Introduction of Australian Diploid Cotton Genetic Variation into Upland Cotton

L. Ahoton, J.-M. Lacape, J.-P. Baudoin, and G. Mergeai*

ABSTRACT

Genetic barriers often prevent exploiting diploid cotton germplasm for the improvement of tetraploid cotton. The objective of this study was to investigate mating schemes to achieve introgression of *Gossypium sturtianum* J.H. Willis and *G. australe* F. Muell diploid cottons into tetraploid *G. hirsutum* L. *Gossypium hirsutum* × *G. sturtianum* and *G. hirsutum* × *G. australe* hexaploids were backcrossed to *G. hirsutum* to produce BC_1 pentaploids and BC_2 and BC_1S_1 euploid and aneuploid plants. The use of *G. hirsutum* × *G. australe* pentaploids as male parent in backcrosses with *G. hirsutum* allowed the production of an important progeny of BC_2 self fertile plants that were euploid or carried one chromosome of *G. australe* in addition to the 52 chromosomes of *G. hirsutum*. The only two BC_2 fertile plants issued from *G. hirsutum* × *G. sturtianum* hybrids were monosomic addition materials. Both of them were obtained with the pentaploid as female parent in the backcross to cv. Stam F. These results confirm that cotton male gametes are more limited than female gametes in the number of supernumerary chromosomes they can carry. This provides a means of developing alien monosomic addition lines in the *G. hirsutum* background from all the diploid species of *Gossypium* whose chromosomes do not carry genes preventing their individual male transfer. The analysis of the monosomic addition plants produced from the *G. hirsutum* × *G. australe* hexaploid with simple sequence repeat (SSR) markers allowed us to distinguish seven lines carrying different single chromosomes of *G. australe*. These lines constitute valuable materials with which to carry out fundamental and applied genetic investigations.

Hexaploid F_1 hybrid cottons from crosses between tetraploid (4*x*; AADD) *G. hirsutum* and either diploid (2*x*,C_1C_1) *G. sturtianum* or (2*x*,G_2G_2) *G. australe* have desirable agronomic traits such as glandless-seed and glanded-plant, high fiber strength, improved lint fractions, cold and drought tolerance, and possible tolerance or resistance to certain plant pests (Brubaker et al., 1996; Demol et al., 1978; Muramato, 1969; Ndungo et al., 1988). These characters might be used for improving commercial cotton if they could be readily transferred to the genomes of the cultivated types. To reach this goal, hexaploids can be used either directly through recurrent backcrossing to the tetraploid parent (Brown and Menzel, 1950) or indirectly through the development of trispecific allotetraploid hybrids with A- or D-genome diploid bridging species (Deodikar, 1949). The trispecific pathway is interesting because in such allotetraploid combinations either the A or the D chromosomes have no autosyndetic partners and theoretically should pair with the chromosomes of the wild Australian species *G. sturtianum* and *G. australe* (Mergeai et al., 1998). However, the successful use of AADC or DDAC synthetic tetraploids requires a large effort to produce fertile progeny and to eliminate the undesirable genetic material contributed by the diploid donor and bridge species (Mergeai et al., 1997; Vroh bi et al., 1999). Although the frequency of homeologous recombination between the Australian chromosomes and the A or D chromosomes may be lower in bispecific derivatives than in trispecific derivatives, the bispecific pathway theoretically offers the possibility of generating more progeny in the same amount of time and, thus, to capture more homeologous recombination events. Moreover, in case of direct exploitation of bispecific hybrids through backcrossing the hexaploids to *G. hirsutum*, recombinant chromosomes are far more likely to be incorporated into fertile plants. Because of strong hybridization barriers in the first backcross generation (Dilday, 1986; Muramato, 1969; Altman et al., 1987), the direct exploitation of *G. hirsutum* × *G. sturtianum* hexaploids is likely to demand more effort than the use of *G. hirsutum* × *G. australe* hybrids whose pentaploid derivatives obtained by Brubaker et al. (1999) presented a rather good level of male fertility. The objective of this study was to investigate mating schemes to achieve introgression of *G. sturtianum* and *G. australe* diploid cottons into tetraploid *G. hirsutum*.

MATERIALS AND METHODS

First (6*x*/1)[1] and second (6*x*/2)[1] generation *G. hirsutum* × *G. sturtianum* hexaploids (G3542, G394)[2] and first (6*x*/1)[1] and second (6*x*/2)[1] generation *G. hirsutum* × *G. australe* hexaploids (G411, G430)[2] from Gembloux Agricultural University (GAU) cotton collection (Maréchal, 1983) were backcrossed at Gembloux, Belgium, in 1998 and 1999 to *G. hirsutum* cultivar Stam F originating from Togo, West Africa, to produce BC_1 pentaploid derivatives. The G354[2] and G394[2] *G. hirsutum* × *G. sturtianum* hexaploids contain the genomes of *G. sturtianum* accession G4[2] and of *G. hirsutum* cv. C2 (G107)[2] originating from the Democratic Republic of Congo. The G411[2] and G430[2] *G. hirsutum* × *G. australe* hexaploids contain the genome of *G. australe* accession G319[2] and of *G. hirsutum* cv. NC8 (G173)[2] originating from the Democratic Republic of Congo. The first pentaploids obtained from backcrossing these hexaploids to Stam F and the *G. hirsutum* × *G. sturtianum* pentaploids already available in the GAU collection (G235, G372)[2] were either selfed or backcrossed as male and female parent to Stam F to produce BC_1S_1 and BC_2 seeds at Gembloux in 1999.

