating to presence or absence of polymorphic bands were entered into an Excel spreadsheet.

Genetic analysis of AFLP inheritance

The *G. sturtianum*-specific AFLP markers were putatively assigned to linkage groups based on the segregation of the markers among the 13 BC₁ *G. sturtianum* multiple chromosome addition lines. It was expected that the *G. sturtianum* chromosomes would be inherited largely unrecombined, and therefore genetically identifiable as sets of cosegregating loci, identified by manually sorting the data in an Excel spreadsheet (Brubaker and Brown, unpublished). Each co-segregating set of loci was labelled alphabetically.

Fusarium wilt resistance in *G. sturtianum* x *G. hirsutum* lines

The synthetic *G. hirsutum* x *G. sturtianum* allohexaploid (Gos 5271) used to generate the chromosome addition lines and its parents (*G. hirsutum*, CPI138969, and *G. sturtianum*, Gos 5275) were germinated in the glasshouse and challenged with *Fov* by root-dip inoculation in a conidial suspension, two weeks after germination. Twenty uninoculated and twenty inoculated plants were used for each line. Host-pathogen resistance of each line was assessed using plant height (PH) (relative to the unchallenged control plants) and foliar disease rating. Similarly, 23 BC₂F₂ multiple chromosome addition lines were scored for Fusarium wilt resistance based on foliar symptoms (FS) and vascular browning index (VBI) using a standard 0-5 scoring system. Forty plants were inoculated for each multiple chromosome addition line, and the *G. hirsutum* parent (CPI 1389069) was included as the control.

Results

AFLP inheritance among the chromosome addition lines

The *G. hirsutum* and *G. sturtianum* AFLP inheritance was additive in the hexaploid (Gos-5271) and in the pentaploid (Hyb 629). A total of 2036 *G. sturtianum*-specific AFLPs were resolved, 861 of which segregated among the thirteen BC₁ chromosome addition lines. Segregation analysis identified 19 distinct sets of cosegregating markers, or linkage groups, designated A to S (Table 1). Because *G. sturtianum* contributed 13 chromosomes to the chromosome addition lines, the identification of more than 13 linkage groups suggests that at least some *G. sturtianum* chromosome restructuring has occurred. The complete absence of one large linkage group suggests that only 12 of the 13 *G. sturtianum* chromosomes are segregating in the chromosome addition lines.

Table 1. Distribution of cosegregating sets of *G. sturtianum* AFLPs among 13 BC₁ *G. hirsutum* x *G. sturtianum* chromosome addition lines.

Linkage groups	No. of AFLPs	BC ₁ Plants													No. of linkage groups per individuals
		645	651	685	686	687	710	711	712	734	735	736	741	742	
A	78														
B	9					X									1
C	36					X		X X			X X				5
D	40				X										1
E	57			X				X			X X				4
F	37			X X					X						3
G	46		X			X X X X					X X X				8
H	57		X X X				X				X X				6
I	59	X				X X X X					X X				7
J	67	X X				X X X X					X X				9
K	54	X X X				X				X					5
L	17	X X X					X								4
M	33	X X X X						X							5
N	49	X X X X					X X	X X X X							10
O	49	X X X X X							X X						7
P	6	X X X X X								X					6
Q	7	X X X X X					X X X				X X				10
R	36	X X X X X					X X X X X X				X X X				11
S	19	X X X X X					X X X X X X				X X X X				12
Totals	756	11	12	12	12	11	8	11	9	7	10	9	2	0	114

Table 2. AFLP inheritance among 114 BC₂ *G. hirsutum* x *G. sturtianum* from 11 BC₁ chromosome addition lines families.

Linkage groups	BC ₂ Plants													No. of linkage groups in BC ₁ s	BC ₁ %	No. of linkage groups in BC ₂ s	BC ₂ %
	645	651	685	686	687	710	711	712	734	735	736	741	742				
A														0	0	0	0
B					2									1	8	2	29
C					1			2		5				5	42	8	33
D														1	8	0	0
E			1				2		4	5				4	33	12	55
F		1	1					3						3	25	5	42
G					3	29	1	8		3		1		8	67	45	49
H			1	1		20				4	3			6	50	29	45
I					3	33	2			4	3			7	58	45	63
J	3				5	42		4		5	3			9	75	62	76
K	2		1		4				10					5	42	17	74
L			1				2							4	33	3	100
M	1		1				2							5	42	4	67
N			1	1		27	2	3	12	7	3			10	83	56	64
O	1		1	1	6			5	11					7	58	25	76
P			1		3				12					6	50	16	80
Q	1		1		3	38	2	5		6	4			10	83	60	71
R	2		1	1	7	45	2	8	10	7	3			11	92	86	98
S	3		1	1	7	50	2	9	11	7	3	1		12	100	95	92
No. of Chr BC ₁	11	12	12	12	11	8	11	9	7	10	9	2	0	114	8-92 (100)		
No. of Chr BC ₂	3-7	-	12	6	5-9	1-8	9	1-7	2-7	5-10	1-7	2	-			570	29-98 (100)
Ave	4.3	-	12.0	6.0	6.3	5.6	8.5	4.7	5.8	7.6	5.5	0.1	-				

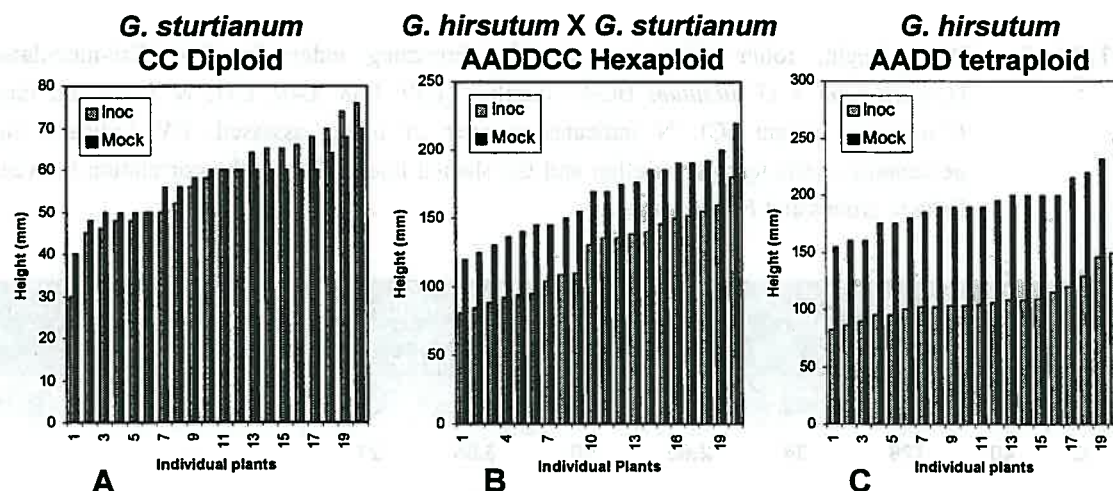
Frequency and fidelity of alien chromosome transmission

One hundred and fourteen BC₂ aneuploid families comprising eleven of the thirteen BC₁ aneuploid parental lines were evaluated to estimate the frequency and the fidelity of *G. sturtianum* chromosome transmission. Twenty-four *EcoRI/MseI* primer combinations were employed—577 *G. sturtianum*-specific molecular markers were screened. Segregation analysis suggests that each of these BC₂ families contains a subset of the *G. sturtianum* chromosomes in their respective BC₁ parent (Table 2).

Fusarium wilt resistance

The Fusarium wilt resistance assay of the *G. hirsutum* x *G. sturtianum* allohexaploid (Gos 5271) and its two parental lines suggested that *G. sturtianum* was markedly less susceptible to Fusarium wilt-induced stunting than *G. hirsutum* and that this effect was also evident in the *G. hirsutum* x *G. sturtianum* hexaploid, albeit at a level intermediate between *G. sturtianum* and *G. hirsutum* (Figure 1). This suggests that *G. sturtianum* has a heritable level of *Fov* resistance, but whether this reduced stunting translates into robust field resistance to *Fov* has not been confirmed and is the subject of ongoing studies.

Figure 1. Height distribution of parental (A & C) and synthetic allohexaploid *G. hirsutum* x *G. sturtianum* (B) plants. Light grey indicated height of *Fov*-inoculated plants; dark grey bars indicate height of un-inoculated plants.



Twenty-three *G. hirsutum* x *G. sturtianum* BC₂F₂ aneuploid families were tested for *Fov* resistance. The Hyb-710- derived families, L30, L51, and L56, performed better than the other 20 families based on plant height, foliar score, and vascular browning index (Table 3). While these results are not conclusive, they are promising, suggesting that *G. hirsutum* x *G. sturtianum* hybrids may offer improved resistance. The performance of these families will be tested further by examining the correlation between the occurrence of specific

G. sturtianum chromosomes within the Hyb-710 family and individual plant scores. In this preliminary analysis, *G. sturtianum* linkage groups J and S were shared by four of the five most resistant lines, suggesting that improved resistance originates from *G. sturtianum*, and that linkage groups J or S may be carrying the gene(s) of interest.