L. Ahoton, Faculté des Sciences Agronomiques, Université Nationale du Bénin, B.P. 526, Cotonou, Republic of Benin; J.-M. Lacape, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Avenue Agropolis, F-34398, Montpellier Cedex 5, France; J.-P. Baudoin and G. Mergeai, Unité de Phytotechnie tropicale et d'Horticulture, Faculté Universitaire des Sciences Agronomiques, 2 passage des Déportés, B-5030 Gembloux, Belgium. Received 18 Jan. 2002. *Corresponding author (mergeai.g@fsagx.ac.be).

Published in Crop Sci. 43:1999–2005 (2003).

677 S. Segoe Rd., Madison, WI 53711 USA

[1] (6*x*/1), (6*x*/2): first and second generation of hexaploid after chromosome doubling.

[2] Accession numbers of seed stock in the GAU cotton collection.

Abbreviations: SSR, simple sequence repeat.

Table 1. Production of BC_1 seeds from synthetic allohexaploids.

Female parent	Male parent	Pollinations	BC_1 seeds	Seed pollination^{-1}	BC_1 seeds planted	BC_1 plants	BC_1 plants
		Number					%
G. hirsutum × *G. sturtianum* (6*x*)	*G. hirsutum* cv. Stam f	63	128	2.0	76	38	50
G. hirsutum × *G. australe* (6*x*)	*G. hirsutum* cv. Stam f	123	202	1.6	9	2	22

$G235^2$ and $G372^2$ pentaploids were obtained by backcrossing the same hexaploid ($G185^2$), issued from a cross between *G. sturtianum* $(G4)^2$ and *G. hirsutum* cv. C2 $(G107)^2$, with C2 and NC8, respectively. The BC_1 pentaploids and a portion of the BC_1S_1 and BC_2 hexaploid created in Belgium were grown at Cotonou, Republic of Benin, West Africa, from November 1999 to April 2000 to produce BC_1S_1, BC_1S_2, BC_2, and BC_2S_1 materials. A portion of some of the BC_2S_1 progenies from *G. hirsutum* × *G. australe* and *G. hirsutum* × *G. sturtianum* hexaploids were cultivated at Gembloux in 2000 and 2001. In Belgium, the new materials obtained in the framework of this work were planted each year in early May and cultivated year round in glasshouses under natural light conditions. In Cotonou, plants were cultivated in field conditions.

The backcrossing scheme was accomplished in the following manner. Flowers were emasculated the afternoon before anthesis and the stigma was covered by a small plastic bag. Pollen was applied to stigmas between 0800 and 1100 h the following morning. To avoid capsule shedding, a small piece of cotton wool containing a drop of the growth regulator solution (100 mg L^{-1} naphtoxyacetic acid + 50 mg L^{-1} gibberellic acid) recommended by Altman (1988) was applied on the ovary just after pollination. Self pollination was forced by clipping the flower bud at candle stage. Hybridization results were pooled by hybrid type to facilitate their interpretation and because no substantial variation among accessions of a same hybrid formula was evident.

Pollen grain fertility was assessed at Gembloux according to two methods, acetocarmine staining (15 g carmine L^{-1} of acetic acid) and the germination method proposed by Barrow (1981). For both methods, 1000 pollen grains produced from two freshly opened flowers were used. Only large, bright red grains were considered fertile when observed after 30 min in acetocarmine solution. Any evidence of pollen tube growth was used to identify fertile pollen grains.

Young flower buds were collected between 0800 and 1100 h according to weather conditions and fixed in fresh Carnoy solution (95% ethanol–chloroform–glacial acetic acid, 6:3:1, v/v/v). The fixing solution was replaced by 70% (v/v) ethanol after 48 to 72 h and the buds stored at 4°C until evaluated. Metaphase I squashes were obtained by macerating and grinding with a scalpel a few anthers in a drop of acetocarmine solution on a microscope slide, removing the debris, adding a cover slip, differentiating the chromosomes with mild heat and flattening pollen mother cells with pressure on the cover slip. Observations were made with a Nikon Eclipse E800 photomicroscope (Nikon, Tokyo, Japan) under oil immersion.

For all the plants analyzed with SSR markers, DNA extractions were performed at Gembloux by the protocol developed by Vroh bi et al. (1996). The SSR markers used to characterize the hexaploid *G. hirsutum* × *G. australe* (G411), its parents, and part of its BC_2S_1 progeny were derived from a repeat-enriched cotton genomic library developed by B. Burr at Brookhaven National Laboratory (Upton, NY). Clone sequences used for primer construction are available at http://demeter.bio.bnl.gov/acecot.html; verified 2 June 2003. The SSR analysis conditions were as described in Risterucci et al. (2000), with a 5′ end labeling of the forward primer with γ-[^{33}P] ATP and a 55°C annealing temperature. The SSRs reported were initially chosen for their ability to yield polymorphic PCR products between the two parents of a 'Guazuncho 2' (*G. hirsutum*) × 'VH8' (*G. barbadense* L.) BC_1F_1 population, as well as for their mapping position on the tetraploid genetic map (Lacape et al., 2003). Each of the 13 pairs of homeologous A and D chromosomes of the map were represented by a minimum of three SSRs. Totally, 86 SSRs were tested on 20 DNAs including the first generation following chromosome doubling to form the *G. hirsutum* × *G. australe* hexaploid, 13 monosomic addition BC_2 lines of *G. australe* on *G. hirsutum*, *G australe* accession G319, as well as C2, NC8, and Stam F, and two BC_2S_1 plants carrying 25 bivalents and two univalents.

Table 2. Pollen fertility of synthetic hexaploid and BC_1 pentaploid hybrids.

Genotype	Stainable† pollen grains	Germinated pollen grains
	%	
G. hirsutum cv. Stam f	97.2	95.5
G. sturtianum	97.4	95.4
G. australe	96.3	96.0
G. hirsutum × *G. sturtianum* (6*x*)	70.2	75.6
G. hirsutum × *G. australe* (6*x*)	37.2	42.8
G. hirsutum × *G. sturtianum* (5*x*)	6.0	1.0
G. hirsutum × *G. australe* (5*x*)	15.6	19.7

† Acetocarmine stained pollen.

RESULTS

Obtaining BC_1 pentaploid seeds by backcrossing *G. hirsutum* × *G. sturtianum* and *G. hirsutum* × *G. australe* hexaploids with *G. hirsutum* was moderately difficult because these crosses resulted in an average of only 2 and 1.6 seeds, respectively, per pollination (Table 1). Both hexaploid hybrids showed a moderate level of male fertility (Table 2). However, a high proportion of the BC_1 seeds produced (50 and 78%, respectively) did not germinate or were empty, lacking a well developed embryo (Table 1).

The BC_1 pentaploids of both families grown to reproductive maturity were large robust plants, but to have enough material to carry out our hybridization program, we had to graft 10 scions from the first two *G. hirsutum* × *G. australe* pentaploid plants obtained in 1998 onto cv. NC8 rootstock. The estimates of male fertility using acetocarmine staining and pollen germination gave similar results (Table 2). With 15.6% of pollen grains stained and a pollen grain germination rate of 19.7%, the *G. hirsutum* × *G. australe* BC_1 pentaploid was more fertile than the *G. hirsutum* × *G. sturtianum* BC_1 pentaploid, whose pollen grain stainability and germination rates were only 6 and 1%, respectively. Among the pentaploids, only 0.3 BC_2 seeds were obtained per pollination when *G. hirsutum* was used as male parent in the backcross. When the pollen of the pentaploids was used in the backcross to Stam F, seed production

Table 3. Production of BC_2 and BC_1S_1 seeds from synthetic BC_1 allopentaploids.

Female parent	Male parent	Pollinations	BC_2 or BC_1S_1 seeds	BC_2 or BC_1S_1 seeds pollination^{-1}	BC_2 or BC_1S_1 seeds planted	Established plants from BC_2 or BC_1S_1 seeds		Self fertile plants from established BC_2 or BC_1S_1 plants	
		Number				Number	%	Number	%
G. hirsutum × *G. sturtianum* (5*x*)	*G. hirsutum* cv. Stam f	435	139	0.32	56	21	38	2	9
G. hirsutum cv. Stam f	*G. hirsutum* × *G. sturtianum* (5*x*)	381	25	0.06	8	6	75	6	100
G. hirsutum × *G. sturtianum* (5*x*)	*G. hirsutum* × *G. sturtianum* (5*x*)	34	0	0.00	—	—	—	—	—
G. hirsutum × *G. australe* (5*x*)	*G. hirsutum* cv. Stam f	195	53	0.27	24	10	42	1	10
G. hirsutum cv. Stam f	*G. hirsutum* × *G. australe* (5*x*)	807	1043	1.29	366	316	86	310	98
G. hirsutum × *G. australe* (5*x*)	*G. hirsutum* × *G. australe* (5*x*)	104	11	0.11	11	3	27	0	0

was higher with the *G. hirsutum* × *G. australe* pentaploid (1.3 BC_2 seeds per pollination) than with *G. hirsutum* × *G. sturtianum* pentaploid (0.1 BC_2 seeds per pollination). The frequency of successful hybridization was consistent with the respective male fertility levels of both pentaploid hybrids (Table 3). No BC_1S_1 seed was produced on selfing the *G. hirsutum* × *G. sturtianum* pentaploids while a few were obtained when selfing the *G. hirsutum* × *G. australe* pentaploids (0.1 BC_1S_1 seeds per pollination). Because of the risk of after ripening seed dormancy, only BC_1S_1 or BC_2 seeds harvested at least 40 d before the sowing time were planted in Cotonou. The rates of adult plant establishment were much better with the BC_2 seeds obtained with *G. hirsutum* as the female parent (Table 3).