Discussion

Analysis of the distribution of *G. sturtianum*-specific AFLP markers among 13 BC₁ multiple chromosome addition lines revealed 19 sets of cosegregating *G. sturtianum*-specific AFLPs. Five of these linkage groups were small (<19 AFLPs), while 14 large linkage groups comprised 33 to 78 AFLPs. Brubaker and Brown (unpublished) indicated that the tertiary-gene-pool *Gossypium* species chromosomes would be "meiotically static" in the *G. hirsutum* background and inherited largely unrecombined, thus 13 *G. sturtianum* chromosomes should correspond to 13 *G. sturtianum*-specific AFLP linkage groups. This suggests that of the 169 *G. sturtianum* chromosomes available (13 chromosomes x 13 BC₁ individuals), 114 (68%) were transmitted to the first generation aneuploids (Table 2). The data presented in Table 1 also showed that one of the 13 chromosomes present in *G. sturtianum* was not transferred (linkage group A). The number of chromosomes present in these first generation aneuploids varied from two to twelve chromosomes, while the transmission frequency of the 19 linkage groups varied from 1 to 12.

Table 3. Plant height, foliar score and vascular browning index for five *Fov*-inoculated *G. sturtianum* x *G. hirsutum* BC₂F₃ families (L30, L38, L48, L51, & L56) and their *G. hirsutum* parent (C). N indicates number of plants assessed, CV indicates the percentage coefficient of variation and the shaded lines indicates the correlation between linkage groups and *Fov* resistance.

Family	Height			Foliar Score		Vascular Browning Index		Linkage Group									
	N	Mean	CV	Mean	CV	Mean	CV	G	H	I	J	N	Q	R	S		
C	40	129	34	2.65	50	3.55	21										
L30	40	144	37	1.60	70	2.70	42			x	x						x
L38	30	129	32	1.63	50	2.60	33										x
L48	29	131	33	1.55	63	2.76	32	x		x	x				x	x	
L51	36	145	37	1.53	73	2.36	53				x						x
L56	40	147	31	1.48	80	2.88	28				x						x

To establish the fidelity of the *G. sturtianum* chromosome transmission, 114 BC₂ lines were genotyped using 24 *EcoRI/MseI* AFLP primer combinations. The analysis of the distribution of *EcoRI/MseI* *G. sturtianum*-specific AFLPs among 114 BC₂ aneuploids revealed that of the approximately 925 *G. sturtianum* chromosomes available, 570 (62%) were transmitted to the second generation aneuploids (Table 2). More linkage groups were identified among the BC₂ individuals suggesting that further *G. sturtianum* chromosome fragmentation had occurred.

The relative rate of chromosome loss versus the frequency of homoeologous interactions between the *G. hirsutum* and *G. sturtianum* chromosomes is the critical parameter estimating the probability that resistance genes from *G. sturtianum* can be introgressed into cotton cultivars. In these first two generations, the level of *G. sturtianum* chromosome fragmentation suggests that some introgression may have occurred, but further genetic analyses are required to confirm this.

The increase in Fusarium wilt across the Australian cotton growing regions has increased interest in the genetics of Fusarium wilt resistance. Ongoing screening of the native *Gossypium* species (unpublished data) and the data presented here suggest that *G. sturtianum* may indeed represent a source of novel *Fov* resistance genes. However, the genetic analysis of the *G. hirsutum* x *G. sturtianum* chromosome additions lines highlights the difficulties in introgressing these genes into cotton cultivars. Over the next year, the priority is to identify specific *G. sturtianum* chromosomes that carry genes that improve the *Fov* resistance of *G. hirsutum*. If this is successful, more efficient introgression strategies can be employed. An alternative strategy that might be considered would be to identify the major gene(s) responsible for *G. sturtianum*'s superior resistance to *Fov* and introduced them into the commercial cotton cultivars using transformation technology.

Acknowledgment

We thank Greg Constable, Peter Reid, Warwick Stiller, and Dave Shann of the CSIRO Cotton Breeding Unit, Narrabri for increasing the *G. hirsutum* x *G. sturtianum* chromosome addition families. This research is funded by the Cotton Research and Development Corporation (CRDC).

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