Among the BC_2 and BC_1S_1 progeny of the two allohexaploids, most of the self fertile plants were BC_2 materials produced with the pentaploid as male parent in the backcross with Stam F (Table 3). Only two fertile plants were obtained in the BC_2 progeny of the *G. hirsutum* × *G. sturtianum* pentaploid when it was used as a female parent. These two plants were phenotypically different from each other and from *G. hirsutum* (data not shown). The six other fertile *G. hirsutum* × *G. sturtianum* BC_2 plants, coming from the backcross of the pentaploids to Stam F, were phenotypically similar to *G. hirsutum*. The 311 self-fertile *G. hirsutum* × *G. australe* BC_2 plants were distributed in 18 distinct phenotypic classes. All the plants grouped in a class presented similar qualitative morphological traits (color and shape of the leaves, color of the flowers, relative position of the stigma and the staminal column, size and shape of the capsules). Most of the classes (17 out of 18) came from seeds produced using the pentaploids as male parent and one class came from the backcross to *G. hirsutum* of the pentaploid *G. hirsutum* × *G. australe* used as female parent. Among these 18 phenotypic classes, the one that presented by far the highest number of individuals (249 plants out of 311) showed a very high level of self-fertility (98% of the control seed production) and qualitative traits similar to those of *G. hirsutum*. Cytogenetic analysis performed in Belgium on the progeny of this class confirmed the euploid nature of these plants ($2n = 4x = 52$ chromosomes). The frequency of appearance and the fertility of the 17 other phenotypic types were variable. The phenotypic segregation observed in the progeny of 13 of the 18 BC_2 phenotypic classes was coherent with the distribution that is expected to be obtained from monosomic addition stocks. Indeed, in the progeny of these 13 phenotypic classes, three types of individuals were found almost systematically: (i) materials that were phenotypically similar to their mother plant (i.e., putative $4x + 1$ monosomic addition plants with 53 chromosomes), (ii) individuals with a very restricted level of fertility (sterile or producing less than five seeds per plant) showing an accentuation of some of the mother plant traits (i.e., putative $4x + 2$ disomic addition plants, with 54 chromosomes), and (iii) individuals totally similar to *G. hirsutum* (i.e., putative 4*x* euploid plants, with 52 chromosomes). The cytological observations performed in Europe on the progeny of these materials confirmed the presence of one additional alien chromosome in all the putative monosomic addition stocks (Fig. 1, Table 4). These 13 monosomic addition lines were designated by G_2 followed by a Latin number from I to XIII.

Among the 86 SSRs used to confirm the origin of the supernumerary chromosome of the 13 monosomic addition lines, we found 26 monomorphic SSRs, 28 SSRs from *G. australe* that were absent in all monosomic addition lines, and 32 SSRs that revealed the presence of a *G. australe* specific allo-allele present in at least one monosomic addition line (Fig. 2, Table 5).

The presence of *G. australe* specific SSRs in the monosomic addition lines and the fact that these markers were mapped and assigned to chromosomes or homeologous chromosome pairs of the tetraploid genome led us to infer specific chromosomal assignments for each of the monosomic addition lines. Among the 13 monosomic addition lines we isolated in the BC_2 progeny of *G. hirsutum* × *G. australe* hexaploid, homeologies were

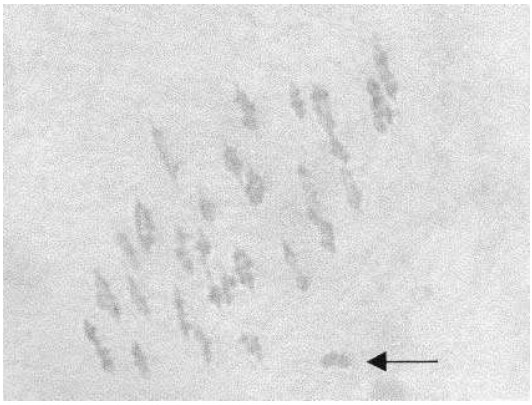

Fig. 1. Meiotic metaphase I cell from plant $G_2V(1)$ showing 26 bivalents and one univalent (arrow head).

Table 4. Results of the cytological observations carried out in the BC_2S_1 progeny of the *G. hirsutum* × *G. sturtianum* and *G. hirsutum* × *G. australe* hexaploids on plants showing the same phenotype as their BC_2 parents.

Genotype†	Chromosome	Univalent	Bivalent	Observed cells
	Number			
C_1I (1)	53	1	26	5
C_1II (1)	53	1	26	6
G_2I (1)	53	1	26	37
G_2II (1)	53	1	26	7
G_2III (1)	53	1	26	34
G_2IV (1)	53	1	26	6
G_2V (1)	53	1	26	28
G_2VI (11)	53	1	26	29
G_2VII (2)	53	1	26	5
G_2VIII (1)	53	1	26	39
G_2IX (2)	53	1	26	31
G_2X (2)	53	1	26	5
G_2XI (3)	53	1	26	35
G_2XII (1)	53	1	26	6
G_2XIII (2)	53	1	26	32

† The plants on which cytogenetic analysis were carried out are designated by the symbol of the phenotypic families, followed by the identification number of the plants analyzed in the BC_1S_1 progeny put between parentheses.

found with eight distinct linkage group pairs of the tetraploid genetic map of Lacape et al. (2003). The supernumerary *G. australe* chromosomes of lines G_2I, G_2V, and G_2VI present homeology with c10-c20, c12-c26, and c3-c17 linkage groups respectively. The line G_2XII shows specific *G. australe* amplicons that are homeologous with markers mapped on c5-D04 and A01-c18 linkage groups. The two SSR (BNL3029 and BNL852) mapped on c5 and D04 are separated by less than 10 centimorgans (cM) while the three SSR of the A01-c18 pair cover about 75% of the length of these chromosomes (150 out of 200cM) (Lacape et al., 2003). The G_2XII monosomic addition line carries thus the *G. australe* chromosome homeologous to A01-c18 linkage groups and has been introgessed by a fragment of the *G. australe* chromosome homeologous to c5-D04 pair. The other monosomic addition lines can be assigned to three groups according homeologies inferred by SSRs. Lines G_2IV and G_2XI carry the same supernumerary chromosome of *G. australe* that is homeologous to c7-c16 pair. The *G. australe* specific amplicon corresponding to BNL3008 is not present in line G_2XI; this could mean that a part of the *G. australe* supernumerary chromosome was deleted in this line. Lines G_2II, G_2VII, G_2IX, and G_2X carry the same *G. australe* chromosome homeologous to c9-c23 pair (about half of the length of the chromosome is covered by the four SSR markers) (Lacape et al., 2003). For the line G_2IX, the presence of three other SSR markers corresponding to the A02-D03 pair, equally covering an important length (130 out of 225 cM) does not allow to conclude about the type of genetic material exchange that occurred in this material (addition, substitution or recombination). In line G_2X the *G. australe* specific amplicons corresponding to SSR markers BNL1317 and BNL4053 are not present. This may be due to the deletion of a portion of the supernumerary chromosome of *G. australe* in this line. Lines G_2III, G_2VIII, and G_2XIII show four SSR markers covering the total length of a *G. australe* chromosome that is homoleogous to c6-c25 pair (Lacape et al., 2003). In

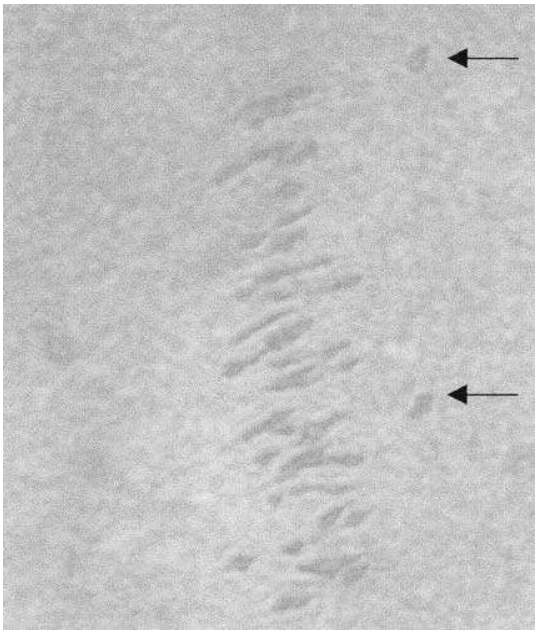

Fig. 2. Meiotic metaphase I cell from plant G_2XI (intro 2) showing 25 bivalents and two univalents (arrow heads).

the line G_2VIII, one can observe an additional homeology with c5-D04 pair (three SSR covering 75cM in the central part). The case of marker BNL3436, mapped on c25 and present as expected on lines G_2III and G_2VIII but also on line G_2V (homeologous to c12-c26) is probably due to confusion between the alleles of two duplicated loci of same migration (homoplasy). Two plants issued from line G_2XI and carrying two univalents (Fig. 3) were designated by symbols G_2XI(intro1) and G_2XI(intro2) because they were thought to be introgressed by fragments of the supernumerary chromosome of G_2XI line. These two plants showed systematically *G. australe* markers found on the line G_2V (addition of the homeolog of c12-c26 pair) on the one side, and on lines G_2II, G_2VII, and G_2IX (addition of the homeolog of c9-c23 pair), on the other side. These data let us suppose that the plants G_2XI(intro1) and G_2XI(intro2) have been the object of a double substitution by two *G. australe* chromosomes homeologous to *G. hirsutum* chromosomes c9-c23 (4 SSR covering about the half of the chromosome length) and c12-c26 (5 SSR covering 130 out of 180 cM). Another hypothesis explaining these results is the substitution of a chromosome of *G. hirsutum* by the homeolog of c12-c26 pair and the recombination of a segment of another chromosome by the homeolog of c9-c23 pair. In both cases, a natural cross must have occurred in Benin between the plant of G_2XI line that produced these two genotypes and another plant carrying these chromosome fragments in its genome.

The SSR data put in evidence that some of the 13 monosomic addition lines identified in the BC_2 progeny of the *G. hirsutum* × *G. australe* hexaploids carries the same supernumerary *G. australe* chromosome and that some lines were introgressed by fragments of two distinct alien chromosomes. The seven groups for which

Table 5. Polymorphism observed for 32 *G. australe* specific mapped SSR markers in the 13 monosomic addition lines issued from *G. hirsutum* × *G. australe* hexaploids and in two plants presenting 25 bivalents and 2 univalents (G_2XI intro1 and G_2XI intro2).

SSR	Linkage groups	Loci No.	G_2 I†	G_2 II	G_2 III	G_2 IV	G_2 V	G_2 VI	G_2 VII	G_2 VIII	G_2 IX	G_2 X	G_2 XI	G_2 XII	G_2 XIII	G_2 XI intro1‡	G_2 XI intro2‡
3563	c10	2	X														
3838	C20	1	X														
946	C20	2	X														
3031	c09-c23	2		X					X		X	X				X	X
2847	c09	1		X					X		X	X				X	X
1317	c09-c23	2		X					X		X					X	X
4053	c09bot-c23	2		X					X		X					X	X
2569	c06	2			X										X		
3436	c25	2			X		X			X						X	X
3103	c25	2			X					X					X		
2884	c06	1			X					X					X		
1604	c07	2				X							X				
1694	c07	2				X							X				
3008	c16	1				X											
1045	c12-c26	2					X									X	X
3537	c26	1					X									X	X
1673	c12	1					X									X	X
3599	c12-c26	2					X									X	X
3261	c12	>2					X									X	X
834	c03-c17	1						X									
3259	c03-c17	2						X									
3029	c05sup-D04	2												X			
852	c05sup-D04	2												X			
2448	c05-D04	2								X							
3992	c05	>2								X							
1671	D04	1								X							
3556	A02	2									X						
3474	A02-D03	2									X						
1646	A02-D03	2									X						
2571	A01-c18	2												X			
3479	c18	2												X			
2652	A01-c18	2												X			

† G_2I to G_2XIII: symbolic representation of the 13 different monosomic addition lines isolated from *G. hirsutum* × *G. australe* pentaploid.
‡ G_2XI intro1 and G_2XI intro2: sympolic representation of two plants issued from the line G2XI presenting signs of introgression.

the additional chromosome of *G. australe* has been assigned unequivocally to a pair of *G. hirsutum* homeologs were designated G_2, followed by a capital letter (from A to G): G_2A (line G_2I), G_2B (lines G_2II, G_2VII, and G_2X), G_2C (lines G_2III and G_2XIII), G_2D (lines G_2IV and G_2XI), G_2E (line G_2V), G_2F (line G_2VI), and G_2G (line XII). The latter was also introgressed by a small fragment of the *G. australe* homeolog to c5-D04 pair. The line G_2IX, present *G. australe* specific SSR amplicons mapped on c9-23 and A02-D03 pairs of *G. hirsutum*. It is probable that this line carries a complete supernumerary chromosome of *G. australe* and that it has also been introgressed by a large fragment of another *G. australe* chromosome at the pentaploid stage. It is however impossible with the data gathered so far to assign with certainty the homoelogies between the complete supernumerary chromosome and the introgressed fragment. The line G_2VIII is introgressed by large fragments of chromosomes homeologous to c6-c25 and c5-D04 pairs.

DISCUSSION

As observed by Dilday (1986), Koto (1989), Altman et al. (1987), and Brubaker et al. (1999), obtaining pentaploids from *G. hirsutum* × *G. sturtianum* and *G. hirsutum* × *G. australe* hexaploids is easier than producing aneuploid plants from these pentaploids. The success of the latter operation depending on whether the pentaploid plant is selfed or used as male or female parent in backcross with *G. hirsutum*. Our data confirm that the *G. hirsutum* × *G. australe* pentaploids are more fertile than those obtained from crossing *G. hirsutum* × *G. sturtianum*. We did not observe large differences in the number of seed per cross when we pollinated both pentaploids with *G. hirsutum* pollen, but the success rate when *G. hirstum* × *G. australe* pentaploid plants were used as male parent in backcrosses to *G. hirsutum* was better. Similar to Brubaker et al. (1999), we were not able to produce selfed seeds from *G. hirsutum* × *G. sturtianum* pentaploids but, unlike Koto (1989), we could with *G. hirsutum* × *G. australe* materials. The sterility barriers existing in our pentaploid hybrids seemed to be less important than in the materials used by Altman et al. (1987) and embryo rescue was not necessary to produce backcross progeny from both of them.

The use of *G. hirsutum* × *G. australe* pentaploids as male parent in backcrosses with *G. hirsutum* allowed the production of novel progeny, among which most of the plants exhibited good fertility levels. These self fertile plants were generally euploid ($2n = 4x = 52$) or carried one chromosome of *G. australe* in addition to the 52 chromosomes of *G. hirsutum*. Similar results were obtained by Koto (1983) with the *G. hirsutum* × *G. longicalyx* Hutch. & Lee pentaploid. On the contrary, the use of both *G. hirsutum* × *G. sturtianum* and *G. hirsutum* × *G. australe* pentaploids as female parent in backcrosses with Stam F or when self-pollinated gave rise to plants with low fertility levels. Only two fertile plants were produced from the *G. hirsutum* × *G. sturtia-*

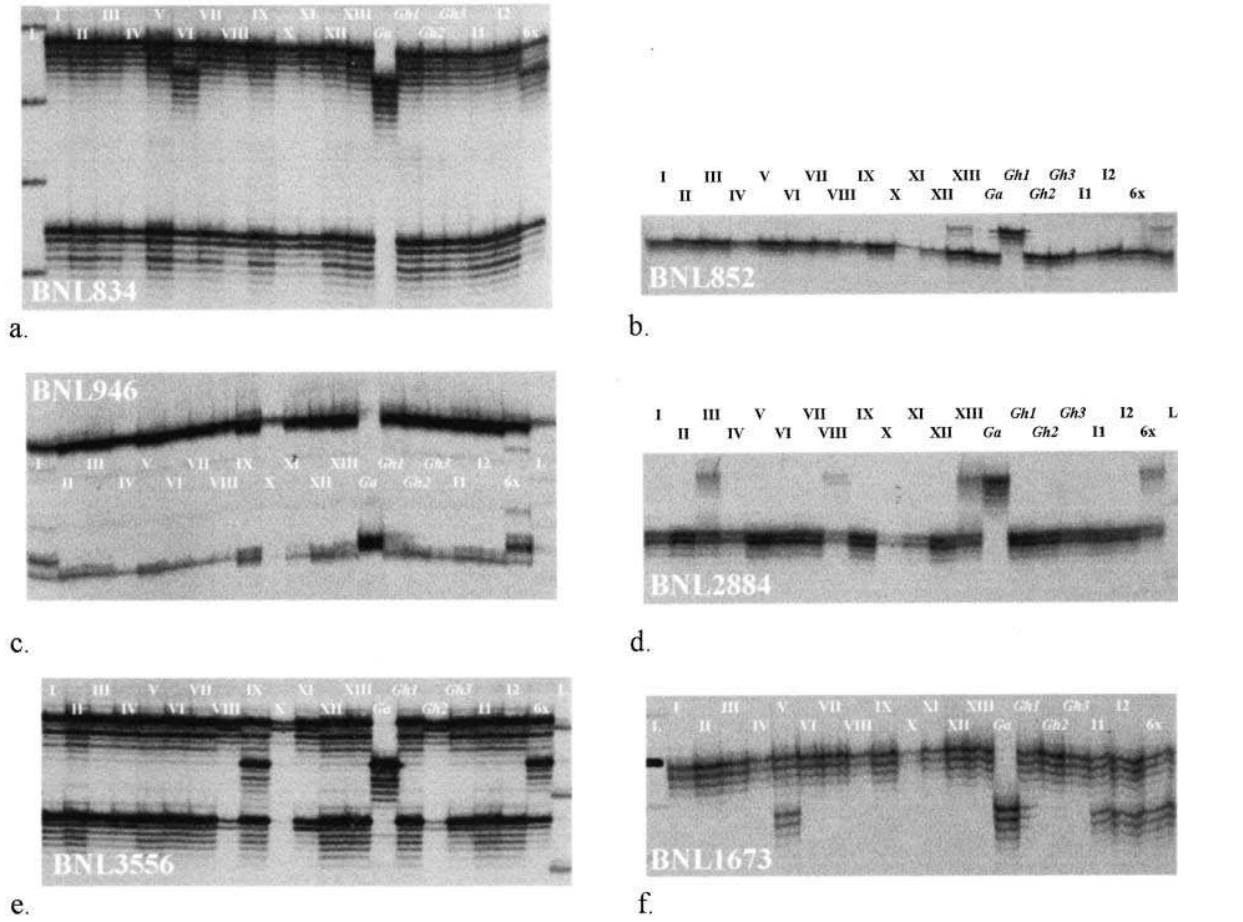

Fig. 3. ***Gossypium australe*** **specific amplicons polymorphism generated by six SSR markers in the hexaploid** ***G. hirsutum*** **×** ***G. australe*****, its parents and the 13 monosomic addition BC_2 lines issued from this hexaploid. I,II,III,IV,V,VI,VII,VIII,IX,X,XI,XII,XIII: phenotypic groups of the 13 monosomic addition plants analyzed.** ***Ga*****: *Gossypium australe*, 6x: *G. hirsutum* × *G. australe* hexaploid, *Gh1*: cv. Stam F *G. hirsutum*, *Gh2*: cv. NC8 *G. hirsutum*, *Gh3*: cv. C2 *G. hirsutum*. I1 and I2: G_2XI(intro1) and G_2XI(intro2) plants issued from line G_2XI carrying 25 bivalents and 2 univalents. a. BNL834: *G. australe* (*Ga*) co-allele present in line G_2VI, b. BNL852: *Ga* co-allele present in line G_2XII, c. BNL 946: *Ga* co-allele present in line G_2I, d. BNL2884: *Ga* co-allele present in lines G_2III, G_2VIII, and G_2XIII., e. BNL 3556: *Ga* co-allele present in line G_2XI,,, f. BNL 1673: *Ga* co-allele present in line G_2V.**

num pentaploid with the pentaploid as female parent and both of them were monosomic addition materials. The same observation was made by Poisson (1970), André and Verschraege (1984), Koto (1983), Altman et al. (1987), and Brubaker et al. (1999) with bispecific pentaploid hybrids involving *G. hirsutum* and B, C, E, G, or F genome diploid species. The autosterile plants obtained in the backcrossed progeny of these pentaploids generally carried several alien chromosomes. Our data indirectly confirm the better tolerance of female gametes to multiple alien chromosome addition in their nucleus. They also confirm the observation made by Hau (1981), Koto (1983), Poisson (1970), and Schwendiman (1978) on the better competitiveness of cotton male gametes carrying only one additional alien chromosome compared with pollen grains carrying several alien chromosomes. The behavior of *G. hirsutum* × *G. australe* hybrid did not conform to the general expectation that in cotton the best crossing successes are obtained when the highest ploidy material was used as the female (Beasley, 1941) and also did not reflect traditional recommendations concerning the critical role of ploidy ratio of endosperm and zygote for successful embryo development (Beasley, 1940; Stephens, 1942). Our results prove that the isolation of a large number of monosomic addition lines from a diploid species in a *G. hirsutum* background is possible. The use of an adequate growth regulator formula to prevent capsule shedding and an efficient embryo rescue technique may play an important role in success with the most recalcitrant hybrids.

The SSR markers have been very useful to confirm the chromosomic status of the different monosomic addition lines we isolated. Genomic homeologies between *G. australe* (genome G_2) chromosomes with those of *G. hirsutum* (genome A_hD_h) were revealed thanks to the SSR flanking sequences conserved in the two species. The SSR data showed that only seven *G. australe* chromosomes were added in single copies to the *G. hirsutum* genome among the 13 monosomic addition families we isolated in the BC_2 progenies of *G. hirsutum* × *G. australe* hexaploids. These genetic stocks constitute very valuable materials that can be used for fundamental and

applied genetic investigations. We plan to use them first to distinguish effects of specific alien chromosomes of *G. australe* and *G. sturtianum* and then to conduct chromosome specific introgression.

ACKNOWLEDGMENTS

This work was funded by the contract 2.4536.99 of the "Fonds de la Recherche fondamentale collective" from Belgium.

REFERENCES

Altman, D.W., D.M. Stelly, and R.J. Kohel. 1987. Introgression of the glanded-plant and glandless-seed trait from *Gossypium sturtianum* Willis into cultivated upland cotton using ovule culture. Crop Sci. 27:880–884.

Altman, D.W. 1988. Exogenous hormone applications at pollination for in vitro and in vivo production of cotton interspecific hybrids. Plant Cell Rep. 7:257–261.

André, B., and L. Verschraege. 1984. Amélioration du cotonnier *Gossypium hirsutum* L. par hybridation interspécifique: Utilisation de l'espèce *G. areysianum* Delf. Hutch. Bull. Rech. Agron. (Gembloux) 18:151–164.

Barrow, J.R. 1981. A new concept in assessing cotton pollen germinability. Crop Sci. 21:441–444.

Beasley, J.O. 1940. The production of polyploids in *Gossypium.* J. Hered. 31:39–48.

Beasley, J.O. 1941. Hybridization, cytology and polyploidy of *Gossypium.* Chron. Bot. 6:394–395.

Brown, M.S., and M.Y. Menzel. 1950. New trispecies hybrids in cotton. J. Hered. 41:291–295.

Brubaker, C.L., C.G. Benson, C. Miller, and D.N. Leach. 1996. Occurrence of terpenoid aldehydes and lysigenous cavities in the glandless seeds of Australian *Gossypium* species. Aust. J. Bot. 44:601–612.

Brubaker C.L., A.H.D. Brown, J. McD. Stewart, M.J. Kilby, and J.P. Grace. 1999. Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. Euphytica 108:199–213.

Demol, J., L. Verschraege, and R. Marechal. 1978. Utilisation des espèces sauvages en amélioration cotonnière. Observations sur les caractéristiques technologiques des nouvelles formes allohexaploïdes. Coton Fibres Trop. 33:327–333.

Deodikar, G.B. 1949. Cytogenetic studies on crosses of *Gossypium anomalum* with cultivated cottons. I. (*G. hirsutum* × *G. anomalum*) doubled × *G. hirsutum.* Indian J. Agric. Sci. 19:389–399.

Dilday, R.H. 1986. Development of cotton plant with glandless seeds and glanded foliage and fruiting forms. Crop Sci. 26:639–641.

Hau, B. 1981. Lignées d'addition sur l'espèce *G. hirsutum*. II. Description phénotypique de quelques lignées d'addition monosomique. Coton Fibres Trop. 36:285–296.

Koto, E. 1983. Tentative d'utilisation de l'espèce sauvage diploïde Gossypium longicalyx pour l'amélioration de l'espèce cultivée tétraploïde G. hirsutum L. par la méthode des lignées d'addition et de substitution. Ph.D. thesis. University of Orsay, France.

Koto, E. 1989. Tentative de transfert du caractère "retard à la morphogénèse des glandes à gossypol" I. Caractéristiques des hexaploides *G. hirsutum* × *G. sturtianum* et *G. hirsutum* × *G. australe*. *En* Tome I. 167–173. 1re Conférence de la recherche cotonnière africaine, Lomé, Togo. 31 jan.–2 fév., 1989. IRCT, Montpellier.

Lacape, J.-M., T.-B. Nguyen, S. Thibivilliers, B. Bojinov, E.C. Matz, M. Blewitt, B. Courtois, R.G. Cantrell, B. Burr, and B. Hau B. 2003. First combined RFLP-SSR-AFLP map of tetraploid cotton based on a *Gossypium hirsutum* × *Gossypium barbadense* backcross population. Genome 42:612–626.

Maréchal, R. 1983. A collection of interspecific hybrids of genus *Gossypium.* Coton Fibres Trop. 38:240–246.

Mergeai, G., J.P. Baudoin, and I. Vroh Bi. 1997. Exploitation of trispecific hybrids to introgress the glandless seed and glanded plant trait of *Gossypium sturtianum* Willis in *G. hirsutum* L. Biotech. Agron. Soc. Environ. 1:272–277.

Mergeai, G., I. Vroh Bi, J.P. Baudoin, and P. du Jardin. 1998. Use of randomly amplified polymorphic DNA (RAPD) markers to assist wide hybridization in cotton. p. 121–139. *In* Y.P.S. Bajaj (ed.) Biotechnology in Agriculture and Forestry, Vol. 42, Cotton. Springer Verlag, Berlin.

Muramato, H. 1969. Hexaploid cotton: Some plant and fiber properties. Crop Sci. 9:27–29.

Ndungo, V., J. Demol, and R. Maréchal. 1988. L'amélioration du cotonnier *G. hirsutum* L. par hybridation interspécifique. 3. Applications et résultats obtenus. Bull. Rech. Agron. (Gembloux) 23: 283–316.

Poisson, C. 1970. Contribution à l'étude de l'hybridation interspécifique dans le genre *Gossypium*: Transfert de matériel génétique de l'espèce sauvage diploïde *G. anomalum* à l'espèce cultivée *G. hirsutum*. Ph.D. thesis. University of Orsay, France.

Risterucci, A.M., L. Grivet, J.A.K. N'Goran, I. Pieretti, M.H. Flament, and C. Lanaud. 2000. A high density linkage map of *Theobroma cacao* L. Theor. Appl. Genet. 101:948–955.

Schwendiman, J. 1978. L'amélioration du cotonnier *Gossypium hirsutum* par hybridation interspécifique: Utilisation des espèces *G. barbadense* et *G. stocksii*. Ph.D. thesis. University of Orsay, France.

Stephens, S.G. 1942. Colchicine-produced polyploids in *Gossypium*. I. An autotetraploid Asiatic cotton and certain of its hybrid with diploid species. J. Genet. 44:272–295.

Vroh bi, I., A. Maquet, J.P. Baudoin, P. du Jardin, J.M. Jacquemin, and G. Mergeai. 1999. Breeding for "low-gossypol seed and high-gossypol plants" in upland cotton. Analysis of tri-species hybrids and backcross progenies using AFLP and mapped RFLPs. Theor Appl Genet. 99:1233–1244.